Differential Effects of Courtship and Mating on Receptivity and Brain Metabolism in Female Red-Sided Garter Snakes (*Thamnophis sirtalis parietalis*)

Mary T. Mendonça, Dena Daniels, and
Constance Faro
Auburn University

David Crews
University of Texas at Austin

In the female red-sided garter snake (*Thamnophis sirtalis parietalis*), the loss of receptivity following intromission during mating can be prevented by injection of a local anesthetic (tetracaine) in the cloacal region prior to courtship and mating. Females that were courted and then mated had significantly higher uptake of radio-labeled [^{14}C]2-fluoro-2-deoxyglucose (2-DG) in the preoptic area (25%) and significantly lower uptake in the ventromedial hypothalamus (-20%) compared with females that were courted but not mated. Tetracaine-treated females had accumulation patterns similar to courted but unmated females and to females exposed only to other females. These results suggest that in the female red-sided garter snake, sensory input from the cloaca during mating alters patterns of metabolism in those brain areas most often associated with female sexual behavior.

The neuroendocrine reflex is initiated by a defined stimulus transmitted by a specific neural pathway that results in change in physiology and behavior. These reflexes, such as mating-induced reflex ovulation in rabbits and pheromone-induced maternal behavior in sheep, were among the first demonstrations of how an environmental stimulus can transition the organism through reproductive states. Neuroendocrine reflexes have obvious adaptive significance in the life history of the organism, making them particularly interesting and physiologically relevant. Surprisingly, examples of such neuroendocrine reflexes in animals other than domesticated mammals have not been forthcoming.

Red-sided garter snakes (*Thamnophis sirtalis parietalis*) in Manitoba, Canada mate upon emergence from hibernacula after a hibernation period that lasts approximately 8 months (Crews, 1990). Males emerge by the thousands and congregate for 1 to 3

weeks around small limestone dens. Female red-sided garter snakes emerge singly a few days later and because of the presence of a pheromone in their skin, are perceived as attractive by the males and vigorously courted (Mason et al., 1989). This results in mating balls that can have 50 to 100 males courting a single female. A female garter snake will not mate until, after some varying length of courtship, she actively gapes her cloaca and allows one male of the ball to intromit (Joy & Crews, 1985, 1988). Mating lasts 10 to 15 min, and most females leave the den area as soon as mating ends (Whittier & Crews, 1986b). Immediately after mating, the female becomes unattractive and unreceptive to male courtship. Unreceptive females, when exposed to new courting males, will display various stereotypical behaviors (e.g., swift zigzag movement, barrel rolling) to elude males that may still find her attractive (Mendonça & Crews, 1996; Whittier, Mason, & Crews, 1985). Mating induces a neuroendocrine reflex in the female that initially is marked by a surge in circulating concentrations of prostaglandins (PG); administration of exogenous PG-F$_{2a}$ appears to enhance the switch from the receptive to the nonreceptive state (Whittier & Crews, 1986a, 1989).

Sexual receptivity in female vertebrates is known to be integrated by specific hypothalamic nuclei, such as the ventromedial nucleus (VMH) and medial preoptic area (mPOA), whereas the mPOA and anterior hypothalamus appear to be important in male sexual behavior (Crews & Silver, 1985; Pfaff, Schwartz-Giblin, McCarthy, & Kow, 1994). The mPOA is sexually dimorphic in size and structure in many vertebrates, but with the exception of whiptail lizards, few studies have indicated a sex difference in the size of the VMH (Crews, Wade, & Wilczynski, 1990; Godwin & Crews, 1997). Previous research has shown that lesions of the mPOA in male red-sided garter snakes inhibited courtship behavior (Friedman & Crews, 1985; Krohmer & Crews, 1987b), and courting males accumulated more [^{14}C]2-fluoro-2-deoxyglucose (2-DG) in the POA than did noncourting males (Allen & Crews,

1992). However, there have been no studies on areas affecting receptive behavior in female garter snakes.

The goals of this study were to investigate (a) the neural substrate underlying mating-induced shifts in physiology and behavior and (b) the importance of cloacal stimulation in these mating-induced changes in neural activity.

