Chem. Senses 36: 137–147, 2011

doi:10.1093/chemse/bjq104
Advance Access publication October 18, 2010

# Alteration of Mouse Urinary Odor by Ingestion of the Xenobiotic Monoterpene Citronellal

**Kazumi Osada[1], Masaaki Hanawa[2], Kenji Tsunoda[2] and Hiroshi Izumi[1]**

[1]Department of Oral Physiology, School of Dentistry, Health Sciences University of Hokkaido, Ishikari-Tobetsu, Hokkaido 061-0293, Japan and [2]Department of Research and Development, Daiichi Sankyo Healthcare Co. Ltd, Tokyo 103-8541, Japan

*Correspondence to be sent to: Kazumi Osada, Department of Oral Physiology, School of Dentistry, Health Sciences University of Hokkaido, Ishikari-Tobetsu, Hokkaido 061-0293, Japan. e-mail: osadak@hoku-iryo-u.ac.jp*

*Accepted September 12, 2010*

## Abstract

Body odors provide a rich source of sensory information for other animals. There is considerable evidence to suggest that short-term fluctuations in body odor can be caused by diet; however, few, if any, previous studies have demonstrated that specific compounds can directly mask or alter mouse urinary odor when ingested and thus alter another animal's behavior. To investigate whether the ingestion of citronellal, a monoterpene aldehyde that produces an intense aroma detected by both humans and mice, can alter mouse urinary odor, mice (C57BL6J) were trained in a Y maze to discriminate between the urinary odors of male donor mice that had ingested either citronellal in aqueous solution or a control solution. Trained mice could discriminate between urinary odors from the citronellal ingestion and control groups. A series of generalization tests revealed that citronellal ingestion directly altered mouse urinary odor. Moreover, trained mice that had successfully discriminated between urinary odors from donor mice of different ages failed to detect age-related changes in urine from male mice that had ingested 50 ppm of citronellal. This study is the first to show that ingestion of a xenobiotic can alter mouse urinary odor and confuse the behavioral responses of trained mice to age-related scents.

**Key words:** citronellal, odor masking, solid-phase microextraction, the scent of age, Y-maze

## Introduction

Body odors provide a rich source of chemical sensory information for other animals; for example, individual body scents, present in urine, are influenced by differences in genetically encoded species information (Yamazaki et al. 1994; Isles et al. 2001), sex (Novotny et al. 1985, 1986; Swaney et al. 2007; Ramm et al. 2008), individual identity (Yamazaki et al. 1976; Yamaguchi et al. 1981; Hurst et al. 2001), and kinship (Yamazaki et al. 2000; Broad et al. 2006) of the owner, as well as information about the animal's current reproductive status (Novotny et al. 1999; Zhang et al. 2008; Osada et al. 2009), virus infection status (Yamazaki et al. 2002), and age (Kokko and Lindstrom 1996; Ferkin 1999; Osada et al. 2003; Muller et al. 2006; Osada, Curran, et al. 2008), all of which may influence other animal's behavior and mate selection. It is generally believed that each of these characteristics must be relatively stable over a considerable time period in an individual animal in order to influence body odors so that the individual can be recognized in multiple behavioral and social contexts.

Nevertheless, there is considerable evidence that short-term fluctuations in body odor can result from dietary changes (Leon 1975; Beauchamp 1976; Ferkin et al. 1997; Kwak et al. 2008). Some reports suggest that dietary changes can mask genetically determined odortypes, preventing recognition of individuals (Brown et al. 1996). In a previous study by our group, a Y-maze olfactometer was used to demonstrate that age-related changes in urinary odor enable C57BL/6J (B6) mice to determine the ages of other mice by sensing changes in the amounts of several specific volatile compounds (Osada et al. 2003). In another study, we showed that aged B6 male mice developed an aging-associated odor that was attractive to female mice in an experimental setting and that this attraction was due to an increase in 3,4-dehydro-exo-brevicomin (DB), 2-*sec*-butyl-4,5-dihydrothiazole (BT), and 2-isopropyl-4,5-dihydrothiazole (IT) (Osada, Tashiro, et al. 2008). Another of our recent studies suggested that ingestion of mugwort and mushroom extract decreased the intensity of odors associated with aging in mice by

decreasing specific urinary chemicals that normally increase with age, although we found no evidence to suggest that xenobiotic chemicals from these edible herbs were released into the urine (Osada, Curran, et al. 2008).

Few, if any, studies have demonstrated that specific odorous compounds found in food directly mask or alter urinary odor when ingested and thus alter animal behavior. However, previous studies have not examined whether orally ingested odorous compounds are directly released into bodily fluids so that they can be detected by other animals, or if these exogenous compounds can mask age-related differences in mouse chemosignals embedded in the urine. The experiments described in this study have been designed to address these questions.

Citronellal was chosen as the experimental exogenous odorant for several reasons: it is a monoterpene aldehyde that produces one of the most intense aromas for humans (Appell 1974) and mice (Krautwurst et al. 1998), it is present at high concentrations in Japanese pepper (Jiang and Kubota 2004), and it is commonly used to mask unpleasant fatty and fishy flavors in broiled eel; large amounts of citronellal are detected in citrus fruit, lemon balm (*Melissa officinalis* L.), and lemon gum (*Eucalyptus citriodora*) (Choi and Sawamura 2002; Nhu-Trang et al. 2006); it is an aromatic, harmless (Gomes-Carneiro et al. 1998), and ubiquitous volatile compound found naturally in food; and it can be ingested by both humans and wild rodents.

