gs-3). Our studies show that mice also develop cell-mediated responses against antigens on histocompatible cells infected with endogenous mouse-tropic C-type viruses.

We have not been able, as yet, to rule out the possibility that these responses might be directed against antigens shared by both mouse-tropic and xenotropic viruses or against virus-induced cellular antigens. Nor have we determined what class of lymphocytes is responsible for these cell-mediated responses. In any event, it appears that at least some strains of mice may be exposed to antigens associated with C-type viruses at an early age, prior to detectability of infectious virus in these animals, and that they can respond with both humoral and cell-mediated immune responses against these antigens. Variations in the production of these immune responses may markedly influence the likelihood of C-type virus replication and subsequent oncogenesis. For example, cell-mediated virus elimination could be facilitated by cytotoxic antibodies or, conversely, inhibited by enhancing antibodies. Similarly, it has been suggested that production of certain antibodies to viral antigens might decrease the likelihood of neoplasia while increasing the chance of immune-complex glomerulonephritis (*2*). Depression of cell-mediated responses with antiserum to lymphocytes or cyclophosphamide in the presence of chronic antigenic stimulation, on the other hand, may increase the amount of endogenous C-type virus replication (*9*). Virus, once activated, may itself further depress host responses, either directly (*10*) or indirectly by induction of cell clones with autoaggressive reactivity (*4*). Thus, the interactions among endogenous C-type viruses, host immune responses, and neoplasia are complex. The unraveling of these complexities will require longitudinal studies in various mouse strains of specific humoral and cell-mediated responses to both viral and tumor antigens. Understanding these relationships may well provide a rational basis for eventual vaccination programs against endogenous oncogenic viruses.

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Vomeronasal Organ: Critical Role in Mediating Sexual Behavior of the Male Hamster

Abstract. *Sexual behavior in male hamsters is totally abolished by bilateral removal of the olfactory bulbs. This operation eliminates sensory input from both the olfactory and the vomeronasal systems. We previously demonstrated that peripheral destruction of the olfactory receptors caused anosmia but did not impair male hamster mating behavior. Here we demonstrate that peripheral deafferentation of the vomeronasal system produces severe sexual behavior deficits in approximately one-third of the treated animals. Combined deafferentation of both the vomeronasal and the olfactory systems eliminates copulation in 100 percent of the animals. This is the first experimental demonstration of a functional role for the vomeronasal organ in a mammalian species.*

Many aspects of reproductive behavior and physiology are influenced by the sense of smell (*1*). In the male rat, removal of the olfactory bulbs produces a deficit in mating behavior, whereas in male mice and hamsters, copulation is completely abolished (*2*). Olfactory bulb ablation interrupts sensory input from both the olfactory and vomeronasal receptors, and additionally destroys central components of these two systems. Attempts have been made to determine which of these variables is most relevant to the olfactory regulation of various behavioral processes (*3*).

We have previously reported that passing a 5 percent zinc sulfate solution through the nasal cavities of male hamsters reliably eliminates their ability to detect amyl acetate and female hamster vaginal odor, but does not impair their mating behavior (*4*). According to other studies, however, hamsters made peripherally anosmic fail to copulate (*5*). Consequently the importance of peripheral olfactory stimulation in this species remains unclear and the extent to which interference with the vomeronasal system might have contributed to these behavioral results has not been established. The experiments reported here differentiate between olfactory and vomeronasal organ effects on sexual behavior and for the first time provide experimental evidence for a functional role of the vomeronasal organ system in mammalian species.

