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Changes in plasma levels of testosterone during male-male interactions in the song sparrow, *Melospiza melodia*: time course and specificity of response

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Summary. Previous investigations have shown that male-male interactions over territory boundaries result in an increase in plasma levels of testosterone (T). In order to determine the time course of this increase following an agonistic challenge, male song sparrows, *Zonotrichia* (= *Melospiza*) *melodia*, were exposed to simulated territorial intrusions. Responding males were captured at intervals after onset of the intrusion for up to 60 min. Plasma levels of T were elevated by 10 min after onset of the challenge, and remained high in birds sampled 10–60 min after onset of the intrusion. In addition, plasma levels of luteinizing hormone (LH) increased and were significantly elevated after 10 min of stimulation. The results suggest that at least the prolonged elevation of T secretion precipitated by a male-male interaction was mediated via an increase in LH release. However, the initial surge of T levels in plasma may be LH independent.

Investigations of captive birds confirmed that circulating LH and T levels increased when male song sparrows were challenged by a conspecific male. Challenges involving a male house sparrow, *Passer domesticus*, had no effect suggesting that responses to male intrusions were species characteristic and not a result of general arousal. Further, auditory stimuli (vocalizations) alone, and visual stimuli (a devocalized male) alone were less effective in stimulating an increase in secretion of T than a combination of auditory and visual stimuli.

Key words: Testosterone – Plasma levels – Song sparrow – *Melospiza melodia* – Male-male interactions

Introduction

In the central Hudson Valley of New York State (42° N), male song sparrows, *Zonotrichia* (= *Melospiza*) *melodia* arrive on the breeding grounds in early spring (March) and establish breeding territories. This period is marked by heightened aggressive interactions as males establish territory boundaries, and is accompanied by a transitory increase in plasma levels of luteinizing hormone (LH) and testosterone (Wingfield 1984a). Although increasing baseline levels of LH and testosterone (T) in spring are regulated by increasing photoperiod (Wingfield and Moore 1987), the early vernal peak of T (well above the level induced by photoperiod) is stimulated by behavioral cues during agonistic interactions (Wingfield 1984b, 1985). However, plasma levels of LH do not increase consistently in response to an agonistic challenge despite reproducible elevations of T. This inconsistency has raised some questions concerning the mechanism by which behavioral stimuli may influence endocrine function. To clarify this issue, the experiments described below were designed to investigate the time course by which plasma levels of T increase after an agonistic challenge, and to determine if LH levels respond in a similar manner.

Further experiments were conducted to determine whether the hormonal response to a challenging male is specific, or if general arousal resulting from the introduction of a heterospecific male could also elicit the response. A third set of experiments were designed to distinguish between different sensory modalities important for mediating this endocrine response. During a male-male interaction it is possible that information from such a social cue can enter the central nervous system via a chemical route, tactile, visual or auditory modalities, or a combination of all or some of these. Elucidation of these pathways is a critical step for investigating possible neural pathways for environmental signals that regulate neuroendocrine and endocrine secretions.

Materials and methods

Field investigations. Adult and territorial free-living male song sparrows were used as experimental subjects at sites located in Dutchess

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Abbreviations: DHT dihydrotestosterone; *LH* luteinizing hormon; *T* testosterone

County, New York (for details see Wingfield 1984a). All birds were captured in mist nets and traps baited with seed between the hours of 07.00 and 12.30. Blood samples were collected immediately (approximately 300 μl from a wing vein and within <5 min) in the field, stored on ice until returned to the laboratory, centrifuged, and plasma frozen at −20 °C until assay (see Wingfield and Farner 1976).

Agonistic challenges (territorial intrusions) were simulated by placing a caged adult male song sparrow in the center of a free-living male's territory and playing conspecific song through a loud speaker placed alongside the cage. A territorial male will almost always attack the decoy male in an attempt to repel the apparent intruder. Responding males then can be caught at intervals after onset of the intrusion and blood samples collected. For analyses, sampling periods were divided as follows: 1–4 min; 5–10 min; and 10 min+(10–60 min). Controls were captured in traps (baited with seeds) as they foraged and thus were not involved in any agonistic interaction with other males at the time of sampling. For procedural details of 'simulated territorial intrusions' see Wingfield (1985).

