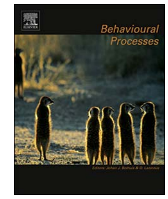
Behavioural Processes 163 (2019) 5–12

Contents lists available at ScienceDirect

Behavioural Processes

journal homepage: www.elsevier.com/locate/behavproc



Review

The sensitive period for auditory-vocal learning in the zebra finch: Consequences of limited-model availability and multiple-tutor paradigms on song imitation

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ARTICLE INFO

Keywords:
Songbird
Birdsong
Speech
Bilingual
Language
Taeniopygia guttata
Sensitive period
Critical period

ABSTRACT

Male zebra finches, *Taeniopygia guttata*, acquire their song during a sensitive period for auditory-vocal learning by imitating conspecific birds. Laboratory studies have shown that the sensitive period for song acquisition covers a developmental phase lasting from 25 to 65 days post hatch (dph); formation of auditory memory primarily occurs between 25 and 35 dph. The duration of the sensitive period is, however, dependent upon model availability. If a tutor is not available early in development, birds will learn from an adult male introduced to their cage even after they reach 65 dph. Birds who are exposed to a second tutor as late as 63 dph can successfully adjust their song 'template' to learn a new song model. However, if second-tutor song exposure occurs after 65 dph, learning of a new tutor's song will not occur for most individuals. Here, we review the literature as well as novel studies from our own laboratory concerning sensitive periods for auditory memory formation in zebra finches; these behavioral studies indicate that there are developmental constraints on imitative learning in zebra finches.

1. Introduction

Some behaviors are more likely to be learned during a 'sensitive' period early in development, such as binocular visual processing in felines, filial imprinting in geese, and auditory processing in mice (Barkat et al., 2011; Lorenz, 1937; Wiesel and Hubel, 1963). We can speak of a 'critical' period if the learning that takes place is essential for normal development and cannot be acquired later in life, such as with filial imprinting (Knudsen, 2004). Another example of behaviors learned during a sensitive period early in life are the vocalizations made by songbirds. Like humans, zebra finches (*Taeniopygia guttata*) are not born with innate songs, but have to learn their song by exposure to members of their own species (song 'tutors') (Immelmann, 1969; Marler, 1970; Thorpe, 1958). Learning happens during a sensitive phase in which juveniles first memorize the songs of adult conspecifics, and subsequently practice and develop their own song. When zebra finches start practicing their song, the elements are poorly formed and highly variable with little to no discernable song phrasing ('subsong') (Arnold, 1975; Immelmann, 1969; Zann, 1996). Around 50 days post hatch (dph) the variability in song elements begins to decrease and the song starts to resemble that of adult song ('plastic song') (Arnold, 1975; Immelmann, 1969; Zann, 1996). By 60 dph almost all of the elements of the bird's final song are recognizable but the order in which the elements (or 'syllables') are sung is still flexible, and by 90 dph the song contains elements that are sung in a stereotyped manner in regards to form and sequence (Arnold, 1975; Immelmann, 1969; Zann, 1996). While the normal song learning process has been well characterized at the behavioral level, few studies have explored flexibility in this process when the timing of availability of suitable song models is experimentally altered. Here, we review the developmental constraints on imitative learning in zebra finches, for both learning a single song and learning from multiple song tutors. We examine the data on sensitive periods for song learning, discuss the impact of social behavior on song learning, provide evidence for a sensitive period for learning a second song, and finish with ideas on how these studies may relate to sensitive periods for language acquisition in humans.

2. The sensitive period for song learning

While some bird species are capable of learning song throughout their life ('open-ended learners'), for many species, song learning occurs during a relatively brief period early in life ('closed-ended' or 'age-limited learners'). Both domesticated and wild zebra finches learn their song during a sensitive period for song acquisition that seems to occur

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http://dx.doi.org/10.1016/j.beproc.2017.07.007
Received 4 April 2017; Received in revised form 30 June 2017; Accepted 17 July 2017
Available online 23 July 2017

between 25 and 90 dph (Immelmann, 1969). No song learning occurs before fledging (around 20 dph); nestlings swapped between the nests of various males before the young reached 18 dph learned their song exclusively from their foster father (Arnold, 1975), and birds that were exposed to song recordings between 9 and 17 dph failed to recognize these songs in an operant procedure (Braaten, 2010). The sensitive period for song learning consists of two phases that overlap to some degree: the sensory memorization phase, during which an auditory model is formed, and the sensorimotor learning phase (Doupe and Kuhl, 1999; Immelmann, 1969; Liu et al., 2004). Because of this overlap it has been challenging to determine the boundaries for each phase of song development. Below, we discuss the outcomes of laboratory experiments that manipulate the availability of song models and what they reveal about the sensitive period for song learning.