Based on the above evidence, we hypothesized that citronellal may be an "edible perfume" capable of altering or masking a mouse's urinary odor. The most straightforward technique for discriminating between 2 chemosignals (i.e., whether they contain different information) is to test whether a sensor mouse can be trained to tell them apart. Hence, in this study, a Y-maze paradigm was used (Yamazaki et al. 1979; Yamaguchi et al. 1981; Beauchamp and Yamazaki 2003) in which mice were trained to discriminate between odor samples that differed only in the presence or absence of citronellal (studies 1 and 2) or in the age of the donor mouse (study 3), using a water reward for water-deprived odor sensor mice. The study covered a period of 10 months and involved approximately 3000 training and testing trials with urine and aqueous odor samples using a standard Y-maze system.

## Materials and methods

### Mice

Odor donor and trained odor sensor C57BL6J mice were bred at the Health Sciences University in Hokkaido. Animals were maintained in a room at 22 °C with a photoperiod of 12:12 (L:D nonreversed) and provided with ad libitum access to food (Lab Chow, MF, Oriental Yeast) and water. The animals were cared for in accordance with the NIH Guide for the Care and Use of Laboratory Animals, and the Animal Ethics and Research Committee of the Health Sciences University of Hokkaido approved the experimental protocols prior to experimentation.

### Administration of citronellal and collection of urine samples

To collect urine samples for use in the behavioral and chemical experiments conducted in studies 2 and 3, urine donor mice ingested citronellal solution (0, 10, or 50 ppm in drinking water; $n = 5$ per group) for 12 days. Urine samples were collected from young adult (Adult: 4–5 months old) and aged adult (Aged: 17–19 months old) male B6 mice housed in metabolic cages. For the behavioral studies, the test samples consisted of pooled urine samples collected from the experimental groups. To avoid diurnal fluctuations in urinary chemical content, urine collection was performed between 18:00 and 8:00. Urine sampling was conducted on days 1, 6, and 12. Voided urine samples were collected from Adult and Aged B6 mice using gentle abdominal pressure, diluted 5×, and used in training trials for study 3. Urine samples and the citronellal solutions were stored at –20 °C.

The citronellal solutions employed in this study had citronellal concentrations similar to those found naturally in plants (Nhu-Trang et al. 2006). Citronellal (10 and 50 ppm) and control (0 ppm citronellal) solutions were prepared as follows: undiluted citronellal (Sigma-Aldrich Corp.) was dissolved in ethanol to make a 10% solution and was then mixed with deionized water. To avoid a neophobic response by urine donor mice to the distinct odor of citronellal (Epple et al. 2004), 1.7% sucrose was added to the solutions. The control solution contained 1.7% sucrose and a volume of ethanol equivalent to that of citronellal in the corresponding experimental solution.

Urine donor mice ingested citronellal or control solution as the sole source of drinking water for 12 days. The average daily intake of the solutions was 9.5 ± 1.0 mL (50 ppm), 9.5 ± 0.4 mL (10 ppm), and 9.8 ± 1.8 mL (0 ppm), respectively; thus, citronellal neophobia was successfully prevented by the addition of sucrose. However, these intake levels were more than twice the normal tap water intake ($n = 3$: 4.2 ± 0.5 mL). There was no significant difference in fluid intake between the Adult and Aged mouse groups.

### Y maze

A custom-made Y maze (long arm length: 450 mm, short arm length: 400 mm, arm width: 100 mm) was constructed from Plexiglas (1 cm thick). The design and operation of the Y maze used in this study are described in a previously published report (Yamaguchi et al. 1981) and in the legend for Figure 1. Briefly, the 2 arms of the maze were scented by air currents conducted through chambers containing odor source materials in petri dishes.

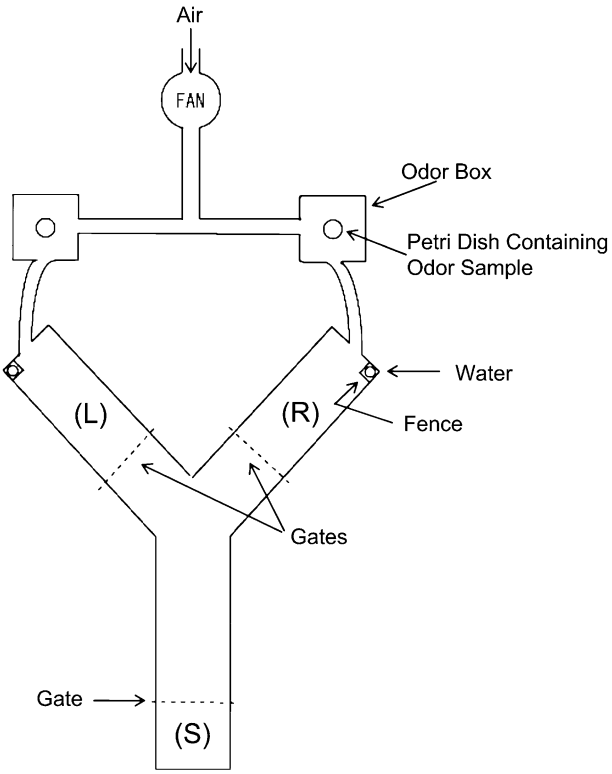


**Figure 1** Y maze. Air is drawn by a fan through a tube with an inlet near the input vent supplying the laboratory and is conducted through the left and right odor boxes. Each odor box has a lid to allow a petri dish containing the odor source to be placed. Air currents then pass through the left (L) and right (R) arms of the maze, which have transparent lids. Each arm of the maze is fitted with a plastic tube perforated at the bottom to make one drop of water available. Each water tube is blocked by a fence that is raised only if the mouse enters the arm scented by the odor consistent with its training. Each arm of the maze is fitted with a gate that is lowered once the mouse has entered. If the choice is incorrect, the fence is not raised, and the mouse is returned to the starting compartment (S). If the choice is correct, the fence is raised to give access to the drop of water. The time interval in the starting compartment is set at 30 s to allow the petri dishes in the odor boxes to be changed and to replace the drop of water if needed; after this, on a timed signal, all 3 gates are raised to commence the next trial. Left–right placing is determined by a series of random numbers. Trained mice make a choice in 2 or 3 s. Mice vary in their activity patterns prior to choosing a maze arm: sometimes the choice is made without pause and other times after sniffing at the entrance to the arms or sometimes with brief retracing from one arm to the other.

