Effect of Acoustic Cue Modifications on Evoked Vocal Response to Calls in Zebra Finches (*Taeniopygia guttata*)

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Besides their song, which is usually a functionally well-defined communication signal with an elaborate acoustic structure, songbirds also produce a variety of shorter vocalizations named calls. While a considerable amount of work has focused on information coding in songs, little is known about how calls’ acoustic structure supports communication processes. Because male and female zebra finches use calls during most of their interactions and answer to conspecific calls without visual contact, we aimed at identifying which calls’ acoustic cues are necessary to elicit a vocal response. Using synthetic zebra finch calls, we examined evoked vocal response of male and female zebra finches to modified versions of the distance calls. Our results show that the vocal response of zebra finches to female calls requires the full harmonic structure of the call, whereas the frequency downsweep of male calls is necessary to evoke a vocal response. It is likely that both female and male calls require matching a similar frequency bandwidth to trigger a response in conspecific individuals.

**Keywords:** call, information coding, playback experiment, songbird, *Taeniopygia guttata*

Songbirds produce two categories of vocalizations: songs and calls (Catchpole & Slater, 2008). Songs are usually complex signals used for mate attraction and intrasexual competition. In these contexts, songs convey multiple pieces of information such as species identity, individual identity (Becker, 1982; Falls, 1982), or motivation of the emitter (Dabelsteen & Pedersen, 1988). Different acoustic parameters in songs are available to the receiver: notes’ syntax, temporal pattern of frequency or intensity, as well as frequency spectrum (Becker, 1982; Holland, Dabelsteen, & Lopez Paris, 2000; Mathevon & Aubin, 2001; Mathevon et al., 2008). Calls are usually shorter signals that consist of a single note and play various roles (Marler, 2004). Indeed, most species show a broad repertoire of calls produced in a great diversity of contexts such as social grouping, information sharing about resource location or aggressive interactions. Although crucial for the understanding of acoustic communication in birds, few studies have explored information coding in calls (Charrier, Lee, Bloomfield, & Sturdy, 2005; Charrier & Sturdy, 2005; Freeberg, Lucas, & Clucas, 2003; Sharp, McGowan, Wood, & Hatchwell, 2005).

Zebra finches *Taeniopygia guttata* are gregarious flock-forming birds native to Australia. Only male zebra finches sing, but both sexes utter a full repertoire of single-syllable calls used in social communication (Zann, 1996). Distance calls, also known as “long calls,” are frequently produced by both males and females, especially when they lose visual contact with their social partners (Price, 1979; Runciman, Zann, & Murray, 2005; Vicario, Naqvi, & Raksin, 2001; Vicario, Raksin, Naqvi, Thande, & Simpson, 2002; Zann, 1984, 1996). The female distance call consists of a harmonic series with a relatively unmodulated fundamental frequency of 400–500 Hz. The male distance call also shows a harmonic structure, but with a typical downsweep frequency modulation and an elevated fundamental frequency of 600–1000 Hz. Although male and female distance calls display different acoustic structures, both allow recognition of conspecifics and individual discrimination of mates (Vignal, Mathevon, & Mottin, 2004; Vignal, Mathevon, & Mottin, 2008; Zann, 1984). Vicario et al. (Vicario et al., 2001) showed that males but not females exhibit gender discrimination based on calls, but it remains to be investigated which call-specific acoustic cues are necessary to elicit a vocal response. By analyzing the behavioral response of male and female zebra finches to the playback of modified calls, we thus tested two predictions based on the dimorphic acoustic structure of the calls: (1) the typical high-pitched downsweep of the male call, and (2) the typical low-pitched plateau of the female call are critical in eliciting a vocal response from conspecific individuals.

**Method**

**Subjects**

Thirty-four adult zebra finches (14 females and 20 males) were bred in a mixed group in an aviary (2 m × 2.3 m × 2.8 m) and put...
in individual cages during the experiment (12L/12D photoperiod, food (seeds and fresh vegetables), water, and nest material provided ad libitum; temperature 23–25 °C). During the tests, temperature, food and water conditions were the same as in the aviary. All birds were naïve to testing procedures. Experiments were performed under the authorization n°42-218-0901-38 SV 09, (ENES Lab, Direction Départementale des Services Vétérinaires de la Loire), and were in agreement with the French legislation regarding experiments on animals.

