Female Sex Behaviors in the South African Clawed Frog, *Xenopus laevis*: Gonadotropin-Releasing, Gonadotropic, and Steroid Hormones

Darcy B. Kelley

Department of Psychology, Princeton University, Princeton, New Jersey 08544

The goals of this study were to characterize sex behaviors of female South African clawed frogs, *Xenopus laevis*, and to explore the behavioral effects of endocrine manipulation. The responses of females to clasp assaults by sexually active males were observed. Two patterns of female responses predominated. In one, females exhibited extreme leg extension and ticking vocalizations when clasped (unreceptive behaviors). In the other, females responded to being clasped by adduction of the thighs and increased flexion at the knee; ticking vocalizations were absent (receptive behaviors). When the female was unreceptive, clasps by males generally lasted less than 1 min. With a receptive female, on the other hand, amplexus could last up to 2 days. In intact females, injection of human chorionic gonadotropin (HCG) or of luteinizing hormone-releasing hormone (LHRH) into the dorsal lymph sac results in significant increases in receptivity. These hormones do not promote receptivity in ovariectomized females. Neither estradiol (E) nor progesterone (P) when administered alone was effective in restoring receptivity to ovariectomized females. In combination, E + P increased sexual receptivity. The releasing hormone, LHRH, when given to ovariectomized, E + P-treated females, further increased receptivity and led to the prolonged amplexus otherwise observed with an HCG-injected intact female. The behavioral effects of LHRH may be independent of action on the pituitary since they are not mimicked by gonadotropin.

One requirement for successful reproduction is the synchronization of female receptivity with the sexual activity of males. Such synchronization is especially important when environmental conditions favorable for procreation are only briefly available. The anura, frogs and toads, require wet or moist conditions and warm temperatures for larval development. In many species, transient changes in temperature and water availability are accompanied by profound alterations in reproductive physiology (Lofts, 1974). Hypothalamic, pituitary, and gonadal hormones serve as internal messengers of these changes. While the physiological

1 Present address: Department of Biological Sciences, Columbia University, New York, New York 10027.
effects of these hormones in frogs have been extensively studied, much less is known of their effects on behavior. We have been investigating the hormonal basis of reproductive behavior in the South African clawed frog, *Xenopus laevis*. In males, clasp ing is abolished by castration and restored by treatment with androgen but not estrogen (Kelley and Pfaff, 1976). The goal of the present study is to investigate the effects of gonadotropin releasing, gonadotropic, and steroid hormones on female sex behaviors.

**GENERAL METHODS**

*Subjects and Maintenance*

Laboratory bred, reproductively mature, male and female *Xenopus laevis* were obtained from the Nasco Company, Fort Atkinson, Wisconsin. The mean weight of females was 65 g (range: 50–93 g); of males, 44 g (range: 34–55 g). Frogs were maintained at 22°C (±5°C) on a 14-hr light/10-hr dark cycle (light 7AM to 9PM), and were fed earth worms *ad libitum* three times weekly and kept in standing tap water (see Kelley and Pfaff, 1976, for details). Experimental females were maintained in groups of six ("cohorts") in 35 × 44-cm Nalgene home tanks. There was no manifest evidence of disease in the colony during the course of behavior testing.

