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Short communication

Sociosexual stimuli affect ER- and PR-mRNA abundance in the hypothalamus of all-female whiptail lizards

Vesta Hartman [1], David Crews [*]

Department of Zoology, University of Texas at Austin, Austin, TX 78712, USA

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Abstract

Hormone-dependent sociosexual behaviors of the displaying individual regulate the abundance of estrogen receptor- and progesterone receptor-mRNA in sex steroid hormone-concentrating brain areas of the partner. This effect of behavior on gene expression in the brain is independent of the gonads.

Keywords: Steroid hormone receptors; Ventromedial hypothalamus; Reptile

Coordination of the internal and external environments both within the individual as well as between the sexes is critical to successful reproduction [3,17]. There are now many examples of the influence of hormone-dependent behaviors of one sex on the behavior and endocrine physiology of the opposite sex [6]. Introduction of estrogen-treated females into a population will prolong the breeding season of conspecific males in both birds and monkeys [24,26]. Since behavior is an expression of brain activity, it is not surprising that behavioral interactions influence brain activity. In the hamster and the rat, exposure to sexual behavior of the opposite sex induces expression of the immediate-early gene c-*fos* in those brain regions that mediate sexual behavior [2,16,18].

Given that the dynamic relationship between steroid hormones and their receptors in the brain is a prerequisite for reproductive behavior in vertebrates, one would predict that the behavioral facilitation of reproduction occurs by regulating the expression of genes coding for peptide hormones, steroidogenic enzymes, and/or steroid hormone receptors. While obvious, this link has only recently been documented.

In this study, unisexual (all-female) *Cnemidophorus uniparens* captured in and around Portal, Arizona were transported to the University of Texas at Austin where they were maintained in environmental chambers under breeding season conditions as described previously [25]. The present experiment was conducted following an artificial hibernation consisting of a 9L:15D photic cycle at 27°C from October through January; during that 3-month period lizards were fed crickets or mealworms once a week.

To eliminate the gonads as a variable, all animals were ovariectomized one week prior to emergence from hibernation. Experimental animals were given a subcutaneous injection of 0.06 μg of estradiol benzoate (EB; Sigma) one week after emergence from hibernation and again 5 days later (24 h before sacrifice). This dosage was chosen because it upregulates estrogen and progesterone receptor (ER and PR, respectively) mRNAs in the brain [27]. The first injection simulated the natural changes in ovarian estrogen secretion following emergence from hibernation and to facilitate behavioral interactions with the stimulus animal. The second injection assessed the behaviorally facilitated neural sensitivity to exogenous estrogen.

The experimental individuals were housed in pairs with a stimulus animal throughout hibernation and the experimental period until sacrifice. Three stimulus conditions were represented: Group I ($n = 6$ pairs) – behaviorally inactive, untreated ovariectomized females [12]. Group II ($n = 6$ pairs) – male-like stimuli consisting of ovariectomized females treated with 10 mm subcutaneous Silastic implants containing testosterone (T) to induce male-like pseudocopulatory behavior [9]. Group III ($n = 7$ pairs) – female-like stimuli consisting of ovariectomized females

* Corresponding author. Fax: (1) (512) 471-6078 or 471-9651; E-mail: crews@bull.zo.utexas.edu

[1] Present address: Neurobiology and Behavior, University of Washington, Box 357750, Seattle, WA 98195-7750, USA.

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treated with 0.5 μg EB injections separated by 2 days to induce female-like receptive behavior [27]. Stimulus animals received hormone treatment immediately following ovariectomy. Behavioral observations confirmed that the stimulus animals behaved in their respective roles.

Experimental animals were sacrificed by rapid decapitation. The brain was quickly removed, frozen on dry ice and stored at −80°C until cryosectioning. The in situ hybridization and silver grain quantification procedures were performed bilaterally on one brain section per animal, 10 silver grain clusters per side, as described previously [27]. Mean silver grain densities (grains per cluster) were compared across the three treatment groups by one-way analysis of variance using Systat 5.1 for the Apple Macintosh. Homogeneity of variance among treatment groups was assessed using Bartlett's test. Following detection of significant differences by ANOVA, treatment group means were compared using Fisher's LSD test. Although not necessary to achieving statistical significance, one value from Group I for PR-mRNA in the VMH was excluded based on the results of an outlier test (Dixon's test, $P < 0.05$; [22]).