**Playback Procedure**

For playback tests, each tested bird was moved from the aviary and placed in an experimental cage (240 × 50 × 50 cm, equipped with perches) in a soundproof chamber one night before the start of the test. Another cage containing two same-sex companion birds was placed near the experimental cage and in sight of the test subject to provide a social context; social isolation induces stress in zebra finches, and the social context greatly affects the response of zebra finches to playback tests (Vignal, Andru, & Mathevon, 2005; Vignal, Mathevon, & Mottin, 2004). As the identity of the companion pair changed in each trial, we do not expect the social context to confound test subjects’ responses. All birds (recorded birds used as stimuli, audience birds, tested males and females) were bred in the same aviary, but stimuli originated from birds that never mated with audience birds or subjects. We chose to use familiar stimuli to avoid unusual behavioral responses potentially elicited by nonfamiliar voices.

All the experiments were conducted between 8 a.m. and 10 a.m. The playback stimuli were broadcast through two high fidelity speakers (Triangle Comete 202) placed at both ends of the cage, connected to a DAT recorder (SONY DTC-ZE 700) and an amplifier (Yamaha AX-396). During each test, the playback stimuli were broadcast through one randomly chosen speaker (sound level: 70 dB at 1 m; each stimulus was rescaled to match the root mean square amplitude of the control signal). The tested bird was presented with five series of experimental stimuli and one series of control stimuli, broadcast at random (series duration: 5.0 seconds; interval between series: 30 seconds). Each series of stimuli consisted of the succession of identical calls played at natural rates (1 call/s for female calls and 2 calls/s for male calls).

**Playback Signals**

The zebra finch distance call is a complex sound showing a fundamental frequency associated with several harmonics (see Figure 1). This sound is modulated over time in spectral and intensity domains. The female distance call shows a fundamental frequency around 400–500 Hz which is relatively unmodulated. The female call duration varies from 100 up to 500 ms (Simpson & Vicario, 1990; Vicario et al., 2001; Vignal, Mathevon, & Mottin, 2004; Zann, 1984). It can be divided into three segments of different durations (Figure 1A): the initial segment defined as a short and loud ascending frequency modulation, the stable part defined by a long and loud plateau with no frequency modulation, and a third segment defined by a short and weak descending

*Figure 1. Acoustic structure of female (A) and male (B) distance call in the zebra finch. Distance calls are complex sounds with a fundamental frequency associated with several harmonics. (A) The female distance call can be divided into an initial part (short and loud ascending frequency modulation), a stable part (a long and loud second part with no frequency modulation), and a final part (short and soft descending frequency modulation). (B) The male distance call can be divided into a tonal part (short and weak ascending frequency modulation followed by a short stable segment), and a final downsweep part (long and loud descending frequency modulation).*
frequency modulation (Vignal, Mathevon, & Mottin, 2004). The male distance call shows a higher fundamental frequency (600–1000 Hz) than the female call. It is typically described as a 100ms-long frequency modulated downsweep (FM downsweep; Simpson & Vicario, 1990; Vicario et al., 2001; Zann, 1984). In our population of zebra finches, the typical male distance call can be divided into two segments of different durations (Figure 1B): an initial segment defined as a short and weak ascending frequency modulation followed by a short stable part (defined by Zann (1984) as the tonal component), and a second segment defined by a long and loud FM downsweep (defined by Zann, 1984, as the noise component).

Control Stimuli

To control every acoustic feature in the stimuli, we built synthetic copies of natural calls. In order to avoid pseudoreplication (McGregor et al., 1992; Schank & Koehnle, 2009), natural calls originated from recordings of different birds (three females and two males). The sample size of the set of natural calls was small, but it allowed us to perform the same modifications of the acoustic cues in two sets of calls with typical male and female structures. In this way, frequency, temporal, and intensity ranges were comparable across stimuli of one sex.

To record natural calls, each bird was isolated in a soundproof chamber. Seven to 10 distance calls were recorded using a Sennheiser MD42 microphone, placed 0.3 m above the cage, connected to a Marantz PMD690/1B recorder with a 22.050 Hz sampling rate. According to the analysis of the spectral, temporal, and intensity cues of the calls ([Vignal, Mathevon, & Mottin, 2004] for females; [Vignal, Bouchut, & Mathevon, 2008] for males), a synthetic copy of one distance call (selected at random) of each recorded bird was then created using the graphic synthesizer module of Avisoft-SAS Lab-Pro software (version 4.16, 2002). Because the spectrum of the natural calls used in this experiment was mainly confined to one fundamental frequency and seven harmonics, all synthesized copies showed seven harmonics.

