February 1980

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Neuroendocrine responses play a critical role in reproduction in every mammalian species, including voles (Richmond & Stehn, 1976). Disruption of these normal responses can result in: (1) abnormal sexual maturation; (2) abnormal or absent female cycles; (3) pseudopregnancy; (4) blocked pregnancies; or (5) the total absence of courtship and mating. Each of these factors in turn plays a considerable role in population dynamics, especially population density. Therefore, mechanisms which disrupt normal neuroendocrine function could affect population dynamics and reduce population density by affecting changes in one or many of these reproductive processes.

Each of the above neuroendocrine responses can be elicited or are modulated by conspecific odors, with urine or specialized scent glands being excellent odor sources in many species. However, work from a number of laboratories has suggested that at least some of the compounds which elicit hormonal changes are large and/or have low volatility (Müller-Schwarze and Silverstein, in press). These results call into question the assumption that olfaction as is traditionally viewed (limited to the first cranial nerves) serves as the primary receptor mechanism for the compounds in question. Instead, the vomeronasal organ may be the primary receptor modulating hormonal responses (Johns, in press; Wysocki, 1979). This receptor organ has neuroanatomical connections with central nervous system structures which exert considerable influence upon neuroendocrine mechanisms. Indeed, recent results have demonstrated involvment of the vomeronasal organ in: (a) female cyclicity (Johns, in press); (b) odor induced ovulation (Ingersol & Lee, 1979); and (c) pregnancy block (Reynolds & Keverne, 1979).

Results from our laboratory have demonstrated that, unlike the olfactory system, the vomeronasal organ (a tubular structure located near the external nares) is not limited to highly or moderately volatile compounds (Wysocki et al., 1980). Investigation of conspecific urine by guinea pigs results in the transport of the urine in liquid form to within the vomeronasal organ. Thus, large and/or "nonvolatile" compounds readily reach the chemoreceptors in the vomeronasal organ, thereby providing the prerequisite for chemoreception; stimulus - receptor contact.

With this as background, we speculated that similar mechanisms exist for pine and meadow voles, viz.,: (a) low- or nonvolatile compounds have access to the vomeronasal organ; and (b) the vomeronasal organ is involved in neuroendocrine events. If true, distruction or disruption of normal vomeronasal organ function could reduce vole populations through its influence upon the reproductive/endocrine system. This report describes our studies of: (a) above; behavioral situations in which "nonvolatiles" have access to the vomeronasal organ.

To date we have explored a variety of behaviors; investigation of con- and heterospecific urine, grooming and feeding. We asked

whether large and/or "nonvolatile" molecules reach the vomeronasal chemoreceptors during these behaviors. Our methodology was straightforward and similar in all situations. A flurochrome, rhodamine B, which is considered to be a nonvolatile dye, was placed in a stimulus prior to its presentation to a vole. For studying the investigation of a socially relevant stimulus, a small quantity of vole urine was mixed with rhodamine (1% in dH20) on a cotton swab and given to the vole for 6 min. For studying grooming, carboxymethylcellulose was mixed with the dye (0.01% or 0.1%) and applied to the animal's fur. The vole was given 5-6 min to groom. For studying feeding, pieces of apple were soaked in 0.1% rhodamine and given to hungry voles that were allowed 6 min to ingest the apple. In all control situations, the stimulus was either not presented or presented without rhodamine.

At the end of the test each vole was killed and the vomeronasal organs were carefully dissected free, frozen, sectioned, mounted on glass slides and viewed with epifluorescence microscopy. The results of these observations are presented in Figure 1.

