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ARTICLE

Vomeronasal organ ablation elicits chemosensory dysfunction and abnormal behavior in mice

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Abstract This study aimed to examine whether the vomeronasal organ (VNO) is a prerequisite in mice to acquire essential information from various social odors and whether long-term VNO dysfunction can elicit behavioral and physiological changes in mice. We used binary choice tests and habituation–dishabituation tests to measure the abilities of male mice to recognize social odors. We found that males with the VNO ablation failed to show olfactory preferences between the odors of mate versus non-mate females, offspring versus non-offspring pups, or opposite-sex conspecifics versus predators (cats or rats), but were capable of discriminating between the two treatments in each of the paired odors, suggesting that male mice with VNO ablation might smell out the chemical differences of the two types of odors, but could not extract the biological information contained in the odors. Furthermore, prolonged VNO deficiency resulted in a reduction in crossing behavior in a light/dark box, the frequency of urine marking, and the time spent in the center in an open field. These results indicate that chronic VNO dysfunction led to anxiety-like or submissive behavior. In addition, males with VNO ablation had atrophic adrenal glands and hypertrophic preputial glands, suggesting that VNO dysfunction could damage the physiological conditions to buffer the stress and that pheromone perception deficiency might enhance self-odor production in mice.

Keywords Vomeronasal organ · Predator odor · Offspring odor · Female mate odor · Anxiety-like behavior

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Introduction

An increasing body of evidence suggests that the main olfactory and vomeronasal systems overlap in detecting pheromonal compounds in mice (Sam et al. 2001; Luo et al. 2003; Lin et al. 2005; Xu et al. 2005; Spehr et al. 2006). However, the olfactory preference for opposite-sex conspecifics over same-sex conspecifics does rely on the vomeronasal organ (VNO) in mice (Wysocki et al. 1982; Del Punta et al. 2002; Stowers et al. 2002; Pankevich et al. 2004, 2006; Brennan and Zufall 2006; Keller et al. 2006; Zhang et al. 2008a). VNO deficiency elicits a range of deficits in sexual behaviors in response to sex pheromones (Clancy et al. 1984; Meredith 1986; Pfeiffer and Johnston 1994; Keverne 2004; Brennan and Zufall 2006). Such VNO-dependent perception of pheromones occurs only in rodents, and not in other mammals such as rabbits, sows, goats, and ferrets (Hudson and Distel 1986; Dorries et al. 1997; Gelez and Fabre-Nys 2004; Woodley et al. 2004).

According to our knowledge, few studies have examined whether the perception of chemical signals is VNO-dependent in rodents, apart from sex pheromones. Although it is revealed that the perception of alarm pheromones in male rats, the suppression of sperm motility of subordinates by the dominance pheromone in male mice,

and the extension of estrous cycles of female rats by mink odor depend on the VNO (Voznessenskaya et al. 1992; Koyama and Kamimura 2003; Kiyokawa et al. 2007), direct evidence that mice with VNO ablation are incapable of distinguishing unpleasant odors from pleasant odors is unavailable.

Furthermore, individual odor discrimination or recognition is also involved in the VNO in mice and golden hamsters (Johnston and Peng 2000; Brennan 2004; Brennan and Zufall 2006). As early as the mid-1980s, Lepri et al. (1985) found that VNO ablation does not differentiate the rates of conception, litter size, pup growth, pup recognition, or maternal behavior of female mice. In male mice, vomeronasal chemoreception does not affect the excretion of the puberty-acceleration chemosignals. At present, however, we do not yet know whether VNO is necessary for mice to show olfactory preferences between opposite-sex mates versus non-mates, or offspring versus non-offspring, or whether long-term dysfunction of the VNO has other behavioral and physiological effects on mice.

In this study, we conducted a series of experiments to examine the effects of VNO ablation on males' olfactory recognition and discrimination of cats and rats, which are natural predators of mice (Apfelbach et al. 2005; D'Arbe et al. 2002; Loggi et al. 1996; Malick 1975; Rylov 1985; Yang et al. 2004), and on offspring and female partners by using binary tests and habituation–dishabituation tests. We also used an open field and a light/dark box to examine whether chronic VNO ablation elicites anxious behavior in male mice. Finally, we killed and autopsied the mice to measure the changes in relevant organs, especially preputial glands, to explore whether long-term VNO dysfunction affects pheromone production in mice.

