Sexual responses of female canaries at the onset of song stimuli

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Abstract. The salience of a short conspecific song phrase versus a long one or a short heterospecific song phrase was tested in females of the common domesticated canary (*Serinus canaria*). All phrases were computer-edited. The copulation solicitation displays (CSD) served as an index of female responses. The results support the hypothesis that female canaries are able at the onset of a song phrase stimulus: 1) to respond to a conspecific one by displaying high levels of CSD and 2) to discriminate a conspecific from a heterospecific one. Proximal explanations examine the link between perceptual processes and phonology in the canary. Functional explanations investigate the relations between female responsiveness and the process of pair formation.

Key words. Song stimuli; syllable phonology; sexual responses; female canaries; pair formation.

Female songbirds respond preferentially to particular attributes of song when song is presented in the absence of males. Their choices have played a role in song evolution[1]. Male songs can thus be studied as a display partly resulting from reproductive isolation and selective mating. Evidence of reproductive isolation is gained when females are able to discriminate between conspecific and heterospecific songs[2,3]. Evidence of selective mating has been demonstrated by the fact that a larger repertoire and greater song diversity have been shown to be important for females in eliciting higher levels of copulation solicitation displays (CSD)[4-8].

In captive female canaries (*Serinus canaria*) both song conspecificity and song diversity have been shown to enhance sexual behavior. Females build nests faster and lay more eggs when they are exposed to a large rather than to a small repertoire[9]. The information made available by song can influence the overall responsiveness of recipients by engendering gradual changes in endocrine states that affect a broad spectrum of 'tonic' reproductive behavior. When using CSD, which is a 'phasic' sexual behavior, as an index of song potency, the discrimination between conspecific and heterospecific songs appears to be very strong when the alien and conspecific stimuli have a very different syntactical structure[10]. However, species and even breed recognition do not appear to be so acute as in other wild or domesticated species. Females do display CSD when tested with song phrase stimuli from alien breeds and alien species whose framework shares the general features of the domesticated canary's song, i.e. partition into discrete repetitive trills[11].

Observations of these CSD displays during our prior experiments demonstrated that their onset occurred in 90% of cases during the first part of a song phrase stimulus playback (before 5 s). Consequently, we carried out experiments to demonstrate that female canaries do recognize and discriminate between song stimuli at their onset: that a short conspecific song phrase is sufficient to elicit high levels of CSD, and by contrast, a short heterospecific song phrase elicits weak responses.

Materials and methods

The basic procedure was to treat captive female canaries with estradiol and to present them with different computed and manipulated male 'song phrase stimuli'. The amount of sexual response shown by the females was evaluated by measuring the number of CSD to these stimuli. The CSD only appears during song playback. We measured the occurrence of displays: scoring the responses and awarding 0 for no display, 0.5 for an incomplete display, 1 for a complete display. These displays were scored as separate events if they were separated by more than 1 s of normal posture. Scores for the responses of each female were summed separately over each entire 'song bout' for statistical analysis.

The strain of canary used in this study, locally called 'common canary', is an outbred form, of heterogenous genetic background. Eight one-year-old female canaries were housed together in an aviary for one month at least, prior to treatment, with a 15 : 9 (L/D) photoperiod. Birds were sexed by the size of the cloacal protuberance. Each female was then implanted with crystalline 17β-estradiol contained in a silastic tube (7 mm long, 5–6 mm of hormone, 1.45 mm inner diameter, 1.925 mm outer diameter) with both ends plugged by silastic adhesive (Dow Corning). Implants were placed under the skin on the lateral surface of the body just anterior to the thigh. As soon as they had received implants, the birds were caged in sound attenuation chambers for the entire testing period. They were observed through one-way glass windows to avoid disturbance. More housing details can be found in Kreutzer and Vallet[11].

