Vomeronasal and Olfactory System Modulation of Maternal Behavior in the Rat

Abstract. *The onset of maternal responsiveness by virgin female rats to foster pups was observed after (i) complete vomeronasal nerve cuts, (ii) partial olfactory bulb cuts, or (iii) the combined procedures. Although both vomeronasal nerve cuts and olfactory bulb cuts resulted in a more rapid onset of maternal care, relative to control animals with sham operations, animals sustaining the loss of both sources of olfactory input exhibited the shortest response latency. These findings are discussed in terms of the probable distinct functions of the two olfactory systems in the control of maternal behavior in the rat.*

The virgin female rat typically avoids neonatal foster pups during the first few days of exposure to them (*1*), but if given continuous exposure to pups (for a week or more) she will eventually retrieve and lick them and adopt a nursing posture over them, much like a normal postparturient mother (*1–3*). Fleming and Rosenblatt (*4*) suggested that the initial hesitancy of the virgin to care for the young is a temporary aversive response to novel olfactory cues emanating from them. Specifically, the elimination of pup odors through the production of anosmia by either intranasal infusion with zinc sulfate, bilateral olfactory bulb removal, or transections of the lateral olfactory tract abbreviates this aversive-withdrawal phase, and virgin females quickly become maternal.

In addition to the primary olfactory system, a vomeronasal or accessory olfactory system processes olfactory information within the rat brain (*5*, *6*). These two systems are morphologically and embryologically similar but have anatomically distinguishable receptors in the nasal mucosa and different sites of termination in the pyriform lobe (*5–7*). Powers and Winans (*6*, *8*) have suggested that the main and accessory olfactory systems of the hamster are also functionally distinct. Moreover, they report that disruption of the primary system often results in the inadvertent destruction of the accessory system.

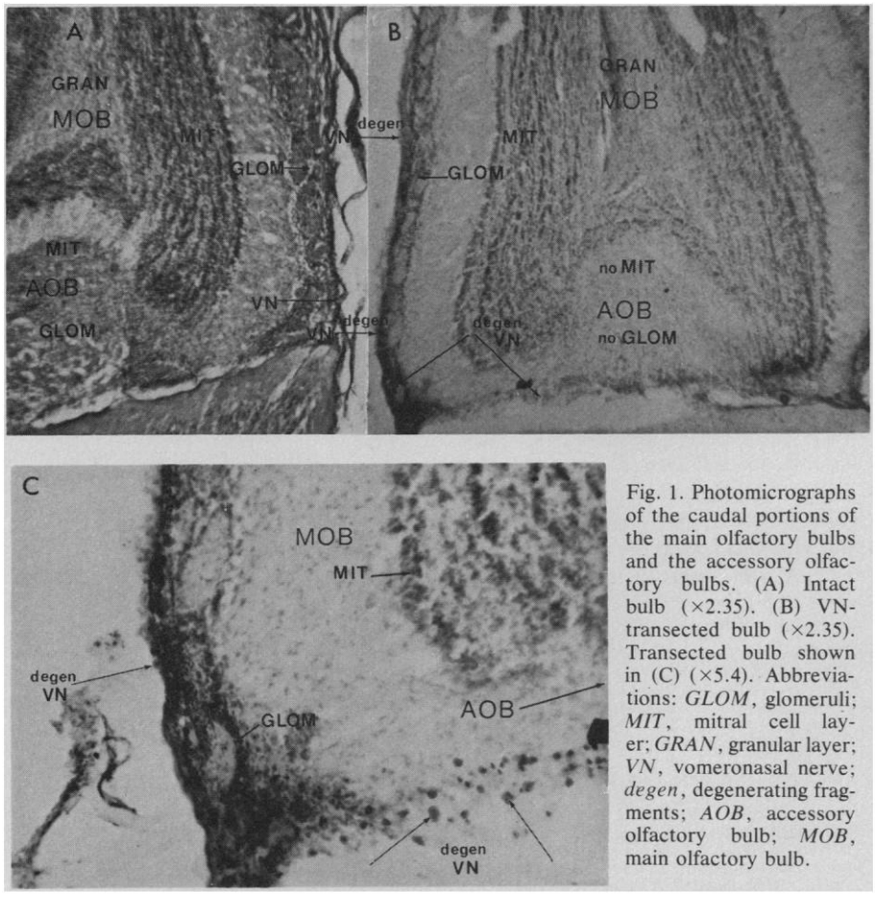

Fig. 1. Photomicrographs of the caudal portions of the main olfactory bulbs and the accessory olfactory bulbs. (A) Intact bulb (×2.35). (B) VN-transected bulb (×2.35). Transected bulb shown in (C) (×5.4). Abbreviations: *GLOM*, glomeruli; *MIT*, mitral cell layer; *GRAN*, granular layer; *VN*, vomeronasal nerve; *degen*, degenerating fragments; *AOB*, accessory olfactory bulb; *MOB*, main olfactory bulb.

