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Short communication

Electrolytic lesions to the ventromedial hypothalamus abolish receptivity in female whiptail lizards, *Cnemidophorus uniparens*

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Abstract

Lesions to the ventromedial hypothalamus (VMH) inhibit receptive sexual behavior in the whiptail lizard, *Cnemidophorus uniparens*, an all-female species. All lesions to the VMH that effectively abolished receptivity specifically damaged the dorsal lateral VMH, an area containing high concentrations of estrogen receptor in this species. These data further emphasize conservation of the VMH as a brain area critical to the expression of female sexual behavior in vertebrates.

Keywords: Brain lesion; Receptivity; Reptile; Sexual behavior; Ventromedial hypothalamus

The ventromedial hypothalamus (VMH) is a brain area critical to the regulation of sexual receptivity in female vertebrates. Specifically, studies have shown that estrogen acts on the VMH to facilitate receptive behavior, while damage to this area diminishes the female-typical response in mammals [2,5,7].

Studies of *Cnemidophorus* lizards have shown that intracranial implantation of estrogen into the VMH will elicit receptivity [8] and that the VMH is metabolically more active during the expression of receptive behavior in these animals [6]. The present study investigates the effects of VMH lesions on the female-typical receptive behavior of the whiptail lizard *Cnemidophorus uniparens*.

Thirty *C. uniparens* were collected in Arizona in the late spring. All technical procedures — housing, lizard care, gonadectomy, brain surgery, and stimulus animal preparation — were performed according to the techniques outlined by Wade and Crews [8] and Friedman and Crews [3]. To insure that differences in receptivity were not due to differences in endogenous hormone levels, all animals were ovariectomized 2 weeks prior to lesion surgery. Electrolytic lesions were made with an electrode with a 250 μm tip exposed. A current of 8–14 milliamps was delivered for 30 s to each target area. The caudal point of the intersection between the parietal scales served as a zero point for the coordinates of the VMH. Twenty-six lizards received lesions using coordinates approximately 0.8-mm posterior to the zero point, and 3.8-mm ventral to the dorsal surface of the brain. Our experience has shown that approximately 30% of the lesions will miss the target, so to insure that our sample of lizards with non-VMH lesions was large enough, four lizards received lesions 1.5-mm ventral to the brain's dorsal surface and 0.8-mm posterior to the zero point. Lizards with lesion damage outside of the VMH serve as lesioned controls.

Receptivity tests were conducted with testosterone-treated stimulus animals, which were paired randomly with subject animals for each behavior test. Previous experiments have established that subcutaneous injections with 0.5 μg 17β-estradiol-3-benzoate (EB) (Sigma Co.) suspended in 10 μl of steroid suspension vehicle (NIH) will consistently induce receptivity in *C. uniparens* [9]. Two days after lesion surgery, each subject was injected with EB. Testing for receptivity began the following day and continued for a total of 3 days (one test per day). One week later the animals were again injected with EB and tested for another 3 days. All behavior tests were conducted for 15 min, without the knowledge of subject animals' treatment group.

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Although many female vertebrates display obvious receptive postures such as lordosis, *C. uniparens* do not. However, it is very clear when a female is not receptive because she actively refuses to copulate. Typical rejection behavior of this sort can range from acting aggressively to running away. Therefore, two types of measures were recorded as a reflection of female receptivity: the male-like stimulus animal's level of copulatory 'success' as well as the experimental animal's response to courtship. Subjects were categorized as receptive or non-receptive on the basis of their reactions to the stimulus lizard's courtship. Receptive behavior was characterized by the absence of struggling during mounting attempts and was usually accompanied by pseudocopulation. The avoidance response included fleeing from the stimulus lizard's attempts to mount, while the aggressive response included more defensive behavior such as fighting with or biting the male-like stimulus animal; however, both of these reactions were categorized as non-receptive behavior.

The second measure of receptivity involved the animals' copulatory performance as a pair. If the experimental animal allowed the stimulus animal to remain on her back for 30 s or more, then she was considered to be receptive. The trial was terminated and the subject animal was categorized as non-receptive if the two animals locked into biting one another for 25 s. In addition, trials were terminated and the experimental lizard was classified as receptive if the testing pair achieved copulation.

To assess the attractivity of the experimental animal, three latency measures were recorded: latency to approach, latency to mount, and latency to copulation. If any of these behaviors were not displayed, a latency score of 900 s (15 min) was assigned for that measure.

All subjects were sacrificed by rapid decapitation on the final day of behavior testing and their heads placed in Kolmer's fixative [4] for eight days. Each brain was then dissected from the skull, dehydrated, and embedded in paraffin. Coronal sections were sliced at 12 μm thickness, mounted on glass slides and stained with Cresyl violet. The location of each lesion was determined without knowledge of the subjects' behavioral results. Statistics were performed according to Zar [10].

