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Research Report

Sex steroid hormones and sexual dimorphism of chemosensory structures in a terrestrial salamander (*Plethodon shermani*)

*Sarah K. Woodley**

Department of Biological Sciences, Duquesne University, 600 Forbes Ave., Pittsburgh, PA 15282, USA

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ABSTRACT

The volume of the vomeronasal organ (VNO) in the terrestrial salamander *Plethodon shermani* was approximately 1.7 times larger in adult males compared to adult females, even though male body size was, on average, slightly smaller than female body size. VNO cell density, however, was the same in adult males and females. The sex difference in VNO volume was found in sexually immature animals as well, indicating that the increase of plasma androgens that occurs at sexual maturity does not produce the sex difference in VNO volume. There was no difference in VNO volume between reproductive and nonreproductive adult females, despite differences in plasma estradiol (E2) levels. The volumes of the main olfactory epithelium and muscles regulating diameter of the external nares were similar between males and females, indicating that the VNO per se, and not other aspects of the nasal cavity, was sexually dimorphic. To conclude, the sex difference in VNO volume appears to be a permanent sex difference that develops before sexual maturity. Future studies will examine the functional consequences of this structural sexual dimorphism in a peripheral sensory organ, the VNO.

1. Introduction

In species in which the male, rather than the female, searches for and locates potential mates, Darwin hypothesized that sexual selection should enhance males' ability to detect and perceive female-derived sensory cues (Darwin, 1871). Although there are numerous well studied examples of sex differences in motor output systems and central nervous system structures (Adkins-Regan, 1988; Bass and Baker, 1990; Gahr, 1994; Kelley, 1988; Meisel and Sachs, 1994; Nottebohm, 1981; Wade, 1998; Zakon and Dunlap, 1999), fewer examples of obvious sex differences in peripheral sensory organs exist (Adkins-Regan, 2005). One exception is the sexually dimorphic antennal olfactory receptive organ in moths. The organ is much larger in males, due to an increase in the surface area of sensilla, which are sensory hairs that contain olfactory sensory neurons (Shields and Hildebrand, 2001). Males, but not females, possess a particular morphological type of sensilla and express odorant receptors for female sex pheromones (Nakagawa et al., 2005; Shields and Hildebrand, 2001). These male-specific traits allow males, but not females, to detect female sex pheromones and thereby find receptive females (Wyatt, 2003). Another example of a sexually dimorphic peripheral sensory organ is the vomeronasal organ (VNO). The VNO is a sensory neuroepithe-

* Fax: +1 412 396 5907.
E-mail address: woodleys@duq.edu.

Abbreviations: DHT, dihydrotestosterone; E2, estradiol; MOE, main olfactory epithelium; NAM, naris accessory muscle; NCM, naris constrictor muscle; NDM, naris dilator muscle; T, testosterone; VNO, vomeronasal organ

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lium found in tetrapod vertebrates that typically detects nonvolatile chemosensory stimuli, including many pheromones (Baxi et al., 2006; Eisthen and Wyatt, 2006). In rats, reed voles, some primates (greater bushbabies), and plethodontid salamanders, the VNO is larger in males than in females (Dawley, 1992b; Dawley, 1998; Segovia and Guillamon, 1993; Smith et al., 2005; Tai et al., 2004). In rats, the sex difference in VNO volume was due to sex steroid hormones acting early in development (Segovia and Guillamon, 1982). Adult levels of sex steroid hormones were also important in maintaining the height of the vomeronasal neuroepithelium and the nuclear size of the VNO sensory neurons in both male and female rats (Segovia et al., 1984). In addition, there is evidence for sex differences in vomeronasal receptor expression (Alekseyenko et al., 2006; Herrada and Dulac, 1997), aspects of VNO signal transduction (Murphy et al., 2001; Thompson et al., 2004), and vomeronasal sensory neuron responses (Brann and Fadool, 2006).

In rats, interpretation of the sexual dimorphism in VNO volume is complicated because VNO size increases as body size increases, and males are larger in body size than females (Weiler et al., 1999). Thus, the VNO is proportionally the same size in male and female rats (Weiler et al., 1999). In contrast, in plethodontid salamanders, male body length is generally the same or even smaller than female body length (Bruce, 2000). Therefore, plethodontid salamanders represent a valuable vertebrate model for studying sexual dimorphism in a peripheral sensory organ. The sex difference in VNO volume has been particularly well studied in the red-backed salamander *Plethodon cinereus*, a species in which the volume of the VNO is almost twice as big in males compared to females (Dawley, 1992a; Dawley, 1998; Dawley and Crowder, 1995). VNO cell density does not differ between males and females, implying that males have more VNO cells than females (Dawley and Crowder, 1995). The function of this striking sexual dimorphism is unknown.

