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Testosterone Stimulates Ultrasound Production by Male Hamsters

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Rates of ultrasound production by male hamsters were observed before, during, and after presentations of stimulus females and synthetic ultrasounds. Following castration, oil-treated control males showed decreased rates of ultrasound production, especially in tests with females. In contrast, castrates treated with 200 μg/day of testosterone propionate called at rates which equaled or exceeded their own preoperative levels and which consistently exceeded the postoperative call rates of the oil controls. These results show that ultrasound production by male hamsters is stimulated by gonadal androgens, and they support the interpretation of these signals as courtship displays.

Behavioral evidence suggests that ultrasonic calls by male hamsters function in courtship and at other stages of reproductive behavior. For example, males emit pure ultrasounds (all components above 20 kHz) during male–female interactions, but produce only wide-band, partly audible, vocalizations during male–male fights (Floody and Pfaff, 1977a; Sales, 1972). Finer differences in ultrasound structure and rate depend on the type of heterosexual contact experienced by a male (Floody and Pfaff, 1977a; Floody, Pfaff and Lewis, 1977). Ultrasounds produced by males during contact with estrous females in lordosis are quite constant in frequency and amplitude. The removal of an estrous stimulus female causes a shift to a more complex type of call with a higher incidence of rapid changes in amplitude and frequency. Furthermore, male call rates after a sexually receptive female has been removed tend to be lower than those in the female's presence. Nevertheless, male ultrasound rates following exposure to estrous females consistently are greater than those following contact with nonestrous females.

Additional evidence supporting the interpretation of male ultrasounds as reproductive signals is provided by the responses of females to natural male calls and to synthetic ultrasounds that resemble male calls (Floody and Pfaff, 1977b). A courtship role for male ultrasounds is suggested by the ability of ultrasounds to stimulate approach and vocalization by estrous females. A role at a more advanced stage of copulation is suggested

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strongly by data showing that natural and synthetic ultrasounds can prolong lordosis by estrous females.

If we are correct in interpreting male hamster ultrasounds as reproductive behaviors, then these signals should depend on a male's capacity to reproduce, and, in turn, on his hormonal state. Thus, the primary goal of this study was to investigate the hormonal dependence of ultrasounds produced by males in response to a highly effective stimulus, i.e., an estrous female. In addition, we studied the effects of stimulus ultrasounds on male call rates. This was intended to investigate possible interactions between the effects of hormonal state and type of extraneous stimulation, and to help define the chain of communications controlling hamster reproduction (see Floody and Pfaff, 1977b).

METHODS

Animals. Subjects were 28 male golden hamsters (Lak:LVG) purchased from the Lakeview Hamster Colony at 60 days of age and first tested at 100–104 days. Stimulus females were 12 ovariectomized adults of the same strain in hormone-induced estrus. All animals were housed individually in 24 × 18 × 18-cm metal cages with *ad libitum* food and water. The colony room was maintained at 24–26°C and on a reversed 14:10 hr bright:dim cycle. All tests were conducted during the second half of the dim period.

Test procedures. Males were tested for ultrasound production in clean 40 × 20 × 24-cm glass aquaria housed in a sound-attenuating chamber (Industrial Acoustics). Three types of tests were used. Each included a 2-min adaptation period and three or four observation periods distinguished by the type of experimental stimulation (Table 1). During each minute of every observation period, male ultrasounds were tabulated using a Holgate Ultrasonic Receiver tuned to 35 kHz (Floody and Pfaff, 1977a).

Two types of tests used synthetic ultrasounds as stimuli (Table 1). These signals were produced by an audio oscillator (Hewlett–Packard 204D) gated through an electronic switch, and consisted of 100-msec pulses of 35 kHz sine waves repeated at a rate of 1/10 sec. In frequency and duration, these sounds are similar to natural hamster calls (cf. Floody and Pfaff, 1977a). In other respects, e.g., the lack of variability in call structure and rate, they clearly differ from the vocalizations they are intended to mimic. However, identical signals have been shown to stimulate rapid approach by male and female hamsters and high rates of calling by estrous females (Floody and Pfaff, 1977b). Though less effective than natural calls in at least some respects, synthetic ultrasounds were used here because of the significant advantages they offer in the ease with which an observer can distinguish them from the ultrasounds they elicit from a test animal.

