ELSEVIER

Brain Research 663 (1994) 163–167

BRAIN RESEARCH

Short communication

The bisexual brain: sex behavior differences and sex differences in parthenogenetic and sexual lizards

Matthew S. Rand *, David Crews

Institute of Reproductive Biology, Department of Zoology, University of Texas at Austin, Austin, TX 78712, USA

Accepted 2 August 1994

Abstract

The parthenogenetic lizard *Cnemidophorus uniparens* alternates in the display of male-like and female-like sexual behavior, providing a unique opportunity for determining the neuronal circuits subserving gender-typical sexual behavior within a single sex. Here we report a 6-fold greater [^{14}C]2-fluoro-2-deoxyglucose uptake in the medial preoptic area of *C. uniparens* displaying male-like behavior in comparison with *C. uniparens* displaying female-like receptivity. The ventromedial nucleus of the hypothalamus showed greater 2DG accumulation in receptive *C. uniparens* than in courting *C. uniparens*. When a related sexual species (*C. inornatus*) was compared to the unisexual species, the anterior hypothalamus in *C. inornatus* males exhibited significantly greater activity.

Keywords: 2-Deoxyglucose; Anterior hypothalamus; Medial preoptic area; Reptile; Ventromedial hypothalamus

Female-typical and male-typical sex behavior are known to be integrated by specific hypothalamic nuclei in the vertebrate brain [6,18,22]. The ventromedial nucleus of the hypothalamus (VMH) and the medial preoptic area (mPOA) are involved in sexual receptivity in females and both the mPOA and anterior hypothalamus (AH) play an important role in the regulation of copulatory behavior in males [6,18,19,22]. The majority of this work has been done on mammals or other gonochoristic species (gonadally separate male and female individuals). Many species of whiptail lizards, genus *Cnemidophorus*, are also gonochoristic. However, approximately one third of the species in this genus are unisexual, consisting of females only and reproducing via parthenogenesis [5].

Cnemidophorus uniparens is parthenogenetic and originally evolved through hybridization between *C. inornatus* (the maternal ancestor) and another *Cnemidophorus* species [9,15]. Individual *C. uniparens* exhibit male-like (heterotypical) and female-like (homotypical) pseudosexual behavior identical to the mating behavior of male and female members of the ancestral species, *C. inornatus* [7,17]. The aims of this study were to determine: (1) if specific regions in the brains of parthenogenetic and gonochoristic whiptail lizards exhibit sexually dimorphic metabolic activity, as measured by the accumulation of [^{14}C]2-fluoro-2-deoxyglucose (2DG) in the brain during mating behavior, and (2) if these dimorphisms complement previous findings based on implant, lesion and morphometric studies in the brains of the same species.

Experimental animals were housed individually in partitioned glass aquaria for at least 2 months prior to testing. All lizards used in this study were gonadectomized under cold anesthesia at least two months prior to testing. Lizards that were tested for male-like behavior or were used as male-like stimulus animals received a subcutaneous implant (1–2 months prior to testing) of crystalline testosterone packed into a 10 mm length of Silastic® tubing (i.d. 1.47 mm, o.d. 1.96 mm). Receptivity was induced by a single subcutaneous injection of estradiol benzoate (1.0 μg in 10 μl steroid suspension vehicle NIH) 24–72 h prior to testing. The stimulus animals were introduced into the experimental animal's home tank.

Each test animal received an intraperitoneal injection of 0.8 μCi [^{14}C]2-fluoro-2-deoxyglucose suspended in 10 μl saline (American Radiolabeled Chemicals,

* Corresponding author. Fax: (1) (512) 471-6078 or 471-9651. Bitnet address: zoom666@utxvm or crews@bull.zo.utexas.edu.