These findings raise the question of whether the rapid induction of maternal behavior by peripheral or central manipulations of the olfactory system was due to the loss of either the primary or the accessory system. We therefore sought to distinguish the relative importance of these two systems in mediating the temporary inhibition of maternal behavior by subjecting virgin rats to (i) complete vomeronasal deafferentation, (ii) partial olfactory deafferentation, or (iii) the combined procedures; we subsequently observed responses to foster pups. If either system played a role in suppressing initial maternal responses by virgins, its disruption should decrease the latency for the onset of maternal care by these females. Our results suggest that both chemosensitive systems contribute to the suppression of maternal behavior, perhaps by transmitting different aspects of the odor complex associated with pups.

Subjects were 60- to 90-day-old virgin female Wistar rats, obtained from Woodlyn Laboratories, Guelph, Ontario. They were housed in clear plastic 47 by 25 by 15 cm cages with a wood shaving bedding. All females had free access to laboratory chow (Purina) and water. Experimental virgin animals and lactating donor females and their pups were housed in separate rooms maintained on an illumination cycle of 12 hours of light and 12 of darkness at 22°C.

Forty-eight females were divided into six groups. One group received vomeronasal nerve cuts (group VN) and another group was subjected to the sham operation (group sham VN). A third group received bilateral olfactory bulb cuts (group OB) and its control group received a sham operation (group sham OB). In groups 5 and 6, vomeronasal nerve cuts were combined with bilateral olfactory bulb damage of increasing extent (groups VN+ and VN-OB, respectively). In groups VN, VN+, and VN-OB, we attempted to cut the vomeronasal nerves where they pass along the dorsomedial surface of the main olfactory bulbs toward their termination in the accessory bulbs. We removed the frontal bone over the anterior portion of the main olfactory bulbs while the animal remained under Nembutal anesthesia. This procedure exposed the large venous sinus located in the midline between the two bulbs. Scissors (Tieman Ultramicro) were positioned over the sinus and lowered 3 to 4 mm. They were opened and closed twice and then withdrawn. Animals receiving a sham operation (sham VN) were treated identically except that

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only the sinus was cut, sparing the vomeronasal nerves situated below. To section the olfactory bulb (groups OB and VN-OB), a rectangular 2.5-mm surgical blade was lowered bilaterally into the anterior third of each olfactory bulb and 3 mm lateral to the midline. The blade was lowered 3 to 4 mm to the base of the skull and then withdrawn. Sham-operated females (group sham OB) were subjected to the same procedure, with the exception that the cuts were not made.

Tests for the induction of maternal behavior were begun 2 to 5 days postoperatively. Each day, four 5- to 10-day-old foster pups were placed in one corner of the cage. During successive 5-second intervals of the subsequent 12 minutes, we recorded each of the following pup-directed behaviors (*3*): approaching, sniffing, licking, retrieving, and adopting a nursing posture. In addition, the time spent grooming and nest building were recorded. Pups remained in the cage for 24 hours and were replaced each day with a new litter. Observations were made daily for 10 days or until virgins displayed maternal behavior. Animals were said to be maternal when they retrieved pups and adopted a nursing posture over them on two consecutive days.

Within 6 weeks after induction tests, animals in all groups were perfused for histological verification of cuts to the vomeronasal nerve (groups VN+ and VN-OB). Horizontal frozen sections were cut at 40 μm, and alternate sections were stained with a cresyl violet. Two observers provided independent evaluations of the presence and location of neural damage.

Transection of the vomeronasal nerves was assessed by (i) the presence of degenerating nerve fragments along the midline of the olfactory bulbs and (ii) an obvious reduction in the size of the mitral and glomerular layers in the accessory bulbs, which occurs as a result of their deafferentation (*6*). Gross damage to the main olfactory bulb along the midline of the bulbs was also noted. Because the granular layer of the main olfactory bulb contains primarily unmyelinated fibers and lacks compact cell layers, cuts within the granular portions of the bulbs in groups OB and VN-OB were difficult to detect; they were seen in only three animals.

Of the 15 animals receiving VN surgery, 11 clearly sustained cuts to the vomeronasal nerves. Of these 11, five showed minimal damage to the olfactory bulb (now designated group VN) and six showed more extensive bulb damage (now designated group VN+). The caudal portions of the main olfactory bulbs and the accessory bulbs with degenerated VN nerve fragments are shown in Fig. 1. Of the eight animals receiving VN-OB surgery, five sustained clear damage to the vomeronasal nerve and midline portions of the main olfactory bulbs.