To make sure that the birds’ responses did not differ between synthetic calls and natural calls, we performed simple preliminary playback tests where females (n = 4) were presented with both synthetic and natural male calls and males (n = 5) were presented with both synthetic and natural female calls. This preliminary experiment followed the same protocol as the actual experiment. During the playback, we scored NC5 (the number of distance calls produced during the broadcasting of the stimulus), NC10 (the total number of distance calls produced within 10s after the start of the stimulus), and LR (the latency of the vocal response in seconds). All tested birds showed similar behavioral responses to synthetic calls and natural calls (natural vs. synthetic call (mean ± 0.95 confidence interval), Wilcoxon’s paired signed rank test: Tests on females: NC5: 4.5 ± 1.69 vs. 3.25 ± 1.67 p = .09, NC10: 5.5 ± 1.26 vs. 4 ± 1.96 p = .18, LR: 0.32 ± 0.16 vs. 0.67 ± 0.88 p = .87; Tests on males: NC5: 12 ± 6.47 vs. 10.8 ± 4.49 p = .58, NC10: 17 ± 9.62 vs. 15.2 ± 7.21 p = .58, LR: 0.29 ± 0.03 vs. 0.36 ± 0.10 p = .44).

To investigate the role of spectral, temporal, and intensity cues, we built modified stimuli out of the synthetic control calls. We performed comparable modifications of male and female calls as much as possible. However, due to the strong sexual dimorphism of zebra finch calls, some of the modifications could not be performed in both sexes; in addition, the same modification could result in a different effect in male and female calls. We adjusted the intensity level of each experimental call to the intensity level of the control calls using the Root Mean Square average (RMS).

Call Modifications in the Spectral Domain

Question 1: How Many Harmonics Are Necessary To Elicit a Behavioral Response in Conspecifics?

We created six experimental stimuli with reduced numbers of harmonics (Figure 2, A and B). Harmonics were removed by zeroing their amplitude in the synthetic call. The order of removal was according to the initial intensity of the harmonics. The harmonic with the lowest amplitude was removed first whereas the harmonic with highest amplitude was the last remaining. Consequently, the order of harmonic removal was not identical in male and female calls: the most intense harmonic is usually the 5th harmonic in female calls whereas it is the 3rd in male calls. The fundamental frequency presents the lowest amplitude in both sexes. These experimental signals (H1 to H7, Figure 2, A and B) are thus made up with respectively seven harmonics (harmonics 1 to 7 without the fundamental frequency, H7), six (harmonics 2 to 7, H6), five (harmonics 3 to 7 in female calls or harmonics 2 to 6 in male calls, H5), four (harmonics 3 to 6 in female calls or harmonics 2 to 5 in male calls, H4), three (harmonics 4 to 6 in female calls or harmonics 2 to 4 in male calls, H3), two (harmonics 4 and 5 in female calls or harmonics 3 and 4 in male calls, H2), or one harmonic (harmonic 5 in female calls or harmonic 3 in male calls, H1). It should be noted that the fundamental frequency was removed in all experimental stimuli (H1 to H7), so the importance of the fundamental frequency in evoking a response could be assessed by comparing the behavioral responses of subjects between H7 and control calls. The differential response of subjects to stimuli H1 to H7 can be attributed to differences in spectral contents. In these experimental signals, we maintained the natural temporal structure (envelope and frequency modulation, duration).

Question 2: Is the Absolute Pitch of the Call Needed To Elicit the Behavioral Response of Conspecifics?

We performed positive and negative shifts of the call’s fundamental frequency. Because our aim was not to assess perceptual capacities of birds but to elucidate the role of the pitch in eliciting a behavioral response, the values were chosen just outside the limits of the range of variation in our zebra finch population (99th percentile). In female calls, these values were ±60 and −60 Hz (Figure 2C). Because male calls show a larger range of variation between individuals than female calls do, the chosen values were ±80 and −80 Hz (Figure 2D). In these experimental signals, we maintained the natural temporal structure (envelope and frequency modulation, duration).
Question 3: Is the Frequency Modulation of the Calls Necessary To Elicit a Behavioral Response in Conspecifics?