After Immelman's 1969 study, one of the first experimental studies that investigated the sensitive period for song memorization was by Eales (1985), who removed juvenile male zebra finches from their fathers at 35, 50, or 65 dph, after which they were housed in single-sex groups of 6–10 juveniles and kept in visual and acoustic isolation from all adult song. Male zebra finches that were separated from their father at 35 dph were unable to develop normal song, with seven of the nine male isolates developing highly abnormal and unpatterned song, whereas males that were isolated at 50 or 65 dph developed typical song. The proportion of shared elements between father and son increased with the time spent together, with those that were isolated at 65 dph having learned all of their father's song. All of the males that were isolated at 35 dph were subsequently placed with normal adults at 6 months of age. These males, who up until this point sang abnormal isolate song, were able to modify their song to some degree into a species-typical pattern that consisted of normal song phrases, but a detailed quantification of these data was not provided by Eales (1985). In a separate experiment, females raised their clutches alone until 35, 50, or 65 dph, when the father was re-introduced to the cage. While birds that were with their father from 35 dph onwards produced better imitations of his song, birds that were housed with him later in development, either from 55 dph or 65 dph onwards, still learned significant parts of the father's song (Eales, 1987b). Thus, if a suitable tutor is not available between 35 and 65 dph, the sensitive phase can be extended until a suitable song model becomes available.

In subsequent studies, juvenile males were also deprived of a tutor between 35 and 120+ dph; these birds' adult songs consisted of elements of the father's song learned before 35 dph, and their songs were only slightly modified upon exposure to adult male tutors after 120 dph (Slater et al., 1992). It was even shown that juvenile male zebra finches are able to completely memorize their father's song by 35 dph (Böhner, 1990). Juvenile males who were not kept in social isolation but were deprived of an adequate song tutor between 35 and 65 dph had songs that were similar to those from the tutor to which they were exposed prior to 35 dph (Eales, 1989; Jones et al., 1996). It is possible that the earlier result by Eales (1985), which showed a lack of tutor-song learning before 35 dph, occurred due to the fact that the juveniles were housed in single-sex groups after separation from the father. Although Eales (1985) does not explicitly mention convergence in songs, it is possible that these birds influenced each other's song development, as untutored group-reared juveniles can imitate song elements from one another (Volman and Khanna, 1995). Unpublished data also shows higher similarity to the brothers' songs than to the tutor when juveniles were kept in same-sex sibling groups from 42 dph onwards ($N = 7$; similarity tutor: $18 \pm 5.2\%$, similarity brother: $48 \pm 13.8\%$; $t(6) = 6.04$, $p = 0.001$; data collected by SMHG at Utrecht University), or when clutches were manipulated in a way that only one brother had access to the song tutor between 35 and 42 dph (Deregnaucourt and Gahr, 2013). In addition, the completeness of song imitation is dependent on the number of male siblings in a clutch, with increased numbers of male siblings leading to shorter song motifs and decreased similarity to their father's song due to decreased copying of song syllables from the father (Tchernichovski and Nottebohm, 1998). Together, these studies show that experience with a song tutor before 35 dph is sufficient to copy the tutor's song and develop a species-specific song pattern, while the presence of brothers during the sensorimotor learning phase could interfere with accurate tutor-song imitation.

But how much exposure to song is necessary to imitate a song? By removing the father from the brood before fledging, around 12 dph, and then reintroducing the father between either 15–20 dph, 15–25 dph, 25–30 dph, or 25–35 dph, Roper and Zann (2006) investigated the onset and minimum duration of tutoring needed for song learning. Control birds were housed with their father between 15 and 35 dph as in the studies by Böhner (1983); Böhner (1990), Eales (1989) and Jones et al. (1996). Males who were exposed to their fathers from 25 to 35 dph produced songs with similarity to their fathers' songs significantly greater than those in any of the other treatment groups and learned a significantly greater portion of the more complex song elements. The level of song-copying exhibited by males that were tutored between 25 and 35 dph was not significantly different from that of the control subjects. Thus, juvenile males are able to learn song starting at 25 dph, and 10 days of experience with a tutor in their cage is sufficient to learn the father's song.

In addition, zebra finches that were raised in isolation and subsequently (from 30 dph onwards) restricted in the number of self-elicited song playbacks (max. 25 motifs per session with 2 sessions per day) learned more than zebra finches that had unlimited access to a song model (Tchernichovski et al., 1999). Remarkably, only 75 s of cumulative tutor song exposure over the course of a 2 h period is sufficient for initiation of the song learning process (Deshpande et al., 2014). Similarity scores of adult song in birds that only received a single tutoring session at 35 or 45 dph indicate significant learning as compared to isolates, but not when tutoring happened at 65 dph (Deshpande et al., 2014). Furthermore, the similarity scores of adult birds who were tutored at either 35 or 45 dph were positively correlated with the degree of vocal changes occurring on the day of training (Deshpande et al., 2014). Limited exposure to a song tutor early in development therefore seems sufficient for successful song imitation but is no longer sufficient for accurate song imitation towards the end of the sensitive phase.

While zebra finches are commonly regarded as closed-ended (or limited-time) learners, who sing a single stereotyped song in adulthood, increases in song stereotypy have been reported between 4 and 15 months of age (Pytte et al., 2007). This indicates that there is some plasticity for adult song, raising the question of whether the sensitive period for motor learning can be experimentally extended into adulthood. Exposure to loud noises, such as white noise, prevents birds from crystallizing their song, and can even disrupt adult song in a similar manner to that observed after deafening. In a study by Funabiki and Konishi (2003), juvenile zebra finches were reared with their fathers until 35 dph, at which time they were isolated and exposed to white noise until 102–200 dph. After cessation of white noise, the birds were able to develop a song that gradually increased in its similarity to the tutor's song, which indicates that a stored template of tutor song is encoded before 35 dph and can be utilized to direct song development at a stage that is well outside the normal period for sensorimotor learning.

