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Research report

Lesions of the vomeronasal organ disrupt mating-induced pair bonding in female prairie voles (*Microtus ochrogaster*)

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Abstract

The prairie vole (*Microtus ochrogaster*) is a highly social, monogamous species and displays pair bonding that can be assessed by the presence of selective affiliation with the familiar partner versus a conspecific stranger. In female prairie voles, exposure to a male or to male sensory cues is essential for estrus induction, and the subsequent mating facilitates pair bond formation. In the present study, we examined the role of the vomeronasal organ (VNO) in estrus induction and pair bonding in female prairie voles. VNO lesions did not alter olfaction mediated by the main olfactory system, but did prevent male-induced estrus induction. We by-passed the necessity of the VNO for estrus induction by estrogen priming the females. Despite the fact that all subjects displayed similar levels of mating, social contact and locomotor activities, VNO lesioned females failed to show mating-induced pair bonding whereas intact and sham-lesioned females displayed a robust preference for the familiar partner. Our data not only support previous findings that the VNO is important for estrus induction but also indicate that this structure is crucial for mating-induced pair bonding, suggesting an important role for the VNO in reproductive success in prairie voles. © 2001 Elsevier Science B.V. All rights reserved.

Theme: Neural basis of behavior

Topic: Hormonal control of reproductive behavior

Keywords: Vomeronasal organ; Olfaction; Estrus induction; Monogamy; Social behavior

1. Introduction

Prairie voles (*Microtus ochrogaster*) are among the few mammalian species in which long-term pair bonds are formed between males and females. Following mating, pair-bonded prairie voles display a well-characterized suite of behaviors including selective affiliation with the familiar partner (partner preference) and aggression toward unfamiliar conspecifics [5,15]. Although pair bonds can be formed after a long period of cohabitation, mating facilitates the formation of pair bonds [19,20,58,61]. This is consistent with the notion that pair bonding may enhance reproductive success [40].

Unlike rats and mice, the reproductive axis in female prairie voles remains quiescent until activated by environmental cues, in particular those associated with adult males [47]. Exposure to a male and/or male associated stimuli for relatively short periods of time (minutes to 1 or 2 days) results in reproductive activation, characterized by increased uterine and ovarian weights [7,10,30], elevated levels of ovarian estrogen and lutenizing hormone [9,12], and the onset of behavioral receptivity [10,63]. It has been shown that the primary stimuli associated with reproductive activation in voles are olfactory/pheromonal stimuli [8].

In rodents, the olfactory pathway is comprised of two distinct systems that differ in anatomy, physiology and function [2,26,37,48]. Receptors for the main olfactory system are located in the nasal epithelium and project to the olfactory bulb, whereas receptors for the accessory olfactory system reside within the vomeronasal organ (VNO) and project to the accessory olfactory bulb [1]. The main olfactory system is responsible for odor discrimination, while the accessory olfactory system is primarily involved in pheromonal communication [52,64]. The role of olfaction, in particular the VNO, in the mediation of

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endocrine and behavioral functions has been well established. The VNO is extremely sensitive to putative pheromones, with detection thresholds as low as 10^{-11}M [29]. Pheromones have been implicated in the onset of puberty, estrous induction/synchronization, and social behaviors in a variety of species, and lesions of the VNO disrupt many of these processes [21–23,32,38,43,44]. In voles, it has been demonstrated that interruption of the olfactory system disrupts reproductive activation [7,30,63]. As mating facilitates pair bond formation, olfactory disruptions, such as olfactory bulbectomy, also result in the absence of pair bonding between male and female prairie voles [59].

Although the study by Williams et al. [59] indicates the importance of olfaction in pair bond formation, removal of the olfactory bulbs disrupts both the main olfactory system and the accessory olfactory system, making it difficult to ascertain which system mediates the formation of pair bonds in prairie voles [59]. In the present study we selectively lesioned the VNO, leaving the main olfactory system intact, in female prairie voles and then tested them for the presence of mating-induced partner preferences. Our data support the hypothesis that the VNO plays an important role in the mediation of pair bonding in female prairie voles.

2. Materials and methods

2.1. Subjects

Subjects were sexually naive female prairie voles (*Microtus ochrogaster*) that were offspring of the F3 generation of a laboratory breeding colony originating from wild-caught animals from Illinois. Breeding pairs were housed in plastic cages (47×25×20 cm) containing corncob bedding with hay as nesting material. Ad libitum food (rabbit chow supplemented with sunflower seeds) and water were provided. After weaning at 21 days of age, offspring were housed in same-sex sibling pairs in plastic cages (29×18×13 cm). All cages were maintained under the same conditions of temperature (21°C) and photoperiod (14/10 h light:dark, lights on at 07:00 h). At 70–80 days of age, subjects were randomly assigned to one of four treatment groups: VNO lesioned group, two sham-lesioned groups, and an intact control group.

2.2. Surgical procedures

In general, the surgical procedures used previously in hamsters [36] and voles [30,63] were followed. Female prairie voles were anesthetized using pentobarbital (1 mg/10 g body weight). The VNO was approached via the roof of the mouth after the animal was placed supine on a head holder. A midline incision through the soft tissue of the palate was made to expose the incisive bone. Blunt dissection was used to expose the VNO capsule. A small burr was then used to enlarge the incisive foramen, through which the entire VNO capsule was removed in most cases. In a few cases when the VNO capsule was damaged, care was taken to examine the area for removing the remaining neural tissue. After VNO lesions, the soft tissue of the palate was closed using a single absorbable suture, followed by tissue adhesive. For 3 days after surgery, animals were provided with powdered rat chow with added water to produce a wet mash, in addition to their regular chow. Two weeks of post-surgical recovery were allowed prior to subsequent behavioral testing.