We built synthetic calls in which the natural frequency modulation was changed into a time-constant frequency pattern (no frequency modulation). The time-constant frequency of each harmonic was equal to the initial mean frequency of the harmonic while maintaining the natural envelope and duration of the call (no FM, Figure 3, A and B).

The fundamental of a natural male call is a frequency modulated downsweep. To confirm that the direction of the FM sweep in the male call is important for behavioral response, we constructed a synthetic call that reversed the frequency sequence while maintaining the natural envelope and duration of the call (rev, Figure 3C).

Figure 2. Spectrograms of frequency modifications of female and male calls used in playback experiments. (A) Female and (B) male calls used as control stimuli are composed of a fundamental frequency and seven harmonics, whereas experimental stimulus H5 is composed of five harmonics (harmonics 3 to 7 for female calls and harmonics 2 to 6 for male calls), experimental stimulus H3 is composed of three harmonics (harmonics 4 to 6 for female calls and harmonics 2 to 4 for male calls), and experimental stimulus H1 contains one harmonic (harmonic 5 for female calls and harmonic 3 for male calls). (C) Examples of shifted female call (−60Hz, +60Hz) and (D) shifted male call (−80Hz, +80Hz).

Question 4: Which Temporal Part of the Call Is Required To Elicit Behavioral Response in Conspecifics?

To assess the importance of the different temporal parts of a natural call to elicit a behavioral response, we constructed call variants by zeroing the amplitude in the synthetic call in different time segments. Boundaries of the variants were chosen according to the call temporal structure. For female calls, we created four variants: the rapid initial segment of the call (initial part, Figure 3D), the long stable part (stable part, Figure 3D), the first 50% of the stable part (50, Figure 3D), and the rapid final segment of the call (final part, Figure 3D). For male calls, we built five variants: the initial segment of the call (tonal part, Figure 3E), the final noise downsweep component of the call (downsweep part, Figure 3E), the first 50% of the downsweep component (1st 50, Figure 3E), and the last 50% of the downsweep component (2nd 50, Figure 3E).
Figure 3. Spectrograms of temporal modifications of female and male calls used in playback experiments. (A) Female and (B) male calls without frequency modulation. (C) Reverse version of the downsweep component of the male call. (D) Temporal cutting of female call: short initial segment, long stable part, first 50% of stable part and short final segment. (E) Temporal cutting of male call: tonal component, final downsweep component, first 50% of final component and last 50% of final component. (F) Female and (G) male calls without amplitude modulation; waveforms were added to illustrate the presence or absence of amplitude modulation. (H) Spectrograms of two male calls produced at a natural rate (2 calls/s) and of broadband noise stimulus mimicking this male rhythm.
Question 5: Are the Relative Energy Levels of Harmonics an Important Cue To Elicit a Behavioral Response in Conspecifics?

We removed the natural amplitude modulation (AM) of each harmonic and of the fundamental frequency (no AM, Figure 3, F and G). The first consequence is that the amplitude of each harmonic was maintained at a constant energy level during the entire call duration. The second consequence is that we changed the relative intensity levels of the harmonics at each time step. The spectrum of the call is modified without changing the absolute frequency values of the harmonics.

Question 6: Is the Rhythm of Call Emission Sufficient To Elicit a Behavioral Response in Conspecifics?

To test whether zebra finches could react only to the particular rhythm of male call emission without any other spectral and temporal information, we built a stimulus showing the natural male call rate (2 calls/s) without any natural frequency characteristic (white noise, Figure 3H). Computer-generated white noise was bandpass-filtered in the range of the natural male distance call and cut into blocks of the same duration as calls. Because females do not produce calls with a noticeable rhythm, this parameter was not tested using female calls.

Response Criteria

During playback tests, we recorded vocal and locomotor activity of the tested bird with a video recorder (SONY DCR-TRV24E). A single trained observer (C.V.) scored from the video the latency of the vocal response (LR in seconds), the number of distance calls produced during stimulus broadcasting (NC5), representing the immediate response of the bird, and the total number of distance calls produced within 10 s after the start of the stimulus (NC10), representing the late response of the bird. In order to test the stimulating effect of the experimental signals, we started the playback when the test subject showed relatively low levels of spontaneous activity (no vocalizations produced during the minute preceding the experiment).