The vomeronasal organs in most mammals are bilaterally paired tubes which lie on either side of the septum in the ventral portion of the nasal cavities (*6*). The tubes are only open anteriorly, near the nares. Axons from sensory receptors in the luminal epithelium of the organ project via the vomeronasal nerves to the accessory olfactory bulb (*7*), whereas the primary olfactory receptors innervate the main olfactory bulb. Main and accessory olfactory bulbs, in turn, maintain anatomically separate connections with nuclei of the amygdaloid complex and other forebrain areas (*8, 9*). Because the amygdaloid nuclei which receive input from the vomeronasal system in turn project to hypothalamic regions known to influence sexual behavior

(*10*), it has been suggested that interfering with this system could have profound effects on hamster copulation (*8, 11*). If this were true, the presence or absence of mating behavior in peripherally anosmic hamsters might be related to whether or not the technique for producing anosmia affected the vomeronasal system. Accordingly, hamsters treated by our zinc sulfate procedure might continue to mate because their vomeronasal system remained intact.

To test this hypothesis male hamsters weighing approximately 100 g were obtained from Con Olson (Madison, Wisconsin) and housed individually with food and water freely available under a 12-hour light, 12-hour dark illumination cycle. We treated six hamsters with zinc sulfate according to the technique described previously (*12*). Two days later we perfused four animals for histological analysis of the vomeronasal and olfactory epithelia. The remaining two males were killed after 4 days for Fink-Heimer preparations. The histological results (of which the details are in preparation) showed that the zinc sulfate application produced extensive coagulation necrosis of the main olfactory epithelium but spared the vomeronasal sensory receptors. Fink-Heimer preparations confirmed this differential effect by showing heavy terminal degeneration over the glomeruli of the main olfactory bulb but none in the glomeruli of the accessory olfactory bulb. Because these findings were consistent with our hypothesis, its behavioral implications were tested directly.

We planned first to deafferent only the vomeronasal system and subsequently in these same animals to deafferent the primary olfactory system by our zinc sulfate procedure. This would allow us to determine the effects of vomeronasal deafferentation alone, and the effects of a combined vomeronasal and olfactory deafferentation in the same animal.

Four sexual behavior tests were given over a 2-week period; animals failing to show normal mating were eliminated. The procedures used for measuring male sexual behavior were similar to those described previously (*13*). Forty-four hamsters were divided into three groups with equivalent means on pretest performance. In one group ($N = 26$) the vomeronasal nerves were cut where they pass along the medial surface of the main olfactory bulbs toward their termination in the accessory olfactory bulb. Two additional groups received sham operations; these controlled for the effect of surgical trauma (sham 1; $N = 12$) and the slight damage to the main olfactory bulbs (sham 2; $N = 6$) which occurred while cutting the vomeronasal nerves (*14*). Beginning 2 days after surgery, three behavioral tests were given at 3- or 4-day intervals.

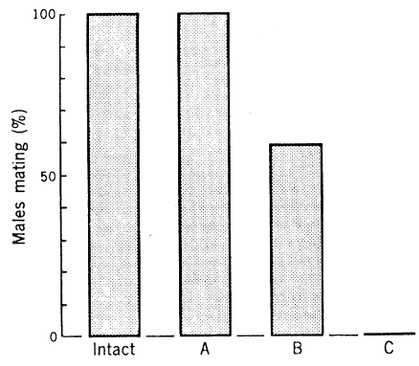

Fig. 1. Destruction of the olfactory epithelium with zinc sulfate (A) did not alter mating behavior; cutting the vomeronasal nerves (B) caused a severe and persistent deficit in 38 percent of the males. The combined treatments (C) eliminated mounts, intromissions, and ejaculations in all males.

The importance of the vomeronasal system became apparent when 10 of 26 males showed a severe deficit on these mating tests after vomeronasal nerve cuts alone. Four of these 10 animals stopped mating entirely; each of the other 6 showed mating behavior only once, and only 4 ejaculated. Therefore, these 10 males were never given zinc sulfate, but behavioral testing was continued. Their behavioral deficit persisted (fuller details in preparation). In contrast, the other 16 hamsters with vomeronasal nerve cuts (62 percent) continued to mate normally after surgery. Ten of these 16 animals then received intranasal zinc sulfate. When tested 2 days later, none of them displayed mounts, intromissions, or ejaculations. The remaining 6 males were given intranasal saline and all achieved two ejaculations in the standard mating test. The two groups of sham-operated hamsters continued to mate normally. Subsequent treatment with zinc sulfate had no effect on their sexual behavior; two ejaculations were achieved by all 18 animals of the sham-operated groups. Figure 1 summarizes the results obtained with the experimental and control groups.