Table 1. Maternal behaviors of virgin rats reported as mean number of intervals during which the behavior occurred (± standard error of the mean). Data were analyzed with two-tailed Mann-Whitney U tests.

Group	Behaviors				
	Sniff	Lick	Retrieve	Nursing posture	Self-groom
VN	23.2 ± 5.5	18.9 ± 5.8	4.2 ± 1.3	7.3 ± 3.4	17.0 ± 11.1
VN+	11.4 ± 3.4	15.1 ± 5.6*	11.3 ± 4.2	14.7 ± 13.9	23.6 ± 9.2
Sham VN	16.1 ± 3.3	43.3 ± 8.1*†	5.0 ± 0.9	20.9 ± 10.9	10.9 ± 6.2
OB	19.1 ± 2.9	38.4 ± 9.1	3.8 ± 0.4	6.3 ± 4.5	22.8 ± 4.0
Sham OB	22.6 ± 2.4	54.5 ± 4.0†	5.5 ± 1.3	10.7 ± 4.1	15.4 ± .4.7
VN-OB	21.7 ± 1.9	29.5 ± 4.9†	4.5 ± 0.3	11.5 ± 5.3	17.8 ± 8.1

*Group VN+ compared with group VN, $P < .05$. †Group VN-OB compared with the combined sham groups, $P < .05$.

Figure 2 shows the additive effects of vomeronasal nerve cuts and olfactory bulb cuts on the facilitation of maternal behavior in the virgin rat. Sham control groups (each $N = 11$) required approximately 8 days of continuous exposure to foster pups before they began to respond maternally; VN and OB ($N = 10$) groups each required from 4 to 6 days, and VN+ and VN-OB animals required only 1 to 2 days of exposure.

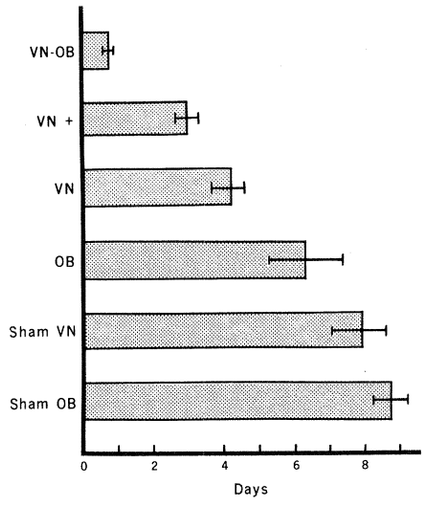

Fig. 2. Mean latency to become maternal (in days ± standard error of the mean) in groups sustaining cuts to the vomeronasal nerves (VN), olfactory bulbs (OB), or both systems (VN+ and VN-OB). Groups sham OB and sham VN are controls. Differences between groups VN and sham VN, VN+ and sham VN, VN-OB and the combined control groups (sham VN and sham OB), and VN+ or VN-OB and both OB and VN were significant (Mann-Whitney U test, $P \leq .05$, two-tailed). Differences between groups OB and sham OB were not significant ($.05 < P < .10$).

The groups also differed in more subtle aspects of their behavior. Animals sustaining VN cuts (groups VN, VN+, and VN-OB) spent less time licking pups than did controls on the day that they became maternal as well as on the following day, although they did not differ from other groups in other maternal behaviors (Table 1).

Disruption of either the vomeronasal system (with minimal olfactory bulb damage) or of the main olfactory system thus reduces the latency to the onset of maternal responsiveness. When the two systems are both more extensively damaged, still greater facilitation is seen, and animals respond as they do after being made anosmic by either zinc sulfate treatment, bilateral bulbectomy, or lesions to the lateral olfactory tract.

The anatomy of the main and accessory systems suggests a plausible route via the limbic system by which both olfactory and vomeronasal information could influence hypothalamic mechanisms mediating maternal behavior. Although the two systems project to distinct nuclear groups within the amygdala (*6*, *9*), efferents from these nuclei are thought to converge in their projections to hypothalamic preoptic structures implicated in the expression of maternal behavior in the rat (*10*, *11*). That the amygdala constitutes part of a system mediating an olfactory and vomeronasal inhibition of maternal behavior is further supported by evidence that lesions to the amygdala or its primary efferent pathway, the stria terminalis, mimic the facilitatory effects of lesions of the olfactory and vomeronasal systems on maternal behavior in the virgin female rat (*12*).

These observations suggest that under normal circumstances, both the vomeronasal and the main olfactory systems contribute to the tonic inhibition of maternal behavior in the virgin animal. Al-

though these two systems probably both convey information relating to the odor characteristics of pups, we believe that they may be responding to somewhat different aspects of the odor complex. Our data showing that animals sustaining cuts to the vomeronasal nerves (groups VN, VN+, and VN-OB) showed reduced licking of pups, whereas animals receiving OB cuts alone did not, support the hypothesis of Winans and Powers (*6*) that in rodents the vomeronasal system is normally activated by nonvolatile substances transmitted through the nares when the animal comes into direct contact with the odor source.

