



## Short Communication

Effects of hypothalamic lesions on courtship and copulatory behavior  
in sexual and unisexual whiptail lizardsPaul A. Kingston<sup>a</sup>, David Crews<sup>b,\*</sup><sup>a</sup> Interdepartmental Neuroscience Program, Yale University, New Haven, CT 06520, USA,<sup>b</sup> Institute of Reproductive Biology, Department of Zoology 60900, University of Texas at Austin, Patterson Room 30, Austin, TX 78712, USA

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## Abstract

Lesions of the anterior hypothalamus-medial preoptic area (AH-MPOA) impair courtship and copulatory behavior in male *Cnemidophorus inornatus*, a sexually reproducing species of whiptail lizard, and male-like pseudosexual behaviors in *C. uniparens*, an all-female species. These results suggest that, since *C. inornatus* is a direct ancestor to *C. uniparens*, the neural structures responsible for mediating male-typical courtship and copulatory behavior in male whiptails are conserved in the evolution of the all-female parthenogen.

**Key words:** Anterior hypothalamus; Brain lesion; Medial preoptic area; Reptile; Sexual behavior

The anterior hypothalamus-medial preoptic area (AH-MPOA) is an area critical to the regulation of sexual behavior in males of all vertebrate classes [2,11]. The whiptail lizards (genus *Cnemidophorus*) offer a unique animal model system with which to study the evolution of the neuroendocrine mechanisms controlling such sexual behavior. Of the *Cnemidophorus* species, approximately one-third consist solely of parthenogenetic females; one such species, *C. uniparens*, is the unisexual descendant of a hybrid union between the gonochoristic, sexually reproducing *C. inornatus* and another *Cnemidophorus* species [4].

In the sexual ancestral species, *C. inornatus*, males court and mount females while females are receptive to male courtship; heterotypical behaviors (e.g., females mounting other lizards) are rarely observed. Sexual behavior in male *C. inornatus* is dependent upon testicular androgens; a proportion of castrated male *C. inornatus* will respond also to exogenous progesterone with mounting and copulatory behavior [7]. Although they are parthenogenetic and therefore asexually reproducing, *C. uniparens* females consistently and reliably mimic the sexual behaviors of both male and

female members of ancestral whiptails [1]; male-like 'pseudosexual' behaviors coincide with peak progesterone levels and can be induced with exogenous progesterone [6]. Intracranial administration of androgens to the AH-MPOA will induce male-typical sexual behavior in both *C. inornatus* [10] and *C. uniparens* [9]. Collectively this evidence suggests that the neural circuitry integral to male-typical courtship and mounting behavior includes the AH-MPOA and has been conserved in the evolution of *C. uniparens* from *C. inornatus* [1]. The current study investigates the behavioral effects of lesions to the AH-MPOA in both *C. inornatus* and *C. uniparens*.

Male *C. inornatus* were collected in West Texas, while male-like *C. uniparens* were obtained in Arizona. Animals were housed and fed as described by Rozen daal and Crews [10]. Each animal received a testosterone implant after gonadectomy according to the procedures outlined by Lindzey and Crews for whiptail lizards [7]. After a recovery period of no less than 5 days, animals were tested daily for 5 days for male-typical courtship and mounting behavior. The subjects' sexual behavior was recorded for each test and ranked against the behavioral scale developed by Lindzey and Crews, where a no response = 0; approach and snout-to-body contact = 1; 'swiggle-walking' = 2; mounting = 3; riding = 4; copulation or moving into 'doughnut'

\* Corresponding author. Fax: (1) (512) 471-6078 or 471-9651. Bitnet address: zoed414@utxvm or crews@utxvms.cc.utexas.edu.

position (to achieve intromission) = 5 [7]. Only reliable, high-intensity courters were included in the study. A high-intensity courter was defined as a subject that mounts (achieves a behavior score of 3) on at least three of the five trials [8].