We assessed our lesions by examining Nissl-stained horizontal sections through the main and accessory olfactory bulbs (*15*). In all sham-operated animals, the nerves were easily visualized running along the medial surface of the main bulbs and terminating in the glomerular region of the accessory olfactory bulbs. In animals receiving nerve cuts, only degenerating nerve fragments could be seen; the glomerular regions of the accessory bulbs had disappeared and heavy gliosis covered the accessory bulbs' surface. All hamsters subjected to the nerve-cut procedure were classified by two independent observers as having the vomeronasal nerves unequivocally cut bilaterally on the basis of histological analysis. Cutting the vomeronasal nerves caused minimal damage to the main olfactory bulbs. At least 85 percent of the glomeruli of both main bulbs remained intact in all but four animals. In each of these animals only one of the main bulbs showed greater than 15 percent tissue loss. There was no tendency for animals with relatively more olfactory bulb damage to be disproportionately represented in the group which showed severe behavioral deficits after the vomeronasal nerve cuts alone.

Our experiments demonstrate that the vomeronasal organ system plays a crucial role in regulating male hamster sexual behavior. Although the anatomical connections between this sensory system and hypothalamic regions which influence sexual behavior and gonadotrophin function have suggested an important role for the vomeronasal organ in reproductive processes (*8, 11*), this study is the first to clearly establish a behavioral function for this system in mammals.

The finding that a significant number of hamsters stopped mating after vomeronasal nerve cuts alone, combined with the fact that olfactory epithelium destruction by our zinc sulfate technique does not affect sexual behavior, suggests that input from the vomeronasal is relatively more important than input from the primary olfactory system. It would appear that inputs from these systems summate in some fashion to reach thresholds for the arousal of sexual activity. If the vomeronasal system contributes proportionately more than the olfactory system, and arousal thresholds vary among individual ani-

mals, then it would be expected that in some hamsters stimulation from the olfactory system alone, after vomeronasal deafferentation, would be insufficient to achieve threshold levels. However, the converse would not be expected, because relatively less interference with the total system is effected by olfactory deafferentation only.

The results of our previous experiments with zinc sulfate suggested that the loss of mating behavior following bilateral bulbectomy was not due to peripheral anosmia (*4*). It is now apparent that peripheral deafferentation can mimic the effects of bulbectomy on male hamster sexual behavior if both the olfactory and vomeronasal systems are rendered nonfunctional. Bilateral bulbectomy produces this effect because both the main and accessory olfactory bulbs are typically removed. Even if the accessory bulb is spared, the vomeronasal nerves are inevitably damaged in the process of removing the main olfactory bulbs. Our results emphasize the necessity for determining to what extent both the primary olfactory and the vomeronasal systems are impaired by techniques designed to induce peripheral anosmia.