Sensorimotor learning of a memorized tutor song may be delayed well past the closure of the typical sensitive period, but acquisition of auditory memory for a new song in adulthood appears to be limited. Juvenile zebra finches who had no experience of hearing song, and who were prevented from developing crystallized song until 137 ± 34 dph (range 101–200 dph) through exposure to white noise, did not learn the song of the tutor that they were exposed to after cessation of white noise (Funabiki and Funabiki, 2009). Similarly, when the songs from adult zebra finches were disrupted due to long-term exposure to white noise, birds did not learn or incorporate the syllables of a novel tutor song when presented with this song after cessation of white noise (Zevin et al., 2004). The recovery of pre-treatment song was also limited, although some aspects of song were re-acquired.

Although there appears to be a limit on an adult zebra finches' ability to acquire an entirely new song from an auditory model, adjustment of the existing motor patterns for song and incorporation of a small number of new song elements has been shown to occur in adulthood when birds are reared under conditions that are suboptimal for song learning. Zebra finches which were raised either in visual isolation from conspecifics or complete isolation were able to copy notes from a tutor which was presented at 120 dph (~2.7 notes per bird for complete isolates, ~4.4 notes per bird for visual isolates), despite the fact that they had already developed stable isolate songs (Morrison and Nottebohm, 1993). Elimination and modification of stable notes also occurred, with the songs of complete isolates containing fewer unusual isolate note types after tutoring (Morrison and Nottebohm, 1993). Another example of plasticity in vocal production has been described for song syntax, in birds that sing several repetitions of the same syllable in their motif ('serial repeaters' or 'stuttering'). In birds that were tutored by serial repeaters, and subsequently moved to an aviary room at 135–150 dph where they were exposed to the songs of non-repeaters, these tutored repeaters showed a progressive reduction in the mean number of repeated syllables per song motif, as well as a reduction in the variability of the number of repeated syllables per motif (Helekar et al., 2003). Vocal changes upon introduction to a common aviary (> 100 dph) have also been observed in other studies in which model availability was manipulated during the sensitive phase (Deregnaucourt et al., 2013; Gehrold et al., 2013). In summary, if a suitable tutor is available early in development, a tutor song template can be acquired as early as 25 dph and with minimal song exposure; some novel song models may still be copied at 120 dph, but the extent of imitation is quite limited. Modifications that only involve sensorimotor learning are somewhat more flexible even past 200 dph, although the flexibility in sensorimotor learning decreases over the normal time course of development (Pytte and Suthers, 2000).

3. Impact of social interaction on song learning

Field studies over three breeding seasons have shown that about 60% of juvenile zebra finches in the wild copy large parts of the song directly from their father (Zann, 1990). Zebra finches also prefer to learn the song of their rearing fathers in laboratory studies, even when cross-fostered by Bengalese finches (Immelmann, 1967, 1969). When deprived of the opportunity to hear adult conspecifics, they develop aberrant adult songs with self-improvised elements but with species specific temporal patterning (Immelmann, 1967, 1969). When juveniles raised in an aviary have the choice to learn from other adult males than their father, they preferentially learn from those tutors with whom they have a greater number of social interactions (Mann and Slater, 1995; Williams, 1990). Thus, social interaction with a song tutor early in development is critical for proper cultural transmission of birdsong, but how critical are the different aspects of social interaction, such as vocal or visual interaction, for the song imitation process?

The combinatorial effects of visual and vocal interactions on the outcome of the song imitation process were first studied by Eales (1989). Juvenile male zebra finches were isolated from their fathers at 35 dph and subsequently exposed to an unrelated tutor and his mate, allowing for either vocal and visual interaction, vocal interaction only, or no social interaction at all. In this experiment, one group of males had their tutor and his mate housed in an adjoining cage that was separated from them by a metal lattice allowing for both visual and vocal interaction to occur between the tutor and the juvenile males. A different group of males had an opaque screen separating them from their tutor, allowing for vocal but not visual interaction. In the last group, the tutor and his mate were kept in a separate soundproof box, and their vocalizations were relayed to the juvenile males through a microphone, amplifier and sound speaker. The juvenile males in this group could therefore hear their tutor but could not see or interact with him in anyway. All except one juvenile that could both see and interact with the tutor successfully learned his song, while juveniles in the other groups showed significantly less learning from the song tutor. Only half of juvenile males that could hear and interact with their tutor, but not see him, learned his song. The other birds in this group sang songs composed of elements of their father's song that were memorized before 35 dph (see also 'Learning from more than one tutor' below). Juvenile males that could hear their tutor but could not interact with him were unable to learn the tutor's song. Half of the males in this group sang songs that were comprised of elements from their father's song, while the other half sang songs that did not resemble the tutor's or their father's song. Similarly, when tutees were individually housed in soundproof chambers (vs. in groups with other juveniles as in the study by Eales, 1989), and could either interact vocally, or visually and vocally with their tutors, those juveniles that could see and hear their tutor produced better song imitations (Chen et al., 2016). Thus, visual and vocal interactions between the juvenile zebra finch and his tutor are essential for accurate tutor song imitation (Chen et al., 2016; Eales, 1989).