Method

Female red-sided garter snakes (*Thamnophis sirtalis parietalis*) were captured emerging from hibernacula located in the Interlake region of Manitoba, Canada, in the spring of 1989 and 1990. They were returned to our field laboratory within the hour where they were individually marked with nontoxic paint on their dorsum and weighed. All females underwent behavior testing within 2 to 4 hours of capture. Each female received a 20-μl subcutaneous injection in the neck of radio-labeled 2-DG (1 micro-Curie/g body weight) before it was placed in the testing arena. All animal experiments were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee at the University of Texas at Austin.

Female red-sided garter snakes are both attractive and receptive to male courtship upon emergence from winter hibernation. However, there is no clear way to predict when she will allow intromission. It is clear that males cannot force females to mate: Females must actively gape their cloacas for males to intromit. Therefore, we quantified *receptivity* as a post hoc phenomenon (e.g., whether females actually allowed copulation). Once females mate, they become unattractive to males and unreceptive to further courtship. Unreceptive behavior was readily detectable: If unreceptive females are courted by males (which is infrequent), they exhibit sudden and rapid darting movements and barrel rolls to escape the males' attentions.

Behavior testing consisted of placing a single, potentially receptive female red-sided garter snake in a 1-m square nylon testing arena with 10 males that had been previously categorized as vigorous courters. After behavior testing, females were killed by rapid decapitation. The average time interval between injection and time of death was slightly less than 1 hr and did not differ between years ($M = 54.1$ min in 1989 vs. 42.9 min in 1990), $t(24) = 1.45$, $p = .16$. Brains were removed quickly (< 2 min) and frozen in a Dewar flask containing liquid nitrogen. Brains were transported back to the laboratory where they were sectioned in the coronal plane at 20 μm with a cryostat (-20 °C). Sections were placed onto individual cover slips. These sections were immediately placed on a hot plate (50 °C) where they rapidly dried. Once dried, the cover slips, along with standards of known ^{14}C concentrations, were opposed to X-ray film (Kodak SB5) in a film cassette and stored at room temperature in a sealed plastic bag filled with Drierite (Cole-Parmer Instrument Company, Vernon Hills, IL).

After 5 to 6 days, the film was removed and developed for autoradiography using methods published previously (Allen & Crews, 1992; Rand & Crews, 1994). Briefly, histological sections stained with Cresyl violet were used to identify brain nuclei, and the autoradiograms were used to compute density measurements of exposed film images. To transmit autoradiographical images, we used a COHU black and white camera (Model JE2362A, Meyers Instruments, Houston, TX) on manual setting mounted on an Olympus stereoscope over a DC light source (Trulite 100, NAL, Houston, TX). Histological sections were transmitted by a Sony CCD digital camera (Model 390, Frederick, MD) mounted on an Olympus 4BH scope (Drexel Instruments, San Antonio, TX). Both sets of images were captured by a Scion frame grabber card (Model LG3, Frederick, MD) in a Macintosh Centris 650. Histological sections were superimposed on their corresponding autoradiographs, and densitometry readings were taken on the whole brain (WB), the optic tract (OT), nucleus sphericus (NS), medial and lateral POA, and the VMH. The fiber-rich OT and the cell-body-rich NS served as control brain areas.

Measurements were taken on 5 to 10 sections through a particular brain region and on sections of the WB throughout the entire hypothalamus. Only one side of a region was measured, and that side was measured through its entire length. A mean density value was calculated using these measurements for each brain region. The average value was then normalized for differences in background gray by dividing it by the average value of the OT (POA value = average POA density measurement / average OT density measurements). These corrected average brain region measurements were then compared with the average WB $\div$ OT measurements of that particular brain, and the percentage of change from the WB was calculated. Thus, if the brain area was denser (thus more active) than the WB section, it would have a positive value; if less active, a negative value would be obtained in relation to the WB.

Female red-sided garter snakes were courted for at least 30 min or until copulation (whichever came first). Most females mated within the first half hour. These females were designated *courted/mated*. In another group, the cloaca of the experimental female was taped to prevent the male from mating with her, insuring that the female underwent an equivalent amount of courtship but could not mate. This group was designated *courted/not mated*. In the 2nd year of the study, courted/mated females were also taped near their cloaca but not directly covering it to preclude any tape effect. No difference was observed in the 2-DG results when these females were compared with females that were not taped, and so the results were combined. A third group of females was placed for an hour with 10 females and did not undergo courtship but still had the experience of interacting with a group of snakes. This group was designated *all females*. Tests of the differing groups were done as simultaneously as possible to account for differing ambient conditions. Behavior tests were only conducted on days in which climatic conditions (temperature, degree of overcast, and wind) were similar.