Experimental animals housed with male-like and female-like stimulus animals had significantly higher ER- and PR-mRNA levels in the ventromedial nucleus of the hypothalamus (VMH) compared to those housed with reproductively inactive stimulus animals (ER: $P < 0.01$ and 0.03; PR: $P < 0.001$ and 0.003, respectively) (Fig. 1). ER-mRNA levels in the posterior hypothalamus (PH) were also significantly higher in lizards housed with a female-like stimulus animal ($P < 0.02$) (Fig. 1). In the periventricular preoptic area (PPOA), the hybridization signal for ER-mRNA did not reach the criterion of 3 × background [27] and hence levels were not quantifiable. PR-mRNA levels in the PPOA and PH did not differ significantly among the groups, although the trend in the PH (Fig. 1) closely resembled ER-mRNA levels in the same nucleus.

This study illustrates that sociosexual stimuli can act independent of the gonads to affect expression of sex steroid hormone receptor-mRNA in the hypothalamus. While sociosexual interactions have been found to influence brain metabolism, most studies focus on protein synthesis and rely on male-female pairs. The latter aspect is important for it introduces difficulties in interpretation as the partner differs in genotype, morphology, physiology, and behavior. Thus, the observed changes could be due to a non-behavioral aspect of the male's phenotype, his behavior, or both. The parthenogenetic whiptail lizard, *C. uniparens*, provides an unusual opportunity to control for this confound of gender, thereby insuring that the effects observed are due to sociosexual stimuli and not the genotype or morphology of the stimulus animal. As demon-