*Testing Procedures*

Pairs of frogs were observed for 2 hr a day (generally 2:30 to 4:30PM) on 2 successive test days (a test sequence). One day before the test sequence, six 10-gallon aquaria were filled with standing tap water and six male frogs were injected with 100 international units (IU) of human chorionic gonadotropin (HCG) into the dorsal lymph sac to insure a high level of sexual activity. On the morning of the first test day, each male was reinjected with HCG and each female was injected with water, hormone, or was uninjected. Pairs of animals were then placed together in individual aquaria and allowed to remain undisturbed until the afternoon testing period. Frogs remained in the testing tank overnight. On the second morning of the test sequence, males and females were reinjected. To insure that each female was observed with a sexually active male, males were interchanged among the tanks at this time. In our previous studies of *X. laevis* sexual behavior, we used a 3-day test sequence. Behaviors observed on the second and third test days were similar. For the present study, therefore, the third test day was omitted. To protect against the possibility of a "ceiling" effect, I retained the behavioral observations on Test Day 1. Following the test sequence, all frogs were returned to home tanks. Females were marked by removal of one toe digit and were re-toe clipped after regrowth. Males used for behavioral testing were allowed a minimum 2-week interval between
successive tests. During observations, all six females in a behavioral cohort were injected with the same substance, either a hormone or water, and observed for the 2 days comprising a test sequence. During the following test sequence, frogs which had received water were injected with hormone and hormone-injected frogs received water. In pilot experiments, the behavioral effects of no injection versus water or saline (0.65%) injection were compared. There were no significant differences in observed female behaviors between the three conditions. To take into account any possible nonspecific effects of repeated administrations, a water injection was used as the control condition in all subsequent experiments. All injections were made into the dorsal lymph sac. Both HCG and LHRH were dissolved in sterile water.

Behavior Scoring

The time of occurrence and duration of every clasp by a male was recorded, as was the number of clasps. One hour into each test day the observer took a 5-min “break.” The timers scoring clasp duration were reset to 0 at the beginning of the “break” and restarted when observations resumed. For each clasp attempt by a male, the response of the female was noted. The degree of extension of the hindlegs was scored as follows: 0, fully flexed; 1, somewhat extended; 2, fully extended (see Fig. 1 for an illustration of characteristic 0 and 2 limb positions). For each female on each test day, a mean hind limb position score was calculated. This score is known as the leg extension score and is directly proportional to sexual unreceptivity. I focused on leg extension instead of leg flexion because the normal resting posture of the unclapped female includes leg flexion. The departure from normal flexion (i.e., leg extension) observed when “unreceptive” females are clasped is more dramatic and more easily scored than the increased flexion of “receptive” females observed in response to a clasp assault. Movements by the female during a clasp and immediately following release were recorded. Test sequences during which the female was not clasped by the male were noted. Since the same females were observed under many different hormone conditions, each female served as its own control. Comparisons of the behavioral scores of individual females under varying conditions were evaluated using nonparametric statistical tests (Siegel, 1956).

SPECIFIC METHODS AND RESULTS

Initial Observations

Methods. Six intact female frogs were observed for three test sequences with HCG-injected males. Each female was observed for one test sequence following water, saline, and human chorionic gonadotropin (HCG) injection. The number of clasps by males, clasp durations, female leg flexions and extensions, female movements and vocalizations were
recorded. Vocalizations of several pairs of frogs were monitored with a Massa Model M-115B hydrophone and taped using a Lockheed recorder. Tapes were analyzed with a Kay Sound spectrograph and Ubiquitous real-time spectral analyzer interfaced with a continuous strip graphic recorder.

Results. After being placed together in the tank, the male and female lie motionless on the bottom, occasionally rising to the surface to breathe. The resting posture of both frogs is characterized by hind leg flexion at the knee and adduction of the thighs. Most HCG-injected males can be heard to vocalize. These vocalizations (mate calls) are repetitive, loud trills emitted while the male is submerged. A movement of the female initiates the next phase of sexual behavior: the clasp assault. The male increases the rate and intensity of mate calling, moves toward and clasps the female. The male frog’s initial assault is usually directed toward one hind leg or toward the inguinal region. The female responds to a clasp assault with one of two behavior patterns. In one, the female remains still and silent maintaining the flexion of the hind legs at the knee and increasing the adduction of the thighs (Fig. 1, upper panel). A female will remain in this position until the termination of oviposition. Females which behave in this fashion are considered to be sexually receptive (see Discussion). Alternatively, a clasped female will abduct the thighs and extend the hind limbs rigidly (Fig. 1, lower panel). The female will swim rapidly around the aquarium. An occasional female will also respond to being clasped by rolling over so that the ventrum is facing the surface of the water. Females exhibiting these behaviors are said to be sexually unreceptive (see Discussion).