SSDI 0006-8993(94)00925-2

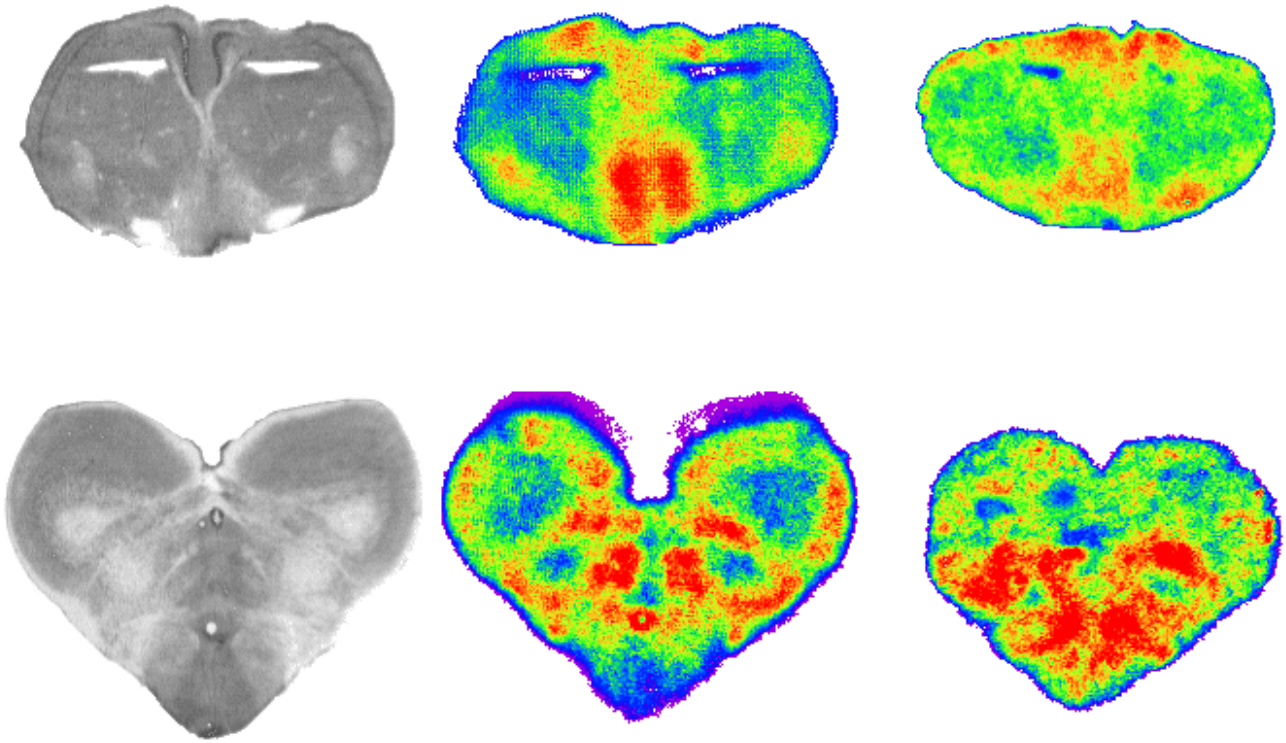

Fig. 1. Brain section images of the medial preoptic area (mPOA) and the ventromedial hypothalamus (VMH) in *Cnemidophorus uniparens*: (A) light photomicrograph of a Cresyl violet-stained section from the level of the mPOA, (B) 2DG image of the mPOA from a courting individual (same as in A), (C) 2DG image of the mPOA from a receptive individual, (D) light photomicrograph of Cresyl violet stained section from the level of the VMH, (E) 2DG image of the VMH from a courting individual (same as in D), and (F) 2DG image of the VMH from a receptive individual. mPOA, medial preoptic area; mCTX, medial cortex; OT, optic tectum; VMH, ventromedial hypothalamus.