Finally, prior to being placed in the testing arena, some female red-sided garter snakes were injected in the cloacal region with either 20 μl of 0.5% solution of tetracaine, a local anesthetic (10 μl on each side of the cloaca) or in a similar manner with a saline solution (tetracaine/mated). Injections of tetracaine occurred approximately 15 min before the injection of the 2-DG. Tetracaine takes effect in approximately 10 to 20 min, numbing the cloacal region. This treatment blocks the initiation of the neuroendocrine reflex that results in an estrogen surge (Mendonça & Crews, 1990). As the tetracaine takes effect, the cloaca of the female tends to relax and open slightly. This side effect allowed almost certain and extremely swift intromission by the males (within 2 min of courtship), removing the female's control of when copulation would occur. To counteract this result, we taped the cloaca of these females shut for about 15 min. This allowed for comparable amounts of courtship to occur in these females relative to the other groups. The tape was then quickly removed to allow the females to be mated.

There was only one tetracaine/not mated female available, and its values for the three brain areas fell within the range of activity found in the courted/not mated group.

This experimental design allowed for three comparisons. First, to determine the effects of courtship of the male red-sided garter snake on neural activity in the female, we compared the values obtained for the all female group with those obtained for the courted/mated, courted/not mated, and tetracaine/mated females. Second, to assess the effect of mating, we compared the values obtained for the courted/mated females with those of the courted/not mated females. Finally, to assess the effect of tetracaine, we compared the values obtained for the tetracaine/mated females with those of the courted/mated females.

Differences in length of courtship and mating periods as well as the interval between injection of 2-DG and time of death were analyzed using an unpaired Student's *t* test. Comparison of the same brain area measurements between years was done by a Mann–Whitney *U* test. Differences in the percentage of change of particular brain areas in relation to the WB $\div$ OT among treatments were analyzed by Kruskal–Wallis test for nonpara-

metric statistics. Scheffé's multiple comparison tests were performed on percentages that were arcsine transformed in order to determine which treatments differed (Sokal & Rohlf, 1981). The comparisons were planned a priori to minimize the number of tests and therefore the possibility of Type I error.

Results

Behavior Tests

There were no significant differences between years either in length of courtship or of actual intromission in either of the mated groups (e.g., 1989 vs. 1990 courted/mated intromission period: 14.0 vs. 16.2 min, respectively), $t(9) = -.057, p = .58$. Nor, when year data were combined, was there a significant difference in length of courtship or mating between groups (e.g., courted/mated vs. tetracaine/mated intromission period: 15.6 vs. 20.3 min, respectively), $t(15) = -1.72, p = .16$. As in previous studies (Mendonça & Crews, 1990), female red-sided garter snakes that had received administration of local anesthetic to the cloacal region prior to mating continued to exhibit receptive behavior to males.

Densitometry Measures

Within treatment categories, there were no significant differences in the densities of WBs between the years (e.g., courted/not mated, 1989 vs. 1990, $Z = -0.22, p = .83$; courted/mated, 1989 vs. 1990, $Z = -0.59, p = .56$). Therefore, densitometry measurements were combined for the same treatment groups of different years. Similarly, there were no significant changes in the OT.

POA

Females that were courted/mated had mean POA values that were almost 20% higher than the mean value of WB background (see Figure 1, top graph). This difference was significantly higher than densitometry readings of the other three groups ($H = 10.21, p = .02$). The values for the POA of courted/not mated, tetracaine/mated, and females alone groups did not differ significantly from one another. However, the females alone group had a mean value for the POA below background for the WB, whereas those of the courted/not mated and tetracaine/mated were marginally higher.

VMH

Females of the courted/mated group had mean relative VMH values that were 11% below the relative values for the WB (Figure 1, middle graph). The mean VMH value for this group was significantly lower than that of the courted/not mated group ($H = 9.1, p = .03$). It was also lower than that of the females alone group and the tetracaine/mated group but not significantly so. The tetracaine/mated group had VMH activity values slightly above WB average, whereas those of the females alone group were closer to the courted/not mated group.

