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Effects of ovariectomy and estrogen replacement on attractivity and receptivity in the red-sided garter snake (*Thamnophis sirtalis parietalis*)

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Abstract Activation of courtship behavior in male red-sided garter snakes, *Thamnophis sirtalis parietalis*, is independent of the presence of sex steroids. The only consistent treatment that stimulates courtship behavior in males is prolonged exposure to low temperature followed by subsequent warming, mimicking the emergence from hibernation. We investigated whether attractivity and receptivity in female red-sided garter snakes is similarly steroid independent.

Female red-sided garter snakes are attractive when they emerge from hibernation and are courted by males; most mate within an hour of emergence. In a series of experiments, groups of females were either ovariectomized (OVEX) in the late spring, fall or while in hibernation. They were tested for attractivity and receptivity upon emergence from hibernation. Females OVEX in the spring were unattractive whereas those OVEX in fall or while in hibernation were attractive. Thus, attractivity appears determined the year before emergence and is dependent on the presence of the ovaries. All OVEX females were unreceptive upon emergence. OVEX females were also given replacement estradiol (E) treatment (either in Silastic capsules or single injections) at various points of their annual cycle. The only treatment that resulted in reinstating receptivity in OVEX females was the injection of E (20 μg) one hour prior to emergence. The effectiveness of E in reinstating receptivity was time dependent: the longer the period between emergence and injection, the less effective the same dosage was in stimulating receptive behavior.

These experiments suggest that sexual behavior in female red-sided garter snakes is, unlike males, dependent on the presence sex steroid hormones. Although E is naturally at its lowest seasonal level upon emergence, the concentration is sufficient to stimulate receptivity. However, it appears that temperature regulates a time-limited window of sensitivity to E.

Key words Ovary · Estrogen · Attractivity · Receptivity · Reptile

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Introduction

Courtship behavior in male red-sided garter snakes, *Thamnophis sirtalis parietalis*, is independent of the short-term activational effects of sex steroids (Camazine et al. 1980; Gartska et al. 1982; Crews et al. 1984). When males emerge from hibernation in the spring, they exhibit courtship at a time when gonads are regressed and circulating concentrations of androgens are low. Males that have been gonadectomized and maintained in the laboratory up to three years will still exhibit courtship after emergence from low temperature (4°C) dormancy simulating hibernation. To date, the only methodology capable of inducing courtship behavior in the laboratory is this hibernation and warming regimen (Camazine et al. 1980; Gartska et al. 1982; Crews et al. 1984). Males which do not hibernate do not court. However, although independent of androgenic modulation of courtship behavior in the relative short term (i.e. 1–2 years), males do require periodic exposure to testosterone (T) for the continued seasonal expression of behavior (Crews 1991). For example, males that have been gonadectomized 3–4 years no longer exhibit courtship behavior even after exposure to hibernation conditions. If, however, longterm castrates are implanted with T the summer *before*

hibernation, they will court upon emergence (Crews 1991). Therefore, some exposure to testosterone is necessary to maintain the seasonal expression of sexual behavior, but the short-term activation of courtship appears dependent on the males experiencing a certain temperature regimen.

The role of steroids in the modulation of sexual behavior in female red-sided garter snakes has not been rigorously addressed. Upon emergence from hibernation in the field, all females are attractive and a large percentage mate almost immediately (Gartska et al. 1982; Whittier and Crews 1986). In the laboratory, females are attractive and will either mate within a few days of emergence or not mate at all (M. Mendonça and D. Crews, *pers. observ.*). In both instances, at the time of mating, ovarian follicles are pre-vitellogenic and steroid levels are at basal levels for this species at this time (Halpert et al. 1982; (Whittier et al. 1987; Mendonça and Crews 1990).

Estrogen appears to play a role in inducing attractivity: unattractive females can be made attractive by daily injection of pharmacological doses of 17β-estradiol (Crews 1976: Kubie et al. 1978; Gartska and Crews 1985). However, the dependence of *receptive* behavior on steroid hormones has not been tested. Bona Gallo and Licht (1982) demonstrated that female garter snakes become attractive but not receptive if maintained at high temperatures (28 °C) and fed during the period they normally hibernate. These females did not appear to undergo ovarian development (Bona Gallo and Licht 1982). These results argue that 1) different mechanisms control attractivity and receptivity in female garter snakes, and that 2) sexual behavior in females, as in males, is affected by changes in temperature regimen rather than changes in circulating sex steroid levels. This paper addresses the following questions: 1) is the receptive behavior of females independent of the activational effects of sex steroids as is the courtship behavior of males? and 2) what is the relationship of attractivity to receptivity in the female red-sided garter snakes? We examined the effects of ovariectomy and estrogen replacement treatment at various points in the ovarian cycle on the subsequent expression of attractivity and receptivity on emergence from hibernation.