Materials and methods

Experimental animals

Thirty sex-naïve male CD1/ICR/albino mice at 8 weeks of age were purchased from the Weitong-Lihua Experimental Animal Company, Beijing, China, and acclimated for 4 weeks prior to use. The mice were housed individually in plastic cages (25 × 15 × 13.5 cm each). The room had a reversed 14L:10D light/dark photoperiod (light on at 1900 hours) and 23 ± 2°C in temperature. Food (standard mouse chow) and water were provided ad libitum. The procedure of animal handling complied with the institutional guidelines for animal use and care at the Institute of Zoology, the Chinese Academy of Sciences.

After the 4-week acclimation, we performed a surgical operation to damage the VNO (VNOx) or a sham-operation (VNOi) on mice. From the postoperative 20th day, the VNOi and VNOx males underwent behavioral tests by using binary choice, habituation–dishabituation test, the open field, and the light/dark box. On the postoperative 46th day, VNOi and VNOx males were paired with respective VNOi and VNOx females. This resulted in the birth of offspring on the postoperative 64th day. From postoperative 70–74th days, we tested the responses of VNOi and VNOx males to the odors of the pups between offspring and non-offspring, or of the mates and non-mates.

Surgery

For VNO ablation, anesthetized males ($n = 15$) were placed supine with head down on a sloping small table with holders. We made a midline incision on the roof of the mouth, gently opened the lower jaw, and exposed and cut the parts (about 2–3 mm long) of the underlying vomer bones. We then removed them by forceps, and closed the incision using the medical sutures (Wysocki et al. 1982; Johnston 1992). To control for the surgery, a group of males ($n = 15$) went through the same procedure except for the removal of the vomer bones. VNO ablation may cause bleeding and obstruction of the nasal cavity, resulting in behavior patterns similar to that of olfactory-deficient mice, which show a reduction in sexual and aggressive behavior (Kimchi et al. 2007). So we used alcohol sterilized swabs or cotton threads to clean the oral and nasal cavity to avoid these complications during the surgery (Zhang et al. 2008a). All animals were allowed 2 weeks for recovery prior to behavioral experiments. After all behavioral trials, we killed all males and anatomized the nasal cavity of VNOx to confirm the disconnection of the VNO under microscope (Zhang et al. 2008a).

Odor collection and sample preparation

To collect the urine of adult mice, we placed each donor in a clean mouse cage with a wire grid floor (Zhang et al. 2007a, 2008a). Upon the animal's urination, the urine was immediately absorbed and transferred to a vial by a disposable glass capillary (i.d. 1.8 mm, 15 cm long) for behavioral and chemical tests. We obtained rat and cat urine using the same method.

An additional 5 male and 5 female mice at 3 months of age, purchased from abovementioned animal supplier, were used as urine donors for either sex. Five male rats aged 5 months and two male cats aged 2 years were used as predator urine donors, respectively. These collected odors were specially used for testing chemosensory recognition and discrimination of sexes and predators. To test sex recognition and discrimination, we randomly paired each male and female mouse urine samples for presentation of odor stimuli in binary tests. In testing the responses of

male mice to urine odor between female mouse and predator, we mixed (equally) the urine samples collected from female mouse, cat and rat donors, respectively. In addition, only estrous female urine was used. To collect pup odor, we used the tip of a glass rod (20 cm long and 4 mm in diameter each) to rub against the anogenital area of a pup for about 20 times.

Presentation of odor sample

Capillary method

We injected 2 μl of each aqueous sample into a disposable glass capillary from one opening (i.d. 1.1–1.2 mm, o.d. 1.3–1.4 mm, 15 cm length). We then sealed the other opening of the capillary with odorless gum to suspend the sample aliquot inside the capillary, 1 cm away from the sample-containing end. As a result, test mice could sense only volatiles. The sample-containing end of the capillary was presented to test mice in their home cages, while the other end was held by testers with disposable plastic gloves. We used this method in all behavioral binary choice and habituation–dishabituation tests except the presentation of pup odors (Zhang et al. 2007b).

Glass rod method

This method was only used to deliver odors from the anogenital areas of pups smeared on the tips of glass rods. The sample-containing tips of the rods were presented to test mice in their home cages, while the other tips were held by the testers with disposable plastic gloves (Zhang et al. 2008a).

Behavioral binary choice test and habituation–dishabituation test

We tested the responses of mice to two aqueous odor samples in the dark phase by using binary choice test or habituation–dishabituation test. Immediately prior to each trial, we transferred the cage containing the test mouse to the test room under dim light and temporarily removed its cage-mate and offspring, if there were any, while leaving the test mouse in the cage for the experiment.