Adult and territorial male song sparrows were also challenged with a tape recording of conspecific song to provide auditory cues while eliminating possible tactile, chemical and visual stimuli typical of an attacking male. Another group were exposed to a caged male song sparrow that had been devocalized by placing an incision (approximately 10 mm long) in the interclavicular air sac (see Smith 1977). This procedure is rapid and the air sac heals within 3–10 days restoring full vocal capacity. Presentation of devocalized males provided visual (and possibly chemical) cues but not auditory and tactile stimuli. A third set of males were presented with a devocalized male plus play back of tape-recorded songs thus providing the full repertoire of possible sensory cues (except tactile) associated with a male-male interaction. Control males were bled immediately after capture in Potter traps baited with seeds and had not, as far as we were aware, been involved in any interaction with an intruding male just prior to capture. The different types of stimuli were presented for at least 10 min (range 10–40 min), the responding male captured in a mist net, and a blood sample collected for hormone analysis as described above. The time of presentation was variable because it is not possible to catch each free-living bird exactly on cue. However, this does not invalidate the data (see Wingfield 1985), and the laboratory experiments described below (in which birds were sampled at a uniform time after challenge) provide a check on potential error introduced by variable times to catch each bird after presentation of the stimuli in the field.

To check for possible differences in behavioral response to the variable stimuli presented, the behavior of the responding male was collected for 10 min prior to capture. If defending males respond differently to each type of presentation, then it is possible that any differences in hormonal response may be due to the subject's motivational state, and subsequent behavioral response to the stimulus, rather than to any inherent characteristic of the stimulus itself. Behavioral data collected included number of songs; closest approach to the source of stimulus; time spent within 5 m of the source of stimulus; and number of flights (characteristic movements around the source of stimulus that are regarded as being highly aggressive in context). For further details of the collection of behavioral data see Wingfield (1984b, 1985).

Laboratory investigations. Adult male song sparrows were captured in 'Potter traps' baited with seeds during September and October. Capture sites were within Washington Township, Dutchess County, New York. Males were housed singly in cages (27×30×52 cm) in an indoor aviary (temperature 23±2 °C and natural photoperiod). All birds received moist food (mashed egg and a vitamin supplement) daily; and greens, fruit and meal worms weekly. Seeds, grit and water were available ad libitum.

The following May (when gonadal development was complete), 3 groups of 6 males were transferred to experimental chambers and exposed to a photoperiod of 15L/9D and ambient temperature (25–32 °C, i.e. close to natural local temperatures of May and June). All cages were arranged so that males could see one another and show agonistic displays accordingly. However, physical contact was not possible. Birds were allowed to acclimate to these conditions for 3 weeks and then a blood sample was collected from a wing vein (as described above).

After 7 days an adult, sexually mature male song sparrow was placed in a cage equidistant (1 m) from males in group 1. Care was taken to select a male that the subjects had not interacted with or seen before. Visual observations from behind a screen indicated that introduction of the novel male induced a bout of aggressive displays from the experimental males. Males in group 2 were exposed to an adult, sexually mature male house sparrow, *Passer domesticus*, placed in a cage as described for group 1. Visual observations revealed no obvious aggressive responses compared with those for the introduction of a novel male song sparrow in group 1 (Wingfield, unpublished observations). The house sparrow was chosen as a control stimulus because this species is abundant in central New York, and it is highly likely that all subject male song sparrows had encountered the species at least once. Also, house sparrows and song sparrows do not compete for territories, nest sites, or food (as far as we are aware). The subject male song sparrows were allowed to interact with the 'intruder' for 6 h and then a blood sample was collected for measurement of T level. Introduction of an 'intruder' by the investigator resulted in considerable disturbance of the experimental birds within the confines of the experimental chamber. Thus a longer time interval for the challenge (compared with the field investigations) was required to ensure that any residual effects of the disturbance had abated. All samples were collected between 12.00 and 14.00 h.

