# Hormonal Induction of Male-like Courtship Behavior in the Female Newt, Cynops pyrrhogaster

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# Hormonal Induction of Male-like Courtship Behavior in the Female Newt, *Cynops pyrrhogaster*

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**ABSTRACT**—In the early stage of courtship, the sexually developed male newt (*Cynops pyrrhogaster*) vibrates its tail in front of the female. Administration of androgen and prolactin (PRL) to sexually inert male newts is known to elicit this behavior. The effects of ovariectomy and hormonal treatment on the induction of male-like tail vibration behavior were studied in female newts, which never show such behavior in an intact state. Ovariectomy elicited the behavior with a low incidence and frequency. Estradiol supplementation almost nullified the effect of ovariectomy. Treatment of ovariectomized females with a combination of testosterone propionate and PRL markedly increased the frequency of the behavior.

## INTRODUCTION

In the breeding season, the male red-bellied newt (*Cynops pyrrhogaster*) vibrates its tail in front of the female newt at an early stage of courtship, as in several other species of urodeles [19]. This tail vibration does not usually occur in the female newt. Androgen is reported to be involved in the manifestation of male-typical reproductive behavior in amphibians [1, 6, 16, 17], and we have shown that androgen and prolactin (PRL) are involved in eliciting the tail vibration behavior of male newts [24].

The ability of androgen to induce male reproductive behavior in females has been examined in a few species of Amphibia. In ovariectomized female *Xenopus laevis*, both testosterone and testosterone propionate pellets reliably produce the male sex behavior of clasping [6]. Intact and gravid *Taricha granulosa* females injected with arginine vasotocin (AVT) exhibit egg-laying behavior and oviposition [17]. This behavioral response to AVT is dependent on sex steroid hormones: Androgen-implanted ovariectomized females injected with AVT exhibit male-like amplectic clasping. The aim of this study was to examine whether PRL and androgen are able to elicit the male-like tail vibration in female newts.

## MATERIALS AND METHODS

### Animals

Adult specimens of the newt, *Cynops pyrrhogaster*, of both sexes were obtained from a commercial dealer in late spring and early summer. They were kept in the laboratory and fed daily with dry *Tubifex* worms. Experiments were commenced after confirming that the animals did not show tail vibration for at least 2 weeks under laboratory conditions. Animals were not used in more than one experiment. Ovariectomy was performed by laparotomy under anesthesia with 0.1% *m*-aminobenzoic acid ethylester methanesulfonate (MS222, Sankyo Co.) 7 days prior to the hormone treatment.

### Hormones

Bovine PRL and human chorionic gonadotropin (HCG) were purchased from Teikoku Hormone Mfg. Co. Testosterone propionate (TP), and estradiol were products of Sigma Chemical Co.

### Hormonal treatment

PRL was dissolved in 0.6% NaCl solution. Steroid hormones were dissolved in a minute volume of ethanol and then suspended in 0.6% NaCl. Intraperitoneal injection of each hormone in 0.05 ml of vehicle was performed at 0800 hr daily for 10 days. The daily doses of PRL, HCG and steroid hormones employed were 1 IU, 25 IU and 5 $\mu$g, respectively.

### Behavioral Test

The test was performed according to the method described previously [24]. Ten hours after the hormonal treatment, each female newt was placed in a glass container (20 cm in diameter) filled with water to a depth of 3 cm, and paired with a male or female which had been treated with PRL and HCG to keep them sexually active throughout the experiment [24]. The incidence and frequency of courtship behavior, i.e., vibration of the tail in front of the partner, were monitored for 1 hr/day throughout the experimental period. The incidence was expressed as the percentage of the animals that exhibited the behavior for the first 5-day period (days 1–5), and for the second 5-day period (days 6–10). The frequency was expressed as the mean number ($\pm$SE) of times that the behavior was recorded per test animal for each test period.

### Statistical analyses

Statistical analyses of incidence and frequency were performed using two-tailed Fisher's exact probability test [20] and Dunn's multiple comparison rank sum test followed by Kruskal-Wallis one-

way analysis of variance or the Mann-Whitney U-test [4, 20], respectively. The statistical comparisons were made across the treatments within each 5-day period.

## RESULTS

In the intact females, the tail vibration behavior was never observed throughout the experiment. Ovariectomy elicited the behavior in a few animals (1 and 3 out of 8 test