Binary choice test

Two glass capillaries or rods scented by two kinds of odor samples, respectively, were lowered through the wire lid and kept approximately 2 cm apart. Thus, each test subject was simultaneously presented with a binary choice. We recorded its behavior for 3 min after it showed the initial sniffing response. The durations that the test mouse spent sniffing within 1 cm of the tips or licked the end of the capillaries were recorded with two hand-held stopwatches (Zhang et al. 2007b).

Habituation–dishabituation test

We exposed each mouse recipient to an odor (e.g., the urine of conspecifics of the same-sex) from one odor donor on a series of three or four trials (in order to induce habituation, four times in female mouse urine and predator odor discrimination test, and other discrimination tests three times), each lasting 3 min with a 2-min interval between consecutive trials, and measured the time that the mouse investigated this odor. The other odor was presented on the fourth or fifth trial. On each trial, a fresh odor sample was taken up into the end of a new glass capillary or on to a rod. For testing the responses of males to pup odors, we randomly selected three same-sex pups sired by the males for the first three trials and one same-sex pup sired by other males for the test trial. Any subject that did not respond to the pup odor over the first 3 min was excluded for the day. These procedures have been described in our previous work (Zhang et al. 2007b, 2008a).

Measurement of anxious behavior

Open field test

We measured the anxiety-like behavior of the test mouse by an open field method. The open field box (75 × 45 × 35 cm in dimensions) was made of plexiglass. Its floor was equally divided into 15 grids (15 × 15 cm^2). To test exploratory behavior, we placed the test mouse into a corner of the box and allowed it to explore freely for 10 min. The behavior of the mouse was videotaped for analysis. We scored locomotion (number of floor grids crossed), frequencies of standing on the hind legs, upright, time spent resting (standing on four feet and showing no movement), time spent in the three center grids and other periphery grids, and latency to initially leave the corner grids (Arakawa 2003; Bridges and Starkey 2004).

In addition, we counted the times of urine marking as frequencies in each trial. Here, "urine marking" refers to vigorous urination over the open field in numerous small droplets. In contrast, ''pool urine'' is excreted by mice in a large amounts (Desjardins et al. 1973; Zhang et al. 2008b).

Light/dark box test

We also measured the anxious responses of the test mouse in light/dark box test. The light/dark plastic box consisted of two chambers (35 × 25 × 17 cm) connected through a

tunnel (i.d. 5.5 cm, 5 cm long). The light chamber had a cover of transparent plexiglass and the dark one had a piece of additional opaque paper board on the top of the box. The light area was lit by a lamp (40 W) that was 30 cm above the chamber bottom. The test mouse was initially placed in the light chamber facing away from the dark chamber and allowed to explore both light and dark chambers freely for 5 min. We recorded the latency to first leave (escape) from the light chamber, time spent in the light and dark chambers, and the frequency of crossing between the two chambers with a stopwatch. A mouse was considered to be inside or outside the light chamber when all its four feet were within the light chamber or the tunnel (Bridges and Starkey 2004). Between trials, the box was washed thoroughly with alcohol solution (75%).

Determination of body and organ weights

We weighed the subjects before the day of surgical removal of the VNO and on the postoperative 30th, 45th and 114th days. After all the behavioral experiments were completed; we killed all VNOi and VNOx males between 0920 and 1020 hours by neck displacement. Then we autopsied the carcasses, took out the selected organs (spleens, adrenals, preputial glands, testes, epididymides, and seminal vesicles), cleaned them of excess fat tissue, and weighed them immediately (to an accuracy of 0.1 mg). Relative organ weights were calculated in milligrams of organ weight per 100 g body weight.

Statistical analysis

We first examined the distribution of all raw data by using the Kolmogorov–Smirnov test in SPSS for Windows. We then used either parametric tests (independent t test or paired t test), if the data did not violate the normal distribution prerequisite, or non-parametric tests (Mann–Whitney U test or Wilcoxon matched-pairs signed-rank test), if the data were not normal. All statistical analyses were conducted using SPSS (Version 13.0) with the critical value of $\alpha = 0.05$.

Results

VNO and olfactory sex discrimination

Sham-operated (VNOi) male mice preferred the odor of female urine to that of male urine ($t = 5.653$, $P < 0.001$), while VNO-damaged (VNOx) male mice responded equally between them ($z = 0.175$, $P = 0.861$) (Fig. 1a).