After investigating urine from either female pine or meadow voles, 80-90% of male pine voles exhibited rhodamine fluorescence in their vomeronasal organs (Fig. 1). From this we conclude that during the investigation of urine nonvolatile compounds found within female urine reach the vomeronasal organ, even if the urine donor is not of the

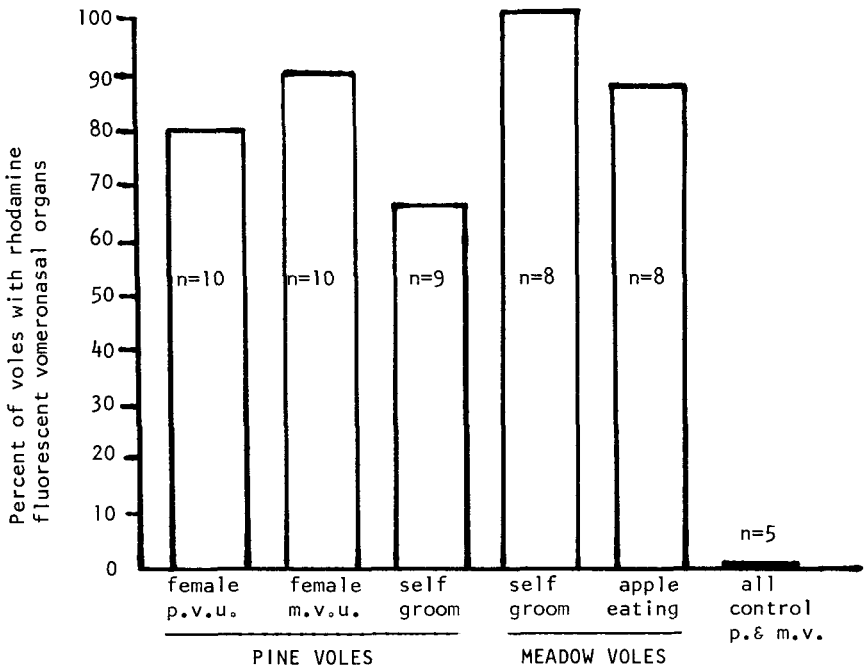

Figure 1. Percent of pine voles (p.v.) and meadow voles (m.v.) exhibiting rhodamine fluorescence in their vomeronasal organs after exposure to dyed urine (u), grooming, or eating of dyed apple.

same species. Whether or not stimulation of the organ occurs is not yet known. The important point however, is the demonstration of access of nonvolatiles in urine to the vomeronasal organ.

After grooming, dye specific fluorescence was found in the vomeronasal organs of 2/3 of the male pine voles and all of the male meadow voles (Fig. 1). The apparent species difference may be due to procedural differences in testing. All of the meadow voles but only half of the pine voles were smeared with 0.1% rhodamine. The remaining pine voles received a less concentrated spread of rhodamine (0.01%). All of the voles that lacked rhodamine fluorescence in their vomeronasal organs were from this latter group. Regardless, the results demonstrate that nonvolatile compounds reach the vomeronasal organ during grooming.

After eating an apple that had been adulterated with the dye, each of the 4 female and 3 of the 4 male meadow voles were found to have dye specific fluorescence in their vomeronasal organs (Fig. 1). Therefore, nonvolatile compounds do reach the vomeronasal organ during feeding. For all behavioral situations no rhodamine fluorescence was detected in any of the control subjects (Fig. 1).

If, as in rats and mice, the vomeronasal system is critical for normal function of the neuroendocrine system, then disruption of the vomeronasal organs in voles could reduce population levels. Our results demonstrate that nonvolatile compounds enter the vomeronasal organ during a variety of behavioral situations. Thus, a mechanism exists by which compounds specificially designed to disrupt chemoreception could be targeted to the vomeronasal organ. For example, substances could be placed in "bait stations" designed to apply the substance to the voles fur. Subsequent grooming would result in the transport of the substance to the vomeronasal organ. Another method could rely upon feeding. A third approach might take advantage of urine investigation.

The tasks remain to demonstrate the import of the vomeronasal organ to pine and meadow vole reproduction and to develop vomeronasal organ - disruptive substances which can be self administered.

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