Materials and methods

Animals

Males and females were collected in the falls of 1988, 1989, and 1990 and in the spring, 1990 from dens in the Interlake region of Manitoba, Canada. Animals were returned to the laboratory, separated by sex and housed in 30 gal aquaria. They were kept at approximately 25 °C and a 12:12 L:D cycle when not in hibernation. Animals were given water ad lib and fed ground fish (frozen and canned) supplemented with vitamins twice a week. To simulate hibernation, all snakes were exposed to 4 °C for 17 weeks from October to February.

Surgery and blood collection

Females were anesthetized with Sodium Brevital (15 mg/kg body mass). Longitudinal incisions were made 45–50 belly scales anterior to the cloaca on the left side and 60–65 belly scales anterior to the cloaca on the right hand side of the animals. The vessels to the gonads and, in Experiment 4, the adrenal glands, were tied off and the organs extirpated using a cautery. The incisions were then sutured. Sham operations involved making the incisions, extruding and then replacing the ovary and suturing the incisions. In several of the experiments ovariectomized females received an peritoneal implant of a Silastic capsule (10 cm long, 1.65 cm o.d. and 70.76 cm i.d.) which contained hormone or was empty.

Plasma was collected by incising the tip of the tail and letting blood drip into a heparinized test tube. Bleeding was stopped by elevating the tail and applying pressure. Blood was then centrifuged, the plasma pipetted and frozen at -20 °C for later radioimmunoassay analysis of circulating E levels.

Radioimmunoassay

Validation of the following procedure is presented in Whittier et al. (1987). Plasma aliquots were incubated for one hour with a known amount radioactively labeled estradiol for later determination of per cent recovery. Plasma was then extracted with ether and snap frozen at −80 °C. The supernate was poured off and dried down with nitrogen gas. The samples were reconstituted with phosphate buffer and known amounts of tritiated E (obtained from New England Nuclear) and 17-β E antibody (obtained from Endocrine Sciences, Tarzana, CA) added. Samples were incubated for 4 h at room temperature and then charcoal suspension aliquots added to remove the unbound steroid. The samples were then centrifuged and the supernate poured off into scintillation vials. Scintillent was added and vials counted in a β counter. Interassay CV averaged 11.2%, intrassay CV averaged 5.4%, and sensitivity equaled 15 pg/ml.

Behavior testing

In males, courtship behavior is clear and unequivocal. It consists of the male aligning his body with that of the female, constantly maintaining contact with her body and actively rubbing his chin against her back while trying to flip her tail up with his tail. In contrast, sexual behavior in female garter snakes is subtle and its nuances are difficult to quantify. A female can only be assessed as "attractive" by the males' reactions to her. Receptivity is, similarly, a post-hoc phenomenon. There is no clear way to predict which female will mate. However, it is clear that males cannot force females to mate: females must actively gape their cloacas for males to effect intromission. Therefore, in order to measure attractivity and receptivity, we quantified 1) the responsiveness of males to a female (i.e. intense chin-rubbing courtship behavior) to a female and 2) whether females actually allowed copulation.

Attractivity tests were conducted by placing a single female in an aquarium with 10 males known to exhibit vigorous courtship behavior. The males' interaction with the female was observed for 10 min and the maximum number of males actively courting the female recorded. Females eliciting courtship from 0–3 males were classified "unattractive", those that received courtship from 6–10 males were "attractive". It was extremely rare to have the intermediate category. In the first week after emergence, females were tested daily for attractivity and then every other day for an additional week.

Receptivity was tested by placing a female in an aquarium with 20 courting males for 3–4 h daily for 10–14 days or until the female mated. Females that had not mated by the end of two weeks of testing were classified as unreceptive. Any female that mated was classified post-hoc as receptive. Mating was determined by the

presence of a cloacal plug which is always left by the male upon completion of copulation. Therefore, mating was not always directly observed but could be readily inferred. Females that had mated also became unattractive and unreceptive, thereby allowing complete certainty of their mated status. Mated females were not usually courted by males and those that were would frequently exhibit sudden and rapid darting movements and barrel rolls to escape the males' attentions.