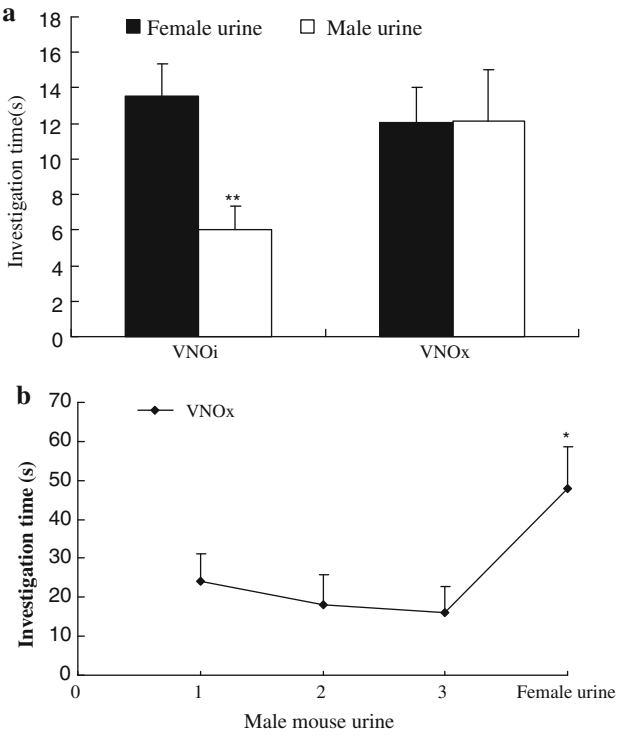

Fig. 1 Preferences (**a**) or discrimination (**b**) of VNOi and VNOx male mice over intact male or female urine (mean ± SE). **a** Seconds spent investigating urine of intact females and males ($n_{VNOi} = 13$, $n_{VNOx} = 13$); **b** a habituation–dishabituation test of the ability of VNOx male mice to discriminate between the two odor types ($n_{VNOx} = 8$). (Paired-samples t test or Wilcoxon signed ranks test was used according to the data distribution; $^{*}P < 0.05$, $^{**}P < 0.01$ in **b** indicate the comparison between the fourth and the third trials in the habituation–dishabituation test)

However, habituation–dishabituation tests revealed that VNOx males were capable of discriminating between male and female urine odor (mouse $♂_3 - ♂_1$: $z = 1.400$, $P = 0.161$; mouse $♀ - ♂_3$: $z = 2.521$, $P = 0.012$; the subscripts indicate the times the odor was presented) (Fig. 1b).

VNO and olfactory predator discrimination

VNOi male mice showed a significant preference for the urine odor of female mice over the urine odor of either of the two predator species ($t = 2.888$, $P = 0.014$ for male cat; $t = 3.067$, $P = 0.010$, for male rat), whereas VNO ablation eliminated such preference in male mice (Fig. 2a, b). However, VNOx males showed the ability to discriminate cat or rat urine odor from female mice urine odor [mouse $♀_4 - ♀_1$: $z = 2.271$, $P = 0.023$; cat–mouse $♀_4$: $z = 2.970$, $P = 0.003$ for cat; mouse $♀_4 - ♀_1$: $z = 1.852$, $P = 0.064$ (marginal insignificance); rat–mouse $♀_4$: $z = 2.830$, $P = 0.005$ for rat; the subscripts indicate the times the odor was presented] (Fig. 3a, b).