animals in the first and second test periods, respectively), although the frequency was low. During the first test period, the estradiol-treated group showed no tail vibration, and during the second period only one specimen exhibited the tail vibration. The PRL-treated, TP-treated and TP plus PRL-treated ovariectomized groups showed a rather high incidence of the behavior, although the values were not significantly different from that in the saline-treated group. During the second experimental period, the frequency of the tail vibration in the TP plus PRL-treated group was markedly increased, whereas the the frequency in the other groups remained low (Fig. 1). To examine whether the sex of the partner affected the performance of tail vibration by the female, each ovariectomized female injected with PRL and TP was kept with either an intact male or female that had received PRL and HCG. Both the incidence and frequency of the behavior were not changed by the sex of the partner (Fig. 2). At the level of analysis used in this experiments, the tail vibration behavior induced in females by ovariectomy and hormonal treatment was not distinguishable from that seen in males. As shown in Figure 3, the female blocked the advance of the partner with her neck, bent her tail, pointing the tail-tip toward the partner's snout, and vibrated the distal half of the tail vigorously.
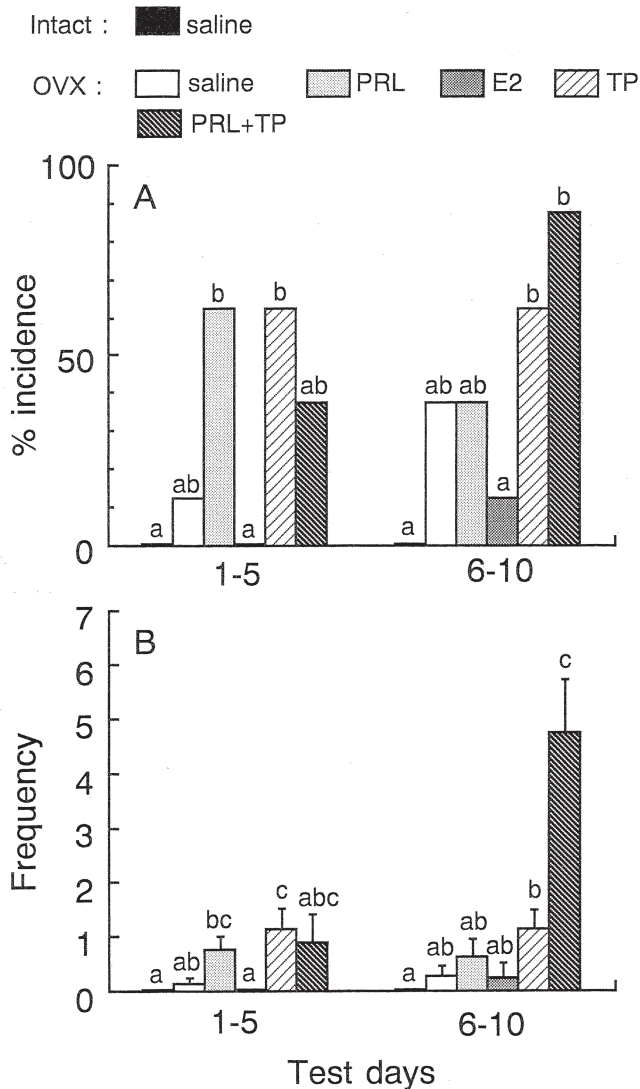


FIG. 1. Effect of PRL and/or sex steroids on male-like courtship behavior in ovariectomized (OVX) female newts. Ovariectomized females were injected with saline, PRL, estradiol (E2), TP, PRL plus TP every day for 10 days (n=8 for each group). Intact females were injected with saline every day for 10 days (n=8). (A) Percentage of females that performed male-like tail vibration behavior during each 5-day period. (B) Mean number (±S.E.) of episodes of male-like behavior per test animal during each 5-day period. Values with the same superscript do not differ significantly from each other at the 5% level during each 5-day period (A, Fisher's exact probability test; B, Dunn's multiple comparison test followed by Kruskal-Wallis one-way analysis of variance).
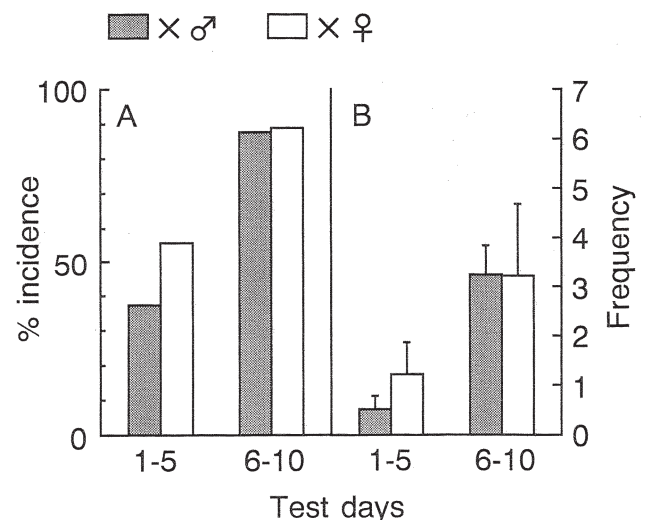