Lesion surgery was performed using the techniques outlined by Freidman and Crews for red-sided garter snakes [5]. The site of the AH-MPOA was approximated relative to an external reference point, namely the junction of the frontal and twin frontal parietal scales. The target coordinates were (length of frontal parietal scale junction minus 0.3–0.4 mm) caudal and 1.85 mm ventral from the surface of the brain.

The effects of the lesions were gauged with a series of postlesion behavior tests initiated within 24 h after lesioning. Each animal was tested at least five times and until it had achieved scores of similar intensity at least 3 days in a row. After testing, animals were weighed and perfused and their brains dissected from the head, rinsed, dehydrated and embedded in paraffin as described by Rozendaal and Crews [10]. Each brain was sliced into coronal sections of 20 mm and the sections stained with cresyl violet. The location and extent of each lesion were determined by examining each section under a microscope and performing image analyses; this was done for each brain without knowledge of species or behavioral data. The NIH Image computer program was used to determine cross-sectional areas, which were then multiplied by the distance between sections to generate volumes. The location of the lesion with respect to the AH-POA was determined using a neuroanatomical atlas created for another teiid lizard [3].

Behavioral data were analyzed with the Kruskal-Wallis (KW) test, the Mann-Whitney *U*-test (MW) and Tukey tests (T). For comparison across behavioral categories, behavioral and latency scores were averaged for each animal and mean pre- and postlesion scores produced for each category before analysis. Percentile data from volume measurements were subjected to arcsine transformations for analysis.

Forty-eight animals completed the testing regimen and appeared healthy (*C. inornatus*  $n = 27$ , *C. uniparens*  $n = 21$ ). Subjects responded following lesion in one of three different ways (Fig. 1). Responders (R) (*C. inornatus*  $n = 12$ , *C. uniparens*  $n = 7$ ) exhibited no significant changes in behavior due to lesion surgery, achieving a minimum score of 3 in at least 60% of post-lesion tests in all cases and attempting copulation at least twice (MW  $P > 0.1$ ). Non-responders (NR) (*C. inornatus*  $n = 8$ , *C. uniparens*  $n = 4$ ) achieved maximum scores of 1 and were often assigned a score of 0 (MW  $P < 0.01$ ). Intermediates (INT) (*C. inornatus*  $n = 7$ , *C. uniparens*  $n = 10$ ) either failed to score a 3 or higher at least three times or never attempted to copulate (MW  $P < 0.05$ ). Mean post-lesion behavioral intensities dif-

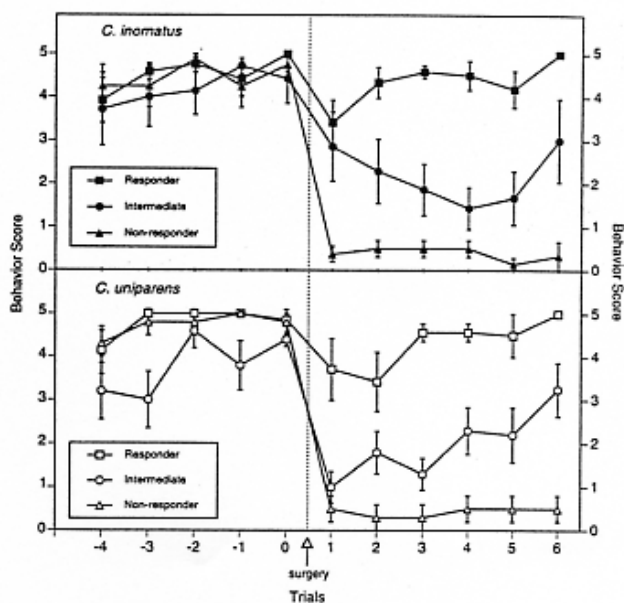


Fig. 1. Mean behavioral intensity scores, before and after lesion, for each experimental group of *Cnemidophorus inornatus* and *C. uniparens*.

fered significantly between groups in both species (KW  $P < 0.001$ ,  $df = 2$ ); R animals achieved higher scores than either INT or NR animals (T  $P < 0.01$ ), while INT animals consistently averaged higher scores than NR animals (T  $P < 0.01$ ).