### Y-maze training and testing

For training and testing in the Y maze, gates were raised and lowered in a timed sequence for up to 48 consecutive trials per day, with the paired odor samples changed for each trial. Reward for the correct response was a drop of water; the odor sensor mice had been deprived of water for 23 h.

In previous studies (Yamazaki et al. 1979, 2002; Yamaguchi et al. 1981; Osada et al. 2003), authors have routinely conducted olfactory learning experiments in Y mazes using 4–6 mice as odor sensors and have produced reproducible results. Therefore, in this study, 5 male adult mice were trained in a Y maze using water as a reward to detect differences in urine samples based on the presence or absence of citronellal (studies 1 and 2) or to detect differences in age (study three).

After successful training (>75% correct for each odor sensor mouse), unrewarded trials were interspersed, at an average frequency of 1 in 4, with rewarded trials to acclimate the mice to the occasional absence of reward after a correct response. The mice performed with comparable accuracy during these trials. The same 5 odor sensor mice were used in all experiments. Mice 1–3 were reinforced to select citronellal samples (studies 1 and 2) and Aged urine samples (study 3), and Mice 4 and 5 were reinforced to select control samples (studies 1 and 2) and Adult urine samples (study 3).

### Generalization

After achieving the criterion response in the training trials (>75% correct for each odor sensor mouse), generalization trials were then instituted to test novel odor samples without reward and thus ensure that the odor sensor mice had learned to distinguish the class distinction rather than the individual samples used during training. The generalization procedure lends itself to the blind testing of coded samples because the operator of the maze is not required to supply a reward for concordant choices. To maintain reinforcement (correct response to the learned scent), unrewarded samples were interspersed uniformly with continued testing of the familiar training samples accompanied by a reward for the correct choice. Thus, odor sensor mice were never rewarded for correct choices during generalization trials, but these were interspersed with rewarded odor sample presentations.

### Testing schedule

This report consists of 3 different studies. Study 1 explored the citronellal odor threshold of odor sensor mice: Mice were trained to distinguish different concentrations of citronellal solution in water and a noncitronellal solution. The citronellal solutions were prepared as a 10% solution in ethanol and then mixed with deionized water. The control solution was made with deionized water mixed with an equal amount of ethanol to that in the citronellal solutions. These training samples were prepared immediately prior to Y-maze training. The training trials were conducted using a 1000 ppm citronellal solution and control solution, and the generalization trials were conducted using the same concentrations of citronellal solution and control solution, but in these trials, the solutions were made approximately 24 h before conducting the Y-maze experiment and stored in closed vial tubes at 22 °C. When odor sensor mice could successfully distinguish between the odor samples, the training and generalization samples were replaced with samples that had been serially diluted 10-fold. The odor thresholds of the sensor mice were designated as the citronellal concentrations between the lowest distinguishable citronellal concentration and the highest indistinguishable citronellal concentration.

Study 2 was conducted to clarify whether orally ingested citronellal was released into the urine and emitted as an exogenous odorant that modified mouse urine odor. Mice trained to discriminate between the urine of mice that had ingested citronellal and those that had ingested control solution were then presented with a choice of odor samples with or without citronellal.

Finally, study 3 was conducted to determine whether urinary citronellal can mask differences in mouse urine odor associated with aging. Mice trained to discriminate between 5-fold diluted urine samples from Aged versus Adult mice were then presented with a choice of undiluted urine samples from Aged versus Adult mice that had both ingested citronellal. For the behavioral studies, the test samples consisted of pooled urine samples collected from the groups.

Statistical tests (Fisher's Exact test) were conducted after combining data from all 5 test mice into a single value.

### Quantitation of volatile urinary chemicals using GC-MS

Chemical analysis by gas chromatography-quadrupole mass spectrometry (GC-MS) was conducted using a QP5000 ver. 2 (Shimadzu), and volatile urinary samples ($n = 5$ for each sample) were prepared using headspace solid-phase microextraction (HS-SPME) as previously described (Osada, Curran, et al. 2008). Briefly, a gas chromatograph was fitted with a Restek Stabilwax column (30 m × 0.32 mm × 0.5 μm) (Restek). The carrier gas was helium, and the column flow rate was 2.4 mL/min. The oven temperature was maintained at 40 °C for 5 min, then increased by 10 °C/min to 200 °C, and then increased by 5 °C/min to 240 °C. The injector temperature was held constant at 230 °C. To concentrate the volatile constituents of the urine, an SPME fiber (2 cm, 50/30 μm DVD/Carboxen/PDMS StableFlex, Supelco) was inserted into the headspace of a 4-mL vial for 30 min with a Teflon septum (Supelco) containing 150 μL of mouse urine that was then saturated with NaCl, warmed to 37–40 °C, and stirred constantly. Identification of citronellal structures and other volatile peaks in urine was performed using both the NIST library and manual interpretation of mass spectra based on comparisons with the literature. In addition, comparison of relative retention times and mass spectra of authentic chemicals was performed using synthetic samples (Tashiro and Mori 1999; Tashiro et al. 2008) or standard chemicals (Sigma-Aldrich). The levels of urinary citronellal were estimated by referring to the standard curve. The chemical concentrations used for the standard curves were 0.1, 1, and 5 ppm.