TABLE 1
Types of Experimental Tests

Test type	Experimental stimuli		Durations of observation periods (min)			
	Stimulus 1	Stimulus 2	Prestimulus	Stimulus 1	Poststimulus	Stimulus 2
1	Female	None	5	1[a]	5	—
2[b]	Ultrasounds	None	5	5	5	—
3	Female	Ultrasounds	4	1[a]	2[c]	2

[a] Call rates are reported only for those periods during which the stimulus female was in lordosis and silent (see Floody *et al.*, 1977).

[b] Statistical analysis of the results from tests of this type were restricted to the last 2 min of the prestimulus period and the first 2 min of each of the remaining two periods.

[c] This period followed removal of Stimulus 1, but was the prestimulus interval for Stimulus 2.

Experimental design. Each male received limited sexual experience during the 9 days preceding preoperative testing. This consisted of three to five 5-min exposures to a receptive female. Male sexual behaviors were not recorded systematically during these sessions.

Each male experienced five preop tests: two with just stimulus females; two with just stimulus ultrasounds; and, one with both stimuli (Table 1). Four-day intervals separated successive tests days. Single-stimulus tests of the two types were conducted on alternate test days and were completed prior to the initiation of dual-stimuli tests. The type of test experienced first by an individual (tests with females vs synthetic calls) was counterbalanced for equal groups of males.

All males were castrated under Nembutal anesthesia within the 3 days following the last preop test. Scores on the preop female-plus-ultrasounds test then were used to assign individuals to one of two matched groups. Experimental males received sc injections of 400 μg of testosterone propionate (TP; ICN Pharmaceuticals) in 0.10 cc of peanut oil on alternate days beginning 6–10 days postop. Control males recieved 0.10 cc sc injections of oil alone according to the same schedule. Postop testing began 13–17 days following castration, so that each male received four injections over a 7-day period prior to his first postop test. The schedule of postop testing was the same as that for preop tests.

Statistics. Call rates were subjected to a logarithmic transformation to reduce heterogeneity of variance (Edwards, 1972). As a result, average call rates summarized here are geometric, not arithmetic, means.

RESULTS

Effects of stimulus females. Rates of ultrasound production by males were observed in two situations involving brief male–female contact (Table 1). The effects of castration, hormone replacement, and exposure to females were similar in these two types of tests. Therefore, only the results of tests using both stimulus females and ultrasounds will be discussed in detail.

These data were subjected to analysis of variance (ANOVA) with hormone treatment as an independent factor and two repeated factors: operative condition (pre- vs postcastration) and test segment (Table 1). This analysis revealed a highly significant hormone $\times$ operation $\times$ stimulus interaction ($F(3,78) = 4.76$, $P = 0.004$, see Fig. 1). Further examination of this interaction using student's t test showed that significant effects of castration and hormone treatment occurred only (a) during male–female contact, (b) postfemale, and (c) during playbacks of synthetic ultrasounds. At each of these test phases, oil-treated males showed significant decreases in call rate following castration ($t(13) \geq 2.94$, $P \leq 0.01$). This effect of castration did not occur in males that were treated postoperatively with TP. As a result, postop scores for TP males consistently