Two types of sham lesions were performed. In one group, an extensive sham lesion was performed, following the same procedures as outlined for the VNO lesion except the incisive foramen was not enlarged and the VNO capsule was not removed. This group controlled for unavoidable damage to the nasopalatine ducts, which are patent in prairie voles [45] and may conceivably play a role in the vole's reproductive biology. In a second group, a small incision was made in the tissue overlying the hard palate, and the incision was immediately closed using tissue adhesive. This group avoided damage to the nasopalatine ducts and controlled for anesthetic and general surgical effects.

2.3. Behavioral tests

2.3.1. Test of the main olfactory system

After 2 weeks of recovery, animals were tested for their ability to find a small piece of apple hidden from view in the cage bedding. This test was used to measure chemosensory ability mediated by the main olfactory system and to determine whether the surgical procedures had interfered with the voles' ability to respond behaviorally to odor cues [30]. For each test, the apple was randomly placed at one of three points along the midline (1/3–1/2 of the length the cage from the perimeter) under the corncob bedding of a clean cage (47×25×20 cm). The animal was introduced and the time it took to find the apple was recorded. Each animal was given a maximum of 5 min to find the apple. Any animal that did not find the apple on a given test day was scored at 300 s. Each animal was tested once on each of three consecutive days and the shortest time needed to find the apple among the three tests for each animal was used for data analysis.

2.3.2. Induction of behavioral receptivity

Two days after the last apple test, a sub-group of animals in each experimental group was tested for the induction of sexual receptivity after exposure to a male prairie vole. Each female was exposed to a male in a cage (29×18×13 cm) that was divided by a wire mesh barrier. The barrier permitted limited physical contact between the two voles but prevented mating. Animals were housed for 48 h, a time period that typically is sufficient to induce

behavioral receptivity in female prairie voles [10]. Thereafter, female subjects were placed in a clean cage, and a sexually experienced male was introduced. The female was observed for lordosis behavior for up to 4 min. If the subject displayed lordosis behavior, the test was terminated to preclude mating. Animals that did not display lordosis within 4 min were considered to be non-receptive. Subjects were given 10–12 days in their home cages prior to being used in partner preference tests.

2.3.3. Partner preference tests

Subjects were injected with estradiol benzoate (EB, 1.0 μg/0.1 ml sesame oil; Sigma) once daily for 3 days. This treatment induced sexual receptivity in a majority of female voles in our previous study [56]. On the fourth day, each female was paired with a sexually experienced male for 24 h and allowed to mate. The cohabitation and mating were videotaped with a Panasonic light sensitive camera, time-lapse VCR and red light to accommodate recording during the dark cycle. As repeated mating during a 24-h period reliably induces pair bonding in female prairie voles [19], only females that mated were further tested for partner preferences.

The apparatus for the partner preference test consists of a central cage (20×25×45 cm) joined by tubes (7.5×16 cm) to two identical parallel cages. One of these latter cages contained the familiar male partner, the other, an unfamiliar conspecific male. The males were tethered to restrict their movements to their separate cages and thus had no direct contact with each other. All cages contained food and water. The female subject was released into the central cage and had free access to all cages. A customized computer program (R. Henderson, Florida State University) using a series of light beams across the connecting tubes was used to monitor movements of the female among the cages. All animals' interactions were also videotaped for behavioral analysis. The partner preference test lasted for 3 h.

2.4. Histological verification

Following the partner preference test, all VNO lesioned and intact control animals were anesthetized with sodium pentobarbital and perfused through the ascending aorta with 0.9% saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PBS, pH 7.4). Brains were removed, immersed in 30% sucrose in PBS, and 40-μm coronal sections were cut through the olfactory bulbs on a cryostat. Sections were mounted on slides and stained with 0.2% thionin. Histological assessments were conducted on coded slides so that the experimenter was not aware of the identity of the specimen. The presence or absence of glomeruli in the accessory olfactory bulbs was examined for each animal [64].

2.5. Data analysis

We first did a preliminary analysis comparing two sham-lesioned groups ($n=6$/group). These groups did not differ in any of the measures, therefore, both groups were combined into a single sham-lesioned group in subsequent analyses. For the apple test, the shortest time among the three consecutive tests was used as the score for each animal, and group differences were examined using one-way analysis of variance (ANOVA). For the lordosis test, group differences in the number of animals that displayed the lordosis behavior were analyzed using a χ^2 test. The frequency of mating bouts and the number of mounts per mating bout received by the female, the amount of time spent in direct physical contact with the male, and the number of contact bouts were scored for the first 6 h of the 24-h cohabitation period using a computerized data acquisition program. Group differences were analyzed using a one-way ANOVA. During the partner preference test, group differences in the time spent in each cage, the frequency of cage entries and the frequency of physical contact with each stimulus animal were analyzed using a one-way ANOVA. In addition, differences in the time spent in physical contact with the partner or the stranger within each group were analyzed using a Wilcoxon signed-rank test.