NS

Females of all four groups had mean relative NS values above background values of the WB, but none of the groups differed significantly from one another ($H = 1.12, p = .77$).

Discussion

Following mating, female red-sided garter snakes become unreceptive to further male courtship. Coincident with this behavioral change is a significant increase in metabolism in the POA and at the same time a significant decrease in metabolism in the VMH. It is the sensations associated with mating that are responsible for this transition in brain and behavior. Administration of a local anesthetic to the cloaca of females prior to mating blocked the changes in 2-DG accumulation due to mating and prevented the loss of behavioral receptivity that typically follows mating.

The largest relative difference in brain area activity associated with the switch from a receptive to an unreceptive state was observed in the POA (24% above the all female group). In the all female group, the activity levels of the POA were below that of background WB levels, whereas in both the courted/not mated and tetracaine/mated groups, the POA had equivalent levels of 2-DG accumulation, higher than that of background WB level but not significantly different from that observed in the all female group. It appears, therefore, that receiving sensory input of courtship behavior alone does not elevate POA activity, but sensory input from the cloaca as a result of mating leads to a significant increase of 2-DG accumulation in this brain area.

The increased POA activity may be associated with the rapid loss of receptivity in the female red-sided garter snake with mating. That is, the POA may be exerting an inhibitory influence on receptivity, and the increased activity following intromission may help initiate the behavioral switch. An inhibitory role of the POA in the control of receptivity has been described in rats. Lesion of the mPOA in female rats enhances receptivity (Powers & Valenstein, 1972) and increases the display of female-typical lordosis behavior in male rats (Hennessey, Wallen & Edwards, 1986).