Accompanying leg extension behaviors is a female vocalization called ticking. Ticking is elicited by the approach of a vocalizing male and by

![Figure 1](image-url)  
**Fig. 1.** *Xenopus laevis* in amplexus. Upper panel: the male (left) is clasping a receptive female. Note the flexion of the female’s leg at the knee and the adduction of the thigh. This leg extension would be scored 0 (no extension). Lower panel: a male clasping a sexually unreceptive female. Note the characteristic extended leg position which would be scored 2 (maximum extension).
the male's clasp. Ticking is composed of a series of regularly emitted clicks. Compared to male mate calls, clicks are softer and are given at a more regular repetition rate. They lack the trill structure and amplitude modulation of the male vocalization. The male may continue to vocalize during this initial assault on the female. During the loud mate calling, the soft ticking of the clasped female can only be heard in the interval between rhythmic trills. After release by the male, the female may continue to tick for a short while.

I. Intact Females

A. HCG

Methods. The responses of intact female frogs to the administration of human chorionic gonadotropin (HCG, Nutritional Biochemicals Co.; 100 IU/frog) were compared to responses to water injection. Twenty-four intact female frogs (four cohorts) were observed for two test sequences; half received the water injection first and half received the HCG injection first.

Results. Injection of HCG increases sexual receptivity in intact females. The mean leg extension scores of intact females when water- and when HCG-injected are compared in Fig. 2. Scores during the gonadotropin-injection condition were significantly less than scores under the water-injection treatment (sign test, $P < 0.05$, Day 1; $P < 0.001$, Day 2). While there was no significant difference between Day 1 and Day 2 scores when females were injected with water, the leg extension of these females when HCG-injected was significantly ($P < 0.01$; sign test) less on Day 2 than on Day 1.

![Graph showing leg extension scores](image)

**Fig. 2.** The effects of injection of water, human chorionic gonadotropin (HCG), and luteinizing hormone-releasing hormone (LHRH) on the leg extension scores of intact females. Each female was observed following injection on each of 2 test days. Both HCG and LHRH significantly reduced leg extension scores. On this and all subsequent graphs, the error bars indicate standard error of the mean.
B. LHRH

Methods. The effect of luteinizing hormone-releasing hormone (human LHRH: 1, 10, or 100 μg/frog; Calbiochem–Behring Corp.) on the receptivity of intact females was evaluated. Each dose level was tested on two cohorts of experimental females. These tests were conducted so that the observer did not know whether an individual female had received water or LHRH.

Results. At high dose levels, LHRH increases female receptivity. The effect of LHRH injections on female leg extension scores was compared to the effects of water injections. There were no significant differences between scores at dose levels of 0 (water), 1, and 10 μg. However, injection of 100 μg (illustrated in Fig. 2) of LHRH significantly reduced the leg extension scores on Day 2 of the test sequence ($P < 0.01$, sign test).

II. Ovariectomized Females

A. HCG

Methods. Twenty-four experimentally naive females were injected with 100 IU of HCG on each of 2 test days and observed with HCG-primed males. One to eleven days later, females were bilaterally ovariectomized through an abdominal incision using previously described surgical procedures (Morrell, Kelley, and Pfaff, 1975). Seven to seventy-three days after ovariectomy, females were injected with HCG as above and observed for one test sequence with HCG-primed males. Six additional females were pretested with HCG and then subjected to a sham operation. The following week they were again injected with HCG and observed with sexually active males.

Results. Ovariectomy eliminated HCG-induced female receptivity. Mean leg extension scores for intact and ovariectomized females are compared in Fig. 3. The results of the preovariectomy test with HCG are similar to results for a separate group described above (Section I.A). On the second test day, the leg extension score of intact females was significantly less than the first-day test score ($P < 0.01$; sign test). In contrast, following ovariectomy, HCG-injection did not reduce leg extension scores. The difference in scores between intact and ovariectomized conditions on Day 2 was significant ($P < 0.02$; sign test). Both before and after sham operation leg extension scores of HCG-injected females were 0.