3. Results

3.1. Verification of VNO lesions

The efficacy of the VNO lesions was assessed by examining the accessory olfactory bulb for the presence of glomeruli [64]. In agreement with previous studies [30,63], glomeruli were visualized in the accessory olfactory bulbs in the intact control voles. Among the eight VNO lesioned voles, glomeruli were absent in six animals. In one animal, a small number of glomeruli remained, and the appropriate tissue was damaged in the final animal.

3.2. VNO lesions did not impair the main olfactory system

The function of the main olfactory system in the female prairie vole was evaluated in an apple-finding test. All animals were able to find the apple on at least one of the three test days. For all groups, the mean time to find the apple was less than 1 min (Fig. 1a). The VNO lesioned animals ($n=9$) did not differ in the amount of time finding the apple when compared to sham-lesioned ($n=12$) or intact ($n=8$) animals ($F_{2,26}=0.16$, $P=0.85$).

3.3. VNO lesions disrupted lordosis behavior

VNO lesions significantly disrupted induction of lor-

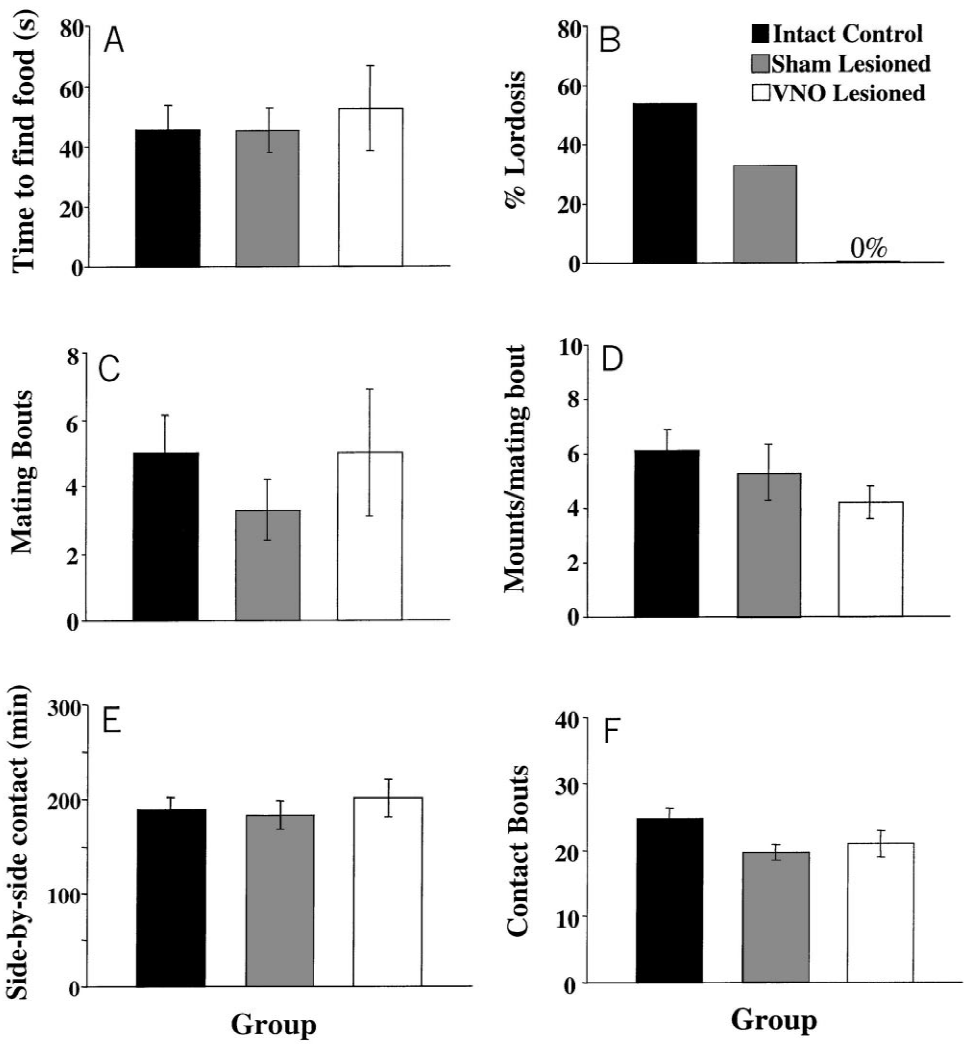

Fig. 1. Effects of VNO lesions on behavior of female prairie voles. Intact control, sham-lesioned, and VNO lesioned voles did not differ in the time of finding a hidden food (A), frequency of mating bouts (C) or number of mounts per mating bout (D), the time spend in body contact with a male (E), or frequency of contact bouts (F). However, lordosis induction by exposure to a male was severely inhibited by VNO lesions (B; $\chi^2=6.48$, $P<0.05$). None of VNO lesioned voles (0/8), but 54% of intact (7/13) and 33% of sham-lesioned (4/12) voles, displayed lordosis behavior. Bars indicate standard errors of the means.

dosis behavior in female prairie voles (Fig. 1b; $\chi^2=6.48$, $P<0.05$). None of the VNO lesioned animals (0/8) displayed lordosis behavior following the 48-h exposure to a male across a wire mesh barrier. In contrast, 54% of intact (7/13) and 33% of sham-lesioned (4/12) females displayed lordosis behavior during the 4 min of the sexual receptivity test.