Statistical Analysis

All measures were taken by the same trained observer (C.V.) from video recordings of the tests. We expected that a strong behavioral response would be characterized by a short LR, a high NC5, a high NC10 or a combination of these criteria. Instead of analyzing the three nonindependent measures of response (NC5, NC10 and LR) separately, we used a principal component analysis incorporating these three parameters to derive a single composite score of response to each stimulus (Holland et al., 2000; McGregor, 1992). The birds’ behavioral response is thus expressed in a simpler, more easily interpretable form. The scores of the first principal component (PC1) were used in further analyses. For each sex, LR, for which low values indicated a strong response to the stimulus, loaded negatively on the PC1, whereas NC5 and NC10, for which high values indicated a strong response to the stimulus, loaded positively on the PC1. To compare the birds’ responses to experimental versus control stimuli, we performed one-way analysis of variance (ANOVA; $\alpha = .05$) followed by Fisher PLSD post hoc tests ($\alpha = .05$; Statistica Software version 6). Because the origin of the stimulus (the identity of the recorded natural call) did not significantly influence the bird’s responses (one-way ANOVA, $F_{4,33} = 0.141$, $p > .05$), we pooled the responses of all birds in the analyses.

Results

Acoustic Cues in Male Calls Eliciting a Behavioral Response

Figure 4 shows relative values of the three parameters describing the behavioral response of birds to experimental stimuli (notice that raw data have been normalized on the figure). Behavioral responses to control stimuli (synthetic copy of natural call) are characterized by a mean Latency of the Vocal Response (LR, mean $\pm$ 0.95 Confidence Interval) of 0.63 $\pm$ 0.34 seconds, a mean Number of distance Calls produced during the stimulus broadcasting (NC5) of 3.07 $\pm$ 0.72, and a mean Number of distance Calls produced within 10 s after the start of the stimulus (NC10) of 4.43 $\pm$ 1.22. A behavioral response weaker than the control is characterized by a longer LR, a smaller NC5, and a smaller NC10. Figure 5 shows the single composite score (first principal component PC1) of responses to each stimulus calculated from the three parameters LR, NC5, and NC10. The first principal component (PC1) explained 81.08% of the variation in the responses. LR loaded negatively on PC1 whereas NC5 and NC10 loaded positively. Positive values of PC1 thus indicate a strong response of the female to the modified male call. Modifications of male distance calls significantly affect PC1 values (one-way ANOVA: $F_{20,78} = 2.36$, $p = .004$), and thus modify female response.

Modifications in the Spectral Domain

Question 1: Male Calls Require at Least Two Harmonics To Elicit a Behavioral Response

Among harmonic-manipulated experimental stimuli, only the H1 stimulus (harmonic 5 alone) did not elicit a response (PC1 significantly lower than PC1 of the control stimulus, Fisher PLSD, $n = 5$, $p = .01$, Figure 5). Responses to experimental stimuli composed of 2 or more harmonics (H2 to H7) did not differ significantly from the response elicited by control stimuli (Fisher PLSD, $n = 5$ for H2 to H4 and $n = 3$ for H5 to H7, $p > .05$, Figure 5). Thus, a minimal structure of two harmonics is sufficient to trigger a behavioral response.

Question 2: Modifications of the Absolute Pitch of Male Calls Do Not Impair Behavioral Response

Behavioral response to shifted signals ($-80 \text{ Hz}$, $+80 \text{ Hz}$) did not differ significantly from the response to control stimuli (PC1 not significantly different from PC1 of the control stimulus, Fisher PLSD, $n = 5$, $p > .05$, Figure 5). However, responses to experimental signals showed considerably more variable latencies (Figure 4A).
Call Modifications in the Temporal Domain

Question 3: Modification of the Frequency Modulation in Male Calls Disturbs Behavioral Response

There was no significant difference between response to experimental male calls without frequency modulation and control signals (PC1 not significantly different from PC1 of the control stimulus, Fisher PLSD, $n = 5$, $p > .05$, Figure 5 no FM). Nevertheless, the latency of response to modified calls was much longer than controls (Figure 4B).