B. Estradiol and Progesterone

Methods. Following the above test sequence, ovariectomized females received an implant of an estradiol silastic capsule or of a progesterone pellet into the dorsal lymph sac. Implant procedures were identical to
those described previously (Kelley and Pfaff, 1976). Estradiol was obtained from Sigma and was inserted into 1.5-mm i.d. (602-235 Dow Corning) silastic capsules (mean length of hormone within pellet: 3.6 mm) whose ends were sealed with Dow-Corning silicone type A medical adhesive. Progesterone was obtained from Sigma and was formed into approximately 3-mm-diameter, 1-mm thick pellets (mean weight: 9.0 mg) with a Parr Instrument Company pellet press. Twelve of the females received the progesterone implant first and 12 the estradiol implant first. Females were observed for two test sequences with HCG-injected males 2 and 4 weeks after hormone implantation. They then received an additional hormone implant (estradiol for progesterone-implanted females and progesterone for estradiol-implanted females) and were observed for two test sequences with HCG-primed males and 2 and 4 weeks after hormone implantation.

I wished to explore the effects of high doses of estradiol on female receptivity. Six of the twenty-four females from the intact experimental group (see Section I.A, B, above) were ovariectomized and, 54 days later, received 10-mg pellet implants of estradiol into the dorsal lymph sac. These pellet implants release more estradiol than do silastic capsules (Kelley and Pfaff, 1976). Females were observed with HCG-primed males 1 and 3 weeks later and then the pellets were removed. Two females died shortly after pellet removal and 2 died a week later. The remaining two frogs were sacrificed after an additional 2 weeks.

Results. Combined treatment with estradiol and progesterone restored receptivity to ovariectomized females. The effects of long-term steroid treatment on the leg extension scores of ovariectomized females are given in Figs. 4 and 5. Treatment with an estradiol silastic alone for 2
Fig. 4. Effects of steroid treatment on leg extension scores of ovariectomized females. Estradiol alone was not effective in promoting receptivity. However, estradiol in combination with progesterone significantly decreased leg extension scores.

or 4 weeks did not significantly reduce the leg extension score compared to postovariectomy values (Fig. 4). Even with a very high-dose estradiol treatment (pellets as opposed to silastic implants), females were not receptive. The mean leg extension scores of estradiol pellet ($N = 6$)-implanted females were 1.0 and 1.6 when tested 1 and 3 weeks (respectively) after pellet implantation. These scores do not differ significantly from each other or from leg extension scores of estradiol silastic-implanted or ovariectomized HCG-injected females.

Fig. 5. Effects of steroid treatment on leg extension scores of ovariectomized females. Progesterone (P) alone did not significantly promote receptivity. However, 2 weeks after estradiol (E) was added to the progesterone regime, receptivity increased significantly. After 4 weeks, leg extension scores of P + E females did not differ from those of P alone.
Adding progesterone to the estradiol silastic regime, however, was effective in reducing leg extension relative to estradiol alone. For test sequences at 2 and 4 weeks after beginning progesterone treatment, mean leg extension scores were significantly reduced relative to estradiol alone ($P < 0.05$; sign test).

Treatment with progesterone alone was ineffective in reducing leg extension scores of ovariectomized females at 2 and 4 weeks (Fig. 5). However, the addition of an estradiol silastic implant significantly reduced leg extension scores after 2 weeks of combined treatment (differences in means of individual animals were compared for each test sequence; $P < 0.02$, sign test). Leg extension scores after 4 weeks of combined treatment did not differ significantly from those observed with progesterone alone ($P > 0.35$).

The effects of estradiol and progesterone in combination on the leg extension scores of the estradiol-first and the progesterone-first groups were compared. At 2 weeks, progesterone-first leg extension scores appeared lower than estradiol-first scores. This difference, however, was not statistically significant (median test; $P = 0.065$, Fisher exact probability test). At 4 weeks, the leg extension scores of the two groups were significantly different (median test, $P < 0.05$, two-tailed; data from either Day 1 or Day 2). Mean leg extension scores of the estradiol-first group were lower than those of the progesterone-first group.

