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Social regulation of plasma estradiol concentration in a female anuran

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Abstract

The behavior of an individual within a social aggregation profoundly influences behavior and physiology of other animals within the aggregation in such a way that these social interactions can enhance reproductive success, survival and fitness. This phenomenon is particularly important during the breeding season when males and female must synchronize their reproductive efforts. We examined whether exposure to conspecific social cues can elevate sex steroid levels, specifically estradiol and androgens, in female túngara frogs (*Physalaemus pustulosus*). We compared plasma estradiol and androgen concentrations in wild-caught females before and after exposure to either natural mate choruses or random tones. After exposure to mate choruses for 10 consecutive nights, estradiol concentrations were significantly elevated whereas there was no significant elevation in estradiol concentrations in the group of females exposed to random tones for 10 nights. Plasma androgen concentrations were not significantly changed after exposure to either natural mate choruses or random tones for 10 consecutive nights. Social modulation of estradiol concentrations may be important in maintaining a female's reproductive state while males are chorusing. To our knowledge, this is the first study to demonstrate social regulation of estradiol concentration in female anurans.

Keywords: Social regulation; Estradiol; Female reproductive behavior; Anuran

Introduction

In order to maximize reproductive opportunity, males and females in many taxa must be able to predict the onset of the breeding season and synchronize their reproductive behaviors to one another. Ultimate factors, such as food availability, weather, competition or predation, favor individuals that reproduce when changes in the environment occur. Therefore, animals must prepare for changes in the environment by responding to proximate cues, which are features of the environment that actually influence physiology or behavior (Hahn et al., 1997). Proximate cues can be predictive, such as changes in photoperiod (Wingfield, 1983), or supplementary, such as changes in weather (Hahn et al., 1997). Predictive cues help regulate sex steroids production, which in turn, regulates reproductive behaviors in a variety of taxa including birds (Noble, 1973; Delville and Balthazart, 1987; Ball and Balthazart, 2002), fish (Tricas et al., 2000, Grober and Bass, 2002), mammals (Hull et al., 2002; Blaustein and Erskine, 2002), reptiles (Alderete et al., 1980; Rhen et al., 1999; 2000; Rhen and Crews, 2000; Godwin and Crews, 2002) and amphibians (Diakow and Nemiroff, 1981; Schmidt, 1984, 1985; Mendonça et al., 1985; Boyd, 1994, Wilczynski and Chu, 2001). Such environmental cues, however, are not the only means by which animals recognize the onset of the breeding season and synchronize their reproductive state. Lorenz demonstrated that the behavior of one individual within a social aggregation could influence the behavior of other animals within the aggregation, thereby demonstrating that social cues can impact an animal's behavior and therefore its survival and fitness (Lorenz, 1970). Since then, it has been shown that social cues also regulate sex steroid production (Dufty and Wingfield, 1986; Chu et al., 1998; Propper and Moore, 1991; Burmeister and Wilczynski, 2000, 2001; Chu and Wilczynski, 2001), function of

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gonadotropin releasing hormone neurons (Propper and Moore, 1991; Dellovade and Rissman, 1994; reviewed in Rissman, 1996; Burmeister and Wilczynski, 2005), gonadal status (Brzoska and Obert, 1980; Bentley et al., 2000; Lea et al., 2001) and survival of new neurons (Lipkind et al., 2002). These cues, whether environmental or social, are clearly a mechanism by which animals coordinate neural, physiological and behavioral responses that enhance the probability of reproductive success.

Social regulation of female reproductive physiology has been demonstrated in classic experiments by Lehrman (1965), in which he showed that the presence of a courting male enhanced the endocrine and behavioral response of the female ring dove (*Streptopelia risoria*). The effects of such stimulation are further enhanced by the presence of both visual and auditory cues provided by the male (Freidman, 1977) as well as by the female's own vocal responses to the male's courting behavior (Cheng, 1992). Further, electrophysiological studies indicate that auditory cues stimulate hypothalamic neurons (Cheng et al., 1998). Similar phenomena in which vocal signals from a sender influence the physiology and neurobiology of a receiver have also been described in anuran amphibians (Wilczynski and Chu, 2001; Wilczynski et al., 2005). This is possible because in anurans, the central auditory system sends projections into the anterior and central thalamic nuclei, which relay auditory information into the anterior preoptic area as well as the ventral hypothalamus (reviewed in Wilczynski et al., 1993). Consequently, hypothalamic neurons respond to acoustic stimulation in male (Wilczynski and Allison, 1989; Allison, 1992) and female (Hoke et al., 2005) anurans. Acoustic cues stimulate the release of gonadal hormones in male anuran amphibians (Burmeister and Wilczynski, 2000; Chu and Wilczynski, 2001). Although exposure to a conspecific mate chorus facilitates oocyte retention in female midwife toads (*Alytes mulentensis*) (Lea et al., 2001), there is no evidence as of yet that conspecific mate signals stimulate endocrine responses in female anurans. Male and female anurans do in fact have very different social interactions, in that males generally remain in choruses exposed to social signals for long periods at a time, while females enter it episodically. Their exposure to calls may be less intense and more sporadic, and it remains unknown whether the stimulatory effect of calls on gonadal hormones seen in males can be generalized to females. The objective of this study is to determine if acoustic exposure to natural, conspecific mate choruses enhances the production of reproductive steroids in a female anuran.