To test whether the FM downsweep is necessary to the behavioral response, we built a synthetic call that reversed the frequency sequence of the downsweep component and thus asked whether sequential high and low frequencies are necessary for behavioral response (rev, Figure 3C). This reverse version with ascending frequency modulation elicited significantly lower responses than control signals (Fisher PLSD, $n = 4$, $p = .05$, Figure 5 rev). The behavioral response to frequency modulated male calls thus requires the presence of a descending modulation.

Question 4: The Whole Male Call Duration Is Not Necessary To Elicit a Behavioral Response

Birds did not respond to male calls showing only the initial component (PC1 significantly lower than PC1 of the control stimulus, Fisher PLSD, $n = 5$, $p = .001$, Figure 5 tonal part). In contrast, the final downsweep component was sufficient to elicit a positive response (PC1 not significantly different from the control stimulus, Fisher PLSD, $n = 5$, $p > .05$, Figure 5 downsweep part).

![Figure 4. Mean values of the behavioral parameters illustrating female responses to male stimuli. For graphic convenience, raw data have been normalized. A response to the experimental stimulus that is stronger than the response to the control is illustrated by a short latency of response (LR) and high numbers of calls (NC5 and NC10). A behavioral response that is weaker than the response to the control is characterized by a longer LR and smaller NC5 and NC10. Dots are means, boxes are SE and bars are 0.95 confidence intervals.](image-url)
In the same way, the first 50% of the final downsweep component (1st 50) and the last 50% of the final downsweep component (2nd 50) elicited a response (PC1 not significantly different from the control stimulus, Fisher PLSD, \( n = 4, p > .05 \), Figure 5, respectively 1st 50 and 2nd 50). However, the latency of response to the 2nd 50 signal was longer than the latency of the response to the control stimulus (Figure 4B). Thus, behavioral response to male call needs at least one portion of the final component presenting the downsweep frequency modulation.

**Question 5: Modification of the Relative Energy Levels of Harmonics in Male Calls Impairs Behavioral Response**

Behavioral response to male calls was disrupted when the natural AM of the call was removed (PC1 significantly lower than PC1 of the control stimulus, Fisher PLSD, \( n = 5, p = .05 \), Figure 5 no AM). When relative energy levels of harmonics are modified, male calls do not evoke behavioral response.

**Question 6: The Rhythm of Call Emission Is Not Sufficient To Evoke a Behavioral Response**

No behavioral response was elicited when the male natural call rate was preserved (2 calls/s) but the stimulus lacked frequency information (PC1 significantly lower than PC1 of the control stimulus, Fisher PLSD, \( n = 4, p = .001 \), Figure 5 white noise).

**Conclusion**

In conclusion, the behavioral response to male zebra finch calls relies on portions of the downsweep frequency modulation of the final component of the call. Two harmonics in the call can be sufficient to trigger a response. A strong modification of the relative energy levels of the harmonics impairs behavioral response.

**Acoustic Cues in Female Calls Eliciting a Behavioral Response**

Figure 6 shows relative values of the three parameters describing the behavioral response of birds to the experimental signals (notice that raw data have been normalized on the figure). The behavioral response to the control stimulus is characterized by a mean Latency of the Vocal Response (LR) of 2.01 ± 1.14 seconds (mean ± 0.95 Confidence Interval), a mean Number of distance Calls produced during the broadcasting of the stimulus (NC5) of 3.4 ± 1.73, and a mean Number of distance Calls produced within 10 s after the start of the stimulus (NC10) of 4.15 ± 1.91. A behavioral response weaker than the control is characterized by a longer LR, a smaller NC5 and a smaller NC10. Figure 7 shows the single composite score (first principal component PC1) of the response to each stimulus calculated from the three parameters LR, NC5, and NC10. It explains 82.41% of the variation in the responses. LR loaded negatively on PC1, whereas NC5 and NC10 loaded positively on the PC1. Thus, positive values of PC1 indicated a strong response of the male subject to the modified female call. The modifications of female distance calls had a significant effect on PC1 values (one-way ANOVA: \( F_{15,101} = 2.54, p = .003 \)). Thus these modifications affect male response (see Figure 7).