C. LHRH and HCG in Estradiol–Progesterone-Implanted Females

Methods. Sixteen ovariectomized females with estrogen and progesterone (see Section II.B, first paragraph) implants were injected with LHRH (Calbiochem, 100 µg/frog) and observed with HCG-primed males. On the 2 days immediately following the LHRH test sequence, they received a water injection and were observed with HCG-primed males. Eight of these females were then injected with HCG (100 IU/frog) for one test sequence followed by an equal volume of water for the next test sequence. All observations were conducted under conditions which insured that the observer was ignorant of which substance (hormone or water) had been injected.

Results. Injection of LHRH but not HCG increased sexual receptivity in steroid-primed ovariectomized females. The effects of injection of LHRH, HCG, or water into estradiol plus progesterone-treated females are shown in Fig. 6. The leg extension scores of females treated with LHRH were lower than scores obtained when these same females were injected with HCG ($P < 0.05$; sign test). This effect of LHRH persisted into the 2-day test sequence (water injection) immediately following the LHRH test sequence. The post-LHRH leg extension scores (water injection) were significantly lower than the HCG-injection scores ($P < 0.05$; sign test) but did not differ significantly from the LHRH-injection
scores. Treatment with HCG did not reduce leg extension scores compared to the last test sequence with estradiol and progesterone alone. LHRH did reduce leg extension scores compared to the last progesterone-plus-estradiol test. This difference was significant for comparisons on Day 1 (P < 0.05; sign test) but did not reach significance on Day 2 (P = 0.055; sign test).

D. Hormone Implant Removal

Methods. All pellets and silastics were then removed. Fourteen to seventeen days after hormone removal, females were injected with water and observed with primed males for one test sequence and then injected with LHRH (100 μg/frog) for the next test sequence.

Results. Injection of LHRH increased receptivity in E + P-treated ovariectomized females. After steroid removal, LHRH was no longer effective. The effects of LHRH and water injection on leg extension scores of ovariectomized females with and without steroid treatment are shown in Fig. 7. The water injection scores of females in the pre-HCG test sequence (Fig. 6) were used instead of the scores of females from the test sequence immediately after LHRH treatment, as the latter scores are depressed due to a lingering effect of the releasing hormone (see above). After steroid removal, the leg extension scores of both LHRH-injected and water-injected ovariectomized females increased (P < 0.005; sign test). In ovariectomized females the LHRH injection versus water injection comparison did not result in a significant difference (P = 0.377; sign test).
Fig. 7. Effects of steroid withdrawal on releasing hormone-induced increase in female receptivity. Injection of LHRH significantly decreased leg extension scores relative to water injection in steroid-replaced females. After steroid removal, leg extension scores increased and LHRH was without effect. E, estradiol; P, progesterone.

All females were sacrificed at the conclusion of testing. Any ovarian regrowth was recorded as was the extent of oviductal development. Five females showed ovarian regrowth ranging from slight (1–5 immature ova: \( n = 4 \)) to significant (>50 mature ova: \( n = 1 \)). This latter female had a robust oviduct; behavioral test data for 3 months prior to the final test sequence were excluded from analysis. All other females showed oviductal atrophy. One female with a few immature ova was tested (LHRH injection; E + P implants removed). The female was not behaviorally receptive. Two females died in the weeks following the conclusion of behavioral testing with estradiol and progesterone. Two females died after the LHRH/water test sequences and one following pellet implant removal.