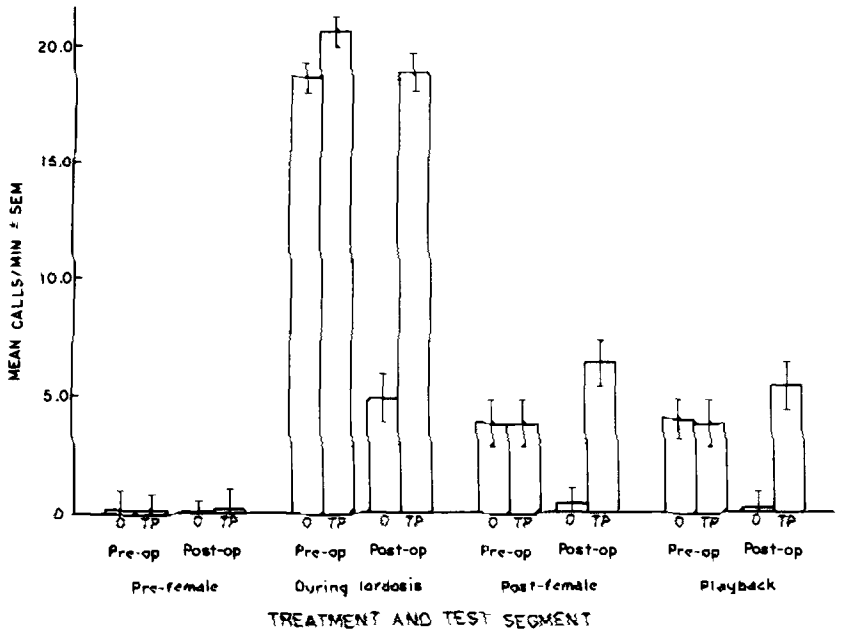

FIG. 1. Average rates of male ultrasound production before, during and after contact with an estrous female in lordosis, and during exposure to synthetic ultrasounds. Oil (O) and TP groups were treated identically preoperatively. Following castration (postop), TP males received 200 μg/day of testosterone propionate, while oil animals received control injections of the oil vehicle.

exceeded those for oil controls in each of these test segments ($t(13) \geq 2.36$, $P \leq 0.034$, matched pairs).

Individual comparisons of average call rates in different test segments confirmed the ordering: female present > postfemale = playback > prefemale. This ordering did not depend on operative condition or type of replacement therapy (for each within-group comparison, $t(13) \geq 2.17$, $P \leq 0.05$).

Effects of Stimulus Ultrasounds

As indicated above, average rates of calling during postfemale and playback segments of the tests using both stimuli did not differ (also see Fig. 1). However, even in these tests, ultrasound rates during the first minute of stimulus playbacks consistently were greater than those in the last postfemale minute in the preop results from oil and TP males ($t(27) = 2.27$, $P = 0.031$) and for the postop data from TP-treated males ($t(13) = 2.53$, $P = 0.025$). These results suggest that (a) males respond to stimulus ultrasounds with increased calling, and (b) this response, like that to stimulus females, is modulated by androgens. These suggestions are supported by the results of other tests using stimulus ultrasounds (below).

Data from tests with just stimulus ultrasounds were subjected to ANOVA with hormone treatment as an independent factor and three repeated factors: operative condition, test day, and test segment. This analysis revealed significant interactions of operative condition with test segment ($F(2,52) = 4.91$, $P = 0.011$) and hormone treatment ($F(1,26) = 3.40$, $P = 0.077$). Further examination of these interactions showed that intact, relatively naive, males produced ultrasounds at significantly higher rates during exposure to synthetic ultrasounds than either before or after stimulus playbacks (for precastration data, $t(27) \geq 3.54$, $P \leq 0.001$; see Fig. 2). Following castration, differences in call rate across test segments were similar, though less consistent ($t(27) \geq 2.04$, $P \leq 0.051$, comparing playback with pre- and postplayback rates). This change in consistency might reflect experiential changes in call rate by TP-treated males (*cf.* Floody *et al.*, 1977), together with a general decline in postop calling by oil-treated controls. In fact, these control males showed significant pre- to postcastration declines in call rate in test segments during and following ultrasound playbacks ($t(13) \geq 2.04$, $P \leq 0.062$). Furthermore, postop call rates by oil-treated controls at each phase of those tests were significantly lower than the comparable rates of TP-treated castrates ($t(13) \geq 1.80$, $P \leq 0.084$).