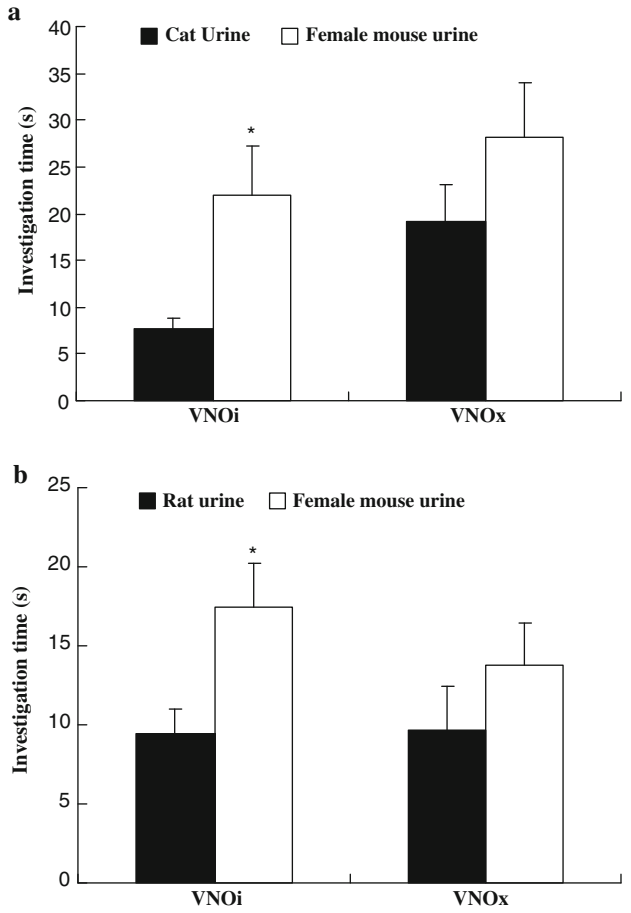

Fig. 2 Preferences of VNOi and VNOx male mice between female mouse urine and cat (**a**) or rat (**b**) urine (mean ± SE, $n_{VNOi} = 13$, $n_{VNOx} = 13$). (Paired-samples t test or Wilcoxon signed ranks test was used according to the data distribution; $^{*}P < 0.05$)

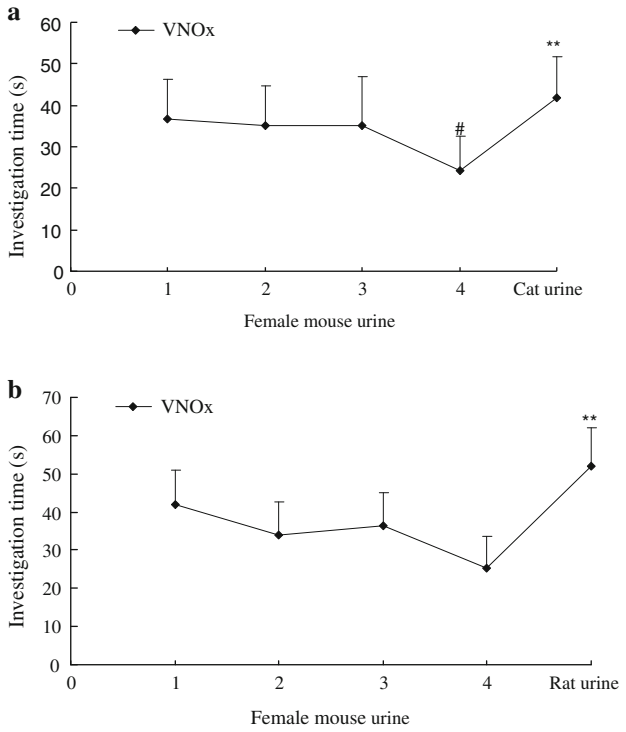

Fig. 3 A habituation–dishabituation test of the ability of VNOx male mice to discriminate between female mouse urine and cat (**a**) or rat (**b**) urine (mean ± SE, $n_{VNOx} = 13$). (Paired-samples t test or Wilcoxon signed ranks test was used according to the data distribution; $^{\#}P < 0.05$, $^{**}P < 0.01$, with $^{\#}$indicating the comparison between the fourth and the first trials, and **indicating the comparison between the fifth and the fourth trials in the habituation–dishabituation test)

VNO and olfactory offspring discrimination

VNOi males spent less time investigating odor from the anogenital area of offspring compared with the same odor of non-offspring ($t = 2.703$, $P = 0.027$), whereas VNOx males responded equally to these two types of odors ($z = 0.267$, $P = 0.790$) (Fig. 4a). However, VNOx males were capable of discriminating between the odors of offspring and non-offspring in the habituation–dishabituation test (offspring$_3$ − offspring$_1$: $z = 1.988$, $P = 0.047$; non-offspring − offspring$_3$: $t = 3.298$, $P = 0.008$; the subscripts indicate the times the odor was presented) (Fig. 4b).

VNO and olfactory mate recognition and discrimination

VNOi males preferred the urine odor of other females to their own female partners ($z = 1.988$, $P = 0.047$), whereas VNOx males responded equally between them ($t = 0.562$, $P = 0.585$) (Fig. 5a). However, the VNOx males were capable of discriminating the urine odor of mates from that of non-mates (mate$_3$ − mate$_1$: $z = 2.275$, $P = 0.023$; non-mate − mate$_3$: $z = 2.824$, $P = 0.005$; the subscripts indicate the times the odor was presented) (Fig. 5b).