Differences in behavioral response corresponded with variations in lesion placement (Fig. 2). *C. inornatus* R animals received significantly less damage to the AH-MPOA than either INT ( $P < 0.04$ ) or NR ( $P < 0.04$ ) animals. No such difference was found between *C. inornatus* INT and NR animals. *C. uniparens* NR animals had significantly greater proportions of the AH-MPOA destroyed than did R animals ( $P < 0.001$ ),

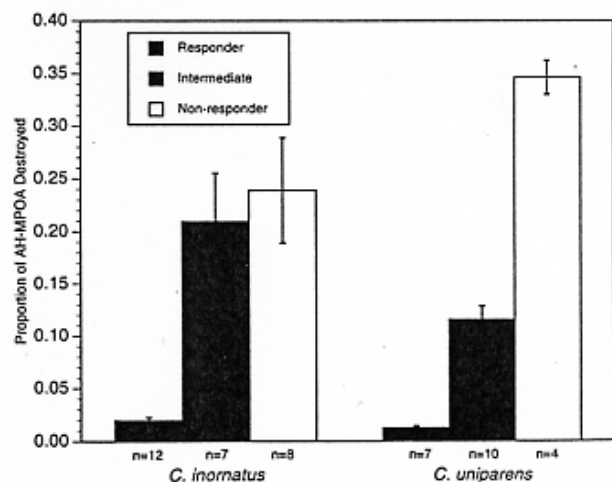


Fig. 2. Mean proportion of the AH-MPOA destroyed in animals of each experimental group of *Cnemidophorus inornatus* and *C. uniparens*.

while INT animals were not significantly different from either R or NR animals ( $P > 0.05$ ).

The relationship between lesion location and behavioral deficit is emphasized by the distribution of animals with significant damage to the AH-MPOA. Only one R animal (*C. inornatus*) was found to have lost more than 25% of its AH-MPOA to lesion damage; such severe AH-MPOA damage was otherwise restricted to the INT (*C. inornatus*  $n = 3$ , *C. uniparens*  $n = 3$ ) and NR (*C. inornatus*  $n = 2$ , *C. uniparens*  $n = 3$ ) categories. No definitive relationship between the size of the lesion and the behavioral deficit was identified.

In both species, the degree of behavioral deficit corresponded with the extent to which the lesion invaded the AH-MPOA. It cannot be argued that lesions produced NR animals due to concurrent destruction of common areas outside the AH-MPOA as a search of surrounding structures found no such common areas.

The current understanding of the role of the AH-MPOA in mediating sexual behavior in male vertebrates assumes that it serves a function crucial to the performance and perhaps the initiation, of male-typical courtship and copulation [11]. Deficits in sexual behavior have been observed in AH-MPOA lesioned males of many vertebrate species; this study shows that significant damage to the AH-MPOA yields pronounced deficits in all aspects of courtship and copulatory behavior in both male *C. inornatus* and male-like *C. uniparens*. Our understanding of the neural control of courtship and copulatory behavior can thus be extended to include male *Cnemidophorus* lizards and female *Cnemidophorus* that express male-typical behaviors.

Additionally, these findings complement those of previous studies showing that intracranial implants of androgen into the AH-MPOA in gonadectomized *C. inornatus* and *C. uniparens* stimulate courtship and copulation [9,10]. With proof that hormone-dependent activation of the AH-MPOA is both necessary and sufficient for the execution of male-typical sexual behavior in *C. inornatus* and *C. uniparens*, it is now clear that the ability of *C. uniparens* to perform these behaviors resulted from a conservation of critical neural circuitry in its evolution from the ancestral *C. inornatus*.

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