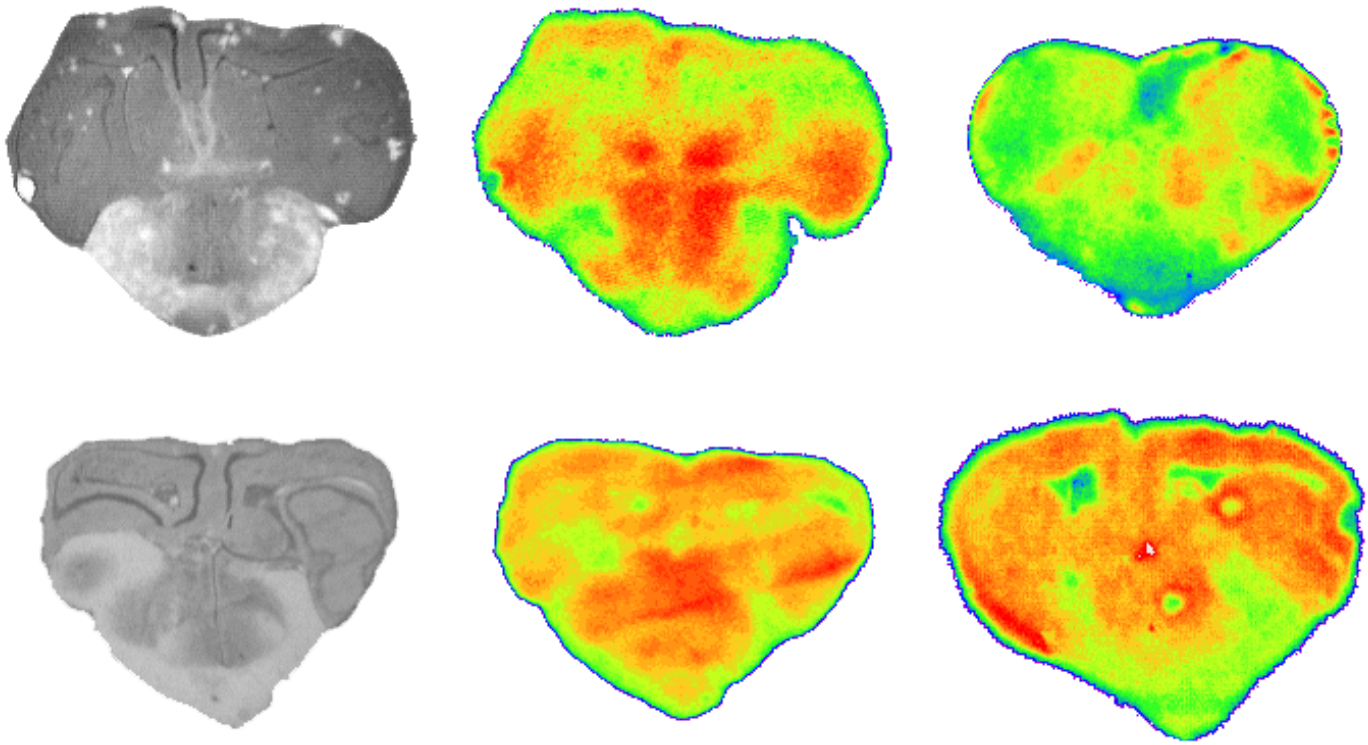

Fig. 2. Brain section images of the anterior hypothalamus in *Cnemidophorus inornatus* (CI) and *C. uniparens* (CU): (A) light photomicrograph of Cresyl violet-stained section from male CI, (B) 2DG image of courting male CI (same individual as A), (C) 2DG image of receptive female CI, (D) light photomicrograph of Cresyl violet-stained section from CU, (E) 2DG image of courting CU (same individual as D), and (F) 2DG image of receptive CU. S, septum; AC, anterior commissure; AH, anterior hypothalamus; AMY, amygdala; OC, optic chiasm.

Inc.), was exposed to a freely interactive stimulus animal, and was killed by rapid decapitation 45 min after injection. Brains were quickly dissected and frozen on dry ice. Coronal sections of 20 μm thickness were cut on a cryostat (−20°C), placed on a glass microscope slide and rapidly dried on a hot plate (50°C). Slides were apposed to imaging film (Kodak Diagnostic Film, Ektascan EB-1) for 3 weeks and then stained with Cresyl violet. Stained histological sections were used to identify brain regions and nuclei to be measured by densitometry of exposed film images. We used Java 1.4 Image Analysis Systems (Jandel Corp.) to analyze all images captured over a DC light source (TrueLite 100) through a Javelin video camera and macro lens. Captured images were corrected by subtracting a normalized background density image and recorded as an absolute gray level (scale 0–255). These values were subtracted from the average gray level of the entire brain section from which each image was captured.

The goal of this study was to determine the neural substrates of male-like and female-like pseudosexual behavior by comparing 2DG images from an all-female species during different behavioral states to males and females of a closely related, gonochoristic species (its direct evolutionary ancestor) exhibiting sex-typical mating behavior. As parthenogenetic whiptails are isogenic, this study also examined the relative activity of the two circuits in the brain during complementary behaviors.

We measured eight different areas of the brain that were hypothesized to show sexually dimorphic glucose metabolism. These included the medial and lateral preoptic area (mPOA, lPOA), septum (S), anterior hypothalamus (AH), anterior dorsal ventricular ridge (ADVR), amygdala (AMY), ventromedial hypothalamus (VMH), and nucleus rotundus (NR). In the all-female *C. uniparens* analysis of variance revealed that dimorphic responses in 2DG accumulation between individuals displaying male-like copulatory and female-like receptive behavior in two different areas of the brain. *C. uniparens* that courted another female showed significant 2DG uptake in the mPOA in comparison to receptive females ($t = -3.39$, $P = 0.008$)(Table 1). The VMH accumulated significantly more 2DG in *C. uniparens* exhibiting receptive behavior compared to *C. uniparens* displaying male-like courtship ($t = 3.61$, $P = 0.006$)(Fig. 1). The only sex difference in 2DG uptake was measured in the AH (Table 2). Courting male *C. inornatus* accumulated significantly more 2DG in the AH than did female *C. inornatus* or *C. uniparens* displaying either male-like behavior or female-like receptive behavior ($F_{3,18} = 3.91$, $P = 0.029$)(Fig. 2).