VNO ablation and anxiety-like behavior of male mice

The 10-min light/dark box tests showed that VNOi males exhibited more frequent crossing between light and dark boxes than did VNOx males ($z = 1.959$, $P = 0.050$; Table 1), while the 10-min open field tests showed that VNOi males appeared to spend more time in the central grids [$t = 2.091$, $P = 0.053$ (marginal insignificance)] and more frequent urine marking ($z = 2.454$, $P = 0.014$) than did VNOx males (Table 2).

Effects of VNO ablation on important physiological organs

Body weights of VNOi and VNOx male mice did not differ on the postoperative 30th, 45th or 114th days from those before surgery (Table 3). At the end of the experiments

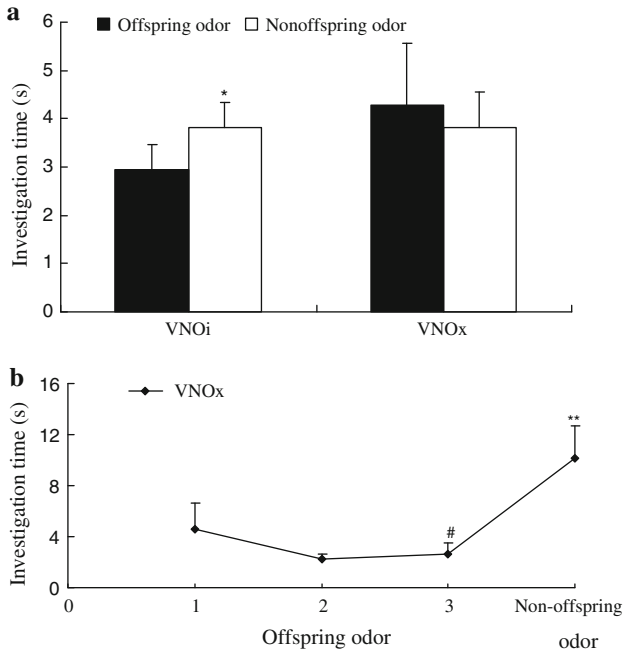

Fig. 4 Preferences or discrimination of VNOi and VNOx male mice between the anogenital area scents of offspring and non-offspring pups (mean ± SE). **a** Seconds spent investigating the two odor types in binary choice tests ($n_{VNOi} = 11$, $n_{VNOx} = 11$); **b** a habituation–dishabituation test of the ability of VNOx male mice to discriminate between the two odor types ($n_{VNOx} = 11$). (Paired-samples t test or Wilcoxon signed ranks test was used according to the data distribution; $^{\#}P < 0.05$, $*P < 0.05$, $**P < 0.01$; in **b**, with $^{\#}$indicating the comparison between the third and the first trials, and **indicating the comparison between the fourth and the third trials in habituation–dishabituation tests)