3.4. VNO lesions did not alter social behavior during mating

The effects of VNO lesions on social interactions were analyzed for the first 6 h of the 24 h of cohabitation/mating with a male. Estrogen treatment induced sexual receptivity in females in all groups. During mating, VNO lesioned animals ($n=9$) had the same number of mating bouts (Fig. 1c; $F_{2,26}=0.73$, $P=0.49$) as did the intact ($n=8$) or sham-lesioned ($n=12$) animals, and the mean number of mounts per mating bout (Fig. 1d; $F_{2,18}=1.03$, $P=0.37$) did not differ between groups. Furthermore, all groups were equivalent in the duration (Fig. 1e; $F_{2,26}=0.32$, $P=0.73$) and frequency (Fig. 1f; $F_{2,26}=2.87$, $P=0.07$) of body contact throughout the first 6 h of mating.

3.5. VNO lesions diminished mating-induced pair bonding

VNO lesions diminished mating-induced partner preferences in female prairie voles (Fig. 2). Intact females spent significantly more time in direct physical contact with the familiar partner (8/9) than with the stranger (1/9), ($Z=2.31$, $P<0.05$). Likewise the sham-lesioned females also spent more time in contact with the partner (10/12) than with the stranger (2/12), ($Z=2.04$, $P<0.05$). In contrast,

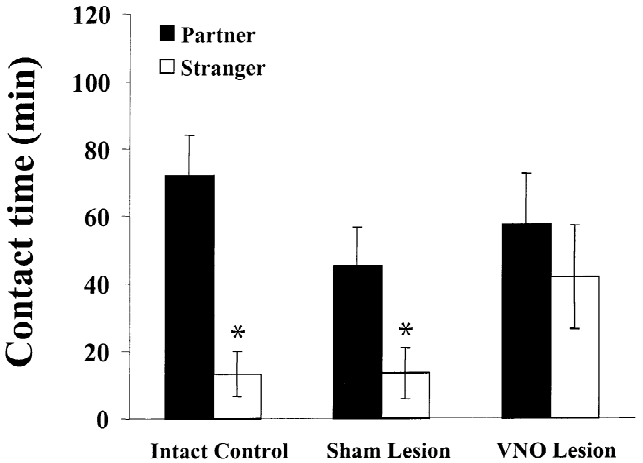

Fig. 2. Effects of VNO lesions on partner preferences of female prairie voles. Intact control ($Z=2.31$, $P<0.05$) and sham-lesioned voles ($Z=2.04$, $P<0.05$) spent significant more time in contact with the partner than with the stranger. However, VNO lesioned voles spent approximately equal amounts of time with either of the stimulus animals. Bars indicate standard errors of the means.

VNO lesioned females were equally likely to spend time in contact with either the partner (4/8) or the stranger (4/8), ($Z=0.42$, $P=0.67$). During the partner preference test, no group differences were found in the number of cage entries ($F_{2,26}=1.12$, $P=0.34$) or in the time that the subjects spent in each cage (Table 1), nor in the frequency of body contact with either of the stimulus animals.

4. Discussion

In female prairie voles, activation of the reproductive system requires exposure to a male or male-related sensory cues [8]. Without such activation, female voles are behaviorally unreceptive and do not mate. Since mating facilitates the formation of pair bonds [19,58,61], activation of the reproductive axis may be a pre-requisite for pair bond formation in this species. Previous studies have demonstrated that interruption of the olfactory system, especially the VNO, disrupts reproductive activation in female prairie voles [31,59,63]. In the present study, we found that VNO lesions disrupt male induction of behavioral estrus in female prairie voles. In addition, after being artificially induced into behavioral estrus by estrogen priming and mating for 24 h, VNO lesioned females failed to display a partner preference, whereas both intact and sham-lesioned females displayed a robust preference for the familiar partner. These data not only support the previous finding that the VNO is important for reproductive activation in voles, but also demonstrate that lesions of the VNO disrupt partner preferences following mating, supporting a role for the VNO in pair bonding.

In agreement with previous findings in voles [31,63] and in other species of rodents [23,38,46], VNO lesions disrupted the male-induced lordosis behavior in female prairie voles, suggesting a role for VNO in estrus induction. However, it is interesting to note that if this role is by-passed (by EB priming in the present study), VNO lesioned voles are able to mate and their mating behavior appears to be similar to that of the intact and sham-lesioned voles. Therefore, it is likely that the VNO is important for the initiation of mating, but is not essential for expression of mating behavior in voles. This finding supports the notion that destruction of the VNO system reduces the arousal necessary for mating but does not impair basal reproductive physiology [57,60]. It is likely that the lesion interferes with the VNO-mediated hormonal surge induced by males and thus disrupts estrus induction [31,33]. Certainly, it is conceivable that VNO lesioned and intact voles differed in some subtle aspects of mating behavior that were not detected by the methods used in our study. This possibility needs to be addressed in further studies.

Following 24 h of mating, female prairie voles display a robust preference for the familiar partner versus a conspecific stranger [19,56,58]. Consistent with this finding, the intact and sham-lesioned voles in the present study showed characteristic partner preferences following mating. VNO lesioned females, however, failed to exhibit partner preferences. These data suggest that the VNO may be involved in the mediation of mating-induced pair bonding in voles. Since pair bonding is a complex social behavior, there are many mechanisms by which VNO lesions might disrupt pair bonding: inhibition of mating, impairment of behavioral interactions, interference with the main olfactory system, destruction of the nasopalatine route, or deficiency in sensory processing.