**Female Attractiveness**

I wished to examine the possibility that the female’s hormonal state influenced the frequency with which the male attempted to clasp. The frequency of clasp attempts on females by males was recorded for all hormonal conditions. The percentage of test days in which at least one clasp assault occurred (percentage clasping) was calculated. Ovariectomy reduced the percentage clasping. The percentage clasping for HCG-injected intact females was 92% and for HCG-injected ovariectomized females was 71% \( (P < 0.05) \). Ovariectomized, estradiol-plus-progesterone treated females received more clasp attempts after LHRH injection, 98%, than after water injection, 72% \( (P < 0.01; \text{ sign test}) \). After steroid removal, the LHRH treatment did not result in a significant increase in
clasping as compared to water injection (75 vs 68%). The reader should note, however, that only test sequences in which the female received at least one assault are included in the foregoing data analysis (Section II.A–D, above).

Coordination of Male and Female Behaviors

In our previous study of male behavior (Kelley and Pfaff, 1976), we observed that the durations of clasps fell into a bimodal distribution. Most clasp durations are very short (less than 2 min) or very long (lasting for the entire observation period). The frequency distribution of clasp durations for all experiments reported here was plotted and was again found to be bimodal.

Analysis of clasp duration data therefore employed the behavioral categories: short and long clasps. The maximum possible number of short clasps per test day was 57 and of long clasps was 5. Any clasping bout which lasted for the entire observation period would be scored as 2 long clasps because the clasp duration timer was reset during the 5-min observation break after the first hour of behavioral observations.

The occurrence of rigid and extreme leg extension in response to a clasp assault was compared to the frequency of short clasps for all experiments. A contingency coefficient (Siegel, 1956) between these two measures was calculated and they were found to be highly correlated across hormone conditions ($P < 0.01$, $\chi^2$). A similar relation between male long clasps and female knee flexion behaviors was obtained ($P < 0.01$, $\chi^2$, contingency coefficient).

To measure the summed results of male and female sexual activities, I calculated the percentage of all females receiving at least one long clasp per test sequence across hormone conditions. These percentages were highest for the preovariectomy HCG-injection test sequence (87%) and for the estradiol plus progesterone LHRH-injection test sequence (86%). After removal of steroid implants, no ovariectomized female participated in prolonged amplexus, even when injected with LHRH. Differences between test sequences were evaluated with the sign test. The HCG-injection pre- and postovariectomy scores were significantly different (87 vs 10%; $P < 0.001$) as were the ovariectomy, estradiol plus progesterone LHRH vs H2O scores (86 vs 30%; $P < 0.005$). Differences between E + P, H2O vs HCG (30 vs 42%), or (after E + P removal) the H2O vs LHRH (0 vs 0%) groups were not significant.

DISCUSSION

In this study I have described several behaviors characteristic of sexually receptive and unreceptive female South African clawed frogs and have shown these behaviors to be under hormonal control. The responses of females to a clasp assault by a male are of two sorts. Under certain
conditions, a clasped female remains silent, passive, and increases the flexion of the legs at the knee and the adduction of the thighs. When a female responds in this fashion, the male’s clasp persists for a long period of time (ranging from hours to days). It seems reasonable to describe females which exhibit these behaviors as sexually receptive. Alternatively, females respond to male clasp assaults by violent movements, extreme extension of the hind limbs, and ticking vocalizations. Such behaviors on the part of the female are accompanied by very short duration male clasps (generally less than 2 min). Again, we may infer that leg extension and ticking are behaviors characteristic of the sexually unreceptive female.

The situations in which ticking is elicited are similar to situations which elicit release calling by females in other anuran species. Release calls by unreceptive female or male frogs and toads result in unclasping by the male. A similar role for ticking has not yet been established. Treated males will clasp during ticking by the female. While the duration of such clasps is shorter than those seen with gonadotropin-treated, silent females, other aspects of the female’s behavior, such as struggling or leg extension, may facilitate the male’s unclasping as well. Female *Xenopus laevis* will also tick during the approach of a vocalizing male. Here ticking could serve to avert a clasp attempt (Long and Kelley, unpublished observations).