These findings are consistent with previous behavioral studies of *Cnemidophorus* lizards that demonstrated specificity of hormone action using other techniques. Implantation of androgen into the AH-POA elicits mounting behavior in gonadectomized male *C. inornatus* [21] and *C. uniparens* [16], whereas implantation of estrogen into the VMH elicits receptive behavior in gonadectomized female *C. inornatus* and *C. uniparens* [23]. Lesions of the AH-POA abolish courtship behavior of male *C. inornatus* and male-like pseudosexual behavior of *C. uniparens* [14].

Table 1
Optical density scores of the medial preoptic area (mPOA) and ventromedial hypothalamus (VMH) in *Cnemidophorus uniparens* exhibiting either male-like pseudocopulatory or female-like receptive behavior

Behavior	(*n*)	mPOA score	VMH score
Courtship (6)	−5.86 ± 0.79	+4.58 ± 1.03	
Receptive (5)	+0.12 ± 1.70	+0.66 ± 0.63	
	+	*	

Lower scores indicate higher 2DG accumulation. The lower the value the denser the image and therefore the higher the glucose accumulation. Negative values indicate a brain structure with greater than average glucose uptake and therefore greater neural activity. + Indicates significance at $P = 0.008$; * indicates significance at $P = 0.006$.

Morphologically, the AH-POA complex is larger in male *C. inornatus* than in females of the same species or *C. uniparens* [8,24]. Similarly, the AH-POA responds to systemic testosterone administration by becoming larger in *C. inornatus* males only; neither *C. inornatus* females nor *C. uniparens* respond to testosterone in like fashion [25]. The VMH is dimorphically smaller in male *C. inornatus* [8,24] and systemic testosterone administration will reduce the size of the VMH in *C. inornatus* males.

One might argue that the metabolic dimorphism seen in the mPOA and VMH of *C. uniparens* was due to the difference in hormone treatment. If this were the case, then one might also expect to see a similar dimorphism in the mPOA or VMH of the sexual species, as these lizards were exposed to an identical hormonal regimen. This was not observed. Further, in their study of neural c-fos, Baum and Wersinger [3]

Table 2
Optical density scores of the anterior hypothalamus relative to whole brain density in two species of whiptail lizard displaying courtship and receptive behavior. CI denotes *Cnemidophorus inornatus* and CU denotes *C. uniparens*

Sex–species–behavior	(*n*)	Anterior hypothalamus score
Male CI courtship	(6)	−8.35 ± 1.31 *
Female CI receptive	(5)	−2.33 ± 1.23
Female CU courtship	(6)	−1.45 ± 1.69
Female CU receptive	(5)	−1.80 ± 1.26

Lower scores indicate higher 2DG accumulation. The lower the value the denser the image and therefore the higher the glucose accumulation. Negative values indicate a brain structure with greater than average glucose uptake and therefore greater neural activity. * Indicates significance at $P = 0.029$.

found that steroid treatment had no effect on the number of immunoreactive fos nuclei in sexually behaving male rats. The immediate-early protein c-fos is detected within 1 h following mating behavior and is independent of sex steroid treatment. Similarly, the sexual dimorphism in the AH observed in *C. inornatus* was most likely not activated by the hormone replacement alone, since the *C. uniparens* that were induced to pseudocopulate did not show elevated 2DG uptake in the AH. This AH sexual dimorphism was strictly related to maleness or genetic sex, not to hormone treatment or to behavior. The higher activity measured in the AH may reflect an organized effect of early or chronic androgen exposure. Jones et al. [13] made a similar finding when they compared the brains of adult female gerbils from different positions in utero. Females that developed between two male siblings had significantly higher cytochrome *c* oxidase activity in the AH than females gestated between two female siblings. Cytochrome *c* oxidase activity provides a relative measure of tissue capacity for activity. Their study suggests that during development, the brains of females exposed to androgens from their male siblings exhibit organized metabolic changes in specific neural substrates.