We examined whether conspecific mate signals stimulate the production and release of the gonadal steroid hormones, specifically estradiol and androgen, in female túngara frogs (*Physalaemus pustulosus*), a Neotropical anuran species that is subject to sporadic favorable breeding environments during the breeding season (i.e., the rainy season). Male túngara frogs gather in lek-like aggregations to form nightly mate choruses during the breeding season, as do many other species of anuran (Wells, 1977). Male choruses, however, are not constant over the rainy season. Males do not form these choruses during heavy rainfall but the nights following a heavy rain typify the most intense mate choruses (Ryan, 1985). Females cycle through periods of high and low receptivity (Lynch et al., 2005) corresponding to cycles in gonadal steroid concentrations (Lynch and Wilczynski, 2005). Females that are gravid and ready to mate will approach these mate choruses; however, it is not clear how long the female is exposed to chorusing males before she actually mates. Therefore, it is unknown whether such acoustic social signals contribute to the regulation of gonadal hormone production in female anurans as they do in chorusing male anurans. Furthermore, the female túngara frog displays asynchronous oogenesis, in which the female túngara retains oocytes in different stages of maturity (stages I–VI, with VI being the most mature). Consequently, túngara frog females are constantly producing new eggs, while other eggs mature, allowing the female to release eggs multiple times during the season with a period of about 4 to 6 weeks (Davidson and Hough, 1969). Although the hormone profile of female túngara frogs show that they experience cyclic fluctuations in gonadal hormone concentrations (Lynch and Wilczynski, 2005; see Table 1 for summary of those results), it is possible that social cues are an additional regulator of gonadal hormone concentrations. We examined this possibility by exposing female túngara frogs to mate choruses or random tones while their gonadal steroid concentrations were at their lowest concentration (approximately midcycle). Elevation in female reproductive hormones as a consequence of social experience would indicate that females can use social cues, specifically male mate choruses, as a signal to continue cycling into the next reproductive cycle during the breeding season, whereas the absence of these social cues may contribute to the female dampening her reproductive capability, an effect that would occur at the end of the breeding season when social signals are no longer present.

Table 1
Summary of results in Lynch and Wilczynski (2005)

Reproductive Stage	Hormones		
	Estradiol	Progesterone	Androgen
Unamplexed	⇩	⇩	⇧
Amplexed	⇧	⇧	⇩
Post-mated	⇩	⇩	⇩

Female reproductive hormones, specifically estradiol, progesterone and androgen concentrations, fluctuate over three different reproductive stages. These reproductive stages mark the transition through a single reproductive cycle, which occurs approximately every 4–6 weeks in the female túngara frog. The unamplexed stage is while the female is alone at the breeding pond and has not mated yet. The amplexed stage is while the female is in the process of mating and the post-mated stage is 10 days after the female has laid her eggs.

Methods

Female túngara frogs were captured in Gamboa, Panama, while they were in the process of mating with males (i.e., amplexus). The female was permitted to release eggs and held for 15 days afterwards because our previous study (Lynch and Wilczynski, 2005) showed that gonadal hormone concentrations are depressed at this time. During the 15-day waiting period, the females were transported to the University of Texas at Austin and housed in 10-gallon aquarium with damp moss in groups of five and fed 1-week-old crickets three times per week. At exactly 15 days post-mated blood was collected via the orbital sinus using a procedure approved by the University of Texas IACUC. The blood was centrifuged, and the plasma layer was removed. The females were then individually placed in an acoustic chamber with water, moss, rocks and artificial plants. The acoustic chambers also included a speaker (RadioShack 277-1008C) and a