Social interaction could encourage the young bird to pay attention to the tutor's song, which positively contributes to the song memorization process. Attentiveness is significantly correlated with song learning: juvenile zebra finches that are more attentive to their tutors when their tutors sing produce better song imitations in adulthood (Chen et al., 2016). When juvenile zebra finches are quiet and awake but not involved in other activities when their tutors sing, they seem to pay more attention to their tutor's song; thus, social interaction may elevate selective attention aiding the acquisition of an auditory model (Chen et al., 2016; Houx and Ten Cate, 1998).

Studies that compare various methods of training young zebra finches have also shown that active involvement in the song learning process enhances the ability for juvenile zebra finches to form accurate song copies. In laboratory studies using taped song recordings to train juvenile zebra finches, juveniles which were given control (self-elicited) over the presentation of these songs developed songs that were more similar to the song stimulus than juveniles that were passively exposed to the recorded songs (Adret, 1993; Deregnaucourt et al., 2013). However, while self-elicited song exposure results in better imitation than passive song exposure, this does not supersede the benefits of having a live tutor, with live tutored birds exhibiting the most complete imitation (Deregnaucourt et al., 2013). Thus, attention and social interaction are closely linked and can enhance the song learning process.

Social setting and interaction do not only influence the extent of learning, but also affect how the song is learned. Zebra finches can utilize a variety of vocal developmental trajectories in order to generate a good imitation of their tutor's song (Liu et al., 2004; Tchernichovski et al., 2001). The two main strategies are (1) a serial repetition strategy, in which dissimilar syllables emerge from successive repetitions of a single syllable, and (2) a motif strategy, in which the juvenile male produces a rough global imitation of the tutor's song 8–10 days after the onset of subsong (Liu et al., 2004; Tchernichovski et al., 2001). Social setting and social interaction influence the type of vocal developmental trajectory that is employed: males from the same clutch often use different strategies in order to learn their tutor's song, possibly as a way to mitigate the inhibitory effects that another sibling's song might have on their ability to acquire an imitation of the tutor song (Liu et al., 2004; Tchernichovski and Nottebohm, 1998).

Additional evidence that social interaction is advantageous for song learning comes from cross-fostering experiments. Male zebra finches cross-fostered by Bengalese finches learned the song of their foster-father, but only if the interaction with the foster father was greater than with other male conspecifics in the colony (Eales, 1987a; Immelmann, 1969). If at 35 dph the juveniles were allowed full social contact with an adult male zebra finch, while the contact with the Bengalese finch foster father was decreased to visual and vocal interactions only, the songs of young males were equally likely to be influenced by the foster father as by adult conspecifics (Eales, 1987a). Zebra finches that were raised by their own species did not show this change in song tutor choice (Eales, 1987a). However, this preference for species-specific song after cross fostering by Bengalese finches may be due to innate limitations on song model selection, which favors zebra finch song based on the intersyllable silent gaps (Araki et al., 2016; Clayton, 1989). This raises the question of whether learning from a second song tutor from either the same or from a different species is limited by social interaction, innate constraints on coding for temporal aspects of song, or by the duration of the sensitive phase.

4. Learning from more than one tutor

In their natural environment, zebra finches are exposed to different songs by multiple potential tutors during development. The process of crystallization results in learning only one song, as reflected by the adult zebra finch singing a song that is highly similar to a single tutor (Zann, 1990). This may be due to the social interactions in the bird's natural environment and independent of neuronal constraints. Can multiple song models be learned during the sensitive period, and can exposure to multiple song models sequentially extend the sensitive period for song learning?