Birds in group 3 were bled between 12.00 and 13.30 h to provide baseline levels of T. After 7 days these males were exposed to tape-recorded conspecific song (i.e. auditory information only) played through a speaker placed equidistant (1 m) from each cage. Play-back began at 07.45 h and ran for 30 min followed by 30 min silence, then 30 min playback etc. until 12.15 h when all males were bled. Within each 30 min play-back sequence, one song type was repeated 10 times at 10 s intervals. The sequence of 10 was followed by 1 min silence and then a sequence of 10 songs of another song type began. A total of 10 song types were repeated during the play-back sequence. These sequences of songs are similar to the patterns of songs given by free living males (see Wingfield 1984b, 1985). Seven days after the playback sequence ended, another blood sample was collected (between 13.00 and 14.00 h) to measure baseline T levels. These males were then exposed 7 days later to a devocalized male (as described for the intact male challenge above). A blood sample was collected after 4 h of exposure (ca. 13.00–14.00 h). In this way it was possible to measure the endocrine responses of mature male song sparrows to auditory stimuli alone, and visual (plus possible chemical) cues alone in comparison to responses to an intact male (combination of all possible cues except tactile) as described in the previous experiment.

Hormone analysis. Plasma levels of LH were measured by radioimmunoassay (Follett et al. 1972, 1975; see also Wingfield 1984a for details and reliability criteria), and plasma levels of testosterone were measured by radioimmunoassay after chromatography on diatomaceous earth/glycol columns (see Wingfield and Farner 1975; Wingfield 1984a for details and assay reliability criteria).

For the field investigations, hormone data were subjected to an analysis of variance to determine heterogeneity of the means, and levels of significance determined by Newman-Keuls multiple range test for unequal samples. Laboratory data were analyzed by Wilcoxon Signed Ranks test, before and after treatment, or by Mann-Whitney U test across treatments. Behavioral data were analyzed by Kruskall-Wallis test and Mann-Whitney U tests.

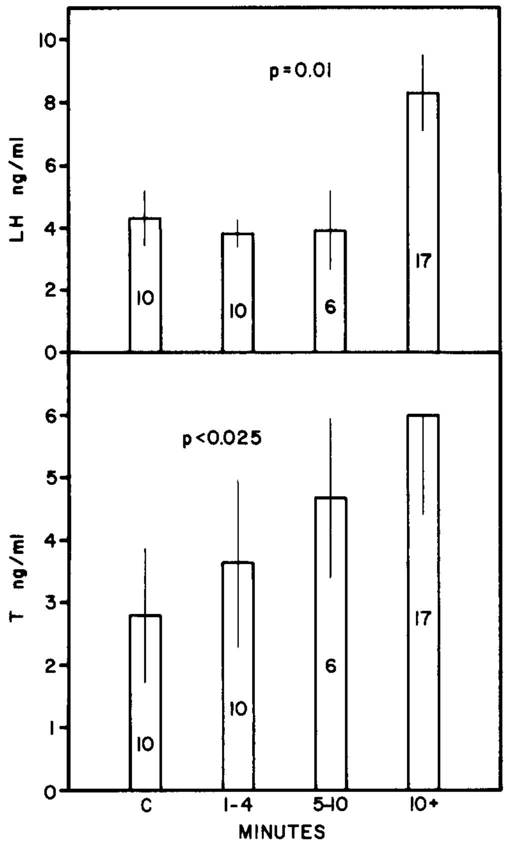

Fig. 1. Plasma levels of luteinizing hormone (LH) and testosterone (T) in free-living male song sparrows, *Melospiza melodia*, exposed to a simulated territorial intrusion. Histograms indicate mean levels of hormones after varying times of exposure to the intrusion. Vertical bars, standard errors; figures within histograms are the sample sizes. C = Control birds captured in Potter traps baited with seed (i.e. no exposure to a simulated territorial intrusion). *P* Values were obtained by Newman-Keuls multiple range test for unequal samples

Results

Time course of the hormonal response to a challenge

The responses of plasma levels of LH and testosterone to simulated territorial intrusion in free-living male song sparrows are presented in Fig. 1. Circulating levels of T and LH in males sampled 1–4 min, or 5–10 min, after onset of the intrusion were similar to those of controls. However, both LH and T levels were elevated after 10 min. These data confirm that the increase of T secretion following an agonistic challenge is accompanied by increasing titers of LH. Note that the elevation of LH does not appear to precede that of T although it is possible that slight increases do occur that are not detectable by the assay system used.

Specificity of response

The field investigations were confirmed by the laboratory experiment in which caged male song sparrows were challenged by a novel male conspecific. Both circulating LH and T were significantly increased after 6 h of interaction with the novel male (Fig. 2). In contrast, group 2 males that were challenged with a neutral stimulus (a male house sparrow) showed no change in plasma LH and T levels (Fig. 3).