Statistics

Behavioral data were analyzed using a Fisher's Exact Test or Chi Square test. Hormone data were tested for heterogeneity of variances. If variances were heterogeneous, data were log transformed and then a repeated measures or independent measure ANOVA used.

Summary

A series of seven experiments on the control of receptivity were conducted in 1989–1990. Animals were ovariectomized or received sham surgery at different stages of their ovarian cycle. They also received estrogen or vehicle treatments at different points of their cycle. All females were tested for attractivity and receptivity upon emergence from hibernation. The experiments are described below and their timeline shown in Fig. 1 in a summary format.

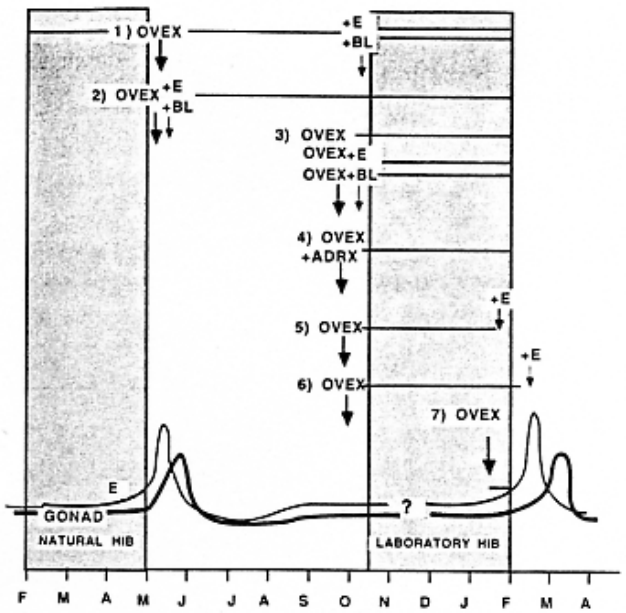

Fig. 1 Timeline of surgical manipulation and exogenous estradiol administration for Experiments 1–7 in female red-sided garter snakes in relation to the natural cycle of ovarian activity and estrogen secretion and hibernation. *Abbreviations* as in Fig 2 and 3

Experiment 1. Effect of ovariectomy in Spring and E replacement in Fall

Females were collected in September 1988, hibernated in the laboratory, and not allowed to mate upon emergence (and thus did not undergo complete ovarian development). These females were either ovariectomized (OVEX) or sham operated (SHAM) in June, 1989 ($n = 20$ and 12 respectively). OVEX females were implanted for two weeks with either 17-β estradiol (OVEX + E) or blank (OVEX + BL) capsules ($n = 10$ group) in September 1989. Females were bled two weeks after ovariectomy and just before implant removal. Animals were hibernated two weeks after the removal of implants. SHAM females were concurrently placed in hibernation.

Experiment 2. Effect of ovariectomy and E replacement in Spring

Females were collected in May, 1990, ovariectomized ($n = 21$) or sham operated ($n = 12$) two weeks after collection in June, as was done the previous year. Simultaneous with ovariectomy, females were implanted with either BL ($n = 10$) or E ($n = 11$) capsules. Capsules were removed four weeks after implantation to mimic the timing of the natural E cycle. All females were bled before surgery and again when implants were removed. Animals were hibernated in October. This experiment was done to 1) eliminate the possibility that prolonged captivity influenced the results obtained in Experiment 1 and 2) provide exogenous E at a time normally experienced by females.

Experiment 3. Effect of ovariectomy and E replacement in Fall

Females were collected in September, 1989, returned to the laboratory and either OVEX ($n = 39$) or SHAM ($n = 9$) within three weeks of collection. A subset of individuals were bled before surgery. OVEX females were then divided into three groups. They either received an E implant (OVEX + E, $n = 16$), a blank implant (OVEX + BL, $n = 10$) or no further treatment (OVEX, $n = 13$). After a two week period, all females were bled and the implants removed. Two weeks after implant removal, all females entered hibernation. Upon emergence, females were tested for attractivity and receptivity. Females that mated were bled as soon as intromission was completed. Females that did not mate were bled approximately one week after emergence.

Experiment 4. Effect of ovariectomy and adrenalectomy in Fall

Females were collected in September, 1989, returned to the laboratory and either OVEX and adrenalectomized (ADRX) ($n = 11$) or SHAM ($n = 11$) within 3 weeks of collection. They were bled one week after surgery. Three weeks after surgery, they were placed in hibernation. Females were bled in the spring following the same protocol as Experiment 3.