The song phrase stimuli (fig. 1) played back here lasted for 9 s and were separated by a 9-s pause. Five song phrase stimuli were repeated, thus making a song bout. Values for the song phrase and pause durations were chosen according to the rules of canary song organization[12,13]. All song phrase stimuli were built with computed song samples. Most of them were chosen from Güttinger's sound library. The songs were constructed using an analysis and synthesis software developed by J.-P. Richard[14]. The program provides editing com-

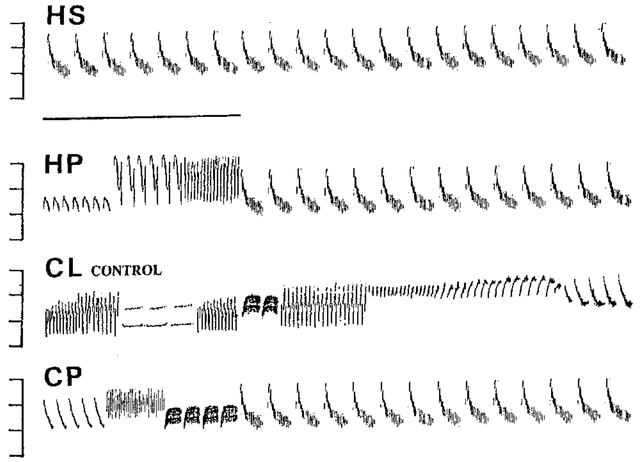

Figure 1. Song phrase stimuli used in playback experiments to test discrimination in female canaries at the onset of a song stimulus. All these stimuli are computer-edited. From top to bottom; 1) HS (heterospecific syllable): repetitive 'harsh sounding' greenfinch syllable; 2) HP (heterospecific phrase), bipartite song: first part (3 s) made of three trills from a greenfinch, second part (6 s) extracted from HS; 3) Control (CL): edited copy of a canary song used in previous experiments; 4) CP (conspecific phrase), bipartite song: first part (3 s) made of three trills from a domesticated canary song, second part (6 s) extracted from HS. Frequency markers show, in ascending order, 0, 2, 4 and 6 kHz. The time interval represents 3 s.

mands that allow manipulations of a digitized signal along time, frequency and amplitude dimensions. The control stimulus (CL: control) was a sequence selected in a song phrase stimulus employed earlier[11]. This 'own strain' common canary song elicited strong reactions in female canaries.

The three other songs were created by assembling natural syllable types into patterns in order to test the saliences of the conspecific and heterospecific song phrase stimuli at the beginning of a song stimulus. Two songs are bipartite: the first part lasts three seconds and the second part six seconds. In one song (CP, conspecific phrase) the first part is built with three conspecific syllables. These syllables were chosen from a song emitted by the same male that produced the control song, outside this control song to avoid responses to CP induced by similarity or generalization from the control. In the other song (HP, heterospecific phrase) the first part is built with three heterospecific syllables (greenfinch: *Chloris chloris*) chosen according to the three most frequent classes of syllables described in greenfinch repertoires[15]. As the most important species-specific song differences between domesticated canary and greenfinch are found in the higher structure of songs, mostly song segmentation[15], the first part of the HP song was built according to conspecific segmentation rules. Thus, syllable phonology is the most important difference between HP and CP at the very beginning of these song stimuli. The second part of these two songs was 'filled' with a greenfinch syllable whose 'harsh sounding' tonal quality was rarely observed in the domesticated canary[13, 15]. In order to control the effect of this syllable we tested it in a fourth song phrase stimulus (HS, heterospecific syllable).

A test session began two days after implantation. The test tapes were played back for several days to the isolated females. The four different song bouts were presented in random order on each day. From one day to another, the 'random order' changed. Testing was carried out between 10 h and 18 h, from April to July 1991. Playback stimuli were played from a Panasonic tape recorder (RQ-P165, 50–14000 Hz) over an Aiwa full-range speaker, power booster (SC-A8, 100–18000 Hz) placed in the sound-attenuation chamber.

For each song bout the CSD scores for the different presentations were summed. Then the four stimuli were compared and tested for significance using the Friedman two-way analysis of variance by ranks for comparison of conditions with a control, having first confirmed overall heterogeneity. The extent of association among the eight rankings for the four-song stimuli was measured using the Kendall coefficient of concordance.