Without the administration of exogenous hormones, intact females in our colony are not sexually receptive. The receptivity of the intact female is increased by gonadotropin-releasing and gonadotropic hormones. After ovariectomy, these same hormones are no longer effective in promoting receptivity. Combined treatment with estradiol and progesterone is instead required. Such steroid-restored receptivity is enhanced by treatment with releasing hormone but not by treatment with gonadotropin. We may thus conclude that the sexual behaviors of female *X. laevis* are under the control of steroid hormones associated with the ovaries (estradiol and progesterone). The data further suggest that gonadotropin-releasing hormone facilitates receptivity through some mechanism that is independent of gonadotropin release itself.

*Previous Studies on X. laevis*

Russell, in his study of hormone effects in male *X. laevis* (1954), made incidental observations on female behaviors. Without gonadotropin injection, his females were sexually unreceptive. Indeed, it is the sexual quiescence of the laboratory-maintained female that allowed the reliable use of this preparation as an assay for responses to human pregnancy urine (Zwarenstein and Duncan, 1944). Russell described the ticking vocalizations and “escape” movements of hormonally untreated females. He noted that while a sexually active male will assault an untreated
female repeatedly, "the clasp . . . is maintained for only a short period" (Russell, 1954, p. 123). These repeated assaults correspond to the short clasps observed in the present study and Russell's data thus support the relation between the occurrence of short clasps and the unreceptive state of the female. Although Russell claimed to observe only one difference between treated and untreated females (namely that treated females are more passive), close inspection of Russell's (1954) Fig. 1 reveals an untreated female in extreme leg extension.

**Female Receptivity in Other Anurans**

Diakow has examined hormone effects on release calling in female *Rana pipiens* (Diakow, 1977, 1978; Diakow, Wilcox, and Woltmann, 1978; Diakow and Raimondi, 1981). In this species, release calling can be inhibited by distention of the abdominal walls, by treatment with the pituitary hormone, arginine vasotocin, and by treatment with prostaglandin E₂. Estradiol did not inhibit release calls. While we did not routinely monitor female unreceptive vocalizations (ticking) in this study, their incidence was high during test sequences after ovarietomy. Injection of HCG into intact females reduces ticking, a reduction not seen in steroid replaced, LHRH-injected ovarietomized females (Long and Kelley, unpublished). Thus leg extension and ticking may differ in endocrine control. In *X. laevis*, PGE₂ is also effective in inducing receptive behaviors (Kelley and Bockman, in preparation).

**Hormone Control of Receptivity in *X. laevis***

Results of this study indicate that HCG and LHRH are effective in inducing receptivity in female *X. laevis*. The sensitivity of this species to gonadotropins of chorionic origin formed the basis of a popular bioassay for human pregnancy earlier in this century (Zwarenstein and Duncan, 1944). Injection of HCG results in ovulation and oviposition in *X. laevis*. The behavioral effects of HCG depend upon the presence of an ovary; HCG is ineffective in gonadectomized females. Such effects could be mediated via increased steroid secretion from the ovary and/or events associated with the release of eggs from the oviduct and their transport to the cloaca.

The doses of LHRH required to induce receptivity were high: females require 100 μg for receptivity whereas male sexual behavior (clasping) can be reliably induced by as little as 1 μg (Kelley and Pfaff, unpublished observations). In other vertebrates, LHRH administered peripherally is rapidly inactivated by peptidases and high dose levels are required to promote female receptivity (Pfaff, 1973; Moss and McCann, 1973). Such high doses may allow enough LHRH to reach the brain and the pituitary where we suspect that behaviorally important targets for LHRH are
found. *X. laevis* has diencephalic immunoreactive-LHRH cell bodies whose axons distribute to the hypophyseal portal system (Doerr-Schott and Dubois, 1976). Peripherally administered LHRH may be mimicking some part of the central action of this hormone.