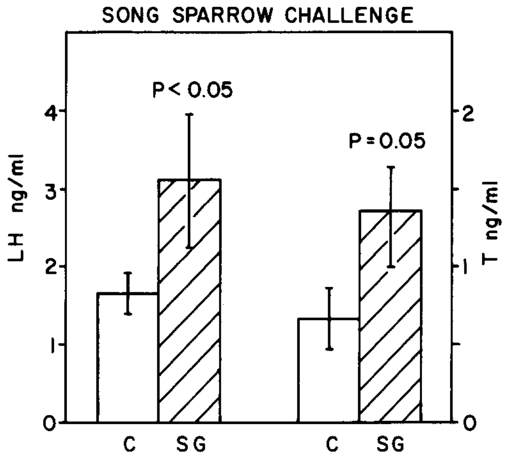

Fig. 2. Plasma levels of luteinizing hormone (LH) and testosterone (T) in response to a challenge from a novel male song sparrow. Vertical bars, standard errors, $n = 7$. C = Control sample (before challenge), SG = sample collected 6 h after introduction of the novel male song sparrow

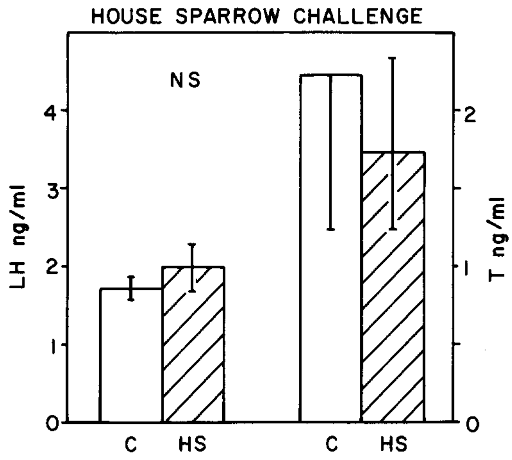

Fig. 3. Plasma levels of luteinizing hormone (LH) and testosterone (T) in response to a challenge from a male house sparrow. Vertical bars, standard errors, $n = 6$. C = Control sample (before challenge); HS = sample collected 6 h after introduction of the male house sparrow

Behavioral responses to partial stimuli in the field

Free-living males exposed to a tape-recording of conspecific song only (i.e. auditory stimuli alone), a devocalized male only (visual and possible chemical cues only), and to both a tape recording and a devocalized male (all stimuli except tactile) showed similar behavioral responses (Fig. 4). The only significant difference found was a greater number of flights given by males responding to the tape-recording only ($P < 0.05$).

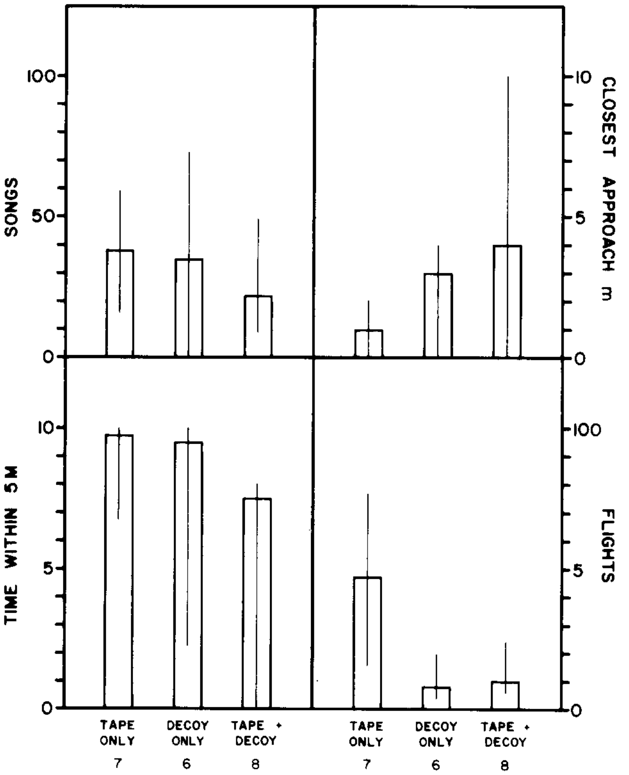

Fig. 4. Behavior of territorial male song sparrows to challenge with a tape recording of conspecific song (tape only), a devocalized male in a cage (decoy only) or a combination of tape recording and devocalized male (tape + decoy). Histograms represent medians, vertical lines are ranges. Numbers below histograms, sample sizes