In the absence of VNO inputs, reproductive activation and mating are severely disrupted in prairie voles [31,63, present study]. However, the absence of pair bonding in our study was not due to lack of mating after VNO lesions,

Table 1
General social behavior during partner preference test

Behavior	Measurement	Treatment group		
		Intact control	Sham-lesioned	VNO-lesioned
In partner's cage	Duration (min)	107.2±10.0	79.0±12.7	81.8±16.4
In stranger's cage	Duration (min)	32.1±11.9	54.0±13.2	62.5±16.9
In neutral cage	Duration (min)	29.4±3.3	35.4±3.8	22.8±3.6
Partner's cage entries	Frequency	39.8±6.0	42.6±6.4	31.9±8.2

as we by-passed this by inducing females into behavioral estrus with estrogen priming. We can also rule out the possibility of impaired behavioral interactions. The VNO lesioned voles did not differ from intact or sham-lesioned voles in mating and social contact towards the male during the first 6 h of cohabitation, nor in locomotor activity (measured by cage entries) during the preference test. These data indicate that the absence of pair bonding in VNO lesioned animals was not a residual effect of altered social behaviors during cohabitation, nor was it an artifact of hyper- or hypoactivity during the preference test. Olfaction plays an important role in the mediation of partner preferences in female prairie voles [59]. However, it is unlikely that VNO lesions interfered with the main olfactory system, as VNO lesioned animals did not show any deficits in their ability to find hidden food, which is in agreement with the previous finding [31]. Finally, nasogenital and possibly orogenital contacts are important aspects of the social interactions in prairie voles and such behaviors are more likely to be initiated by females [14]. It is important to note that the nasopalatine ducts are patent in voles [45]. The patency of this structure may facilitate the flow of substances through the external nares [65] or provide a direct route by which compounds may access to the VNO [34]. It is possible that VNO lesions destroyed the nasopalatine ducts and thus interfered with pair bonding in female prairie voles. However, this possibility can be ruled out as the two groups of sham-lesioned voles, which differed in the amount of damage to the nasopalatine ducts, did not differ in their behavior, nor did they differ from the intact animals. Along the same line, damage to the VNO, but not occlusion of the nasopalatine ducts, induced mating deficits in male hamsters ([36] but see Ref. [33]).

Collectively, the most likely explanation for the lack of pair bonding is that the lesions eliminated VNO-mediated sensory processing thus preventing activation of neuronal structures essential for pair bonding. Neural signals from the VNO are sent to the amygdala and the bed nucleus of the stria terminalis (BST), both of which have been implicated in the control of social behaviors [4,13,25,35,50,62]. In female prairie voles, exposure to male sensory cues increased neuronal activation, as indicated by c-*fos* expression, in the accessory olfactory bulbs and in different aspects of the amygdala [41,53]. Furthermore, lesions of the amygdala impaired olfactory memory for the mate [11]. In male prairie voles, sexual and social experience with a female significantly increased neuronal activation in both the amygdala and the BST [54], while lesions of the corticomedial amygdala reduced pair bonding [27]. It can be hypothesized that some of the brain regions along the accessory olfactory pathway are activated by male-associated cues but not by mating, and activation of specific neurochemical mechanisms in those areas are necessary for pair bonding in female prairie voles. Interestingly, stimulation of the accessory olfactory system results in activation of the mesolimbic dopamine system, specifically, the induction of dopamine release in the nucleus accumbens in rats [39]. Released dopamine in the nucleus accumbens plays an important role in pair bond formation in female prairie voles [16].

Comparisons of results from the present study in which only VNO input was removed with those from previous studies in which all olfactory input was eliminated may reveal which behaviors are mediated by specific parts of the olfactory system. In this context, it is important to note that in the present study, voles were treated with estrogen, which could mask or enhance the similarities and/or differences in behavior discussed below.

Bilaterally bulbectomized and VNO lesioned voles share some behavioral deficits. Voles typically were not induced into behavioral estrus by exposure to a male after either manipulation [30,59,63, present study]. In addition, neither VNO lesioned nor bulbectomized voles displayed partner preferences [59]. Since the accessory olfactory system was disrupted in both types of manipulation, these data indicate that the VNO is likely the source for behavioral deficits in estrus induction and pair bonding of prairie voles [59]. It has been suggested that even minimal afferent input remaining after VNO lesions is capable of supporting chemosensory-induced activation of the female reproductive system [63]. Mating occurred in a small portion of VNO lesioned voles in a previous study [31] but was not observed in the present experiment. This discrepancy might be attributed to the different paradigms used for male cohabitation. The wire mesh barrier used in our study allowed limited physical contact but prevented mating, while no barrier was used in the earlier study [31]. Physical contact with males is important for reproductive activation into behavioral estrus [10]. It is possible that non-vomeronasal sources of chemosensory information obtained during an extensive and prolonged period (60 h) of physical contact were processed by the main olfactory system and were sufficient to cause reproductive activation in some prairie voles [30]. Indeed, if exposure to a male is limited, the reproductive activation is severely disrupted by VNO lesions [63].