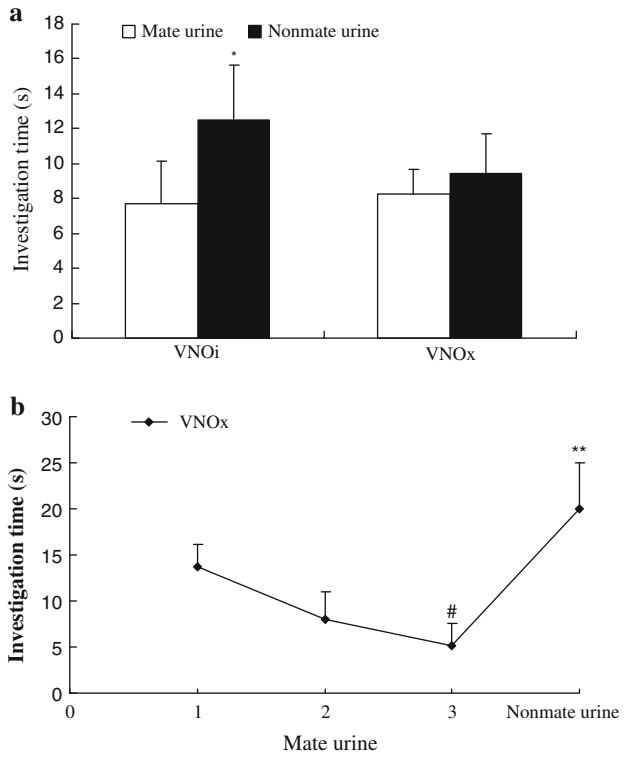

Fig. 5 Preferences or discrimination of VNOi and VNOx male mice between the urine of female mates and other females (mean ± SE). **a** Seconds spent investigating the two odor types in binary choice tests ($n_{VNOi} = 11$, $n_{VNOx} = 11$); **b** a habituation–dishabituation test of the ability of VNOx male mice to discriminate between the two odor types ($n_{VNOx} = 12$). (Paired-samples t test and Wilcoxon signed ranks test was used according to the data distribution; $^{\#}P < 0.05$, $*P < 0.05$, $**P < 0.01$, in **b**, with $^{\#}$indicating the comparison between the third and the first trials, and **indicating the comparison between the fourth and the third trials in habituation–dishabituation tests)

(the postoperative 114th day), we found that VNO dysfunction significantly atrophied adrenal glands and hypertrophied preputial glands in male mice as measured by both absolute weights ($t = 4.101$, $P < 0.001$, for adrenal; $z = 2.077$, $P = 0.038$, for preputial gland) and relative weights ($t = 3.396$, $P = 0.002$ for adrenal; $t = 2.325$, $P = 0.029$ for preputial gland) (Table 4).

Table 1 Comparison of behavioral patterns between VNOi and VNOx male mice in the light/dark box during 10-min tests (mean ± SE, $n_{VNOi} = 9$, $n_{VNOx} = 9$)

Behavior	VNOi	VNOx	Significance	
			t/z	P
Latency (s)	271.6 ± 57.28	381.4 ± 72.70	$t = 1.187$	0.252
Light time (s)	369.4 ± 39.93	415.4 ± 62.21	$t = 0.622$	0.543
Dark time (s)	217.8 ± 36.63	179.4 ± 60.99	$z = 0.539$	0.597
Crossing (times)	13.22 ± 3.609	5.111 ± 2.424	$z = 1.959$	0.05*

Independent samples t test and Mann–Whitney U test were used
* $P < 0.05$

Discussion

Our data show that sexually naïve male mice with VNO ablation exhibited no preference between female and male urine, but the discrimination ability remained, suggesting that the VNO is necessary in olfactory recognition of sex, but not in olfactory discrimination of sex-related odor (Pankevich et al. 2004). Here, discrimination refers to the differences in behavioral responses that individual shows; however, recognition refers to the neural processing that take place when animals classify conspecifics odors (Tang-Martinez 2001). The present results agree with previous findings that sexual preference for the odor of conspecific individuals of the opposite sex does rely on the VNO in the mouse (Wysocki et al. 1982, 2004; Del Punta et al. 2002; Stowers et al. 2002; Pankevich et al. 2004, 2006; Brennan and Zufall 2006; Keller et al. 2006; Zhang et al. 2008a). In addition, the disappearance of sex

Table 2 Comparison of behavioral patterns between VNOi and VNOx male mice in the open field during 10-min tests (mean ± SE, $n_{VNOi} = 9$, $n_{VNOx} = 9$)

Behavior	VNOi	VNOx	Significance	
			t/*z*	*P*
Latency (s)	4.778 ± 1.234	5.000 ± 1.716	$t = 0.105$	0.918
Center time (s)	27.31 ± 3.099	18.64 ± 2.751	$t = 2.091$	0.053
Periphery grids (numbers)	255.0 ± 28.41	266.1 ± 21.08	$t = 0.314$	0.758
Center grids (numbers)	30.44 ± 3.973	29.33 ± 4.634	$t = 0.182$	0.858
Locomotion (numbers)	285.4 ± 31.45	295.4 ± 24.07	$t = 0.253$	0.804
Rearing (times)	102.9 ± 13.29	102.3 ± 8.307	$t = 0.035$	0.972
Urine marking (times)	8.778 ± 1.516	3.778 ± 0.741	$z = 2.454$	0.014*

Independent samples *t* test and Mann–Whitney *U* test were used

* $P < 0.05$

Table 3 Comparison of body weight gain between VNOi and VNOx male mice (mean ± SD, $n_{VNOi} = 13$, $n_{VNOx} = 13$)

Experimental day	Body weight (g)		Significance	
	VNOi	VNOx	*t*/*z*	*P*
0	37.23 ± 3.982	37.75 ± 2.463	$z = 1.566$	0.117
30	38.61 ± 3.587	38.42 ± 2.552	$t = 0.158$	0.876
45	39.59 ± 3.438	39.60 ± 2.938	$z = 0.154$	0.878
112	41.02 ± 4.669	40.29 ± 3.282	$t = 0.449$	0.657

Independent samples *t* test and Mann–Whitney *U* test were used

* $P < 0.05$

preference of VNOx male mice confirms the success of the surgical removal of the VNO in our study. Although male mice with VNO ablation were able to discriminate between female and male odor based on the function of the olfactory epithelium, they did not grasp the biological meaning of the odor without their normal VNO functions.