Mating induces not only a shift from receptive to unreceptive behavior in the female red-sided garter snake but also activates a neuroendocrine reflex. Previous work on this species has found that mated females exhibit a surge in circulating concentrations of PG-F$_{2a}$ (Whittier & Crews, 1989) followed by a sharp increase in plasma levels of estradiol within 4 hr of mating, peaking within 6 to 24 hr (Mendonça & Crews, 1990; Whittier & Crews, 1986b, 1989; Whittier, Mason, & Crews, 1987). Female red-sided garter snakes treated with tetracaine and then allowed to mate do not exhibit this mating-induced estrogen surge (Mendonça & Crews, 1990). It is possible that the mating-induced increase in activity in the POA initiates the release of gonadotropin-releasing hormone (GnRH). GnRH-containing neurons have been identified in the preoptic area of the red-sided garter snake (Sherwood & Whittier, 1988; Smith, Moore, & Mason, 1997). Hence, it is plausible that this species has a neuroendocrine reflex similar to that observed in mammals that are induced ovulators, in which copulation stimulates GnRH release, a luteinizing hormone (LH) surge, and ovulation (Komisaruk & Steinman, 1986; Lin & Ramirez, 1991; Ramirez & Beyer, 1994). Mammals that are spontaneous ovulators can, under varying conditions, also experience an LH surge upon mating (Day, Morales, & Lu, 1988; Gibson, Moscovitz, Kokoris, & Silverman, 1987; Lin & Ramirez, 1991). Furthermore, researchers using c-*fos* as a marker of neuronal activity have found increased activity in the POA in females of several species of mammals in response to mating, especially in neurons that are immunoreactive for GnRH (e.g., mice, spontaneous ovulators, Wu,
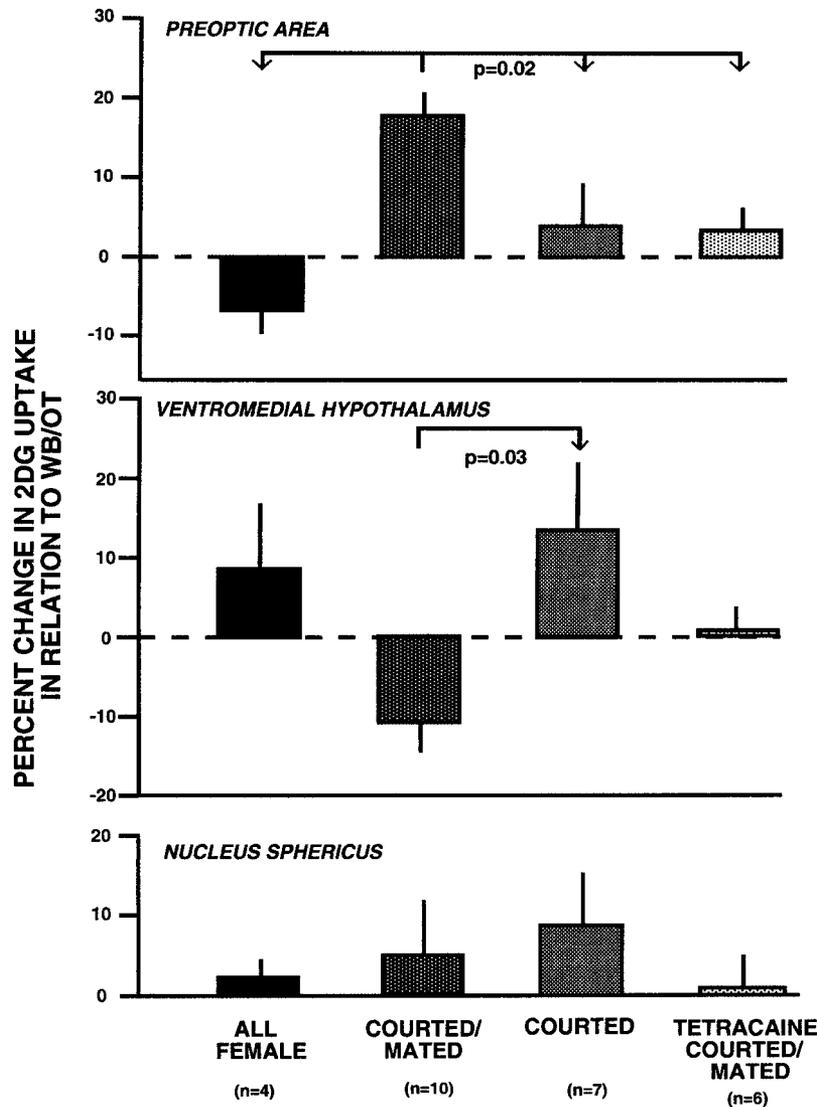
Figure 1. Mean relative change in [^{14}C]2-fluoro-2-deoxyglucose (2-DG) uptake in limbic nuclei of the red-sided garter snake (*Thamnophis sirtalis parietalis*). Values are shown for different treatment groups relative to whole brain/optic tract (WB/OT). The dashed line indicates background brain levels (e.g., zero difference from background). A positive change represents higher accumulation; a negative change, lower accumulation. The vertical lines represent 1 *SE*, and the arrows represent significant differences.

Segal, Miller, Gibson, & Silverman, 1992; ferrets, reflex ovulators, Lambert, Rubin, & Baum, 1992). Together, these studies support the idea that copulatory stimuli increase GnRH secretion. Finally, bilateral transection of the pelvic nerve of female rats that were then mated significantly attenuated the amount of neuronal activity in the mPOA (Wersinger, Baum, & Erskine, 1993). This response mirrors that exhibited in the present study by tetracaine-treated, mated female garter snakes in which POA activity was equivalent to females that were courted but not mated.

However, given the role of the VMH in the control of female sexual receptivity in vertebrates, it is possible that the attenuation of activity in the VMH of mated female red-sided garter snakes underlies the loss of receptivity. It is interesting that the ventro-

medial nucleus of the hypothalamus (VMN, analogous to the snake VMH) of female rats exhibits more neural activity in mated versus unmated individuals (Flanagan-Cato & McEwen, 1995; Wersinger et al., 1993). Additionally, pelvic nerve transection prior to mating results in upregulation of c-*fos* in the VMN of mated females being equivalent to those of unmated individuals (Wersinger et al., 1993). This result suggests it is a neural signal associated with intromission alone that affects the activity in this brain area. Thus, the difference in the response of the VMH to copulatory stimuli may lie in whether vaginal (cloacal) stimulation facilitates sexual receptivity (e.g., hamster: Buntin & Lisk, 1979; frog: Diakow & Nemiroff, 1981; rat: Marrone, Rodriguez-Sierra, & Feder, 1979) or inhibits it (e.g., guinea pig: Irving, Goy, Haning, & Davis, 1981;

Marrone et al., 1979; green anole lizard: Valenstein & Crews, 1977; garter snake: Whittier et al., 1985).