DISCUSSION

These results show that castration causes a decline in ultrasound production by male hamsters, but that TP replacement therapy can maintain

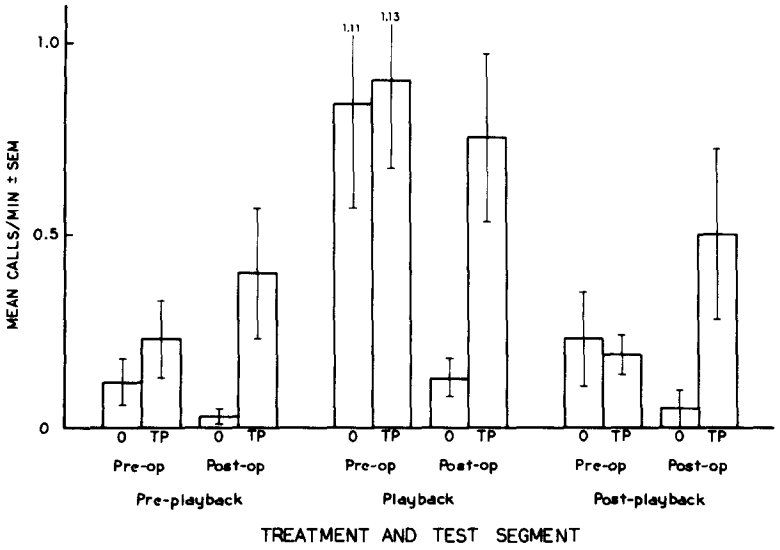

FIG. 2. Average rates of male ultrasound production before, during, and after the presentation of synthetic ultrasounds. Oil (O) and TP groups were treated identically during preoperative testing. Following castration (postop), TP males received 200 μg/day of testosterone propionate, while oil animals received control injections of the oil vehicle.

preoperative call rates. These effects are most dramatic during and after male–female contact, although they also are seen in response to synthetic ultrasounds. The variations in the extent and consistency of hormonal effects observed in the different test situations used in this study probably stem from (a) the relatively low levels of responsiveness by males to stimulus ultrasounds (e.g., compare Fig. 2 to comparable data for females in Floody and Pfaff, 1977b); (b) the matching of oil and TP groups just on the basis of preop scores in female-plus-ultrasounds tests; and (c) the fact that female-plus-ultrasound tests were conducted last, both in pre- and postop testing. This fact provided greater opportunity for the stabilization of preop call rates and also maximized the extent to which control oil-treated animals suffered from what probably is a gradual decline in call rate over days postop. Such a gradual decrease was not readily apparent over the 23 postop test days summarized in this report. Consistent declines might be detectable over longer periods, but probably would simply accentuate differences described here in the behaviors of castrated males receiving TP-replacement versus oil.

These effects of castration and TP treatment suggest strongly that endogenous androgens control rates of ultrasound production by intact male hamsters. Androgens also have been shown to control the duration of postejaculatory ultrasonic "song" by male rats (Parrott, 1976) and rates of precopulatory ultrasounds by male mice and rats (Dizinno and Whitney, 1977; Nyby *et al.*, 1977; Geyer *et al.*, 1978). It is not clear, however, if these androgen effects are direct, or are mediated by aromatization to estrogens. Large doses of estrogen inhibit 22 kHz postejaculatory ultrasounds by androgen-treated male rats, suggesting that the stimulation of male rat ultrasounds by androgens does not require aromatization (Parrott and Barfield, 1975; White and Barfield, 1977). On the other hand, preliminary data from our laboratory suggest that estradiol can stimulate ultrasound production by castrated male hamsters, implying that aromatization may mediate the androgen effects described in this report. The evaluation of this suggestion clearly will require additional studies of the mechanism of androgen action on hamster ultrasound production.

The androgen dependence of ultrasounds by male hamsters is consistent with the interpretation of those signals as reproductive behaviors (e.g., Floody *et al.*, 1977). This pattern of hormonal control also is similar to that shown for male scent-marking behaviors (e.g., Vandenbergh, 1971, 1973) and for the responsiveness of males to female odors (Gregory *et al.*, 1975). These findings are consistent with the view that those behaviors are linked, both functionally and physiologically, in a chain of social communications controlling hamster reproduction (Floody and Pfaff, 1977b).

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