The red-legged salamander, *Plethodon shermani* (formerly *Plethodon jordani*), is an emerging model of the nature and evolution of chemical communication (Feldhoff et al., 1999; Houck, 1998; Houck et al., 1998; Palmer et al., 2005; Rollmann et al., 1999; Rollmann et al., 2000; Schubert et al., 2006; Watts et al., 2004; Wirsig-Wiechmann et al., 2002). Here, I showed that the VNO of *P. shermani* is sexually dimorphic in volume, as has been found in other plethodontid salamanders (Dawley, 1998). Furthermore, I expanded on previous studies of the plethodontid VNO by examining whether the main olfactory epithelium (MOE) and three narial muscles were sexually dimorphic. The main olfactory epithelium detects volatile chemosensory cues, and narial muscles control the opening and closing of each nostril (external naris) (Wirsig-Wiechmann and Ebadifar, 2002; Wirsig-Wiechmann and Holliday, 2002). The presence of sexual dimorphism in the MOE and the narial muscles, in addition to the VNO, would suggest that the nasal cavity as a whole, and not just the VNO, has been subject to selection for sexual dimorphism.

Finally, I examined whether circulating sex steroid hormones influenced the volume of the VNO. In amphibians, as in other vertebrates, sex steroid hormones rise with sexual maturation and change seasonally, peaking during the mating season (Houck and Woodley, 1995; Woodley, 1994). I measured testosterone and its androgenic metabolite dihydrotestosterone in immature males and adult males to determine whether the increase in androgens that occurs with sexual maturity is correlated with changes in VNO volume. I also examined VNO volume in adult females of different reproductive conditions. Individual female *P. shermani*, like other large species of *Plethodon* in north temperate climates, require 2 years to develop a clutch of follicles (Arnold, 1976; Highton, 1962). Thus during the mating season, both reproductive (have large yolked ovarian follicles, are sexually receptive, and will lay a clutch in the next few months) and nonreproductive (have small unyolked follicles, are not sexually receptive, and will not lay a clutch in the next few months) females are present (Arnold, 1976). Comparison of these two types of adult females represents a natural experiment to determine if VNO volume differs according to female reproductive condition.

2. Results

2.1. VNO

Body length was a significant covariate of VNO volume ($F(1,39)=9.8$, $P=0.003$) and hence was included as a factor in all statistical analyses of VNO volume. Initial analyses comparing reproductive and nonreproductive adult females indicated that there were no significant differences in any of the VNO variables measured so adult females were pooled in subsequent analyses. Male VNO volume was significantly greater than female VNO volume (sex: $F(1,39)=59.15$, $P<0.001$). The sex difference was found in both adults and immature animals (no effect of age class: $F(1,39)=0.736$, $P=0.39$) (see Table 1, Fig. 1).

Examination of the VNO throughout its rostral–caudal extent revealed that the sex difference in the size of the VNO was most pronounced in the caudal half of the VNO in both adults and immature animals (Fig. 2). Measurement of VNO epithelial height at 3 separate locations along the rostral–caudal dimension revealed that the epithelial height of the VNO in males was greater than in females (sex: $F(1,38)=28.26$, $P<0.001$) in both adult and immature animals (no effect of age class: $F(1,38)=1.34$, $P=0.25$) (Table 1). The VNO was also slightly longer in males than in females for both adults ($U=36.5$, $N_1=12$, $N_2=19$, $P=0.001$) and immature animals ($U=5.0$, $N_1=6$, $N_2=7$, $P=0.02$) (Table 1).

VNO cell density did not differ between males and females although there was a nonsignificant trend for VNO density to be greater in immature animals than in adults (sex: $F(1,24)=0.42$, $P=0.52$; age class: $F(1,39)=3.7$, $P=0.07$). Likewise, the percent of the epithelial height that consisted of cell bodies (i.e., VNO cell body layer, % of total epithelial height, Table 1) did not differ between males and females (sex: $F(1,36)=1.9$, $P=0.17$) but was greater in immature animals than in adult animals (age class: $F(1,36)=13.03$, $P=0.001$).

2.2. MOE

Body length was almost a significant covariate of MOE volume ($F(1,39)=3.3$, $P=0.075$), and thus body length was included as a factor in all statistical analyses of MOE volume. Initial analyses comparing reproductive and nonreproductive adult