Results

The eight females were tested with the four-song phrase stimuli. No female failed to respond. Four females did not display in response to the heterospecific syllable (HS) song phrase stimulus. As a whole, the song phrase stimuli with conspecific syllables (control and CP) elicited most of the CSD (fig. 2). There is significant heterogeneity in the responses to the four different song phrase stimuli, as assessed by the number of CSD (Friedman, df = 3, Chi-square = 14.55, $p < .01$). The song stimulus CP elicited a similar level of response as compared to the control. In contrast, HS and HP stimuli elicited levels of responses that were smaller than the control (Friedman comparison of conditions with control, $p < 0.05$ for HP, $p < 0.01$ for HS – sums of ranks: control = 11, CP = 16, HP = 24, HS = 29; limit value = 15.08 for $p = 0.01$ and 12.14 for $p = 0.05$). Since low rankings indicate the most behaviorally salient songs, the control and CP phrase song stimuli were the most preferred; the number of CSD elicited by these songs was not significantly different. HP elicited weak responses, not significantly different from those to HS (Wilcoxon two-tailed, $T = 1.5$, $N = 6$, $p > 0.05$), but significantly different from those to CP (Wilcoxon two-tailed, $T = 2$, $N = 8$, $p = 0.02$). Kendall's coefficient of concordance showed that the females ranked the four-song phrase stimuli in a similar way ($W = 0.606$, $p < 0.01$).

Conclusions and discussion

As a whole these results support our hypothesis that female canaries are able: 1) to respond at the onset of a conspecific song phrase by showing high levels of CSD, and 2) to discriminate a conspecific song phrase from a heterospecific one at the very beginning of a song stimulus. As expected, the harsh-sounding syllable, outside domesticated canary phonology, elicited very low levels of responses. Why females are responsive and discriminative at the very beginning of song stimuli raises ques-

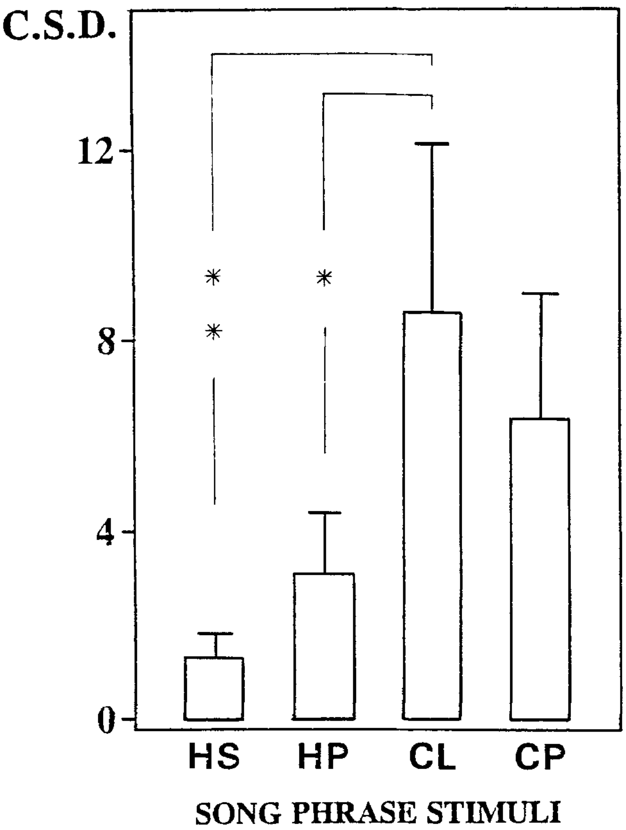

Figure 2. The mean number (+ SE) of CSD elicited in female canaries ($n = 8$) by each song phrase stimulus (abbreviations as in fig. 1). Significant heterogeneity was tested using Friedman two-way analysis of variance by ranks. Labels indicate significant differences (*: $p < 0.05$; **: $p < 0.01$) between control (CL) and two-song stimuli: HS and HP (Friedman comparison of conditions with control).

tions at both proximal and functional levels of explanation.