Experiment 5. Effect of ovariectomy in Fall and E injection before emergence

Females were collected in September, 1989, returned to the laboratory and OVEX ($n = 10$) within 3 weeks of collection. Three weeks after surgery, they were placed in hibernation. An hour *prior* to emergence females were given either a single injection of 20 µg E in steroid suspending vehicle ($n = 6$) or vehicle alone ($n = 4$). Upon emergence, females were tested as in the preceding experiments. We repeated the experiment the next year (September, 1990) with a larger sample size ($n = 11$/treatment).

Experiment 6. Effect of ovariectomy in Fall and E injection after emergence

Females collected in September, 1989 ($n = 14$) were treated as in Experiment 5 with the exception of receiving the injection of 20 µg E

($n = 8$) or vehicle ($n = 6$) 8 days *after* emergence instead of hours *before* emergence. The experiment was repeated the next year with groups receiving a dose of either 20 µg ($n = 8$) or 40 µg ($n = 9$) of E or vehicle ($n = 6$) 6 days *after* emergence.

Experiment 7. Effect of ovariectomy during hibernation

Females collected in fall 1990 underwent hibernation with their ovaries intact. Two weeks before emergence, females were OVEX ($n = 10$) or sham operated ($n = 12$) while in hibernation and then tested as in the other experiments. Blood was collected while females were in hibernation and on the day after emergence.

Results

Experiment 1. Effect of ovariectomy in Spring and E replacement in Fall

None of the females that had been ovariectomized in Spring, 1989 were attractive upon emergence from laboratory hibernation in 1990. Females that were given two weeks of an E implant (OVEX + E) before hibernation and thus had circulating E levels of 10–20 ng/ml in the fall were unattractive as were females that had received a blank capsule (OVEX + BL) and had very low levels of circulating E ($\bar{x} \pm 1$ SE: 34.6 ± 7.4 pg/ml). The majority of sham females, on the other hand, were attractive (Fig. 2A) and had low but significantly higher levels of E than the OVEX + BL ($122.75 + 9.8$ pg/ml; $t = -4.3$, d.f. = 20, $P = 0.001$).

Since very few males courted OVEX females, it was difficult to objectively assess receptivity. However, the behavior of these females (e.g. barrel rolls, sudden and rapid darting away from males) indicated that, in addition to not being attractive, they were not receptive. Although sham operated animals elicited more courtship than any of the OVEX animals (75% of the sham group was attractive vs 0% of either of the OVEX treatments: Fisher's Exact Test $P = 0.00002$ when OVEX treatments are grouped; Fig. 2A), none of the sham treated animals mated. Sham animals also tended to display non-receptive behavior.

Experiment 2. Effect of ovariectomy and E replacement in Spring

Although attractive, SHAM females of Experiment 1 did not mate. Prolonged captivity depresses mating in garter snake females (M. Mendonça, *pers. observ.*). To eliminate this influence, we shortened the females' time in captivity. Females were captured in late May, 1990 and also treated as soon as possible to more closely mimic the species natural cycle.

The percent of SHAM females that were attractive did not differ between Experiment 1 and 2 and, in fact, even declined (75% vs 66%, Fig. 2A and B). The percent of "spring" (June) OVEX + E females scored as attractive the next spring was only marginally greater than those receiving BL implants (36% vs 0%, $P = 0.055$; Fig. 2b). None of the OVEX group, regardless of implant type, mated and all displayed unreceptive behavior. The majority of SHAM females were attractive, but only 44% (4 of the 9 attractive females) mated. However, significantly more of the SHAM group mated compared to the OVEX + E group (Fisher's Exact; $P = 0.026$). Circulating E levels before surgery were elevated and did not differ significantly among the three groups. SHAM females that mated had higher E levels at emergence than those that did not mate ($\bar{x} \pm 1$SD: 580 ± 198 pg ml. $n = 4$ vs. 173 ± 130 pg/ml, $n = 5$).