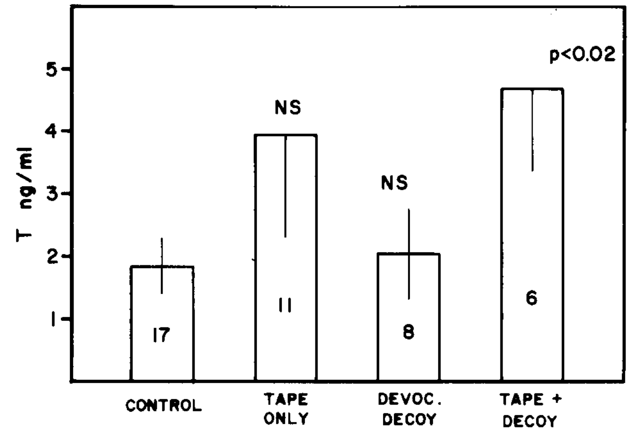

Fig. 5. Plasma levels of testosterone in free-living and territorial male song sparrows exposed to tape recording of conspecific song (tape only), devocalized male in a cage (decoy only), or a combination of tape recording and devocalized male (tape + decoy). Controls were males sampled after capture in Potter traps baited with seed. Histograms represent means, vertical bars standard errors. Figures within histograms are sample sizes. NS = Not significant

Plasma levels of testosterone in response to partial stimuli

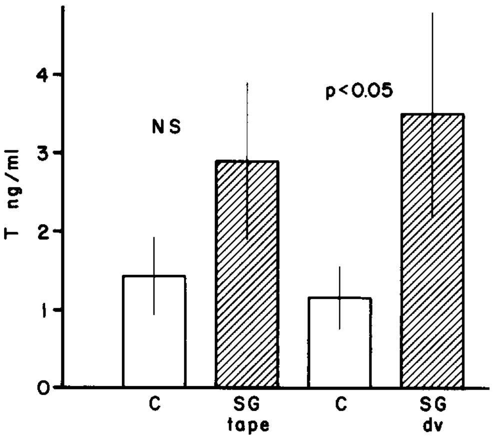

Fig. 6. Changes in plasma levels of testosterone in captive male song sparrows exposed to a tape recording of conspecific song, and a devocalized male. C = Controls sampled in abscence of any external stimulation. Histograms are means, vertical bars standard errors. NS = Not significant, sample size = 6

Although there was a tendency for plasma levels of T to increase in response to a tape-recording of conspecific song, only the T levels in response to both a devocalized male and tape recordings were significantly higher than controls (captured in traps baited with seeds). T levels in males exposed to a devocalized male alone were not different from controls (Fig. 5). Similarly in Fig. 6 it can be seen that males exposed to a tape recording of song did not have significantly elevated levels of circulating T, although exposure to a devocalized male did increase T levels significantly ($P < 0.002$) to a level similar to those in captive males exposed to an intact male song sparrow (Fig. 2).

Discussion

Previous experiments have shown that circulating levels of T rose during male-male interactions in song sparrows, but these changes were not always accompanied by surges in plasma levels of LH. When territorial male song sparrows were given subcutaneous implants of T, to maintain a high level of territorial aggression throughout the breeding season, then neighboring males also tended to have higher levels of T than neighbors of males given control implants. However, plasma levels of LH were not different (Wingfield 1984b). It was proposed that because this was a long term experiment, individual variation in plasma levels of LH, possibly coupled with problems of episodic secretion of this hormone, may have obscured any differences even though T levels were significantly elevated (see Wingfield 1984b, 1985). In separate experiments, simulated territorial intrusions were accompanied by an increase in LH up to 1 h after onset of an intrusion, although details of the time course of that increase were not clear (Wingfield 1985). The data presented here suggest that plasma levels of LH and T are elevated after 10 min of territorial challenge. Since an increase of circulating LH does not occur prior to the rise in plasma T, it is possible that the initial stimulus for T secretion is LH independent, although the mechanisms remain unknown. However, longer term elevation of T during a challenge is accompanied by enhanced LH secretion.

