

## Testosterone facilitates some conspecific song discriminations in castrated zebra finches (*Taeniopygia guttata*)

(birdsong/neuroethology/operant)

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**ABSTRACT** An experiment was designed to test for the influence of testosterone on song discriminations. We found that testosterone did have an effect, which interacted with practice and the nature of the stimuli. Fourteen adult castrated zebra finches (*Taeniopygia guttata*) were grouped into seven pairs. In each pair, one bird was implanted with a testosterone-filled silastic tube and the other was implanted with an empty silastic tube. They were then trained on a *go/no-go* operant task to discriminate between bird songs in six consecutive experiments. The songs to be discriminated were as follows: experiment 1, two canary song segments; experiment 2, the bird's own song and that of the other member of the pair; experiment 3, the same two songs as in experiment 2 but with reversed stimulus–response contingencies; experiment 4, two other zebra finch songs; experiment 5, another two zebra finch songs; and experiment 6, another two canary song segments. There were no reliable learning differences between birds treated with testosterone or with an empty silastic in experiments 1 and 3–6. However, in experiment 2, testosterone-treated birds mastered the discrimination between their own song and the song of the other member of the pair in fewer trials than birds treated with empty silastics. We suggest that a song's ability to control the behavior of male zebra finches is influenced by the nature of the song, prior experience with the training paradigm, and hormone levels.

Adult male songbirds sing to defend a territory and attract a mate (1, 2). The amount of singing is influenced by the bird's reproductive condition, as determined by blood levels of gonadal hormones. Zebra finches offer a good example of this relation between hormone levels and song. Adult males with high testosterone levels sing a lot; those with low testosterone levels sing much less (3, 4). Earlier work has shown that brain areas used for song production also respond to playbacks of conspecific song and thus may be involved in song perception (5–7). Since several of these brain areas are androgen sensitive (8), song perception may be influenced by hormones.

Perception is relatively easy to test in humans because subjects can comment on what stimulus features they can perceive and the relative ease with which two different stimuli can be told apart. Direct reporting of perception is not possible in animals that, however, can be trained to give different responses to different stimuli. The outcome of such training tells us what stimuli can be discriminated. When a bird gives an operant response to one but not to another of a pair of song stimuli, it has mastered a song discrimination. If two stimuli are perceived as very similar, then the discrimination will be difficult, or impossible. However, ease in solving a discrimination test need not result directly or exclusively from perception—for example, it may also be influenced by attention and by the extent to which a partic-

ular stimulus, chosen by the investigator, can be associated with a particular response, also chosen by the investigator (9).

We tested whether the ability of zebra finches to acquire song discriminations was influenced by testosterone. In a companion paper, we report that a male zebra finch acquires a conspecific song discrimination in fewer trials if its own song is one of the stimuli (10). We report here that this effect in adult castrate zebra finches is influenced by testosterone.

### METHODS

**Subjects.** We trained and tested 14 male zebra finches (*Taeniopygia guttata*), all castrated as adults (older than 90 days). Castration was performed by suction of testicular tissue and cautery of the remainder of the tunica albuginea. Castration is known to reduce circulating testosterone to negligible levels (11). Ten of the birds (pairs I–V) had been castrated for over a year, and the others were castrated at least 6 weeks before the beginning of the discrimination training. All birds were experimentally naive. They were maintained on a standard aviary diet and food-deprived 4–6 waking hr before each experimental session. Dry seed was used during experimental sessions to reinforce discrimination behavior. Water and grit were available to the birds at all times. All birds had been on a 12:12 light:dark cycle with full-spectrum fluorescent lights for at least 2 months before the beginning of the experiment. Year-round temperature in the aviaries was maintained at between 21°C and 27°C. Humidity, as measured with a chart recorder, varied from 40% to 80% (median = 60%) in the summer months (when pairs I–V were trained) and from 10% to 60% (median = 30%) in the fall (when pairs VI–VII were trained).

The 14 birds were divided into seven pairs. Members of a pair were kept in the same cage, separated by a wire partition. A 5-mm silastic tube (0.76-mm inner and 1.65-mm outer diameters) was placed beneath the skin of the back of each bird in a pair on the same day, 2–4 weeks before onset of discrimination training. One of the birds in each pair received an empty silastic tube (control); the other received a tube filled with testosterone. The amount of hormone used is known to restore the level of courtship in castrate zebra finches to that of intact controls (12). Pairs I and II were trained and tested in April and May, pairs III–V in July and August, and pairs VI and VII in October and November of 1990.