In order to test the timing of the sensitive period for song learning, male juvenile zebra finches were housed with their father from birth to 35, 50, or 65 dph (Eales, 1985). Some of the birds were then isolated entirely from adult song (see Section 2), and the rest were exposed to tutoring by a different adult male (Eales, 1985). The juveniles that were given a second opportunity for tutoring at 35 dph produced songs that contained no evidence of learning from the father and rather demonstrated learning from the second tutor to which they were exposed (Eales, 1985). On the opposite end, the juveniles that received the second tutoring opportunity at 65 dph showed no signs of learning from the second tutor and their songs indicated complete learning from their father (Eales, 1985). In contrast, the juveniles that were given the second tutoring opportunity at 50 dph learned from both their fathers and their second tutors. However, more was learned from the new models than from the fathers (Eales, 1985). Three separate experiments confirmed that juveniles learn more from tutors that they are exposed to later in development: by introducing a series of tutors for 5 days each between 35 and 65 dph (Slater et al., 1991), or by placing juveniles with three different sets of foster parents, from 0 to 35 dph, 35 to 70 dph, and 80 to 115 dph (Clayton, 1987), or 0 to 35 dph, 35 to 65 dph, and 65 to 100 dph (Slater and Richards, 1990). In the first experiment, it was found that juveniles preferentially learned from the tutors that they were exposed to between 55 and 65 dph (Slater et al., 1991). In the second experiment, when both the first and the second foster father were from the same species (ie. both zebra finch or both Bengalese finch), juveniles learned exclusively from the second tutor that they were housed with from 35 to 70 dph, while when the first two tutors were each from a different species, some of the juveniles learned from both tutors, resulting in an hybrid song, and none of the birds learned from their third tutor that was introduced at 80 dph (Clayton, 1987). The birds in the third experiment learned predominantly from their second tutor with no learning from the third tutor after 65 dph (Slater and Richards, 1990). It should be noted that song similarity in these studies was assessed by the author, or by an additional independent observer by manually comparing sonograms. As such, measures of imitation accuracy may have been less detailed than those achieved using current methods (Tchernichovski et al., 2000). In addition, there are innate limitations to copying songs from Bengalese finches (Araki et al., 2016). Similarity between the first and subsequent tutors was not assessed either, which may have limited the potential to learn from additional tutors. Despite the limitations, these results seem to suggest that the sensitive period may end at 65 dph, with peak sensitivity, and thus most optimal song learning, during the sensorimotor learning phase between 35 and 65 dph.

In another study, male zebra finches were exposed to their first tutor, either a male zebra finch or a male Bengalese finch, from birth to 30 dph, isolated for 30 days, exposed to their second tutor, a male zebra finch, from 60 dph to 90 dph, and then isolated for another 30 days (Yazaki-Sugiyama and Mooney, 2004). Some zebra finches were given the male Bengalese finch as a first tutor to serve as a control for the inherent similarities between zebra finch songs. At 60 dph, the tutees' songs, still largely in development, contained features of their first tutors' songs, indicating that the tutees were in the process of successfully learning from their first tutor. Furthermore, tutees' songs were naturally significantly dissimilar to the second tutor to which they would be exposed. After exposure to their second tutor, tutees' songs became gradually more similar to their second-tutors' songs and by adulthood (115 dph), the tutees' songs were more similar to the second-tutor songs than to the first-tutor songs. Similarity to the first-tutor song either decreased or stayed the same as a result of learning from the second tutor.

We performed a similar experiment with zebra finch tutors only, while replicating exposure to the two different song tutors between 0 and 32 dph (± 1.44 SD) and 60–90 dph (Fig. 1A; Olson et al., 2016). Similarity scores collected at both day 59 and day 91 showed similar behavioral results as seen in the study by Yazaki-Sugiyama and Mooney (2004). Overall, at 59 dph, tutee song was significantly more similar to first-tutor song as compared to the novel song (2nd tutor); at 91 dph, after exposure to both first- and second-tutor song, tutee song was significantly more similar to second-tutor song than at 59 dph, and there was a trend towards decreased similarity to first-tutor song as a result of exposure to a second tutor (Fig. 1A). In both experiments, zebra finches demonstrated learning from two different tutors and the hypothesized sensitive period for sensory exposure resulting in learning was extended beyond day 60, as indicated by significant learning from a second tutor introduced at day 60.

Although these studies have demonstrated that zebra finches are capable of sequentially learning two songs, there are constraints on song learning. We recently exposed juvenile male zebra finches ($N = 10$) for limited time periods to two different tutors (previously unpublished results collected at Wellesley College, approved by IUCAC #1405). Zebra finches were raised by their mother alone and in acoustic and visual isolation of adult males from day 7 (± 1.5 SD) onwards (Fig. 1B). From 33 dph, zebra finches were kept in individual sound isolated chambers and subsequently exposed for two periods of 5 days each to their first tutor between 44 and 49 dph and their second tutor between 68 and 73 dph. Overall, zebra finches learned from their first tutor, as reflected by high similarity scores at day 67, which were collected after exposure to the first tutor and before exposure to their second tutor with Sound Analysis Pro (Fig. 1B; mean ± SD: 64 ± 17% similarity with 1st tutor; paired *t*-test with unfamiliar 2nd tutor: $t(9) = 6.86$, $p < 0.001$). However, there was no evidence that these zebra finches learned from their second tutor (Figs. 1B and 2 , mean ± SD similarity with 2nd tutor before vs. after tutoring: 47 ± 17% vs. 52 ± 19%; paired *t*-test $t(9) = -1.2$, p = n.s.). This suggests that similar to the hypothesized sensitive period for tutor song learning in normally reared zebra finches, this sensitive period may also restrict learning multiple songs when potential song tutors are experimentally separated in time and the new song model does not become available until late in development, after 65 dph.