That the mPOA exhibited higher 2DG uptake in pseudocopulating *C. uniparens* and not male *C. inornatus* may be indicative of the role this structure plays in sex behavior. Though the mPOA is typically thought of as an important structure in the control of male copulatory behavior, evidence has been accumulating supporting its role in female-typical sex behavior as well. Sexual receptivity is facilitated in female rats following lesion of the mPOA [19] and the display of female-typical lordosis increases following mPOA lesions in male rats [12]. A morphometric sex difference in the mPOA was first described in rats in 1978 [11]. This sexually dimorphic nucleus of the POA (SDN-POA) is larger in male rats and presumed homologues have been described to be sexually dimorphic in birds and in mammals, including humans [reviewed in 1 and 22]. Although most of the morphological work on the mPOA has focused on male-favored dimorphic structures, the anteroventral periventricular nucleus of the POA (AVPVN-POA) contains more cells and is larger in female rats than the same nucleus in males [4]. Unfortunately our imaging could not resolve the smaller individual nuclei within the mPOA. Other physiological parameters suggest a sexual function for this area in female vertebrates. For example, there is a larger number of aromatase immunoreactive cells in the anterior region of the POA in female quail [2]. The anterior preoptic nucleus of the female leopard frog (*Rana pipiens*) exhibits increased uptake of 2DG during sex behavior [10]. In addition, we have measured a larger number of tyrosine hydroxylase immunoreactive cells in the anterior portion of the POA in *Cnemidophorus* females compared to males [20].

This study illustrates that not only are there separate neural circuits involved in female-typical (VMH) and male-typical (AH and mPOA) sex behavior, but that it is quite possible that there are separate circuits within the mPOA as well. Viewed on a larger scale, the mPOA could be interpreted as a shared circuit controlling sex behavior in both sexes, possibly having an inhibitory role in one sex and a stimulatory or permissive role in the other (e.g. [19]).

An interesting difference surfaces when the relative activity of the POA and VMH (Table 1; Fig. 1) are compared. In receptive females, the activity of the VMH is similar to surrounding regions with the whole-brain section resulting in a score value near zero (nucleus optical density minus whole section density). The higher positive score for the VMH in the male-like *C. uniparens* indicates a reduction or inhibition of activity in the VMH relative to the surrounding brain areas. It is tempting to posit that the display of male-like courtship and copulatory behavior may inhibit neural activity in the VMH.

At least on the scale at which it is possible to investigate with the 2DG technique, it appears that the AH is the one area involved in sex behavior that reflects a true sexual dimorphism. That is, the AH exhibited increased 2DG uptake only in genetically male lizards. This dimorphism most likely reflects an organized sex difference in brain metabolism, as it is independent of both behavior and activational hormone treatment.

We thank John Godwin, Juli Wade and Larry Young for discussions; Kira Wennstrom for assistance with behavioral testing; and John Branch for technical assistance with the figure images. We wish to extend our gratitude to Ronnie and Sherrie Hall, and Ross and Frank Stavely of Sanderson, TX, for their hospitality and permission to collect specimens on their land. This work was supported by MH41770 to D.C. M.S.R. supported by NIH Training Grant HD07264, and D.C. by Research Scientist Award MH00135.

References

[1] Arai, Y., Nishizuka, M., Murakami, S., Miyakawa, M., Machida, M., Takeuchi, H. and Sumida, H., Morphological correlates of neuronal plasticity to gonadal steroids: sexual differentiation of the preoptic area. In M. Haug (Ed.), *The Development of Sex Differences and Similarities in Behavior*, Plenum, New York, 1993, pp. 311–323.

[2] Balthazart, J., Foidart, A. and Harada, N., Immunocytochemical localization of aromatase in the brain, *Brain Res.*, 514 (1990) 327–333.

[3] Baum, M.J. and Wersinger, S.R., Equivalent levels of mating-induced neural c-fos immunoreactivity in castrated male rats given androgen, estradiol, or no steroid replacement, *Biol. Reprod.* 48 (1993) 1341–1347.

[4] Bleier, R., Byne, W. and Siggelkow, I., Cytoarchitectonic sexual dimorphisms of the medial preoptic and anterior hypothalamic areas in guinea pig, rat, hamster and mouse, *J. Comp. Neurol.*, 212 (1982) 118–130.

[5] Cole, C.J., The evolution of parthenogenetic species of reptiles. In R. Reinboth (Ed.), *Intersexuality in the Animal Kingdom*, Springer, New York, 1975, pp. 340–355.