## Results

### Study 1: odor threshold of sensor mice to citronellal solution

The number of training trials presented in this study represents only those by animals that trained successfully (>75% correct for each odor sensor mice) and participated in the generalization trials. In study 1, the numbers of training trials and generalization trials included all trials by all 5 mice (Table 1). Mice were successfully trained to distinguish between 1000 ppm citronellal aqueous solution and control solution. Correct scores of greater than 80% were obtained for each of the 5 odor sensor mice tested, and this was confirmed in generalization (unrewarded) trials (Table 1, test 1). Various concentrations of citronellal aqueous solutions (from 100 ppm to 100 ppb) were tested on the 5 odor sensor mice in descending concentrations (Table 1, tests 2–5). Mice successfully distinguished the most dilute 100 ppb citronellal solution (70%, $P < 0.01$) but failed generalization trials using a 100 ppb citronellal sample prepared 24 h before the test (50%, not significant [NS]; Table 1, test 5). It is known that most odorants are easily oxidized in air during handling or storage (Oka et al. 2004) and that the composition of citronellal in essential oil (*Citrus tamurana*) is decreased significantly during storage at ambient temperature (Choi and Sawamura 2002). Therefore, the citronellal used in the generalization trials may have undergone decomposition during the 24 h before the test, resulting in the generation of derivatives and a reduction in concentration. Therefore, it was estimated that the odor threshold of trained sensor mice was close to a concentration of 100 ppb or less for the citronellal aqueous solution.

### Chemical analysis of mouse urinary citronellal

From the results of study 1, we determined that sensor mice were able to detect the odor of citronellal aqueous solution at a concentration of 100 ppb or less in the Y-maze system. However, because there are numerous odoriferous chemicals in mouse urine, it is conceivable that if citronellal is discharged into mouse urine following oral ingestion, sensor mice may need a concentration of citronellal much greater

**Table 1** Percentage of correct responses (no. of trials) by trained mice to different citronellal solutions

| Test | Citronellal solution | Training trials | Generalization trials |
|---|---|---|---|
| 1 | 1000 ppm | 88 (136) $P < 0.001$ | 94 (16) $P < 0.01$ |
| 2 | 100 ppm | 84 (183) $P < 0.001$ | 91 (22) $P < 0.01$ |
| 3 | 10 ppm | 84 (187) $P < 0.001$ | 88 (24) $P < 0.01$ |
| 4 | 1 ppm | 78 (133) $P < 0.001$ | 92 (13) $P < 0.05$ |
| 5 | 0.1 ppm | 70 (135) $P < 0.001$ | 50 (14) NS |

Fisher's Exact test was used to determine statistical significance after combining data from all 5 odor sensor mice into a single value. Mice 1–3 were reinforced for citronellal solution, and mice 4 and 5 were reinforced for water. The number of training trials in this study represents only those mice that also underwent generalization trials. In all training trials, each odor sensor mouse performed better than could be expected by chance (50%). This was also the case in generalization trials, except in test 5, in which the response pattern was random.

than the threshold of 100 ppb to distinguish differences in urinary odor due to the presence or absence of citronellal. To determine the concentration of citronellal required for ingestion by urine donor mice to enable detection, chemical analysis was conducted of urine from mice that ingested 0, 10, or 50 ppm citronellal in their drinking water for 1, 6, or 12 days (Figures 2 and 3). The results showed that urine donor mice that had ingested 50 ppm citronellal solution excreted urine containing 600 ppb citronellal in the first day after citronellal ingestion and over 900 ppb on and after day 6. The urine of mice that ingested urinary citronellal concentrations of 10 ppm contained approximately 100 ppb of citronellal (Figure 3). Hence, in study 2, we used the urine of donor mice that had ingested 50 ppm citronellal solution for 12 days as the odor source.

### Study 2: odor detection of urinary citronellal by test animals

In study 2, the number of training trials and generalization trials included all trials by all 5 mice (Table 2). Mice were successfully trained to distinguish between the urine of mice that had ingested 50 ppm citronellal and that of mice that had ingested the control solution (Table 2). The generalization trials confirmed that citronellal ingestion changed the urinary odor of urine donor mice for both the 12 day citronellal ingestion samples (80%, $P < 0.01$; Table 2, test 1), and the 6 day ingestion samples (78%, $P < 0.01$; Table 2, test 2). These data indicated that urinary citronellal could be detected by the sensor mice even though the urinary samples were harvested after different administration time periods. To determine whether the distinction observed was attributable specifically to the novel odor imparted by citronellal, rather than to the effects of other odoriferous urinary compounds, Y-maze generalization assays were performed using control mouse urine samples with 500 ppb citronellal added and control mouse urine containing the same amount of ethanol, which was the solvent used to dissolve citronellal. Test animals discriminated between the presence or absence of citronellal in the urine samples, confirming that the distinction was due to the presence of citronellal (84%, $P < 0.01$; Table 2, test 3). This finding suggests that alterations in mouse urinary odor following citronellal ingestion are due to citronellal and not due to the effects of citronellal metabolites or to alterations in the proportions of endogenous volatile urinary compounds in response to citronellal ingestion. However, in the generalization trials, test animals failed to discriminate between the presence or absence of 500 ppb citronellal in aqueous solution, suggesting that the interaction between citronellal and other urinary odorants altered the odor (58%, NS; Table 2, test 4).

### Study 3: effects of citronellal ingestion on discrimination of urine from aged mice

In order to determine whether test animals could detect age-related differences in the urinary odor of mice that had ingested citronellal, we again used a Y-maze assay. To show the individual differences between odor sensor mice, we have presented the numbers of training and generalization trials separately (Table 3). The preliminary training trial showed

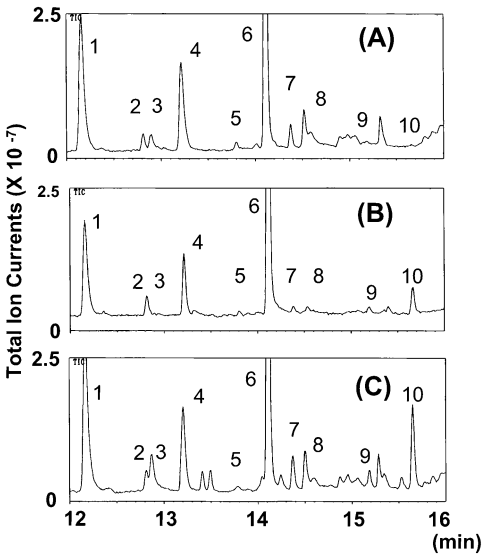


**Figure 2** Chromatograms from GC-MS analysis of HS-SPME of typical urine samples from individual Adult mice that had ingested citronellal solution at 0 ppm **(A)**, 10 ppm **(B)**, and 50 ppm **(C)** for 6 days. Numbers refer to the following compounds: 1, 6-methyl-5-heptene-3-one[a]; 2, IT; 3, 2-nonen-1-ol[a]; 4, DB; 5, acetic acid; 6, BT; 7, 2-ethyl-2-hexanol; 8, unknown; 9, benzaldehyde; 10, citronellal. a: Tentatively identified by mass spectrum (quadrupole).