In a different unpublished study, we have replicated once more that birds significantly learn both first and second tutor song if the second tutor is presented for 10 days between 55–65 dph (Figs. 1C and 3 ; at 54 dph: mean ± SD similarity with 1st tutor 64 ± 15%; paired *t*-test with unfamiliar 2nd tutor: $t(30) = -7.02$, $p < 0.001$; at 91 dph: similarity with 2nd tutor before vs. after tutoring: 42 ± 13% vs. 64 ± 14%; paired *t*-test $t(30) = 7.78$, $p < 0.001$). Therefore, although a period between 68 and 73 days still falls within the

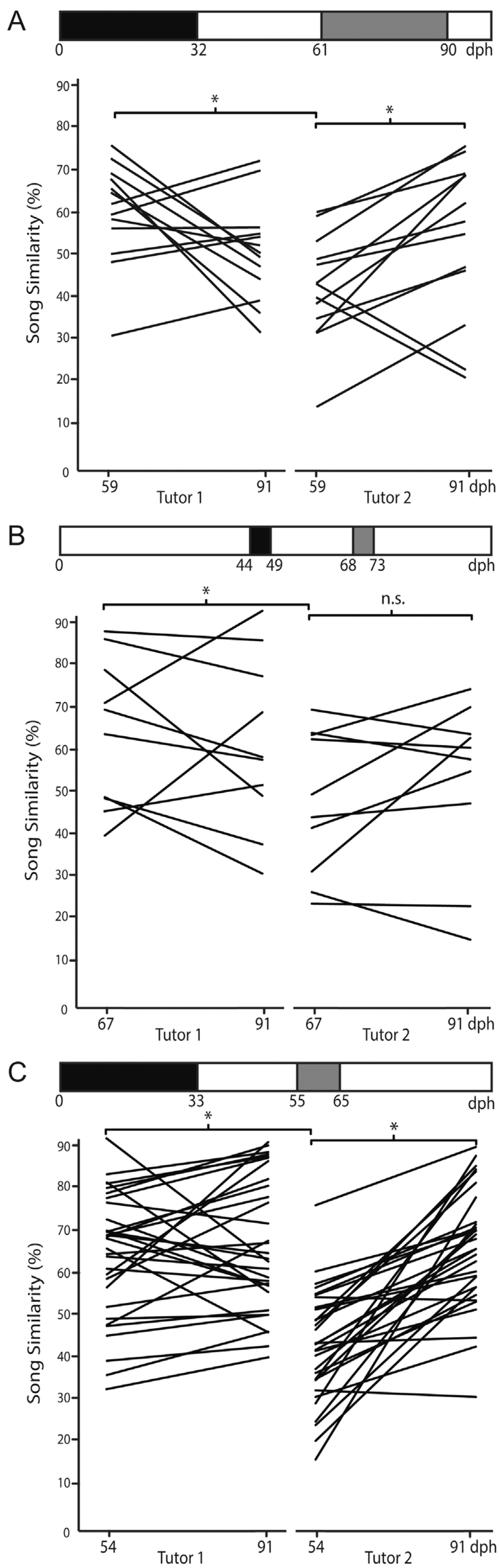

Fig. 1. Experimental timelines of exposure to a second tutor for 30 (A), 5 (B), and 10 (C) days with the duration of the first tutoring period represented in black, the second tutoring period represented in gray and individual isolation in white, and learning outcomes represented by lines connecting similarity scores at two different time points for each bird. Tutoring periods were kept constant, with no variation between experimental birds, with the exception of the first tutoring period in A (32 dph ± 1.44 SD). Song similarity was calculated by comparing 10 motifs of both Tutor 1 (left) and Tutor 2 (right) to 10 motifs of the tutee one day before exposure to the second tutor, and at 91 dph, after exposure to the second tutor, using Song Analysis Pro with a syllable-to-syllable (asymmetric) comparison. Each line represents two data points ('early' similarity to 'late' similarity) from one bird.

A) Birds significantly learned from the first tutor at 59 dph, compared to similarity with the unfamiliar second tutor ($t(13) = 5.37$, $p < 0.001$). Between 59 dph and 91 dph, learning from their second tutor was also significant (paired *t*-test, $t(12) = 2.66$, $p = 0.021$). Adapted, with permission, from Olson et al. (2016).

B) When tutoring periods were limited to 5 days, there was significant learning from the first tutor at 67 dph, compared to similarity with the unfamiliar second tutor ($t(9) = 6.86$, $p < 0.001$). Birds did not learn from their second tutor between 68 dph and 91 dph (paired *t*-test, $t(9) = -1.2$, p = n.s.).

C) Juveniles significantly learned from the first tutor at 54 dph, compared to similarity with the unfamiliar second tutor ($t(30) = -7.02$, $p < 0.001$). Between 55 dph and 91dph, learning from their second tutor was also significant (paired *t*-test, $t(30) = 7.78$, $p < 0.001$).

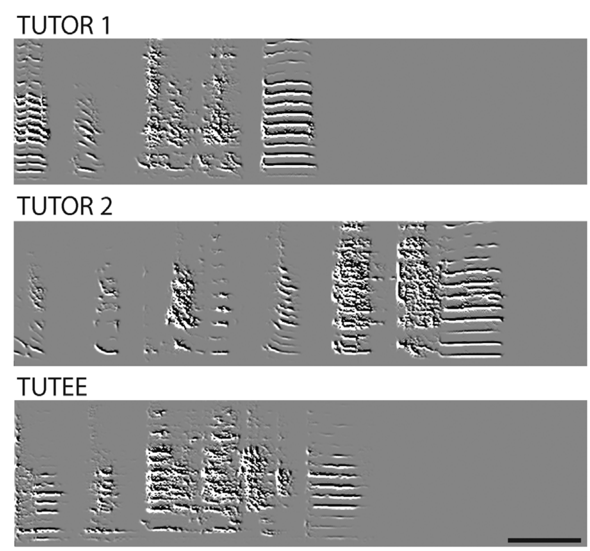

Fig. 2. Sonograms (0–10 kHz) of motifs of two tutors and their tutee. The tutee was exposed to the first tutor from 44 to 49 dph and to the second tutor from 68 to 73 dph. At the end of development (91 dph) the tutee's song resembles the song from the first tutor more (86%) than the song from the second tutor (57%). Scale bar represents 100 ms.