Likewise, VNOx male mice showed no olfactory preference between female mice and predators, offspring and non-offspring pups, or female mates and non-mates. Yet they were still able to discriminate between these pairs of odor sources, suggesting that male mice with VNO ablation might not recognize the biological significance of the odors. The effects of alarm and predator odors on rodents have previously been shown to rely on the VNO of the rodents (Voznessenskaya et al. 1992; Sam et al. 2001; Kiyokawa et al. 2007; Muroi et al. 2006). Our data suggest that the biological significance of individuality-related volatile chemosignals (i.e., offspring vs non-offspring or opposite-sex vs same-sex partners) were also recognized via the VNO. These findings were consistent with the role of the vomeronasal system in recognizing individual odor types (Brennan 2004; Johnston and Peng 2000).

On the other hand, VNO ablation reduced the frequency of urine marking, the crossing behavior in the light/dark box, and the time spent in the central grids in the open field, suggesting that VNO ablation suppressed urine marking of male mice and induced anxiety-like or submissive behavior (Belzung and Griebel 2001). In contrast, golden hamsters with VNO lesion do not change their scent marking in either males or females (Petrulis et al. 1999; Kimchi et al. 2007).

Because adrenal glands release glucocorticoids in response to social stressors such as defeats (Johnston and Mueller 1990), the atrophy of adrenal glands induced by

Table 4 Comparison of organ weights between VNOi and VNOx male mice (mean ± SD, $n_{VNOi} = 13$, $n_{VNOx} = 13$)

Relevant organs	Weight[a]	VNOi	VNOx	Significance	
				t/*z*	*P*
Adrenals	Absolute (mg)	4.554 ± 0.7996	3.185 ± 0.8999	$t = 4.101$	<0.001**
	Relative (%)	11.19 ± 2.219	7.973 ± 2.596	$t = 3.396$	0.002**
Spleens	Absolute (mg)	90.66 ± 12.91	86.99 ± 22.53	$z = 1.103$	0.27
	Relative (%)	221.8 ± 29.2	213.4 ± 46.25	$t = 0.548$	0.589
Testes	Absolute (mg)	270.9 ± 46.48	269.6 ± 24.83	$t = 0.086$	0.932
	Relative (%)	659.5 ± 81.24	668.7 ± 74.21	$t = 0.303$	0.765
Epididymides	Absolute (mg)	113.3 ± 16.50	116.4 ± 11.61	$t = 0.557$	0.583
	Relative (%)	276.8 ± 34.87	287.9 ± 24.22	$t = 0.944$	0.354
Seminal vesicles	Absolute (mg)	566.8 ± 167.3	585.4 ± 167.3	$t = 0.297$	0.769
	Relative (%)	1385 ± 400.6	1439 ± 334.0	$t = 0.370$	0.715
Preputial glands	Absolute (mg)	115.6 ± 30.46	140.6 ± 35.25	$z = 2.077$	0.038*
	Relative (%)	281.4 ± 66.25	346.3 ± 75.69	$t = 2.325$	0.029*

Independent samples *t* test and Mann–Whitney *U* test were used

* $P < 0.05$, ** $P < 0.01$

[a] % = mg/100 g body weight

prolonged VNO dysfunction might damage the stress response of the mouse. Our results were consistent with this idea that VNOx mice had more anxiety-like or submissive behavior than did VNOi mice. Preputial glands are exocrine glands producing pheromones. VNO ablation led to the hypertrophy of preputial glands of male mice, which might imply a compensation of pheromone production for olfactory deficits (Brennan and Zufall 2006).

In conclusion, all the above-mentioned paired odors used in the current study could be discriminated by the male mice with VNO ablation. The mice without a functional VNO might smell out the difference in social odors, but they were unable to recognize the essential biological information contained in these odors. Meanwhile, long-term VNO dysfunction induced some changes in the physiology and behavior of mice. We think our results provide an important insight into the functional divergence between the VNO and the main olfactory system in detecting odor molecules.

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