Despite the similarities, bulbectomized and VNO lesioned prairie voles also show important differences in some behaviors. After bilateral bulbectomy, voles spend significantly less time engaging in social contact or more time in isolation [28,59]. However, VNO lesioned voles showed neither reduced social contact, nor increased time in isolation in our study — results consistent with a lack of deficits in social interactions in a previous study [31]. Together, these data suggest that bulbectomy may interfere with prairie voles' social behavior, making them more non-social — an effect not seen after VNO lesions. Similar results have been reported for hamsters; disruption of main olfactory input, but not the VNO input, significantly reduced social behaviors such as flank marking or urogenital licking of the conspecific female [24,42].

Bulbectomy also causes changes in general behavioral patterns that were not observed after VNO lesions. For

example, in male prairie voles, bilateral bulbectomy caused an increase in locomotor activity [28], which was not found in VNO lesioned females [30, present study]. Therefore, damage to the main olfactory system, but not to the accessory olfactory system, may alter locomotor behavior [28]. Alternatively, the differences in locomotor behavior may simply represent gender differences, as in rats the VNO pathway is sexually dimorphic [49,50]. The latter possibility is supported by the finding that VNO lesions reduced social contact in male [57] but not in female prairie voles [present study].

Although our data suggest that the VNO plays an important role in the regulation of pair bonding, it is by no means clear whether the VNO mediates the formation of a partner preference, the expression of this behavior, or both. In a previous study, dopamine was found to regulate pair bond formation but was not necessary for its expression in female prairie voles [56]. If the VNO plays a role in processing male-related sensory information as discussed above, lesions of the VNO may prevent animals from forming pair bonds. On the other hand, VNO lesions may interfere with individual recognition and thus influence the expression of pair bonding. Like other rodents, female prairie voles are capable of discriminating between individuals. Following mating, exposure to an unfamiliar stranger male, but not to the familiar partner, causes disruption of fetal implantation [17,51]. In this context, VNO neurons may contribute to individual recognition [18]. This is further supported by evidence that VNO lesions impair social recognition in rats [3].

In summary, mating-induced pair bonding in prairie voles has been utilized as a behavioral paradigm for the study of neural, endocrine, and behavioral bases of social attachment [6,55,66]. In the present study, we report that lesions of the VNO in female prairie voles disrupts estrus induction after exposure to a male. In addition, VNO lesioned females do not display pair bonding following estrogen-induced estrus and mating. Despite these deficits, many other behaviors are unaffected. These data, together with previous findings [30,63], suggest that the VNO is crucial for reproductive activation and pair bonding in female prairie voles, and thus may play an important role in the reproductive success of this species. Since many of the neural components of the VNO system interact with the brain regions implicated in social behaviors, our results provide strong justification for further examining the interactions of chemosensory cues and neurochemical processing to fully understand the mechanism(s) underlying social attachment.

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References

[1] P.C. Barber, G. Raisman, An autoradiographic investigation of the projection of the vomeronasal organ to the accessory olfactory bulb in the mouse, Brain Res. 81 (1974) 21–30.
[2] A. Berghard, L.B. Buck, E.R. Liman, Evidence for distinct signaling mechanisms in two mammalian olfactory sense organs, Proc. Natl. Acad. Sci. USA 93 (1996) 2365–2369.
[3] R.M. Bluthe, R. Dantzer, Role of the vomeronasal system in vasopressinergic modulation of social recognition in rats, Brain Res. 604 (1993) 205–210.
[4] P. Brennan, H. Kaba, E.B. Keverne, Olfactory recognition: a simple memory system, Science 250 (1990) 1223–1226.
[5] C.S. Carter, A.C. De Vries, L.L. Getz, Physiological substrates of mammalian monogamy: The prairie vole model, Neurosci. Biobehav. Rev. 19 (1995) 1–12.
[6] C.S. Carter, L.L. Getz, Monogamy and the prairie vole, Sci. Am. 268 (1993) 100–106.
[7] C.S. Carter, L.L. Getz, L. Gavish, J.L. McDermott, P. Arnold, Male-related pheromones and the activation of female reproduction in the prairie vole (*Microtus ochrogaster*), Biol. Reprod. 23 (1980) 1038–1045.
[8] C.S. Carter, L.L. Getz, L. Gavish, J.L. McDermott, P. Arnold, Male-related pheromones and the activation of female reproduction in the prairie vole (*Microtus ochrogaster*), Biol. Reprod. 23 (1980) 1038–1045.
[9] C.S. Carter, D.M. Witt, S.R. Manock, K.A. Adams, J.M. Bahr, K. Carlstead, Hormonal correlates of sexual behavior and ovulation in male-induced and postpartum estrus in female prairie voles, Physiol. Behav. 46 (1989) 941–948.
[10] C.S. Carter, D.M. Witt, J. Schneider, Z.L. Harris, D. Volkening, Male stimuli are necessary for female sexual behavior and uterine growth in prairie voles (*Microtus ochrogaster*), Horm. Behav. 21 (1987) 74–82.
[11] G.E. Demas, J.M. Williams, R.J. Nelson, Amygdala but not hippocampal lesions inpair olfactory memory for mate in prairie voles (*Microtus ochrogaster*), Am. J. Physiol. 273 (1997) R1683–R1689.
[12] D. Dluzen, C.S. Carter, L.L. Getz, Male vole urine changes lutenizing hormone-releasing hormone and norepinephrine in female olfactory bulb, Science 212 (1981) 573–575.
[13] G.D. Fernandex-Fewell, M. Meredith, c-*fos* expression in vomeronasal pathways of mated or pheromone-stimulated male Golden hamsters: contributions from vomeronasal sensory input and expression related to mating performance, J. Neurosci. 14 (1994) 3643–3654.
[14] L. Gavish, C.S. Carter, L.L. Getz, Male–female interactions in prairie voles, Anim. Behav. 31 (1983) 511–517.
[15] L.L. Getz, C.S. Carter, Prairie-vole partnerships, Am. Sci. 84 (1996) 56–62.
[16] B. Gingrich, Y. Liu, C. Cascio, Z.X. Wang, T.R. Insel, Dopamine D2 receptors in the nucleus accumbens are important for social attachment in female prairie voles, Behav. Neurosci. 114 (2000) 173–183.
[17] E.J. Heske, R.J. Nelson, Pregnancy interruption in *Microtus ochrogaster*: laboratory artifact or field phenomenon, Biol. Reprod. 31 (1984) 97–103.
[18] T.E. Holy, C. Dulac, M. Meister, Responses of vomeronasal neurons to natural stimuli, Science 289 (2000) 1569–1571.
[19] T.R. Insel, T.J. Hulihan, A gender-specific mechanism for pair bonding: oxytocin and partner preference formation in monogamous voles, Behav. Neurosci. 109 (1995) 782–789.
[20] T.R. Insel, S. Preston, J.T. Winslow, Mating in the monogamous male: behavioral consequences, Physiol. Behav. 57 (1995) 615–627.
[21] B. Jemiolo, S. Harvey, M. Novotny, Promotion of the Whitten effect