PGs can be produced by the female reproductive tract in response to mating or introduced into the female through the seminal fluid of the male (Kelley, 1981). In species in which mating facilitates female receptivity, exogenous PGs enhance receptive behavior (Buntin & Lisk, 1979; Diakow & Nemiroff, 1981; Marrone et al., 1979). In species in which mating inhibits female receptivity, exogenous PGs render females unreceptive (Irving et al., 1981; Marrone et al., 1979; Tokarz & Crews, 1981; Whittier & Crews, 1986a). PGs have been shown to have direct central nervous system effects in mammals (Rodriguez-Sierra & Komisaruk, 1982). Injection of PG-E_2 has been demonstrated in the leopard frog to enhance receptivity and increase 2-DG uptake in the POA and, to a lesser extent, in the VMH (Diakow, Woicyk, McEacheron, & Adler, 1989). It may be that PG can enhance VMH activity in species that remain receptive after mating and decrease VMH activity in species that become unreceptive. The intermediate levels in 2-DG activity in the VMH and the loss of receptive behavior displayed by tetracaine-treated female red-sided garter snakes may reflect the presence of male-contributed PG deposited in the reproductive tract of the female rather than arising from the neurally mediated response associated with intromission.

Mating induces both a change in receptivity and endocrine physiology in female red-sided garter snakes and, as suggested by the present study, these changes are paralleled by changes in activity in brain areas known to be involved in reproductive behavior and physiology in this and other vertebrate species. Cloacal stimulation as a consequence of mating results in a relative increase in activity in the POA and a corresponding decrease in activity in the VMH. These changes in activity can be blocked by administration of a local anesthetic to the cloaca of females prior to mating. The NS, the homologue of the medial amygdala in mammals, receives direct projections from the accessory olfactory bulbs (Halpern, 1992). Courtship behavior in the male red-sided garter snake is elicited by attractant pheromones (Mason et al., 1989), and lesions of the NS abolish courtship behavior in sexually active males (Krohmer & Crews, 1987a). Previous research indicated that courting male red-sided garter snakes exhibited significantly higher uptake in the NS and in the anterior hypothalamus POA relative to the OT compared with males not presented with females (Allen & Crews, 1992). Thus, the fact that in the present study, the NS did not show significant differences among females suggests that whereas chemosensory information is central to courtship and mating in the male red-sided garter snake, it is cloacal stimulation that is important to the female red-sided garter snake.

References

Allen, E. E., & Crews, D. (1992). Sexual behavior and 2-deoxyglucose uptake in male red-sided garter snakes (*Thamnophis sirtalis parietalis*). *Brain, Behavior and Evolution, 37,* 17–24.

Buntin, J. D., & Lisk, R. D. (1979). Prostaglandin E2 induced lordosis in estrogen-primed female hamsters: Relationship to progesterone action. *Physiology & Behavior, 23,* 569–575.

Crews, D. (1990). Neuroendocrine adaptation. In J. Balthazart (Ed.), *Hormones, brain and behaviour in vertebrates* (pp. 114–126). Farmington, CT: Basel.

Crews, D., & Silver, R. (1985). Reproductive physiology and behavioral

interactions in non-mammalian vertebrates. In N. T. Adler, D. W. Pfaff, & R. W. Goy (Eds.), *Reproduction, handbook of behavioral neurobiology* (Vol. 7, pp. 101–182). New York: Plenum.

Crews, D., Wade, J., & Wilczynski, W. (1990). Sexually dimorphic areas in the brain of whiptail lizards. *Brain, Behavior and Evolution, 36,* 262–270.

Day, J. R., Morales, T. H., & Lu, J. H. K. (1988). Male stimulation of luteinizing hormone surge, progesterone secretion and ovulation in spontaneously persistent-estrous aging rats. *Biology of Reproduction, 38,* 1019–1026.

Diakow, C., & Nemiroff, A. (1981). Vasotocin, prostaglandin and female reproductive behavior in the frog, *Rana pipiens. Hormones and Behavior, 15,* 86–93.