In a similar experiment, Harding and Follett (1979) were unable to show an increase in plasma levels of T and dihydrotestosterone (DHT) in male redwing blackbirds (*Agelaius phoeniceus*) challenged in a similar way. Plasma levels of LH actually were depressed following a challenge. It is possible that the difference in response between the two species may be related to mating system. Male redwing blackbirds are polygynous and spend much of the breeding season defending a territory from other males and mate-guarding receptive females. Thus, plasma levels of LH, T and DHT may be maximal and further stimulation has no effect. In contrast, male song sparrows are monogamous and show considerable parental care. Typically there is a precipitous decline in plasma levels of T once the parental phase is underway. However, these males clearly can respond to a challenge and increase the T level to spring levels if challenged. Circulating concentrations of DHT are much lower than T and do not show any significant responses to a challenge (Wingfield 1984a, 1985). The implications of these differential responses to social stimuli are discussed in more detail by Wingfield and Marler (1988) and Wingfield and Moore (1987).

Under laboratory conditions, a challenge using a novel male song sparrow results in an increase of circulating LH and T levels, at least after 6 h of interaction. These data confirm the field investigations. The purpose of using a house sparrow as a challenger in group 2 was to control for general arousal. Lack of a hormonal response in this group suggests that the increase in LH and T in group 1 was specific to challenges from conspecifics and not a reaction to non-specific disturbance.

The next experiments address the question of how such social stimuli affect hormone (T) secretion and by what possible neural pathways. To begin investigating mechanisms it was necessary first to dissect apart each sensory modality by which information from a social cue could regulate neuroendocrine and endocrine secretion. These sensory modalities include tactile reception (i.e. contact during a fight or chase), visual input (seeing a conspecific intruder), auditory input (hearing vocalizations – especially songs), and chemical cues (possible pheromones). There have been many studies on possible neural pathways by which interactions between male and female birds affect secretion of reproductive hormones (e.g. Lehrman 1965; Hinde 1965; O'Connell et al. 1981a, b; Cheng 1983; see also Wingfield and Marler 1988 for further review), but none have addressed the mechanisms by which male-male interactions influence secretion of T.

It should be noted that presentation of tape recording, devocalized male or both elicited similar behavioral responses from the territorial males under investigation. This is important since it suggests that all males were responding in a similar fashion to all stimuli and thus responses of the neuroendocrine and endocrine systems should be due to differences in components of the stimuli. Males exposed to tape only, did show significantly more flights than males exposed to other stimuli. However, we feel that this is to be expected since a male exposed only to auditory input is likely to 'search' for the intruder more than in the other two groups (see Fig. 4). This difference notwithstanding, we feel that the challenges elicited similar behavioral responses to auditory and visual stimuli and thus the different hormonal responses are due to the type of stimulus rather than due to inherent differences in behavioral response.

From previous experiments, and those presented here, it is possible to exclude an important role for tactile cues affecting T secretion during male-male interactions in song sparrows. Presentation of caged male decoys (thus precluding contact) appears to elicit a similar increase in circulating T as males interacting with other males under natural conditions (in which contact was frequent, e.g. Wingfield 1984a, b, 1985). Presentation of auditory cues alone (play-back of tape recorded songs) did not significantly increase plasma levels of T in responding males either in the field or in captivity (Figs. 5, 6). Although exposure to a devocalized male in the field did not increase T levels, similar treatment in an experimental chamber did elicit a significant rise in T concentrations over baseline levels (note that in this experiment each bird acted as its own control, Fig. 6). Thus it appears that presentation of auditory stimuli alone, and visual cues in the absence of auditory input, do not consistently increase secretion of T. However, presentation of intact males, or a combination of both devocalized male and tape recording (i.e. all stimuli except tactile) consistently resulted in an increase of plasma T (Figs. 2, 5). The possible role of chemical cues is unlikely since exposure of a devocalized male in Fig. 5 did not increase T levels.

From the data presented in Figs. 2, 5 and 6, it appears that tactile components of male-male interactions are not important for mediating the neuroendocrine and endocrine responses that result in an increase in testosterone secretion. Auditory and visual inputs in isolation are also not consistently effective. Rather, it appears that a combination of visual and auditory input is most effective (see also, Wingfield 1984b, 1985). Although it is unlikely that chemical cues play a major role, a possible secondary influence cannot be ruled out at present.

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