in female mice by synthetic analogs of male urinary constituents, Proc. Natl. Acad. Sci. USA 83 (1986) 4576–4579.
[22] M.A. Johns, The role of the vomeronasal organ in behavioral control of reproduction, Ann. NY Acad. Sci. 474 (1986) 148–157.
[23] M.A. Johns, H.H. Feder, B.R. Komisurak, A.D. Mayer, Urine-induced reflex ovulation in anovulatory rats may be a vomeronasal effect, Nature 272 (1978) 446–447.
[24] R.E. Johnston, U.G. Mueler, Olfactory but not vomeronasal mediation of scent marking by male Golden hamsters, Physiol. Behav. 48 (1990) 701–706.
[25] E.B. Kaverne, Pheromonal influences on the endocrine regulation of reproduction, Trends Neurosci. 6 (1983) 381–384.
[26] E.B. Keverne, The vomeronasal organ, Science 286 (1999) 716–720.
[27] B. Kirkpatrick, C.S. Carter, S.W. Newman, T.R. Insel, Axon-sparing lesions of the medial nucleus of the amygdala decrease affiliate behaviors in the prairie vole (*Microtus ochrogaster*): Behavioral and anatomical specificity, Behav. Neurosci. 108 (1994) 501–513.
[28] B. Kirkpatrick, J.R. Williams, B.M. Slotnick, C.S. Carter, Olfactory bulbectomy decreases social behavior in male prairie voles (*M. ochrogaster*), Physiol. Behav. 55 (1994) 885–889.
[29] T. Leinders-Zufall, A.P. Lane, A.C. Puche, W. Ma, M.V. Novotny, M.T. Shipley, F. Zufall, Ultrasensitive pheromone detection by mammalian vomeronasal neurons, Nature 405 (2000) 792–796.
[30] J.J. Lepri, C.J. Wysocki, Removal of the vomeronasal organ disrupts the activation of reproduction in female voles, Physiol. Behav. 40 (1987) 349–355.
[31] J.J. Lepri, C.J. Wysocki, Removal of the vomeronasal organ disrupts the activation of reproduction in female voles, Physiol. Behav. 40 (1987) 349–355.
[32] D.E. Lomas, E.B. Keverne, Role of the vomeronasal organ and prolactin in the acceleration of puberty in female mice, J. Reprod. Fertility 66 (1982) 101–107.
[33] A. Mackay-Sim, J.D. Rose, Removal of the vomeronasal organ impairs lordosis in female hamsters: effect is reversed by luteinizing hormone-releasing hormone, Neuroendocrinology 42 (1986) 489–493.
[34] P.Y. Melese d'Hospital, B.L. Hart, Vomeronasal organ cannulation in male goats: evidence for transport of fluid from oral cavity to vomeronasal organ during Flehman, Physiol. Behav. 35 (1985) 941–944.
[35] M. Meredith, Sensory physiology of pheromone communication, in: J.G. Vandenbergh (Ed.), Pheromones and Reproduction in Mammals, Academic Press, New York, 1983, pp. 199–252.
[36] M. Meredith, Vomeronasal damage, not nasopalatine duct damage, produces mating behavior deficits in male hamsters, Chem. Senses 16 (1991) 155–167.
[37] M. Meredith, Vomeronasal function, Chem. Senses 23 (1998) 463–466.
[38] M. Meredith, G. Fernandez-Fewell, Vomeronasal system, LHRH, and sex behaviour, Psychoneuroendocrinology 19 (1994) 5–7.
[39] J.B. Mitchell, A. Gratton, Mesolimbic dopamine release elicited by activation of the accessory olfactory system: a high speed chronoamperometric study, Neurosci. Lett. 140 (1992) 81–84.
[40] D.W. Mock, M. Fujioka, Monogamy and long-term pair bonding in vertebrate, Trends Ecol. Evol. 5 (1990) 39–43.
[41] C.A. Moffatt, G.F. Ball, R.J. Nelson, The effects of photoperiod on olfactory c-fos expression in prairie voles, *Microtus ochrogaster*, Brain Res. 677 (1995) 82–88.
[42] M.R. Murphy, Sexual preferences of male hamsters: importance of preweaning and adult experience, vaginal secretion, and olfactory or vomeronasal sensation, Behav. Neural Biol. 30 (1980) 323–340.
[43] M.V. Novotny, W. Ma, D. Wiesler, L. Zidek, Positive identification of the puberty-accelerating pheromone of the house mouse: the volatile ligands associating with the major urinary protein, Proc. R. Soc. Lond. B 266 (1999) 2017–2022.
[44] J.B. Powers, S.S. Winans, Vomeronasal organ: critical role in mediating sexual behavior of the male hamster, Science 187 (1975) 961–963.