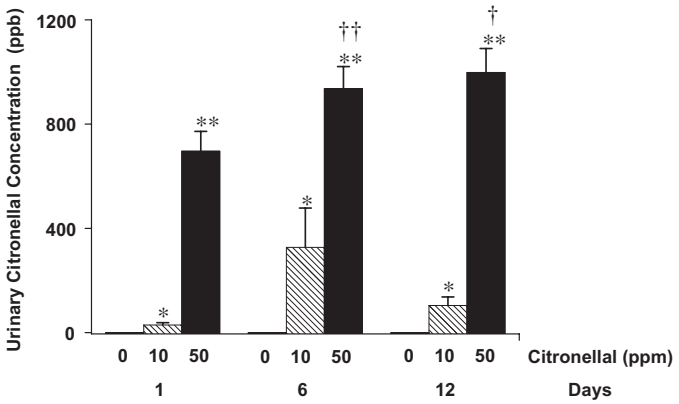


**Figure 3** Changes in urinary citronellal levels over time in male mouse urine. Urine samples were collected from urine donor mice that had ingested 10 ppm or 50 ppm citronellal solution. Five urine donor mice provided urine samples that were used for chemical analysis. These data were compared by 2-way analysis of variance followed by a post hoc test, Tukey's honest significant difference test. Significance is represented by a dagger symbol for comparison between ingestion periods and by an asterisk for comparison of citronellal concentrations (*$P < 0.05$ for 0 ppm, **$P < 0.001$ for 0 ppm and 10 ppm, †$P < 0.05$, ††$P < 0.01$ for day 1).

**Table 2** Percentage of correct responses (no. of trials) by trained mice to mouse urine with or without citronellal

| Test | Training trials | | Generalization trials | |
|---|---|---|---|---|
| 1 | cit-U versus cont-U (12 days) | 79 (155) $P < 0.001$ | cit-U versus cont-U (12 days) | 80 (20) $P < 0.01$ |
| 2 | cit-U versus cont-U (12 days) | 85 (172) $P < 0.001$ | cit-U versus cont-U (6 days) | 78 (27) $P < 0.01$ |
| 3 | cit-U versus cont-U (12 days) | 87 (190) $P < 0.001$ | cont-U + cit versus cont-U + e | 84 (31) $P < 0.01$ |
| 4 | cit-U versus cont-U (12 days) | 83 (180) $P < 0.001$ | water + cit versus water + e | 58 (26) NS |

Mice were successfully trained to distinguish between the urine of mice that had ingested 50 ppm citronellal (cit-U) and that of mice that ingested control solution (cont-U). The number of training trials in this study represents only those mice that also underwent generalization trials. Fisher's Exact test was used to determine statistical significance after combining data from all 5 odor sensor mice into a single value. Mice 1–3 were reinforced for citronellal solution, and mice 4 and 5 were reinforced for water. In all training trials, each odor sensor mouse performed better than could be expected by chance (50%). This was also the case in generalization trials, except in test 4, in which the response pattern was random. Cit, citronellal (500 ppb); e, ethanol (citronellal solvent).

that the mice distinguished between whole voided urine from Aged and Adult mice (80% [correct 239, incorrect 58] $P < 0.001$; data not shown). Because sucrose was added to the citronellal solution, ingestion of citronellal solution was more than 2 times that of tap water. Therefore, it was predicted that the intrinsic urine odor of a mouse that had ingested citronellal would be weaker than usual; thus, in the training trials for study 3, mice were presented with 5-fold (5×) diluted Aged urine versus 5× diluted Adult urine (Osada et al. 2003). These training trials showed that mice were able to distinguish between 5× diluted Aged and Adult voided urine (79%, $P < 0.001$; Table 3); thus, the odor sensor mice could distinguish age based on urinary odor that was 5× weaker than the original voided urine.

Next, to determine whether citronellal ingestion inhibited discrimination between Adult versus Aged urine, we tested whether odor sensor mice initially trained to discriminate between 5× diluted Adult and 5× diluted Aged mouse urine were able to discriminate between urine from Adult and Aged mice that had consumed citronellal (0, 10, or 50 ppm) (Table 3, tests 2–4). The generalization tests supported the hypothesis that urinary citronellal has a significant effect on mouse behavior because it inhibited the ability of trained sensor mice to discriminate between Aged versus Adult urine samples. When mice trained to choose the maze arm scented with Aged urine were presented with a choice of urine from Aged and Adult mice that had ingested 50 ppm citronellal, they failed to show a preference for the correct response (Table 3, test 3).

In contrast, these mice could correctly distinguish urine from Aged and Adult mice that had ingested either vehicle (0 ppm citronellal) or 10 ppm citronellal (Table 3, tests 1 and 2). In study 3, odor sensor mouse 4 died before the last test (10 ppm citronellal) could be completed. However, loss of this mouse had only a limited effect on the results of the study, and the effects of 50 ppm citronellal ingestion in the odor masking test 3 remained when data from mouse 4 were omitted. (training trials 78%, $P < 0.001$; generalization trials: test 1 76%, $P < 0.05$; test 2, 75%, $P < 0.05$; test 3, 41%, NS).