[6] Crews D. and Silver, R., Reproductive physiology and behavioral interactions in nonmammalian vertebrates. In N.T. Adler, D.W. Pfaff and R.W. Goy (Eds.), *Reproduction, Handbook of Behavioral Neurobiology, Vol. 7*, Plenum, New York, 1985, pp. 101–182.

[7] Crews, D. and Fitzgerald, K.T., "Sexual" behavior in parthenogenetic lizards (*Cnemidophorus*), *Proc. Natl. Acad. Sci. USA*, 77 (1980) 499–502.

[8] Crews, D., Wade, J. and Wilczynski, W., Sexually dimorphic areas in the brain of whiptail lizards, *Brain Behav. Evol.*, 36 (1990) 262–270.

[9] Densmore, L.D., Moritz, C.C., Wright, J.W. and Brown, W.M., Mitochondrial-DNA analyses and the origin and relative age of parthenogenetic lizards (genus *Cnemidophorus*). IV. Nine *sexlineatus*-group unisexuals, *Evolution*, 43 (1989) 969–983.

[10] Diakow, C., Woicyk, W., McEachron, D.L. and Adler, N.T., Pattern of [^{14}C]2-deoxyglucose concentration associated with potentiation of reproductive behavior by prostaglandin E_2, *Behav. Neurosci.*, 103 (1989) 1028–1034.

[11] Gorski, R.A., Gordon, J.H., Shryne, J.E. and Southam, A.M., Evidence for a morphological sex difference within the medial preoptic area of the rat brain, *Brain Res.*, 148 (1978) 333–346.

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

[13] Jones, D., Gonzales-Lima, F., Crews, D., Galef, B.G. and Clark, M.M., Effects of intrauterine position on metabolic capacity of the hypothalamus in the gerbil: a cytochrome oxidase study, *Soc. Neurosci. Abstr.*, (1994) in press.

[14] Kingston, P.A. and Crews, D., Effects of hypothalamic lesions on courtship and copulatory behavior in sexual and unisexual whiptail lizards, *Brain Res.*, 643 (1994) 349–351.

[15] Lowe, C. and Wright, J., Evolution of parthenogenetic species of *Cnemidophorus* (whiptail lizards) in western North America, *J. Ariz. Acad. Sci.*, 4 (1966) 81–87.

[16] Mayo, M.L. and Crews, D., Neural control of male-like pseudocopulatory behavior in the all-female lizard, *Cnemidophorus uniparens*: effects of intracranial implantation of dihydrotestosterone, *Horm. Behav.*, 21 (1987) 181–192.

[17] Moore, M.C., Whittier, J.M., Billy, A.J. and Crews, D., Male-like behaviour in an all-female lizard: relationship to ovarian cycle, *Anim. Behav.*, 33 (1985) 284–289.

[18] Pfaff, D. and Schwartz-Giblin, S., Cellular mechanisms of female reproductive behaviors. In E. Knobil and J.D. Neill (Eds.), *The Physiology of Reproduction*, Raven, New York, 1988, pp. 1487–1568.

[19] Powers, B. and Valenstein, E.S., Sexual receptivity: facilitation by medial preoptic lesions in female rats, *Science*, 175 (1972) 1003–1005.

[20] Rand, M.S. and Crews, D., unpublished material.

[21] Rozendaal, J.C. and Crews, D., Effects of intracranial implantation of dihydrotestosterone on sexual behavior in male *Cnemidophorus inornatus*, a direct sexual ancestor of a parthenogenetic lizard, *Horm. Behav.*, 23 (1989) 194–202.

[22] Sachs, R.D. and Meisel, R.L., The physiology of male sexual behavior. In E. Knobil and J.D. Neill (Eds.), *The Physiology of Reproduction*, Raven, New York, 1988, pp. 1393–1485.

[23] Wade, J. and Crews, D., The effects of intracranial implantation of estrogen on receptivity in sexually and asexually reproducing female whiptail lizards, *Cnemidophorus inornatus* and *Cnemidophorus uniparens*, *Horm. Behav.*, 25 (1991) 342–353.

[24] Wade, J. and Crews, D., The relationship between reproductive state and "sexually" dimorphic brain areas in sexually reproducing and parthenogenetic whiptail lizards, *J. Comp. Neurol.*, 309 (1991) 507–514.

[25] Wade, J., Huang, J-M. and Crews, D., Hormonal control of sex differences in the brain, behavior and accessory sex structures of whiptail lizards (*Cnemidophorus* species), *J. Neuroendocrinol.*, 5 (1993) 81–93.