Diakow, C., Woicyk, W., McEacheron, D. L., & Adler, N. T. (1989). Pattern of [^{14}C]2-deoxyglucose concentration associated with potentiation of reproductive behavior by prostaglandin E$_2$. *Behavioral Neuroscience, 103,* 1028–1034.

Flanagan-Cato, L. M., & McEwen, B. S. (1995). Pattern of Fos and Jun expression in the female rat forebrain after sexual behavior. *Brain Research, 673,* 53–60.

Friedman, D., & Crews, D. (1985). Role of anterior hypothalamus-preoptic area in the regulation of courtship in the male Canadian red-sided garter snake (*Thamnophis sirtalis parietalis*): Lesion experiments. *Behavioral Neuroscience, 99,* 942–949.

Gibson, M. J., Moscovitz, H. C., Kokoris, G. J., & Silverman, A. J. (1987). Plasma LH rises rapidly following mating in hypogonadal female mice with preoptic area (POA) brain grafts. *Brain Research, 424,* 133–138.

Godwin, J., & Crews, D. (1997). Sex differences in the nervous system of reptiles. *Cellular and Molecular Neurobiology, 17,* 649–669.

Halpern, M. (1992). Nasal chemical senses in reptiles: Structure and function. In C. Gans & D. Crews (Eds.), *Hormones, brain, and behavior: Biology of the reptilia* (pp. 423–523). Chicago: University of Chicago Press.

Hennessey, A. C., Wallen, K., & Edwards, D. A. (1986). Preoptic lesions increase the display of lordosis by male rats. *Brain Research, 370,* 21–28.

Irving, S. M., Goy, R. W., Haning, R. V., & Davis, G. A. (1981). Prostaglandins, clonidine and sexual receptivity in the guinea pig. *Brain Research, 204,* 65–77.

Joy, J., & Crews, D. (1985). Social dynamics of group courtship behavior in male red-sided garter snakes (*Thamnophis sirtalis parietalis*). *Journal of Comparative Psychology, 99,* 145–149.

Joy, J., & Crews, D. (1988). Male mating success in red-sided garter snakes: Size is not important. *Animal Behaviour, 36,* 1839–1841.

Kelley, R. W. (1981). Prostaglandin synthesis in the male and female reproductive tract. *Journal of Reproduction and Fertility, 62,* 293–304.

Komisaruk, B. R., & Steinman, J. L. (1986). Genital stimulation as a trigger for neuroendocrine and behavioral control of reproduction. In B. R. Komisaruk, H. I. Siegel, M. F. Cheng, & H. H. Feder (Eds.), *Annals of the New York Academy of Science: Vol. 474. Reproduction: A behavioral and neuroendocrine perspective* (pp. 64–75). New York: New York Academy of Sciences.

Krohmer, R. W., & Crews, D. (1987a). Facilitation of courtship behavior in the male red-sided garter snake (*Thamnophis sirtalis parietalis*) following lesions of the septum or nucleus sphericus. *Physiology & Behavior, 40,* 759–765.

Krohmer, R. W., & Crews, D. (1987b). Temperature activation of courtship behavior in the male red-sided garter snake (*Thamnophis sirtalis parietalis*): Role of the anterior hypothalamus-preoptic area. *Behavioral Neuroscience, 101,* 228–236.

Lambert, G. M., Rubin, B. S., & Baum, M. J. (1992). Sex difference in the effect of mating on c-*fos* expression in luteinizing hormone-releasing hormone neurons of the ferret brain. *Endocrinology, 131,* 1473–1480.

Lin, W. W., & Ramirez, V. D. (1991). Effect of mating behavior on

luteinizing hormone-releasing hormone release in female rabbits as monitored with push–pull cannulae. *Neuroendocrinology, 53,* 229–235.

Marrone, B. L., Rodriguez-Sierra, J. F., & Feder, H. H. (1979). Differential effects of prostaglandins on lordosis behavior in female guinea pigs and rats. *Biology of Reproduction, 20,* 853–861.

Mason, R. T., Fales, H. M., Jones, T. H., Panell, L. K., Chinn, J. W., & Crews, D. (1989, July 21). Sex pheromones in garter snakes. *Science, 245,* 290–293.