We did not measure blood testosterone levels but confirmed the effects of castration and testosterone implantation by observing that a secondary sex characteristic and behavior were altered by castration and testosterone treatment. The bills of male zebra finches in reproductive condition are deep red while those of females are orange (13). Following castration, the bills of males acquire a female coloration. Whereas castrates seldom sing to females, intact males in reproductive condition usually engage in vigorous courtship (11). All subjects displayed orange rather than red bills at the

time the silastic tubes were implanted. Seven days after implantation the birds implanted with testosterone, but not the control birds, had red bills and sang within 5 min of being placed in the presence of a female zebra finch.

**Behavioral and Acoustical Apparatus.** The test cages were standard operant chambers with speakers (10). Perch hopping was the operant behavior. An observation perch was placed parallel to the operant panel and in front of the speaker. A similarly equipped response perch was placed at the entrance to the food dispenser. The test cages were placed inside illuminated sound attenuation chambers. Stimulus presentation, experimental contingencies, and data collection were controlled on line by a microcomputer (10).

**Stimuli and Procedures.** The 14 zebra finch song stimuli were single, stereotyped zebra finch songs recorded during non-courtship behavior (14, 15). The 20 canary (*Serinus canaria*) stimuli—song segments from four Waterschlagger strain canaries—were comparable in length to the zebra finch stimuli. The initial recordings were made with a cassette recorder, digitized, and stored on the computer's hard disk (20-kHz sampling rate). The overall loudness level of each stimulus was measured with a sound level meter and then set to 70 dB(A) sound pressure level, as measured at the point in front of the speaker where a bird heard the sound. Food-deprived finches were taught first that hopping to the center (detector zone) of the observation perch provided them with food. This was a shaping procedure that taught them how to operate the equipment. When they learned this, they were placed on a *go/no-go* procedure. Perching in the detector zone on the observation perch began a trial. The computer randomly selected a *go* or *no-go* stimulus with equal probability. A *go* response consisted of moving to the response perch within the 3-s *go* period. A *go* response to the *go* stimulus produced access to food. The same response to the *no-go* stimulus turned off the house light for 15 s, signaling to the bird that it had made an error. A *no-go* response always resulted in the end of the trial after 3 s. Daily sessions were 3–4 hr in duration.

Six experiments were conducted in succession and were completed by all 14 subjects. The order of the experiments was the same for all birds. In experiment 1, the finches in each pair were trained to discriminate between two canary song segments. In experiment 2, the canary song stimuli were replaced with zebra finch song stimuli. For each bird in a pair, the *go* stimulus was his own song; the *no-go* stimulus was the song of the other bird in the pair. Experiment 3 reversed the stimulus–response contingencies of the two zebra finch songs used in the previous experiment. Experiment 4 required that the subjects discriminate between two other zebra finch songs. Experiment 5 required that the birds discriminate between yet another two zebra finch songs. Experiment 6 was a repeat of experiment 1, using two novel canary song segments. The birds had not heard the stimuli used in experiments 1 and 4–6 prior to the study.

## DATA ANALYSIS AND RESULTS

Acquisition of a song discrimination was determined by measuring the mean percent correct responses across blocks of 100 trials. The learning criterion was set at 75%, the midpoint between chance and errorless performance.

The mean number of trials to reach criterion for birds in all experiments is shown in Fig. 1. A hierarchical repeated measures analysis of variance on these data, using the treatment and the seven pairs of birds as factors across the six experiments, showed that there was no overall significant effect for the hormonal treatment,  $F(1, 6) = 5.25$ ;  $P < 0.10$ . However, a test for simple effects showed a significant effect,  $P < 0.05$ , for experiment 2 alone.

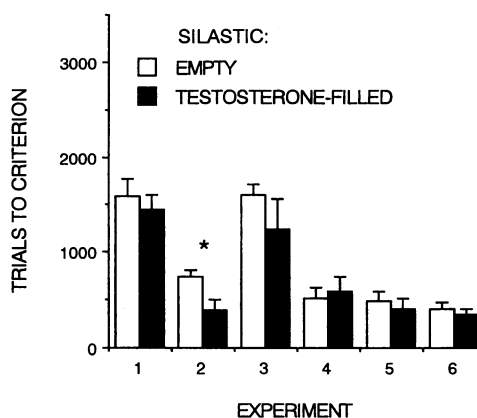


FIG. 1. Mean number of trials to criterion (+ SEM) for each of the six song discrimination experiments, listed in the order in which they were performed: experiment 1, two novel segments of canary song; experiment 2, the bird's own song and that of its cage mate; experiment 3, the same songs as in experiment 2 but with the stimulus–response contingencies reversed; experiment 4, two novel zebra finch songs; experiment 5, two other novel zebra finch songs; and experiment 6, two other novel segments of canary song. Differences between group means reached significance only in experiment 2, as indicated by the asterisk.