**Modifications in the Spectral Domain**

**Question 1: Female Calls Require a Minimum of Four Harmonics To Trigger a Behavioral Response**

The response to the experimental stimuli composed of one to three harmonics (H1, H2, H3) was significantly weaker than the response elicited by the control stimulus (PC1 significantly different from PC1 of the control stimulus, Fisher PLSD, H3 \( n = 13, p = .05, H2 n = 11, p = .01 \) and H1 \( n = 5, p = .01 \), Figure 7). Conversely, the response to stimuli presenting four harmonics or more (H4, H5, H6, H7) did not significantly differ from the control response (PC1 not significantly different from PC1 of the control stimulus, Fisher PLSD, \( n = 4, p > .05 \)). Therefore, a minimum number of four harmonics is necessary for a female call to elicit a behavioral response. However, it should be noted that the latency of the response to the signals H4 and H5 was longer than the control (Figure 6A).
Question 2: Modifications of the Absolute Pitch of Female Calls Do Not Impair Behavioral Response

Female calls shifted $-60$ or $+60$ Hz both triggered behavioral responses (PC1 not significantly different from PC1 of the control stimulus, Fisher PLSD, $n = 8$, $p > .05$, Figure 7). Although the global reaction to female calls was not disturbed by pitch modification, the latency of response to shifted signals was longer than the control (Figure 6A).

Modifications in the Temporal Domain

Question 3: Frequency Modulation in Female Call Is Not Necessary To Elicit a Behavioral Response

There was no significant difference between behavioral response to experimental stimuli with no frequency modulation and to control stimuli (PC1 not significantly different from PC1 of the control stimulus, Fisher PLSD, $n = 8$, $p > .05$, Figure 7 no FM). Female calls do not necessarily need frequency modulation to elicit a behavioral response.

Question 4: The Whole Female Call Duration Is Not Necessary To Elicit a Behavioral Response

The initial segment of the call was not sufficient to trigger a response (PC1 significantly different from PC1 of the control stimulus, Fisher PLSD, $n = 3$, $p = .01$, Figure 7 Initial part). In contrast, the stable part of the female call was sufficient to elicit a behavioral response (PC1 not significantly different from PC1 of the control stimulus, Fisher PLSD, $n = 4$, $p > .05$, Figure 7 Stable part). The first 50% of the stable part or the final part still evoked a response (PC1 significantly different from PC1 of the control stimulus, Fisher PLSD, $n = 5$, $p = .05$, Figure 7: 50 and Final part) but after clearly longer latencies (Figure 6B). Thus, the female call

![Figure 6. Mean values of behavioral parameters illustrating male responses to female stimuli. For graphic convenience, raw data have been normalized. A stronger response to a stimulus than to the control is illustrated by a short latency of response (LR) and high numbers of calls (NC5 and NC10). A behavioral response that is weaker than the response to the control is characterized by a longer LR and smaller NC5 and NC10. Dots are means, boxes are SE and bars are 0.95 confidence intervals.](image-url)
needs at least a portion of the stable part or the final part to evoke a response.

**Question 5: Modification of Relative Energy Levels of Harmonics in Female Calls Impairs Behavioral Response**

Behavioral responses were disrupted by removing the natural AM of female calls (PC1 significantly lower than PC1 of the control stimulus, Fisher PLSD, \( n = 5 \), \( p = .01 \), Figure 7 no AM). Thus, the response to female call is impaired when the relative energy levels of harmonics are modified.

**Conclusion**

In conclusion, a female call needs to show an unmodified energy spectrum and a minimum structure of four harmonics to elicit a behavioral response. The complete female call is not required, since a portion of the stable part or the final part of the call can be sufficient to trigger a response.

**Discussion**

**Significant Acoustic Cues in Male Calls To Elicit a Behavioral Response in Conspecifics**

Our results emphasize that the stimulating value of male zebra finch calls is mainly due to the downsweep frequency modulation of the final component that can be reduced to only two harmonic elements. Behavioral response is sensitive to modifications of the relative levels of energy of the harmonics but not to significant alterations of male call duration.