The receptivity of HCG-injected intact females is abolished by ovariectomy. A combined estradiol-plus-progesterone treatment regime was effective in increasing the receptivity of ovariectomized females. Estradiol alone (even in very high doses) or progesterone alone was not effective in restoring receptivity. In many mammals, the effects of estradiol on receptivity synergize with those of progesterone. In rats, lorderosis can be restored with estradiol alone while in other rodents, such as hamsters, some progesterone is required (see Lisk and Reuter, 1980, for review). The effects of estradiol on female receptivity are thought to be mediated via a system of hormone-sensitive brain neurons (see McEwen, Davis, Parsons, and Pfaff, 1979, for review). The synergistic effects of progesterone may be accounted for by the finding that estradiol can induce progesterone receptors (MacLuskey and McEwen, 1978; Blaustein and Feder, 1979). We have described a system of estradiol-concentrating cells in brain nuclei of *X. laevis* (Morrell et al., 1975). The relation of such nuclei to behavior has not yet been explored. One possibility suggested by these behavioral findings is that there are also CNS progesterone target cells in *X. laevis*. Progesterone receptors in these cells could be inducible by estradiol. In the present study, the estradiol-first treatment group was more receptive than the progesterone-first group when tested 4 weeks after combined steroid treatment was begun.

*Can LHRH Act Independently of Effects on Gonadotropin?*

Amplexus in *X. laevis* is a protracted behavior. Under laboratory conditions, in which both members of a pair are injected with gonadotropin, amplexus can last for days. In the present study, protracted amplexus was typically observed under only two hormone-treatment conditions: (1) after HCG injection of intact females, and (2) after LHRH injection of ovariectomized estradiol plus progesterone-treated females. In the latter instance, LHRH could be observed to increase female receptivity under conditions where HCG was ineffective. These results suggest that the mechanism by which LHRH increases female receptivity may be independent of LHRH-stimulated gonadotropin secretion from the pituitary. A pituitary-independent action of LHRH on female receptivity has been reported in ovariectomized, estradiol-primed, hypophysectomized rats (Pfaff, 1973; Moss and McCann, 1973). How and where does LHRH act "directly" to facilitate female receptivity? Does the behavioral effect of exogenous LHRH mimic some physiological role of releasing hormone within the brain? These questions require further exploration.
Female Attractiveness

The female leg extension or flexion behaviors described here are given in response to a clasp assault by a male. Such assaults are triggered by a movement of the female. Sometimes, however, the female does not receive a single clasp-assault during the 2-hr observation period. Are such females "unattractive" to males and can a female's "attractiveness" be the result of her hormonal condition? Examination of the data suggests that the frequency of assaults by males varies across hormonal conditions. The highest frequencies of clasping occurred with intact HCG-injected females and with LHRH-injected E + P females. The reason for these differences is open to speculation. In certain hormonal states, females may move very little and thus elicit fewer male clasp assaults. Perhaps, also, males are responsive to behavioral or chemical social signals from females, stimulated by hypothalamic-hypophyseal hormones, which convey receptivity.

Relation to Naturally Occurring Changes in Hormonal State

Most of the hormones administered in this study are present in X. laevis. Estradiol-17β is the major estrogen (Gallien and Chalumeau-Le Foulgoc, 1960); it functions to promote vitellogenin synthesis from the ovary (see Shapiro and Baker, 1979, for review). Progesterone is believed to act within the ovary at the membrane of oocytes to promote the final reduction divisions of meiosis and promote ovulation (Baulieu, Godeau, Schorderet, and Schorderet-Slatkine, 1978). Immunoreactive-LHRH is present in central neurons (Doerr-Schott and Dubois, 1976); brain levels are highest during the breeding season (King and Millar, 1979). One way to look at the behavioral effects of these hormones is to regard them as internal signalers of reproductive state. As such they may serve, through action on CNS targets, to insure that behavioral receptivity reliably accompanies readiness for reproduction.

ACKNOWLEDGMENTS

I greatly appreciate the assistance of B. Goun, C. Szmauz, P. Hannigan, and D. Wetzel in conducting observations. I also wish to thank K. McGeady for typing, and R. Lisk for reading, the manuscript. This research was supported by PHS Grant HD12126 and a fellowship from the Sloan Foundation.

REFERENCES