FIG. 2. Effect of sex of the partner on male-like courtship behavior in ovariectomized female newts. Test females were injected with PRL plus TP every day for 10 days (n=8 or 9 for each group). Each treated female was paired with a male injected with PRL plus HCG (n=8) or paired with a female injected with PRL plus HCG (n=9). (A) Percentage of females that performed male-like tail vibration behavior during each 5-day period. (B) Mean number (±S.E.) of episodes of male-like behavior per test animal during each 5-day period. No significant difference in the values between the two groups at the 5% level was noted (A, Fisher's exact probability test; B, Mann-Whitney $U$ test).
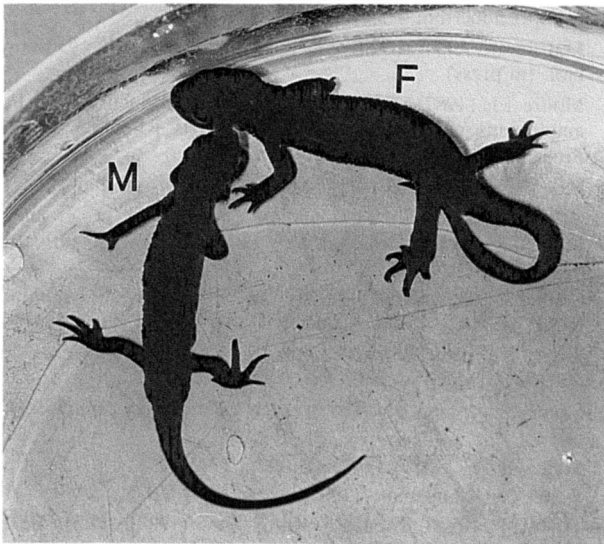
FIG. 3. Male-like tail vibration by an ovariectomized female newt that received PRL and TP in front of a male. F, female; M, male.

## DISCUSSION

We have shown previously that a combination of androgen and PRL elicits tail vibration behavior in castrated male newts [24]. In this experiment, the tail vibration behavior, which is normally never observed in intact female newts, was manifested after ovariectomy, although in a limited number of animals. This suggests that ovaries secrete a tail vibration-suppressing substance(s). It was noteworthy that in estradiol-injected ovariectomized females, no tail vibration behavior occurred except in one specimen during the second test period.

Androgen has been considered to act centrally to elicit sexual behavior in amphibians on the basis of autoradiographic observations suggesting that testosterone-concentrating cells, which are known to be associated with the regulation of sexual behavior in other vertebrates, exist in the brain (reviewed by Kelley and Pfaff [7]). The fact that the implantation of testosterone into the preoptic nucleus of castrated males of the frog, *Rana pipiens*, evokes mating behavior [27] supports this consideration. In *Xenopus laevis*, the distribution of testosterone-concentrating cells was found to be the same in both male and female brains, but different from that of estradiol-concentrating cells [5, 18]. Testosterone-concentrating cells were found in the anterior preoptic area (APOA), ventral infundibular nucleus (VIN), the dorsal tegmental area of the medulla, and the motor nucleus of cranial nerves IX-X. Estradiol-concentrating cells were found in the telencephalic continuum, which includes the ventral striatum, ventrolateral septum and rostral amygdala, in the APOA and VIN, in the ventral thalamus and in the torus semicircularis of the midbrain. Kelley and Pfaff [6] reported that the ability of testosterone to elicit clasping in both male and female *Xenopus laevis* and the

inability of estradiol to restore the male sexual behavior were paralleled by the autoradiographic localization of these sex hormones in the brain.

In the newt, *Triturus cristatus*, the courtship behavior-controlling center is presumed to be located in the preoptic area, since lesions of this area abolish sexual behavior [13]. A pair of Mauthner neurons in the medulla oblongata has been considered to play a role in the tail movement of urodeles as well as that of anuran larvae [22]. Interestingly, development of these neurons in urodeles is dependent on androgen and PRL [15, 23]. In hypophysectomized male newts, testosterone pellet implantation and PRL injections induce significant enlargement of the nucleus of the Mauthner cell [23].

It has been known that androgenization elicits male-like behavior in the female of other classes of the vertebrate, although the ease with which exogenous androgen induces it varies from species to species [7]. This suggests that neuronal system that is involved in the expression of male behavior is not completely altered during the course of sex differentiation.

PRL has been assumed to play an important role in the reproduction of urodeles [10]. This hormone is involved in eliciting courtship behavior [10, 14, 24]. PRL also induces migration into water, where courtship by the male and oviposition by the female take place [3], and elicits morphological and functional changes in the skin for osmoregulation [2, 12]. Furthermore, PRL plus androgen enhances the development of peripheral organs such as the tail fin [9, 21, 26] and cloacal glands [8, 25]. These organs seem to be important in the series of reproductive events occurring in male urodeles. During courtship, the well-developed tail fin may efficiently generate a flow of water to carry a pheromone derived from the cloacal gland [11] to the female. When male and female newts are deprived of both the pituitary gland and the gonad, the height of the tail fin is decreased [9, 10]. Administration of PRL to these animals markedly increases the tail height, but this increase is suppressed by estradiol.

In this study, the combination of PRL and TP markedly increased the frequency of the male-like tail vibration behavior in ovariectomized females during the second experimental period (6–10 days of treatment). This delayed behavioral response suggests that these hormones have organizing effects on peripheral and central organs for manifestation of the tail vibration behavior. In conclusion, male-like tail vibration behavior can be induced in the female newt by ovariectomy followed by treatment with androgen and PRL.

## ACKNOWLEDGMENTS

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