Experimental male calls missing the fundamental and most of the harmonics still elicit a strong behavioral response. Either most harmonics in male calls do not convey information or we can hypothesize as for female calls, that male calls require matching a certain frequency bandwidth to trigger a response. The bandwidth of the two-harmonic male calls might be sufficiently broad (approximately 3200–3500 Hz) to trigger a response. Indeed, due to the steep frequency downsweep, it is similar to the bandwidth of the four-harmonic female calls (approximately 2800–3100) and exactly the same to the five-harmonic female calls (3200 to 3500 Hz) that elicit behavioral response. Because it reduces the spectral bandwidth, the stable plateau of the female call could explain the apparent lack of tolerance of the response to harmonic suppression. Information coding in the spectral bandwidth could be equally important in male and female calls. Contrary to female calls, the broad spectral bandwidth of male calls could allow stronger degradation of harmonic structure without impairing the stimulating value. It is likely that a minimal call bandwidth is necessary to elicit a behavioral response to both male and female zebra finch calls.

Okanoya and Dooling (1991) showed that the discrimination of conspecific vocalizations in zebra finches is not affected by a shift in frequency. Our experiments using shifted signals are in accordance with this previous work. Indeed, behavioral responses tolerate some variations in absolute fundamental frequency. However, the latency of response to shifted stimuli was clearly longer than the response latency to controls. Because the chosen shifted values of the fundamental frequency reflect the limits of the range of variation containing 99\% of our population, the tolerance of birds’ responses to these shifts is likely to be a consequence of the variation of call pitch among individuals. This tolerance might have been further challenged by a higher degree of modification at the edge of the population range.

Our results show that male calls without any frequency modulation elicit some behavioral responses. This may be explained by the fact that a male call without frequency modulation resembles a female call. Nevertheless, the reverse version of the downsweep component of the male call did not elicit any response. This is not surprising as Lohr et al. (Lohr, Dooling, & Bartone, 2006) demonstrated that zebra finches discriminate very fine temporal structures. Thus, sequential high and low frequencies are not necessary to trigger a response: the recognition system is tolerant to steepness of the slope but rejects a reverse slope. This is consistent with the hypothesis that a call has to overlap with a defined frequency range to evoke a response.
Significant Acoustic Cues in Female Calls To Elicit a Behavioral Response in Conspecifics

Our results emphasize that the stimulating value of female zebra finch calls is mainly due to the energy spectrum. Indeed, behavioral responses require a minimum of four harmonics in female calls and are disrupted by modifications of the relative levels of energy of the harmonics. We hypothesize that female calls require matching a certain frequency bandwidth to trigger a response. Conversely, the main temporal cues of female calls are unnecessary to trigger a response: suppression of frequency modulation as well as significant alterations of female call duration do not impair behavioral responses to playback.

Previous experiments using go/no-go operant conditioning procedures showed that zebra finches are able to discriminate between two harmonic structures that differ by only one harmonic (Okanoya, 2000; Uno, Maekawa, & Kaneko, 1997). Thus, birds may perceive a difference between control (seven harmonics with the fundamental frequency) and all the other experimental stimuli containing no fundamental frequency. Nevertheless, the experimental calls elicited behavioral responses that were not significantly different from the control calls as long as they contained at least four harmonics. Female calls may allow behavioral response in spite of the lack of the fundamental frequency and several harmonics.

Shifts of the fundamental frequency of female calls resulting in absolute pitch modifications do not significantly impair behavioral response. Nevertheless, as also observed for male calls, the latency of the response to experimental stimuli was clearly longer than the latency of the response to the control. Here again, this tolerance could have been further challenged by a higher degree of modification at the edge of the population range.

Previous studies using go/no-go operant procedures showed that zebra finches can detect alterations in the timbre of complex harmonic signals (Cynx, Williams, & Nottebohm, 1990; Lohr & Dooling, 1998). Our results show that a drastic modification of the relative energy levels of harmonics—and thus of the timbre—impairs the behavioral value of female calls.

Conclusion

Our results show that the stimulating value of zebra finch calls is mainly due to the broad energy spectrum in female calls and to the down-sweep frequency modulation in male calls. In this study, we showed that male and female zebra finch calls still elicit a strong behavioral response in spite of important modifications of the call acoustic structure. Thus, the stimulating value of the calls does not rely on the fine acoustic structure. Zebra finches are colonial birds that show long-term mate fidelity and face the problem of individual vocal discrimination among many conspecifics. The fine structure of zebra finch calls is likely to convey information on the individual identity of the sender (Vignal, Bouclet et al., 2008; Vignal, Mathevon & Mottin, 2008; Vignal et al., 2004).

References


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