Table 1
Frequency of female *Cnemidophorus uniparens* exhibiting receptive and non-receptive behavior in relation to lesion damage of the ventromedial hypothalamus (VMH)

Lesion result [a]	Receptive	Non-receptive
VMH Damaged	1	9
VMH Intact	8	4

[a] Observed distribution of receptive and non-receptive lizards in relation to the presence of lesion damage is significantly different from an even distribution ($P = 0.009$; Fisher's Exact Test).

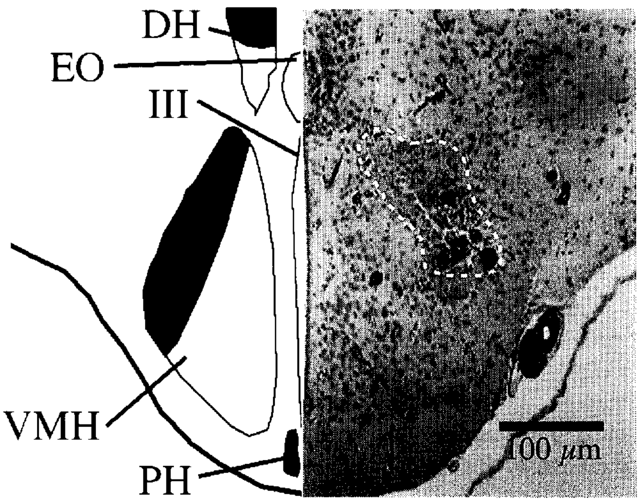

Fig. 1. Composite illustration of the ventral portion of the hypothalamus of *Cnemidophorus uniparens* at the level of the ventromedial hypothalamus (VMH). The left side of the figure shows the outlines of the VMH, dorsal hypothalamus (DH); the ependymal organ (EO); the third ventricle (III); and the periventricular hypothalamus (PH). The black regions represent the location of estrogen receptor message according to Young et al. [9]. The right side of the figure is a photomicrograph of brain tissue stained with Cresyl violet. The dashed white line demarks the tissue damage caused by an electrolytic lesion.

Distribution of receptive and non-receptive lizards in relation to lesion damage (hit or miss) was tested using Fisher's Exact Probability Test. Comparisons of weight change and behavioral latencies between groups were tested with the Mann–Whitney *U*-statistic.

The results of the lesion surgery and the behavioral tests for the experimental lizards are summarized in Table 1. Lizards sustaining lesion damage to the VMH were significantly less likely to exhibit receptive behavior towards male-like stimulus animals ($P = 0.009$; Fisher's Exact Probability Test). Twenty-two lizards survived lesion surgery and thrived during the 2 weeks of behavior testing, of these, 10 had lesions that damaged the VMH and 12 were lesioned outside of the VMH. Nine of the ten VMH-lesioned lizards were not receptive to male-like courtship or mounting behavior. Eight of the 12 lizards that received lesions outside of the VMH exhibited typical receptive behavior. All of these lizards with lesions that missed the VMH sustained damage that was either dorsal ($n = 9$) or lateral ($n = 3$) to the VMH.

Lizards were weighed to test for differences in body weight change between the receptive (+0.92 g) and non-receptive (+0.52 g) females. This insured that individuals in the non-receptive group were not weakened or suffering from a general lethargy due to lesion damage. Significant changes in weight might also indicate an increase or decrease in food intake during the experiment due to hypothalamic lesions. There was no significant difference in weight change between groups (Mann–Whitney $U = 28$; $P = 0.32$). As a measure of attractivity, subjects were tested for differences in latency of the male-like lizard to approach the experimental animal. Lizards with a damaged VMH were not less attractive (mean time to approach = 5.93 s) than lizards with an intact VMH (5.81 s; Mann–Whitney $U = 42.5$, $P = 0.57$).

These data support previous findings indicating that the VMH is an important neural structure in the control of female sexual behavior in this species. Specifically, the VMH has been shown to accumulate significantly more 2-deoxyglucose during receptive behavior than the same area accumulates during male-like pseudocopulatory behavior [6]. This suggests that the VMH has higher metabolic activity and therefore is neurally active during female sexual behavior. Estradiol implanted directly into the VMH has been shown to elicit receptive behavior in gonadectomized *C. uniparens* [8], indicating that estrogen may mediate female sex behavior through binding to receptors within the VMH. Indeed, using in situ hybridization, Young et al. [9] showed that messenger RNA for the estrogen receptor was expressed in the dorsal lateral VMH in *C. uniparens*. Interestingly, in the present study the single lizard that sustained lesion damage to the VMH, but still exhibited receptive behavior, received damage that was restricted to the medial VMH. All other lizards received lesions that damaged the dorsal lateral VMH. Fig. 1 illustrates the location of estrogen receptor expression and the location of a typical VMH lesion, showing the extent of damaged tissue in the dorsal lateral VMH. In addition, Carter [1] and Schwartz-Giblin et al. [7] noted that in female rats, only large lesions were effective in disrupting lordotic behavior, whereas smaller lesions were less effective in diminishing female sex behavior. It may be that the smaller lesions fell short of destroying estrogen receptor rich areas of the VMH, but the larger lesions successfully destroyed these areas.

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