Experiments 3 and 4. Effect of ovariectomy and E replacement in Fall and ovariectomy and adrenalectomy in Fall

All females captured in the autumn and hibernated in the laboratory were attractive and elicited vigorous

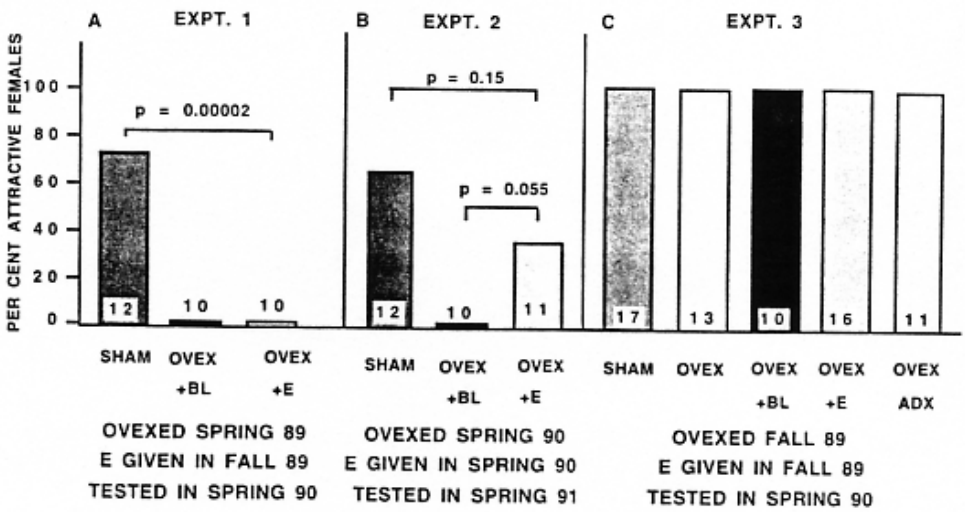

Fig. 2A–C Percent of females scored as attractive after different manipulations. **A** Females were ovariectomized (OVEX) in spring 1989, given estradiol or a blank capsule (OVEX + E/ OVEX + BL) in fall, 1989 and tested in spring 1990 **B** Females were OVEX in spring 1990, given + E/ + BL in spring, 1990 and tested in spring 1991 **C** Females were OVEX in fall, 1989, given + E/ + BL in fall, 1989 and tested in spring 1990. *Numbers* at the base of the histogram indicate sample size

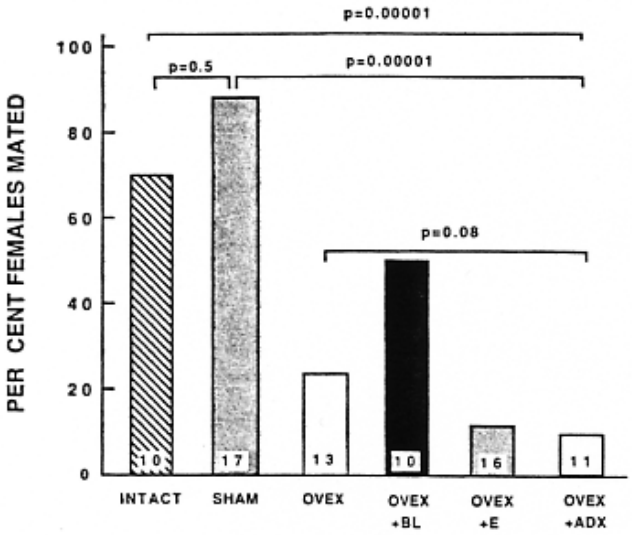

Fig. 3 Percent of females that mated upon emergence from hibernation in spring, 1990. *Numbers* at the base of the histogram indicate sample size. *Abbreviations* as in Fig. 2. OVEX + ADX = ovariectomized and adrenalectomized

courtship from males the next spring, whether they were INTACT, SHAM, OVEX females (regardless of E treatment) or OVEX + ADRX (Fig 2C). Significantly fewer OVEX females (in all treatment categories) mated than intact or sham females ($X^2 = 30.7$, d.f. = 5, $P = 0.0001$; Fig 3). In this experiment, several of the OVEX groups had individuals that mated. Circulating E levels of these individuals were higher than those group individuals that did not mate (Table 1). Upon sacrifice, treated females that had mated were found to have incomplete ovariectomies. All treated females that did not mate had complete ovariectomies.

Experiment 5. Effect of ovariectomy in Fall and E injection before emergence

As in Experiments 3 and 4, all females OVEX in the fall were attractive upon emergence in the spring. This retention of attractivity was the case in every female that had undergone a complete ovarian cycle in the spring and summer and had only been manipulated in the fall regardless of experiment (this result also applies for OVEX females in Experiments 6 and 7).

A significantly higher number of fall OVEX females receiving a single injection of 20 µg E prior to emergence mated than those receiving a vehicle injection in both 1990 and 1991 (Fig. 4).