[45] W.B. Quay, The anatomy of the diastemal palate in Microtine rodents, Misc. Publ. Mus. Zool. Univ. Michigan 86 (1954) 9–13.
[46] T.R. Saito, H. Moltz, Sexual behavior in the female rat following removal of the vomeronasal organ, Physiol. Behav. 38 (1986) 81–87.
[47] D.K. Sawrey, D.A. Dewsbury, Control of ovulation, vaginal estrus, and behavioral receptivity in voles (*Microtus*), Neurosci. Biobehav. Rev. 9 (1985) 563–571.
[48] F. Scalia, S.S. Winans, The differential projections of the olfactory bulb and accessory olfactory bulb in mammals, J. Comp. Neurol. 161 (1975) 31–53.
[49] S. Segovia, A. Guillamon, Sexual dimorphism in the vomeronasal pathway and sex differences in reproductive behaviors, Brain Res. Rev. 18 (1993) 51–74.
[50] R.B. Simerly, Hormonal control of neuropeptide gene expression in sexually dimorphic olfactory pathways, Trends. Neurosci. 13 (1990) 104–110.
[51] R.A. Stehn, M.E. Richmond, Male-induced pregnancy termination in the prairie vole (*Microtus ochrogaster*), Science 187 (1975) 1211–1213.
[52] R. Tirindelli, C. Mucignat-Caretta, N.J.P. Ryba, Molecular aspects of pheromonal communication via the vomeronasal organ of mammals, Trends Neurosci. 21 (1998) 482–486.
[53] M.L. Tubbiola, C.J. Wysocki, Fos immunoreactivity after exposure to conspecific or heterospecific urine: where are chemosensory cues sorted?, Physiol. Behav. 62 (1997) 867–870.
[54] Z.X. Wang, T.J. Hulihan, T.R. Insel, Sexual and social experience is associated with different patterns of behavior and neural activation in male prairie voles, Brain Res. 767 (1997) 321–332.
[55] Z.X. Wang, L.J. Young, G.J. De Vries, T.R. Insel, voles and vasopressin: a review of molecular, cellular, and behavioral studies of pair bonding and paternal behaviors, Prog. Brain. Res. 119 (1998) 479–495.
[56] Z.X. Wang, G.Z. Yu, C. Cascio, Y. Liu, B. Gingrich, T.R. Insel, Dopamine D2 receptor-mediated regulation of partner preferences in female prairie voles: a mechanism for pair bonding, Behav. Neurosci. 113 (1999) 602–611.
[57] K.S. Wekesa, J.J. Lepri, Removal of the vomeronasal organ reduces reproductive performance and aggression in male prairie voles, Chem. Senses 19 (1994) 35–45.
[58] J.R. Williams, K.C. Catania, C.S. Carter, Development of partner preferences in female prairie voles (*Microtus ochrogaster*): the role of social and sexual experience, Horm. Behav. 26 (1992) 339–349.
[59] J.R. Williams, B.M. Slotnick, B.W. Kirkpatrick, C.S. Carter, Olfactory bulb removal affects partner preference development and estrus induction in female prairie voles, Physiol. Behav. 52 (1992) 635–639.
[60] S.S. Winans, J.B. Powers, Olfactory and vomeronasal deafferentation of male hamsters: histological and behavioral analyses, Brain Res. 126 (1977) 325–344.
[61] J.T. Winslow, N. Hastings, C.S. Carter, C.R. Harbaugh, T.R. Insel, A role for central vasopressin in pair bonding in monogamous prairie voles, Nature 365 (1993) 545–548.
[62] R.I. Wood, S.W. Newman, Hormonal influence on neurons of the mating behavior pathway in male hamsters, in: P.E. Micevych, R.P.J. Hammer (Eds.), Neurobiological Effects of Sex Steroid Hormones, Cambridge University Press, New York, 1995, pp. 3–39.
[63] C.J. Wysocki, M. Kruczek, L.M. Wysocki, J.J. Lepri, Activation of reproduction in nulliparous and primiparous voles is blocked by vomeronasal organ removal, Biol. Reprod. 45 (1991) 611–616.
[64] C.J. Wysocki, J.J. Lepri, Consequences of removing the vomeronasal organ, J. Steroid Biochem. Mol. Biol. 39 (1991) 661–669.
[65] C.J. Wysocki, J.L. Wellington, G.K. Beauchamp, Access of urinary nonvolatiles to the mammalian vomeronasal organ, Science 207 (1980) 781–783.
[66] L.J. Young, Z.X. Wang, T.R. Insel, Neuroendocrine bases of monogamy, Trends Neurosci. 21 (1997) 71–75.