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12. One-half milliliter of zinc sulfate (5.0 percent in 0.5 percent saline) was passed through the nasal cavities of etherized hamsters with a bent section of 18-gauge stainless steel tubing attached to a syringe. The fluid was introduced through the nasopharyngeal meatus and aspirated as it exited at the external nares. In some animals saline solution (0.5 ml of 0.5 percent) was administered identically. This was done as a control procedure preceding later behavioral tests, but histological examination of the olfactory and vomeronasal epithelia was not performed.
13. See Powers and Winans (*4*). Mating behavior tests lasted 10 minutes or until two ejaculations had occurred with an ovariectomized female. All mounts, intromissions, and ejaculations were scored on an event recorder. Females had subcutaneous Silastic implants of estradiol and received injections of 500 μg of progesterone 3 to 5 hours prior to use; these females were used only if they showed intense lordosis when placed with stud males.
14. Animals were anesthetized with Nembutal (70 mg/kg); the frontal bone over the anterior portion of the main olfactory bulbs was removed and the large venous sinus in the midline between the two bulbs was visualized. McClure UltraMicro scissors were positioned over the sinus and lowered 3 to 4 mm while open. After they were closed they were withdrawn, and the bleeding was controlled with pressure and Gelfoam. Animals receiving the sham-1 procedure were treated identically except that the scissors were lowered only 1 mm so that the sinus was cut but the vomeronasal nerves, lying below this level, remained intact. For sham-2 treatment, a 22-gauge hypodermic needle was oriented vertically and lowered 3 to 4 mm into the main olfactory bulb just lateral to the midline sinus on both sides. This was designed to produce olfactory bulb damage comparable to that associated with the nerve-cutting procedure, but without damaging the vomeronasal nerves.
15. Hamsters were perfused with 0.8 percent saline followed by 10 percent formalin in 0.8 percent saline. The rostral portion of the skull, from bregma to the external nares, with the brain in situ, was hardened in formalin, decalcified in 25 percent formic acid, washed, and embedded in a mixture of egg yolk and gelatin. Horizontal sections (40 μm) were cut on a freezing microtome and stained with cresyl violet.
16. We thank E. Valenstein for valuable criticism of the experiments and the manuscript. We thank P. Britten for histological assistance and D. Fleming for conducting the behavioral tests. Sponsored by NIMH research grant 2R01MH20811-03 to E. Valenstein.

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Changes in Protein Levels in Perfusates of Freely Moving Cats: Relation to Behavioral State

Abstract. *Perfusates from the brains of freely moving cats, obtained by means of a push-pull cannula, contain high concentrations of proteins. The levels vary in a cyclic fashion and are higher during rapid eye movement sleep than during the waking state. The proteins represent a distinctive class of tissue protein and their changing levels appear to reflect an alteration in the protein content of the extracellular space of brain related to behavioral state.*

Sleep can be induced by perfusing a cat's brain with a perfusate obtained from a sleeping cat by means of a push-pull cannula (*1*). Substances that influence temperature control and food ingestion are found in brain perfusates obtained with similar procedures (*2*). These results suggest that samples of the extracellular medium can be obtained in perfusion experiments with push-pull cannulas and that the perfusates contain regulatory substances.

In the experiments reported here, we analyzed the protein composition of perfusates obtained from the brains of cats during sleep and waking states. We examined proteins because of evidence that a polypeptide may be a sleep-inducing factor (*3*) and because proteins can serve in a variety of situations as regulatory substances. In a more general context, proteins and polypeptides are secretory products of a variety of cell types, including neurons in cultures (*4*) and in the peripheral nervous system (*5*), and it is of fundamental importance to determine whether proteins are present in substantial quantities and whether they can be collected from the extracellular space of brain.

Eighteen cats of either sex (2.5 to 3.5 kg) were implanted with a 17-gauge stainless steel tube that served as a guide for a cannula. Detailed procedures have been described (*1*). The guide tube was aimed at the mesencephalic reticular formation (MRF) but the tip terminated 7 mm above the MRF. Electrodes were implanted in the sensorimotor cortex, around the orbit of the eyes, and in the neck muscles for electroencephalographic, eye movement, and electromyographic recordings, respectively.

The perfusion experiments were started at least 1 week after surgery. At approximately 11:00 a.m., the cat was placed in a large observation chamber in a quiet laboratory room. A two-barrel (20- and 27-gauge) push-pull cannula was introduced into the guide tube and lowered into the MRF and hippocampus. The cannula was connected via polyethylene tubing to syringes driven by a Harvard infusion-withdrawal pump. The brain was perfused continuously with a sterile commercial Ringer solution at 1 ml/hour for 12 to 21 hours. Samples of the perfusate were removed from the tubing each hour by means of a three-way