Experiment 6. Effect of ovariectomy in Fall and E injection after emergence

Unlike the females in Experiment 5, a single 20 µg E injection was not sufficient to instate receptive behavior in females when administered 8 days after emergence (Spring 1990). The next spring, a 20 µg injection 6 days after emergence was again ineffective in stimulating receptivity. However, there was a slight but not significant increase in number of females mating after a single 40 µg E injection (double the original dose) at the same time point (33.3% vs 0%, Fig. 5).

Table 1 Plasma estrogen levels for mated and unmated females in Experiment 3 treatment grouyps. Blood was taken for mated females immediately after copulation ended (+ 0 h). Bloods for unmated females were collected after animals had been courted daily (for a 2 k period) for four days and still had not mated. Numbers are mean, (standard deviation), and sample size

	Mated	Not mated
INTACT	584	167
	(307)	(298)
	10	4
SHAM	390	110
	(410)	(130)
	10	5
OVEX	290	50
	(50)	(30)
	10	5
OVEX + BL	1,600	—
	1,721	
	5	
OVEX + E	220	106
		(134)
	2	5
OVEX + ADRX	—	77
		(57)
		6

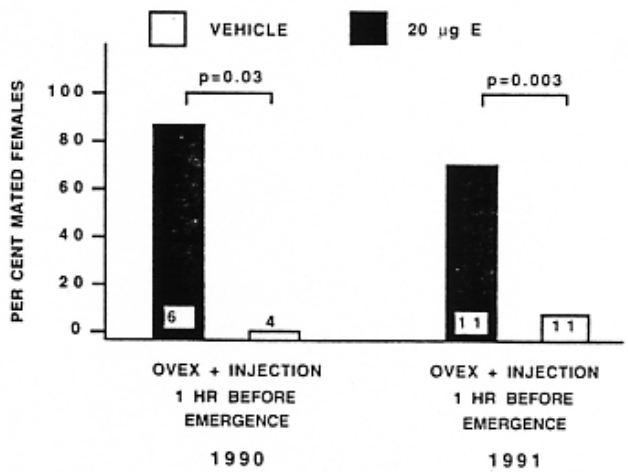

Fig. 4 Percent of females that mated after a single injection of 20 µg estradiol one hour before emergence in 1990 and 1991

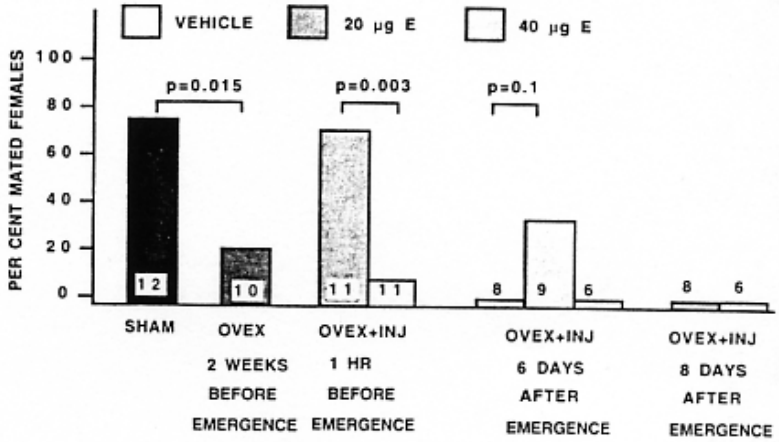

Fig. 5 Percent of females that mated after ovariectomy one week before emergence, ovariectomized in the fall and given estradiol one hour before emergence or were ovariectomized in the fall and given estrogen at two dosages approximately a week after emergence

Experiment 7. Effect of ovariectomy during hibernation

Females OVEX in hibernation also mated in significantly lower frequency than those that had undergone sham operations (Fig. 5). Females had detectable levels of estradiol in hibernation before the surgery (1,302 + 249.3 pg/ml, $n = 7$) and, at two days after emergence, these females had significantly lower E levels (205 + 71.4 pg/ml, $n = 7$; $P = 0.003$)

Discussion

We offer the following summary to put the various results in perspective.

First, females that were manipulated in the spring early summer and, thus, did *not* undergo an ovarian cycle (either because they had not mated or were OVEX before they could begin their cycle) were neither attractive nor receptive the next spring. Treatment with E, either in the late spring (mimicking the natural cycle) or in the fall, was ineffective in restoring either condition (see Experiments 1 and 2). However, every female that underwent a natural ovarian cycle and was *then* OVEX in October or while in hibernation was extremely attractive (i.e. indistinguishable from females in the SHAM or INTACT groups) upon emergence in the spring. This retention of attractivity occurred irrespective of subsequent E treatment (see results of Experiments 3–6).