The caring of the postparturient female for her young without delay or hesitancy, as soon as they are born, may result from multiple effects of her hormones [primarily estrogen (*2*)] acting at a number of different sites in the central nervous system. For instance, the olfactory tubercle, the medial and cortical nuclei of the amygdala, and the medial preoptic region all contain receptors for estradiol (*13*). It is possible, therefore, that endogenous estradiol—which peaks just before parturition—acts simultaneously (i) on the olfactory system to change the female's attraction to pup odors, (ii) on the amygdala to reduce her neophobia (*14*), and (iii) on the preoptic region to facilitate the coordination and integration of the different caretaking behaviors (*11*).

Note added in proof: Using a technique of selectively cauterizing the vomeronasal organ (with no damage to the olfactory bulbs), A. Mayer and J. Rosenblatt have found a significant reduction in latencies to become maternal in virgin female rats of the Charles River strain.

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Fever and Reduced Iron: Their Interaction as a Host Defense Response to Bacterial Infection

Abstract. *When rabbits are infected with* Pasteurella multocida, *the concentration of iron in their plasma decreases and their rectal temperature rises. To determine whether the rise in body temperature (fever) and the fall in plasma iron may be a coordinated host defense response,* Pasteurella multocida *were grown in vitro at various temperatures and iron concentrations. At afebrile temperatures the bacteria grew equally well at low or high concentrations of iron. However, when the temperature of the bath was raised to a febrile temperature the growth of the bacteria was inhibited by the low, but not the high, iron concentrations. These data support the hypothesis that one of the mechanisms behind the adaptive (or beneficial) role of fever is the reduced ability of pathogenic bacteria to grow well at elevated temperatures in an iron-poor medium.*

Many studies have indicated that fever is a host defense mechanism during bacterial (*1, 2, 2a*) and viral (*3*) infections. Many mechanisms have been suggested as being responsible for the beneficial effects of an elevated body temperature on an organism's immune response. Among these have been an increase in leukocyte mobility (*4*), increased leukocyte killing of ingested microorganisms (*5*), and an increased production of interferon (*6*). An area that has recently received considerable attention has been that of "nutritional immunity." This term, coined by Kochan (*7*), refers to the fact that during infection the blood levels of many nutrients become altered. For example, the concentrations of iron and zinc in the serum generally decrease and the concentration of copper generally increases (*8, 9*). It has been suggested by Weinberg (*10*) and by others that the decrease in serum (or plasma) iron might reduce the growth of pathogenic microorganisms. In fact, many studies have shown that certain species of bacteria grow poorly in a medium containing low concentrations of iron and that iron supplements increase the growth of bacteria in vitro and in vivo (*8, 9*).

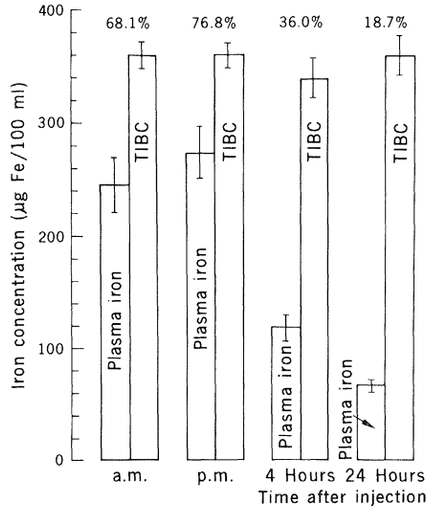

Fig. 1. Plasma iron (PI) and total iron-binding capacity (TIBC) in eight male New Zealand White rabbits. Prior to infection with live pathogenic bacteria *(Pasteurella multocida)*, the rabbits' PI concentrations averaged 247 μg per 100 ml during the morning and 275 μg per 100 ml during the afternoon hours. After infection with bacteria, the PI concentrations fell to 118 μg per 100 ml by 4 hours and to 66 μg per 100 ml by 24 hours. The TIBC did not change during this period. The percentages above each set of bar graphs indicate the percentage saturation of the plasma protein, transferrin.

Garibaldi (*11*) has shown for *Salmonella typhimurium* and Kochan (*9*) has shown for *Escherichia coli* that the ability to produce iron-transport compounds (siderophores) is diminished by small elevations in temperature and, as a result, it has been suggested that the reduction in serum iron, coupled with an elevation in body temperature (fever) is a coordinated host defense mechanism (*9-11*). To further support this contention, recent evidence indicates that the leuko-

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