sensorimotor learning phase, the probability that a new song tutor will be accepted decreases over the course of juvenile development; at the same time, the plasticity of the brain to encode novel song templates may decline.

In addition to these experiments, which show that in the laboratory zebra finches can learn from a second tutor when they are allowed to socially interact with him before 65 dph, Lipkind et al. (2013) demonstrated that juvenile zebra finches are able to acquire a second song using operant conditioning. Juvenile zebra finches were tutored via operant-triggered playback until 63 dph; zebra finches that were most successful at song learning were selected for further training (Lipkind et al., 2013). The artificially modified second song model consisted of either a song in which syllable order was switched or a song in which new syllables were inserted (Lipkind et al., 2013). Nearly half the zebra finches in both groups were able to successfully learn the new songs. In this study, in contrast to other experiments (Olson et al., 2016; Yazaki-Sugiyama and Mooney, 2004), zebra finches were exposed to a second song that had similar elements to the first song model, yet only about half the zebra finches were able to acquire the new target song. Introduction of a second song model at 63 dph may still fall within a plastic phase for vocal learning, but the ability to acquire a new song becomes more limited as the sensitive period nears its end.

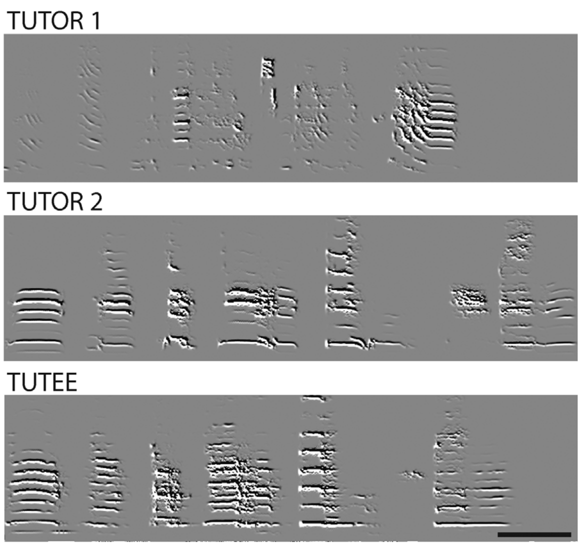

Fig. 3. Sonograms (0–10 kHz) of motifs of two tutors and their tutee. The tutee was exposed to the first tutor from 0 to 32 dph and to the second tutor from 55 to 65 dph. At the end of development (91 dph) the tutee's song resembles the song from the second tutor more (84%) than the song from the first tutor (64%). Scale bar represents 100 ms.

5. Limitations on second-song and second-language learning

There are several neural and behavioral consequences of aging that may set limits on the acquisition of a new song template. Early in development, tutor song is regarded as behaviorally salient, which allows for the zebra finch to pay attention to and gradually learn the tutor's song (Miller-Sims and Bottjer, 2014). When directing song towards pupils, the tutor sings its model song with acoustic modifications, resulting in changes in song structure in the temporal domain, such as longer pauses between motifs and more introductory notes, as well as in the acoustic structure of individual syllables, such as increased goodness of pitch and decreased mean frequency and spectral entropy (Chen et al., 2016). This is similar to “motherese” in humans, a term referring to the acoustic difference in infant-directed human speech, including higher pitch and changes in speech structure (Ferguson, 1964). Human studies indicate that infants prefer this type of speech, which results in greater attention towards speech stimuli as to stimulate learning (Fernald and Kuhl, 1987). Similarly, changes in tutee-directed singing by the tutor may result in maximizing tutee attention towards the tutor song and adding salience to the model song (Chen et al., 2016). There may be a shift that occurs later in development that re-defines which song stimuli are behaviorally relevant in order to protect the neural representation of what has already been learned. In the sensorimotor phase, the zebra finch is practicing its own song and using trial-and-error learning in order to gradually shape the song into one that resembles the one it was exposed to early in development; acquisition of a new sensory representation (or ‘template’) during this phase could conflict with the ongoing sensorimotor learning process, resulting in overall poor learning outcomes, and thus be evolutionary undesirable.