**Table 3** Responses of trained mice to Adult and Aged mouse urine from animals that ingested citronellal drinks.

| Mouse | Dilute voided urine | | | 0 ppm Citronellal | | | 10 ppm Citronellal | | | 50 ppm Citronellal | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Training | | | Test 1 | | | Test 2 | | | Test 3 | | |
| | C | NC | % | C | NC | % | C | NC | % | C | NC | % |
| 1 | 180 | 51 | 78 | 9 | 3 | 75 | 7 | 4 | 64 | 4 | 8 | 33 |
| 2 | 97 | 29 | 77 | 4 | 1 | 80 | 6 | 2 | 75 | 2 | 2 | 50 |
| 3 | 101 | 25 | 80 | 7 | 1 | 88 | 9 | 0 | 100* | 3 | 2 | 60 |
| 4 | 114 | 20 | 85 | 8 | 0 | 100* | | n.t. | | 7 | 5 | 58 |
| 5 | 150 | 43 | 78 | 5 | 3 | 63 | 8 | 4 | 67 | 3 | 5 | 38 |
| Total | 642 | 168 | 79*** | 33 | 8 | 80** | 30 | 10 | 75* | 19 | 22 | 46 |

Mice 1–3 were reinforced for Aged (18 months) male mouse urine, and mice 4 and 5 were reinforced for Adult (5 months) male mouse urine. Tests were performed in this order: 0, 50, 10 ppm. C, correct; NC, not correct; n.t., not tested. Mouse 4 died before all tests could be completed. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$ (null hypothesis 50%) by Fisher's Exact test.

### Chemical analysis of urinary citronellal and aging-associated urinary chemosignals

To determine the effects of 50 ppm citronellal ingestion on urinary composition and urinary citronellal levels, urinary levels of citronellal, DB, BT, IT, and 6-hydroxy-6-methyl-3-heptanone (HMH, which is significantly decreased with age) (Osada, Tashiro, et al. 2008) were measured using HS-SPME in conjunction with GC-MS (Figures 4 and 5). This chemical analysis revealed that citronellal levels in the urine of Aged mice that consumed 50 ppm citronellal were nearly identical to those in young Adult mice that consumed 50 ppm citronellal (Figures 4 and 5A). In addition, although there were significant differences in the urinary levels of DB, BT, and HMH in Adult and Aged samples, there was no difference in content of these chemicals in the citronellal ingestion and control groups (Figures 4 and 5B–D). There was no significant difference in urinary IT between the citronellal and control groups or between the

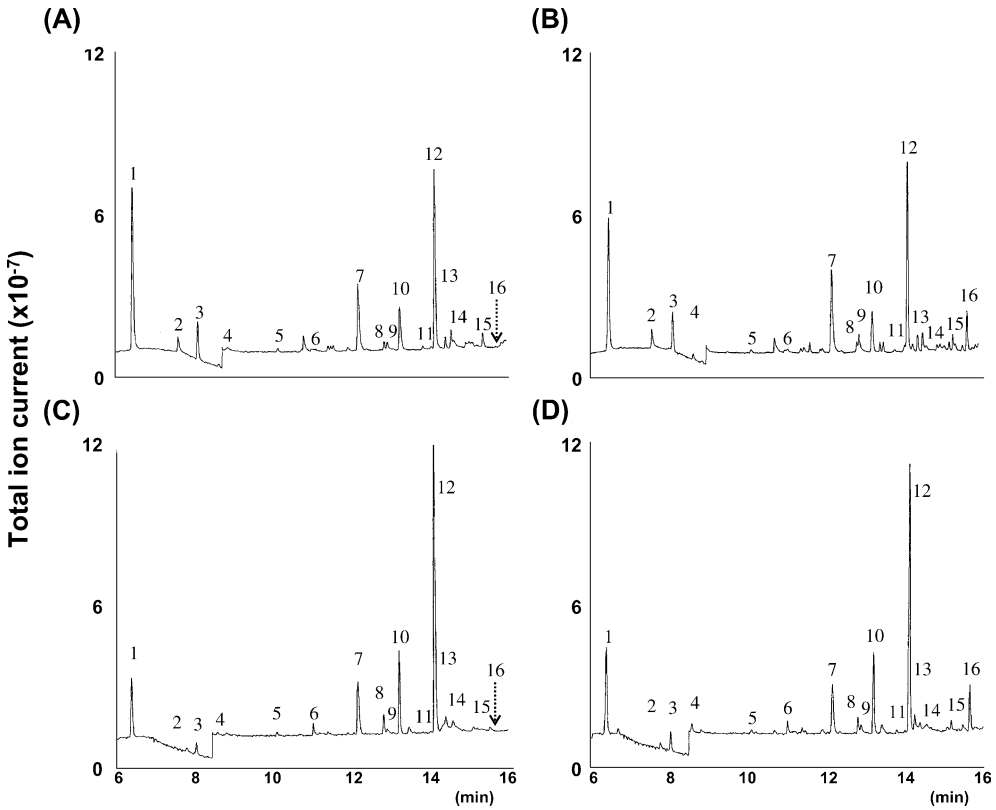


**Figure 4** Chromatograms from GC-MS analysis of HS-SPME of typical mouse urine samples from **(A)** Adult male + control solution intake, **(B)** Adult male+ 50 ppm citronellal intake, **(C)** Aged male + control solution intake, and **(D)** Aged male + 50 ppm citronellal intake for 6 days. Numbers refer to the following compounds: 1, 6-hydroxy-6-methyl-3-heptanone derivative; 2, *p*-xylene; 3, 6-hydroxy-6-methyl-3-heptanone derivative; 4, nitromethane; 5, 5-heptene-2-one[a]; 6, 4-methyl-6-heptene-3-one[a]; 7, 6-methyl-5-heptene-3-one[a]; 8, IT; 9, 2-nonen-1-ol[a]; 10, DB; 11, acetic acid; 12, BT; 13, 2-ethyl-2-hexanol; 14, unknowns; 15, benzaldehyde; and 16, citronellal. a: Tentatively identified by mass spectrum (quadrupole).