Proximal explanations for responsiveness and discrimination would come from studies dealing with perceptual processes and song patterns. In song birds, and especially in the canary, the auditory and vocal production systems appear to be about equally matched in precision. Psychophysical data on auditory perception indicate a well-developed resolving power for frequency, time domain, and intensity. So song variations in temporal patterning, syllable frequency and intensity are well matched to the resolving power of the ear[16]. The great similarity among avian auditory processes suggests that one species does not filter out the vocalization of another species[16]. Thus, acute discrimination seems unlikely to be based on differences in hearing between species. Nevertheless, this does not rule out the possibility that special fine hearing processes may account for recognition and discrimination. For example, one can observe that greenfinch phonology does not match the best capacity of canary hearing very well. Although Güttinger et al.[15] stated that major phonological acoustic cues were similar in the two species, they also underlined some specific differences, especially a higher range of frequency in some greenfinch syllables. This is the case for two syllables used in the first part of HP; syllables of the second and of the third trills. In these syllables, there are high pitched frequency modulation (FM) emitted at similar intensity levels to the low pitched FM. These high pitched FM (6.5 kHz for the second trill and 5.5 kHz for the third) are rarely reached in canary songs. They are partly outside the best absolute threshold sensitivity of canaries (2 to 5 kHz[17]) and could be used by female canaries to identify these FM as heterospecific cues. If so, they play the role of 'rejection markers'[18].

Other processes may account for complex specific sound recognition and discrimination. Attentional mechanisms appear to be critical when song birds are tested with complex species-specific vocalizations. Variables such as prior experience, learning and stimulus salience may serve to focus attention and enhance species recognition at the onset of song stimuli[19]. Moreover, we know that song birds discriminate and categorize conspecific versus heterospecific syllables presented in pairwise comparisons[20]. So acoustic cues, even only a few, allow categorization and discrimination. These perceptual, attentional and 'cognitive' processes are not mutually exclusive, and may help to explain why females so rapidly display high levels of sexual responses at the very beginning of conspecific vocalizations.

From a functional point of view, many observations demonstrate that male song is essential in intersexual communication and pair formation. Temporarily muting male birds in the field may dramatically alter their potency for mate attraction and retention[21]. Moreover, some recent field data strongly suggest that male songs have an important effect on mate choice in the context of extra-pair copulations[22,23]. Some field observations and experiments support an assortative mating hypothesis[24], based on the similarity between the songs of males and their mate females' songs, after testosterone implantation. In the laboratory, estradiol techniques allow females' song preferences to be tested, demonstrating that song syntax is behaviorally salient for intraspecific recognition[25] and that female sexual responses are consistent in certain species or subspecies with assortative mating observed in the field[8]. Choice-experiments carried out in the field or in outdoor aviaries demonstrated that average song bout length and repertoire size are positively correlated with mate choice and male breeding success[23,26]. In the laboratory, preferences for diversified songs and large repertoires were also found[5,8]. Some special parts of the songs are more salient than others in conveying information that is preferred by females[27,28].

However, in contrast to these positive associations between song and female behavior, some experiments under natural conditions demonstrated that females' pairing was not well correlated with their song preferences or their song types as induced by testosterone[29]. Females were also observed mating with males singing slightly 'unfamiliar' songs[30]. Both song and plumage characteristics need to be combined to elicit high levels of CSD in some bunting species[31]. Other criteria than song can be decisive in mate choice: Alatalo et al.[32] reported that territory quality was better correlated with female choice than song repertoire.

In the female canary, the rapid response to conspecific song phrases raises questions about the function of song in pair formation. Because the sexual selection theory hypothetizes that females should be 'choosy', one might expect a long latency before CSD is elicited. How can a female be selective if she is so rapidly responsive? Our experiment raises questions about the relations between the methodology used, female responsiveness and pair formation. As stated by Eens et al.[23], estradiol implantation techniques are powerful since they effectively rule out confounding variables from all other aspects of male or territory quality. However, as Dabelsteen[28] pointed out, hormone treatment actually accelerates processes that naturally last for several weeks at the beginning of the breeding season. Consequently, females invite copulation prior to pair formation. Thus, the ability of male song to influence mate choice may not be demonstrated by experiments of this type, and remains hypothetical. Events which may happen over a period of time, and may be prerequisites for pair formation and copulation, are experimentally erased. In the canary, as in other species well-known for female responsiveness to song, more experiments are needed to demonstrate that during the course of pair formation song is indeed required, and elicits an invitation to copulation.

In conclusion, we have found that female canaries do recognize and discriminate song phrase stimuli at the very beginning of a song. Three seconds of conspecific song are sufficient to elicit high levels of CSD. The function of this rapid response remains a problem for further investigation.

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