When we exposed zebra finches to their second tutor for a period of only 5 days at 68 dph, limiting the amount of visual and social interaction between tutor and tutee, zebra finches were unable to learn from their second tutor (Fig. 1B), indicating that brief exposure to a second tutor late in development does not result in successful song learning. These zebra finches may not have attended to the second tutor song in order to inhibit interference with the ongoing learning process, or the tutor may not have treated these juveniles as ‘tutees’ and neglected to sing tutee-directed song to them that would have promoted the learning process. Alternatively, the behavior of these juveniles may have been more adult-like in the presence of the tutor, similar to changes in song behavior noted in the presence of a female (Kojima and Doupe, 2011). This resulted in maintaining more elements of the first tutor song rather than acquiring those from the second song tutor (Fig. 1B). Sufficient social interaction, or a period of interaction with a second tutor longer than 5 days and/or earlier in development (Eales, 1985; Olson et al., 2016; Yazaki-Sugiyama and Mooney, 2004), appears to be enough to override this selectivity and allow for attention towards and thus learning from a second tutor (Fig. 1A and C). Although it seems as if the hypothesized sensitive period for acquiring a sensory representation of song can be extended by introducing a second tutor, there are developmental constraints, as shown by the differences in second-song learning outcomes in zebra finches tutored for 5, 10, or 30 days at different times in development (Fig. 1).

This potential sensitive period observed for second-song acquisition in zebra finches resembles the hypothesized sensitive period for second-language acquisition in humans: second-language learning declines with age, as second language proficiency is greater if learning occurs during childhood rather than adulthood. Studies have shown that second-language grammar proficiency is indeed linearly correlated with age of acquisition, up to the age of puberty (Johnson and Newport, 1989), but the boundaries of a sensitive period for second-language learning have been debated extensively (Reichle, 2010). An amended version of the study by Johnson and Newport (1989) did not find a correlation during puberty; instead, performance of native Spanish speakers coming to the US before or at the age of 16 was either perfect or near perfect, whereas there existed a correlation between accuracy and age among the later-arriving immigrants, or native Spanish speakers coming to the US after the age of 17 (Birdsong and Molis, 2001). This suggests that puberty cannot be defined as a critical period for second-language acquisition per se, but rather furthers evidence for the effect of age on second-language learning.

We also see a declining sensitivity to second-song learning in birds (see Section 4); in the data we presented here (Fig. 1), it appears that sufficient social interaction with the second tutor, and time to learn and practice the second song before the end of the developmental period, can result in successful song acquisition. Zebra finches are ‘age-limited’ learners, and even after learning two songs sequentially, are only able to produce a single song as adults. As such, second-song learning in zebra finches cannot be seen as analogous to true bilingualism in humans. Human bilinguals use their languages flexibly, with the exception of international adoptees, for whom the ability to speak or understand the first language often gets lost, but neural traces of that language persist in brain regions dedicated to language (Choi et al., 2017; Oh et al., 2010; Pierce et al., 2014). It is possible that a general sensitive period for second-language learning may not exist because just as there are many different features of song, such as tone, rhythm, and syntax, there are many facets of language, and each may be learned along different time scales (Newport, 2006). Furthermore, as seen in our zebra finches and in humans, there exists individual variation in the ability for vocal imitation throughout the developmental learning process, which may be a result of various factors at the neuronal and at the behavioral level (Newport, 2006).

6. Conclusions

In this review, we advance songbirds as a model system to tease apart the different factors that influence acquisition of a second language. While neither sequential learning in zebra finches can be seen as strictly analogous to bilingualism in humans, nor do the experimental manipulations of the early auditory environment resemble the social conditions that zebra finches are exposed to in the wild, this paradigm has the potential to elucidate mechanisms of the template acquisition process when more than one template is acquired. Individual differences in learning outcomes can be assessed after the first song has been learned, and again after exposure to a second song, which may shed light on mechanisms of flexibility in song imitation. Hence, the parallel with individual differences in second-language learning outcomes in humans can be further explored at the neural level.

Since we now have a better understanding of the developmental and behavioral restrictions on learning a second song, the next step is to investigate the neuronal mechanisms underlying second song acquisition; of particular interest are inhibitory circuits, and integration of new neurons into these circuits during acquisition of a second song. Why are some birds more successful in switching to a new song model than others? We have previously shown that left-lateralized expression of the Immediate Early Gene *ZENK* (acronym for *zif-268, egr-1, ngf-Ia* and *krox-24*) is related to successful second-song imitation in a secondary auditory brain region upon re-exposure to the tutor's song (Olson et al., 2016). *ZENK* is commonly expressed in GABAergic neurons (Pinaud et al., 2004). In the premotor nucleus HVC (proper name), inhibitory interneuron activity is high for syllables that have already been learned while low for 'new' syllables (Vallentin et al., 2016). Thus, it will be critical to determine how inhibitory circuits in secondary auditory brain regions regulate second song acquisition. These studies could elucidate the factors that predict successful acquisition of a second song, and thus may inform our understanding of the neural mechanisms underlying second language learning.

Acknowledgments

This work was supported by the Helen Wallace '33 Summer Research Health Professions Fund, Allene Lummis Russell '46 and Paul S. Russell Fund for Neurosciences, Early Career funds from Wellesley College, and by the National Institutes of Health [grant number R15HD085143]. We thank Alexa Pagliaro for assistance with preparing Figs. 2 and 3, Pat Carey and Valery LePage for animal care, and two anonymous reviewers whose suggestions helped to improve a previous version of this manuscript.

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