Adult and Aged groups (Adult control, 0.50 ± 0.06; Adult citronellal, 0.45 ± 0.06; Aged control, 0.61 ± 0.07; Aged citronellal, 0.62 ± 0.21. Total ion currents $\times 10^{-7}$).

## Discussion

The experiments described in this report demonstrate that an exogenous odorant, citronellal, was discharged into mouse urine following its ingestion and that citronellal altered the odor of normal mouse urine. In this study, we found that citronellal was released into mouse urine without undergoing chemical modification (Figures 2 and 3). In addition, we confirmed that animals could be successfully trained to distinguish between urine from mice that had ingested 50 ppm citronellal and from control mice (Table 2, test 3). Although it is known that the scent of mouse urine is created by hundreds of odoriferous chemicals, this study demonstrates that the test mice could recognize a difference in urinary odor due to the presence or absence of only one scent, citronellal (Table 2, tests 1–3).

There is considerable evidence to suggest that short-term fluctuations in body odors can be caused by diet (Leon 1975; Beauchamp 1976; Ferkin et al. 1997; Kwak et al. 2008). Some reports have suggested that dietary changes might mask genetically determined odortypes, thus preventing recognition of individual animals (Brown et al. 1996). The most robust genetic contributors to individual body scents occur at highly polymorphic sites, including the major histocompatibility complex (MHC, or H-2 in mice) on chromosome 17 (Boyse et al. 1983; Schaefer et al. 2002) and the major urinary proteins (MUPs) (Hurst et al. 2001) on chromosome 4 (Bishop et al. 1982). These genes can influence the composition of body odors that enable mice to distinguish one another according to the constellation of alleles carried. However, Kwak et al. (2008) demonstrated that although differences in MHC can be recognized even when obscured by major dietary changes, a significant change in diet has a greater effect on the total urinary odor profile than do MHC differences. In that study, the authors suggested that differences in urinary odor between different diet groups were determined by differences in the relative proportions of many volatile urinary compounds rather than by the presence or absence of specific compounds.

It has also been suggested that MUPs bind and release exogenous compounds. When a radiolabeled industrial chemical, a polychlorinated biphenyl derivative, was administered

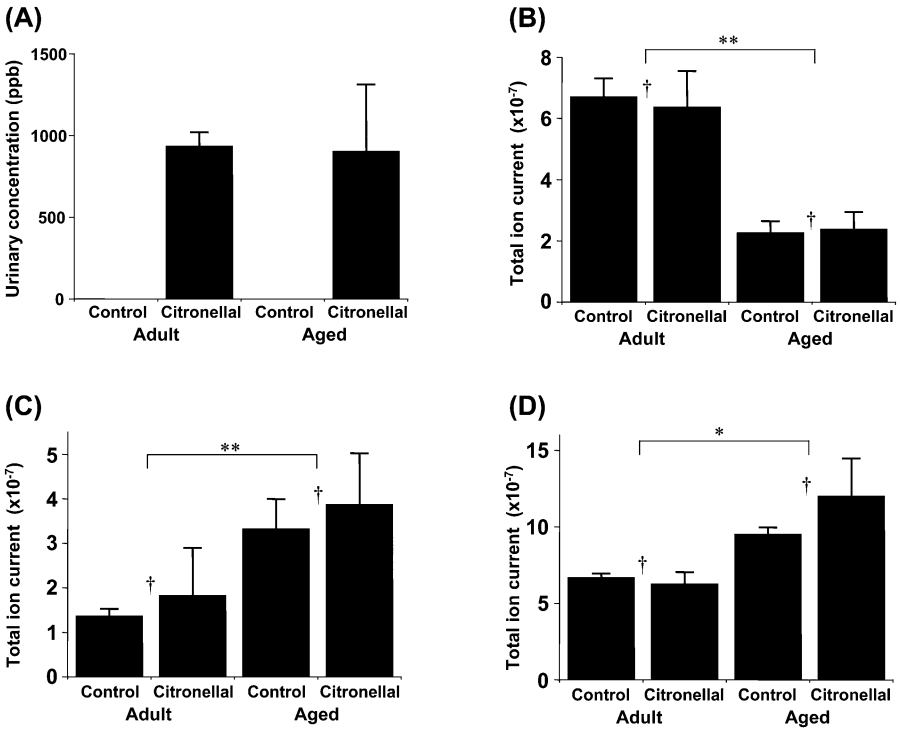


**Figure 5** Urinary levels of **(A)** citronellal, **(B)** HMH, **(C)** DB, and **(D)** BT from Adult and Aged urine donor mice that had ingested 50 ppm citronellal or control solution for 6 days. A panel of 5 donors provided the urine samples used for chemical analysis, and each data point represents the mean of the 5 samples. These data were compared by 2-way analysis of variance followed by a post hoc test, Tukey's honest significant difference test. Significance is represented by an asterisk for comparison of citronellal intake groups (*$P < 0.05$ between Adult and Aged urine donor groups, **$P < 0.01$ between Adult and Aged urine donor groups: †not significant between citronellal and control solution urine donor groups).

to male mice intraperitoneally, significant radioactivity was incorporated into urinary MUPs (Larsen et al. 1990). In addition, Robertson et al. (1998) reported that menadione, when injected subcutaneously, was physically associated with excreted male urinary MUPs. Therefore, it is conceivable that these xenobiotic substances alter the relative proportions of urinary semiochemicals, which are carried by MUPs. However, we are unaware of any previous reports of xenobiotic substances capable of changing urinary chemosignals through their own odor. The behavioral and chemical results of studies 2 and 3 indicate that citronellal was the essential odorant in urine from citronellal-ingesting mice. To our knowledge, this is the first investigation in mice to show that oral ingestion and urinary excretion of a specific chemical can mask urinary odor with its own odor.