Mendonça, M. T., & Crews, D. (1990). Mating-induced ovarian recrudescence in the red-sided garter snake. *Journal of Comparative Physiology A, 166,* 629–632.

Mendonça, M. T., & Crews, D. (1996). Effects of ovariectomy and estrogen replacement on attractivity and receptivity in a dissociated pattern breeder, the red-sided garter snake (*Thamnophis sirtalis parietalis*). *Journal of Comparative Physiology A, 178,* 373–381.

Pfaff, D. W., Schwartz-Giblin, S., McCarthy, M. M., & Kow, L.-M. (1994). Cellular and molecular mechanisms of female reproductive behaviors. In E. Knobil & J. D. Neill (Eds.), *The physiology of reproduction* (Vol. 2, pp. 107–220). New York: Raven.

Powers, B., & Valenstein, E. S. (1972, March 3). Sexual receptivity: Facilitation by medial pre-optic lesions in female rats. *Science, 175,* 1003–1005.

Ramirez, V. D., & Beyer, C. (1994). The neuroendocrine control of the rabbit ovarian cycle. In E. Knobil & J. D. Neill (Eds.), *The physiology of reproduction* (Vol. 2, pp. 1873–1892) . New York: Raven.

Rand, M. S., & Crews, D. (1994). The bi-sexual brain: Sex behavior differences and sex differences in parthenogenetic and sexual lizards. *Brain Research, 663,* 163–167.

Rodriguez-Sierra, J. F., & Komisaruk, B. R. (1982). Common hypothalamic sites for activation of sexual receptivity in female rats by LHRH, PGE_2 and progesterone. *Neuroendocrinology, 35,* 363–369.

Sherwood, N. & Whittier, J. (1988). GnRH from brains of reptiles: Turtles (*Pseudyms scripta*) and snakes (*Thamnophis sirtalis parietalis*). *General and Comparative Endocrinology, 69,* 319–327.

Smith, M. T., Moore, F. L., & Mason, R. T. (1997). Neuroanatomical distribution of chicken I GnRH in the brain of the red-sided garter snake. *Brain, Behavior, and Evolution, 49,* 137–148.

Sokal, R. R., & Rohlf, F. J. (1981). *Biometry.* San Francisco: Freeman.

Tokarz, R. R., & Crews, D. (1981). Effects of prostaglandins on sexual receptivity in the female lizard, *Anolis carolinensis. Endocrinology, 109,* 451–457.

Valenstein, P., & Crews, D. (1977). Mating-induced termination of behavioral estrus in the female lizard, *Anolis carolinensis. Hormones and Behavior, 9,* 362–370.

Wersinger, S. R., Baum, M. J., & Erskine, M. S. (1993). Mating-induced FOS-like immunoreactivity in the rat forebrain: A sex comparison and a dimorphic effect of pelvic nerve transection. *Journal of Neuroendocrinology, 5,* 557–568.

Whittier, J. M., & Crews, D. (1986a). Effects of prostaglandin F2 on sexual behavior and ovarian function in female garter snakes (*Thamnophis sirtalis parietalis*). *Endocrinology, 119,* 787–792.

Whittier, J. M., & Crews, D. (1986b). Ovarian development in red-sided garter snakes, *Thamnophis sirtalis parietalis:* Relationship to mating. *General and Comparative Endocrinology, 61,* 5–12.

Whittier, J. M., & Crews, D. (1989). Mating increases plasma levels of prostaglandin F_{2a} in female garter snakes. *Prostaglandins, 37,* 359–366.

Whittier, J. M., Mason, R. T., & Crews, D. (1985). Mating in the red-sided garter snake, *Thamnophis sirtalis parietalis:* Differential effects on male and female sexual behavior. *Behavioral Ecology and Sociobiology, 16,* 257–261.

Whittier, J. M., Mason, R. T., & Crews, D. (1987). Plasma steroid levels of female red-sided garter snakes, *Thamnophis sirtalis parietalis:* Relationship to mating and gestation. *General and Comparative Endocrinology, 67,* 33–43.

Wu, T. J., Segal, A. Z., Miller, G. M., Gibson, M. J. & Silverman, A.-J. (1992). FOS expression in gonadotropin-releasing hormone neurons: Enhancement by steroid treatment and mating. *Endocrinology, 131,* 2045–2050.