The number of trials required to reach criterion varied across experiments,  $F(5, 30) = 52.48$ ;  $P < 0.01$ . With the exception of experiment 3, the birds required fewer trials to master successive discriminations. This practice effect may have resulted from increasing familiarity with the training situation—the apparatus, the time frame, and the importance of sound stimuli as predictors of the consequences of an operant response. The significant increase in numbers of trials required in experiment 3 was probably due to the nature of a reversal discrimination. In this experiment the birds had both to unlearn the stimulus–response contingencies from experiment 2 and to learn the new ones demanded by experiment 3 (16).

Although humidity has been suggested as a factor in mediating the reproductive state of zebra finches (17), we failed to detect in our experiments, using post hoc *t* tests, any significant effects between pairs trained during different median levels of humidity.

## DISCUSSION

The results of our experiments suggest that testosterone treatment, the nature of the stimuli, and practice with the training paradigm, by themselves or in interactions, affected the difficulty of the task, as defined by the number of trials required to reach criterion.

**Effect of Practice with the Training Paradigm.** A comparison of the outcome of experiments 1 and 6 suggests that a majority of the trials required to reach criterion in experiment 1 were spent learning about the general aspects of that experiment—what stimuli to attend to, what responses were required, and the time frame involved. Learning about these general aspects of the training situation may be affected by the nature of the stimuli used. So, for example, intact zebra finches initially trained on procedures identical to experiment 2—i.e., discriminating between their own song and a cage mate's song—acquired the discriminations in fewer trials than the zebra finches in experiment 1 (compare figure 2 of ref. 10 with Fig. 1 of the present report).

Performance in experiments 4–6, after the birds had had much exposure to the training situation, required comparatively low numbers of trials to reach criterion. From this we infer that at that stage of training the birds found it equally

easy (or difficult) to perceive the difference between pairs of canary or zebra finch songs and memorize these songs and their *go* or *no-go* valence.

**Hormonal Influences on Song Discrimination.** Testosterone made the discrimination between a bird's own song and that of a cage mate significantly easier (experiment 2). The ease with which such a discrimination is mastered has also been shown to change seasonally. Intact adult zebra finches kept on a natural photoperiod and tested during the summer require fewer trials to reach criterion than birds tested during winter (10, 18). The mean number of trials in experiment 2 was 700 for castrated birds with empty silastics and 314 for testosterone-implanted castrates. Likewise, the male birds in our original report of seasonal differences (10) reached criterion in approximately twice as many trials during the winter as during the summer (figure 3 in ref. 10). This seasonal effect may be mediated by testosterone if the testosterone levels of adult male zebra finches are higher during the summer than during the winter—something we do not yet know.

The testosterone effect on song discrimination may be restricted to conspecific song. The initial discrimination between canary song segments, which the birds found difficult to master, was not affected by the testosterone treatment. In addition, when the discrimination was rendered easy by practice, as in our present experiments 4–6, then testosterone treatment did not make it easier. Testosterone treatment helped achieve criterion in a fewer number of trials when the training situation was still relatively novel and one of the stimuli to be discriminated was the bird's own song (experiment 2).

We know from other work (5, 7, 19) that neurons in parts of the song system, such as the high vocal center (HVC), are particularly sensitive to playbacks of the bird's own song and that this sensitivity may be affected by hormones (20). Lesions to these nuclei interfere with prompt discrimination between conspecific songs (18, 21, 22). Cells in HVC and other song nuclei concentrate testosterone. This hormone, acting on androgen-sensitive cells, may affect the attention of a bird to stimuli that resemble its own song and the ease with which such stimuli gain control over an operant response. In addition, testosterone may affect the way in which song is perceived. These various effects could occur directly, by testosterone acting on the cells and circuits involved, or indirectly, by testosterone affecting the incidence of song. Birds that sing more might find it easier to discriminate between conspecific songs—e.g., by comparing them with their own song (3, 4).

**Overview.** Song discrimination—learning to give different responses to different songs—can be affected by testosterone levels. In the present series of experiments this hormonal effect was restricted to discriminating between the bird's own

song and that of a cage mate. Testosterone did not have an effect on other, subsequent discriminations between zebra finch songs or between alien songs, which, as a result of practice, became easier to master. Social significance, practice with the training situation, use of the bird's own song, and the circulating levels of testosterone or its metabolites may act via similar pathways, by helping focus attention on the relevant stimuli.

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