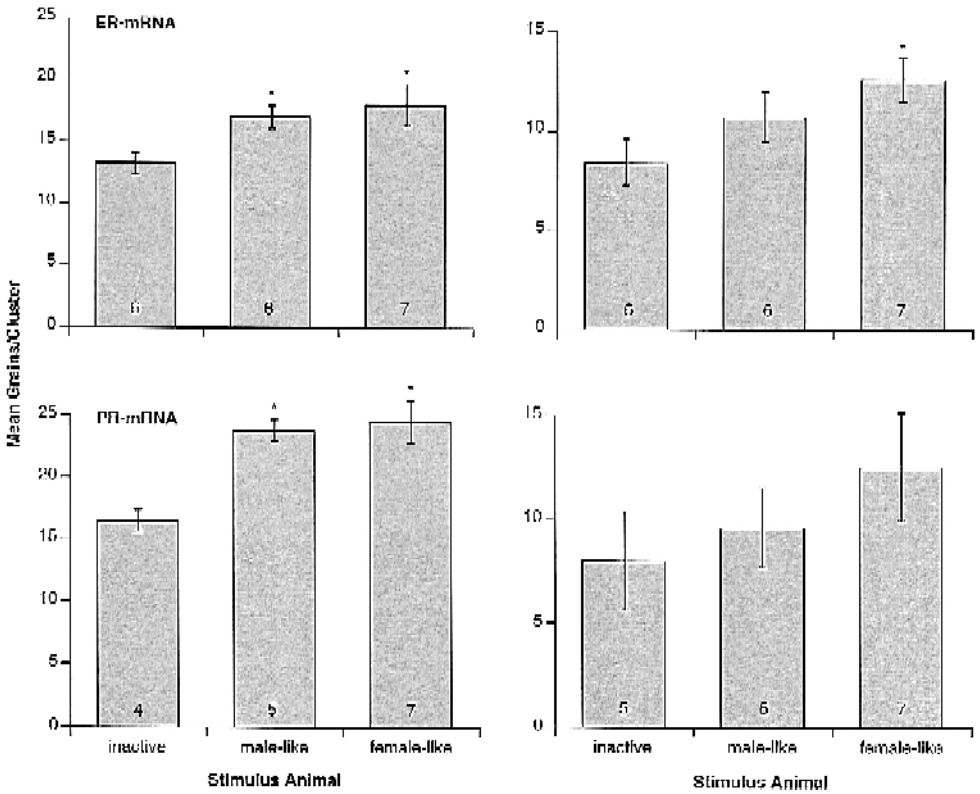

Fig. 1. Abundances of estrogen receptor (ER) and progesterone receptor (PR) mRNA in the brain of parthenogenetic whiptail lizards housed with sexually inactive, male-like, or female-like conspecifics. Left panel: ventromedial nucleus of the hypothalamus (VMH). Right panel: posterior hypothalamus (PH). Mean and standard error shown. Asterick indicates a significance difference fromthe sexually inactive (control) group. Sample sizes are different for the PR- than ER-mRNA counts because a slide was lost during in situ procedures.

strated previously, individual female *C. uniparens* regularly exhibit male-like and female-like pseudosexual behaviors which are indistinguishable from those shown by male and female members of their sexual ancestral species, *C. inornatus* [5,7]. This pseudosexual behavior has functional significance. In the ancestral species, females housed with sexually active males produce more eggs sooner compared to females housed with castrated, sexually inactive males [8,9,12]. In the parthenogen, individuals ovulate sooner and produce more clutches if housed with a cagemate that displays male-like pseudosexual behavior, compared to individuals housed with behaviorally inactive or female-like cagemates [5].

The VMH of *Cnemidophorus* is known to control female-typical receptive behavior [15,19,25]. The increased abundance of ER- and PR-mRNA in the VMH of the experimental animals is similar to the pattern observed during receptivity [27]. A similar, although less pronounced, effect was observed in the PH, which is also involved in female sexual behavior in whiptail lizards [15,19,25]. It should be kept in mind, however, that mRNA abundance and the level of receptor protein do not necessarily correlate in a predictable manner.

It has been assumed that the behavioral facilitation of reproduction in conspecifics occurs indirectly via activation of the hypothalamo-pituitary-gonadal axis. In fish, birds, and mammals, sociosexual interactions increase gonadotropin-releasing hormone (GnRH) content in certain cells in the brain [11,21,28]. Increased GnRH levels trigger upregulation of genes expressing pituitary gonadotropins which, in turn, control the synthesis and release of adrenal and gonadal steroids [13]. However, even in the absence of ovaries, Hnatczuk et al. [14] found elevated levels of circulating estradiol in estrogen-primed prairie voles exposed to extended male cohabitation. This suggests that sociosexual stimuli may induce estrogen secretion from a nonovarian source, possibly the adrenal gland [10] or the brain [20].

The lack of a difference in PR-mRNA levels previously observed in the PPOA of receptive *Cnemidophorus* [27] may be because of the estrogen priming, which would predispose the experimental animals to be receptive and unlikely to exhibit male-like pseudocopulatory behavior [12] toward an estrogen-treated stimulus female (as in Group III). The lack of apparent differences lends support to a direct effect on the hypothalamus vs. an effect via the gonads since in all vertebrates studied to date, the PPOA is involved in the control of gonadotropin secretion via GnRH cells. There is no evidence that pituitary hormones influence sexual behavior in lizards [4], although hypothalamic peptides are potential modulators of courtship behavior [1]. Alternatively, social interactions in general might affect the VMH, while only mating would affect the PPOA, as appears to be the case in Syrian hamsters in which c-*fos* is activated in the VMH of males involved in both agonistic and mating behavior, but only mating behavior increases c-*fos* activation in the MPOA [16]. In female rats, increased c-*fos* expression in ER-immunoreactive neurons in the VMH and other brain nuclei is associated with sexual receptivity in response to vaginal-cervical stimulation [23].

These results suggest an expanded role of sociosexual stimulation in the facilitation of reproduction, and how sensory and hormonal information are integrated at the molecular level in the brain. While the mechanism(s) are unclear, the differential gene expression in the brain observed could be mediated by a number of avenues including, but not limited to, pheromonal cues, tactile or visual stimuli, and sexual experience.

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