Second, these very attractive OVEX females were, however, unreceptive. A two week period of E replacement in the fall was insufficient to stimulate receptivity the subsequent spring. The only OVEX females that mated were ones that exhibited elevated circulating E levels in the spring and, upon later sacrifice, found to be incomplete ovariectomies (see Experiments 3 and 4, Table 1).

Third, the presence of E at emergence was found to be effective in stimulating receptive behavior but there appeared to be a time or temperature-dependent dosage effect. In other words, a dosage that, if given prior to emergence, was effective in stimulating receptivity became ineffective if given approximately one week later. Twice the E dose was required to elicit a behavioral response at a similar time point but it was not effective for all females (see Experiments 5 and 6).

Fourth, females that remained intact and thus had normal circulating E levels throughout hibernation but were OVEX shortly before emergence were also unreceptive. This result seems to again indicate that the presence of E is necessary *at* or *near* the time of emergence rather than its continous and/or elevated presence before or during hibernation (see Experiment 7).

Attractivity

The mechanisms and time frames of the control of attractivity versus receptivity appear quite different. This is not surprising given that attractivity in female garter snakes is determined by the production and deposition of a female sex pheromone (a long-chained methyl ketone lipid) in the skin (Mason et al. 1989), while receptive behavior, presumably, is determined by changes at the neural level.

To be attractive upon spring emergence, females apparently need to undergo a natural reproductive cycle the previous summer. Females ovariectomized *before* undergoing a cycle did not *become* attractive whereas ovariectomy *after* the ovaries undergo a complete cycle had no effect on an animal's attractiveness on emergence from hibernation the subsequent spring. It is not clear, however, which aspect(s) of the cycle is/are important. Elevating E for four weeks in early summer, thus mimicking the normal cycle (Experiment 2) was not a successful treatment, only a small percentage (37%) of the treated females became attractive the

next spring. It may be that we were not truly successful in recreating the time course or exposure levels a female would normally experience (e.g. we did not continue low levels throughout the summer). It may be that four weeks of low E is insufficient to stimulate production of the necessary skin lipid. However, injecting an unattractive, *intact* female with pharmacological doses of E (40 µg/70 g body weight for a week) will cause the female to become attractive (Crews 1976; Kubie et al. 1978). This result indicates that E, in excessive quantities and with an ovary present, can stimulate the production/deposition of female sex pheromone fairly quickly. This injection protocol, however, does not always result in attractive females and male courtship never seems as vigorous in artificially produced attractive females as compared to naturally attractive animals (M. Mendonça and D. Crews, *pers. observ.*). This observation may indicate that the protocol is not totally effective or may not be stimulating the normal pathway for pheromone production. Therefore, the results of Experiment 2 may suggest that changes in E alone are insufficient to induce the production of the necessary skin lipid and other factors (*e.g.* other hormones, either ovarian or non-ovarian; fat stores or diet) may contribute to normal pheremone production and deposition. The experiments suggest that the pheromone lipids are produced and deposited in the skin of the female in the summer in association with the ovarian cycle, remain there at least until the spring and do not require the presence of an ovary (at least in the short term) for its maintenance once deposited. Gartska and Crews (1985) found that male *Thamnophis sirtalis parietalis* tested in the field did not discriminate between OVEX and INTACT, E-primed females (40 µg/70 g body weight for a week) but those in a laboratory colony did *after* 10 days of testing: initially they behaved as the field males did and did not discriminate. This suggests that the pheromone may become depleted without estrogenic support.

Receptivity

Unlike attractivity, ovariectomizing females at any point in the cycle adversely affected the expression of receptivity. This was true even when females were ovariectomized as little as two weeks before emergence and still had detectable levels of E. Females that had incomplete ovariectomies mated. These females had higher circulating E values at emergence than those with complete ovariectomies (Table 1). However, there was some overlap. For example, a female with an E value of 290 pg/ml mated while one at 520 pg/ml did not. Therefore, receptive behavior does not seem to be exclusively determined by some threshold E level but rather a combination of E and the presence of an ovary *at emergence*.

However, when a single, high dose (20 µg of E, which produces a high circulating concentration in relation to naturally occurring levels) was given before emergence, receptive behavior was restored to a level equivalent to that of intact females. This result indicates that a change in ambient temperature appears to mediate responsiveness to E. Females were much more sensitive to the behavioral effectiveness of E shortly after the increase in body temperature. Therefore, there seems to be a temperature/time window when E will have a stimulatory effect on behavior. This effect is expressed extremely rapidly; females given an E injection one hour before emergence could then mate in as little time as one hour after emergence. most mated the first day of emergence (< 24 h after the injection). Very few mated more than 24 hs after emergence (though this did occasionally occur). However, once females reached a certain temperature, they become far less sensitive to E (Mendonça and Crews, data unpublished). Exogenous E, even when given at twice the initially effective dose (an injection of 40 µg of E), was relatively ineffective in stimulating receptive behavior when females had been at room temperature for approximately one week (Fig. 5). This loss of sensitivity may be related to the fact that mating triggers a neuroendocrine reflex which results in a surge in E (Whittier et al. 1987; Mendonça and Crews 1990). In the natural reproductive cycle of the female, elevated levels of E indicate mating has occured and vitellogenesis has begun. Normally, therefore, high levels of estrogen, days after emergence, should *not* stimulate receptive behavior. Females become unreceptive after mating apparently through the mediation of prostaglandins (Whittier and Crews 1986b). Additionally, receptivity itself, even without mating, is a transitory state. If a female does not mate within one week of emergence, she will not mate that spring, though hormone values do not change (M. Mendonça and D. Crews, *unpublished data*).

There have been numerous studies on the time course of exogenous estrogen effects on receptive behavior in several mammalian species (reviewed in Pfaff et al. 1994) but this has not been at all well documented in reptiles (McNicol and Crews 1979; Jones et al. 1983; Moore et al. 1985; Moore 1987). It has been found that, in rats, the minimum length of time estrogen was necessary to "prime" receptive behavior in ovariectomized rats was 30 mins. However, behavior was not seen until progesterone (P) was given three days later (Johnston and Davidson 1979). Receptivity was seen at 24 h when females received two discontinuous one hour exposures to E (but again, only after P injection, Parsons et al. 1982 a,b). In the rat, the minimum time exposure between E treatment and receptivity is 17–18 h (Green et al. 1970; Parsons et al. 1980). In Experiment 4, of the eight OVEX females (injected with pre-emergence E) that mated, five mated within eight hours of emergence, two of these within one hour. Therefore, the minimum time exposure to E for these animals was two hours and behavior followed rapidly. In the field in Canada, females mate very soon (as rapidly as minutes) after

emergence. These observations indicate a fairly rapid response to E, equal to, if not faster than, that seen in in rats when administered E discontinously in two 1 hr segments (which had to be at least 4–13 h apart). Female rats were tested for response to E at least 3.5–8 h into their dark cycle (i.e. within 24 h of E treatment: Parsons et al. 1982a, b). The timing of the response to E in the snakes may indicate a non-genomic mode of action (McEwen 1991). Estrogen can, within minutes, alter neuronal firing rate (Kelly et al. 1976), changes in potassium conduction (Nabekura et al. 1986), and neurotransmitter release/binding (Dluzen and Ramirez 1989; DiPaolo et al. 1986; Levesque and DiPaolo 1988). However, these rapid effects seem to occur in animals that have been previously estrogen-primed. This restriction does not appear to be necessary in female snakes unless the low level of estrogen remaining in ovariectomized animals is sufficient to induce genomic, long-term changes. An alternative mechanism may be that of "gene memory", where estrogen can exert long-term effects on the genome that persist in the absence of the steroid and then elicit a more rapid response when the stimulus is re-instated (Burch and Evans 1986; Hache et al. 1987; Crews 1991; Pfaff et al. 1994).

Whatever the mechanism, receptivity in females is far more dependent on the presence of sex steroids than is courtship behavior in male red-sided garter snakes. Garter snake females do not mate unless a certain level of estrogen is present while males can continue to exhibit courtship behavior after every emergence for three years after gonadectomy (Crews 1991). Aronson (1959) noted that in species which exhibited a decline in sexual behavior after gonadectomy, females always showed the decline far more rapidly than males. The mechanisms stimulating sexual behavior may be under greater selective pressure to maintain a more precise timing (or link to ovarian condition) than those in males. Female garter snakes seem to have a very precise time/temperature window when they are receptive and the apparent non-genomic nature of the mechanism may contribute to this transitory state.

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