In a previous study, it was reported that xenobiotic aldehydes, including citronellal, are degraded by liver microsomal enzymes into unsaturated hydrocarbons (Roberts et al. 1991). In that report, the authors showed that 2,6-dimethyl-1,5-heptadiene, the olefin predicted to result from the oxidative deformation of citronellal, was identified by GC-MS in the headspace gas of a reaction mixture containing citronellal and cytochrome P-450 2B4 (Roberts et al. 1991). Therefore, it is possible that this citronellal metabolite could be responsible for alterations in urinary odor. However, the results of the Y-maze assay in this study in which citronellal was added to or excluded from urine samples clearly indicated that odor sensor mice can distinguish between these samples (Table 2, test 3). In addition, chemical analysis in this study revealed that an infinitesimal quantity of 2,6-dimethyl-1,5-heptadiene was identified in urine samples from mice that had ingested 50 ppm citronellal (data not shown). Although we cannot rule out subtle changes in urine odor by unknown citronellal metabolites as a consequence of citronellal ingestion, these behavioral and chemical data imply that citronellal itself can modify mouse urinary odor.

In previous studies of humans, the odor of amniotic fluid obtained from women who had ingested garlic capsules was judged to be stronger or more garlicky than paired samples collected from women who had consumed placebo capsules (Mennella et al. 1995). In another chemical study, it was shown that allyl methyl sulfide, one of the odoriferous volatile sulfur compounds in garlic, was absorbed from the gut into the bloodstream and then excreted in the alveolar air and urine (Suarez et al. 1999). Therefore, it is possible that citronellal is excreted in a similar manner without undergoing chemical modification.

In addition, this study showed that age-related changes in the urinary odor of male mice that had ingested 50 ppm citronellal were masked by citronellal released into the urine (Table 3: test 3, Figures 4 and 5). Chemical analysis in study

3 showed that urinary citronellal levels were the same in Adult and Aged mice that had ingested 50 ppm citronellal but that citronellal was absent in the urine of control animals (Figures 4 and 5A). In previous Y-maze experiments using voided urine, we revealed that inbred aged male mice developed an age-associated odor that was distinguishable to sensor mice in an experimental setting (Osada et al. 2003). In addition, we identified chemical components present in the urine and showed that aged mice have higher levels of the volatile pheromones DB, BT and IT, and lower levels of the volatile pheromone HMH (Osada, Tashiro, et al. 2008), which play a behaviorally significant role in mediating female recognition of mouse urine from older mice. The present chemical study also clearly indicated significant changes in the levels of these chemicals with age (Figures 4 and 5B–D). There were no significant differences in urinary IT levels between the citronellal ingestion and control groups or between the Adult and Aged groups. Because the urinary concentration of IT was about one-tenth that of DB and BT, it is possible that the higher intake of experimental solutions due to the presence of 1.7% sucrose, resulting in more dilute urine, obscured any differences in urinary IT content in the Adult and Aged groups. Therefore, the behavioral data presented in this report suggest that the odor of urinary citronellal masks age-related changes in the content of major volatile urinary compounds that occur with increasing age.

It is possible that subtle differences in other components of the urine from mice that did or did not ingest citronellal might also impair the discriminability of aged urine in odor sensor mice. However, chemical analysis in study 3 indicated that several natural urinary components known to contribute to aging-related changes in urinary odor, namely HMH, DB, BT and IT, were not altered by oral citronellal ingestion (Figures 4 and 5). Therefore, these chemical data consist with the hypothesis that the odor of citronellal itself can mask age-related changes in urinary odor.

In this study, the same 5 odor sensor mice were used in all Y-maze experiments. Therefore, it is postulated that the failure of animals to discriminate when detectable levels of citronellal should be present (test 3) might be explained by a preservation of previously trained responses to citronellal. However, we know that odor sensor mice can quickly adopt the novel set of training samples and respond accurately to the generalization samples even though they were confronted quite different kind of odor samples (Osada et al. 2003). Therefore we do not think that previously trained responses to citronellal disturb the results of the other series of Y-maze tests. To completely preclude the above possibility, further experimental work is desired to perform the Y-maze test (study 3, test 3) using odor sensor mice that do not know the citronellal odor.

In rodents, older males are often preferred mates because they carry ''good'' genes that account for their longevity (Trivers 1972; Halliday 1978, 1983; Manning 1985; Kokko and Lindstrom 1996; Brooks and Kemp 2001), and females show greater attraction to the odor of male mouse urine when the urine is from aged males (Ferkin 1999; Osada, Tashiro, et al. 2008). Thus, this study suggests that the administration of citronellal to wild mice could result in a decrease in population by inhibiting the attraction of females to male mice without the need for application of toxic substances. Clearly, further experimental work is needed to determine whether citronellal ingestion actually affects the response to age-related odors in male mouse urine by female mice.

Perception of age-related changes in urinary odors was not affected by ingestion of 10 ppm citronellal solution (Table 3, test 2). These behavioral data suggest that the concentration of citronellal in the urine of these mice was insufficient to mask age-related changes in mouse urine.

It is not yet known which of the olfactory systems, the main olfactory system or the vomeronasal system, is mainly responsible for detecting differences in volatile urinary compounds in adult and aged male mouse urine. In a previous study, in vitro electrophysiology of the mouse vomeronasal organ (VNO) revealed that 6 volatile pheromones, including DB and BT, which are increased with age in male mouse urine, induced excitatory responses in single vomeronasal neurons, leading to the generation of action potentials and elevated calcium influx (Leinders-Zufall et al. 2000). However, another study found that DB and BT did not increase Fos protein immunoreactivity (Fos-IR) in the mouse VNO (Kimoto et al. 2005). Therefore, it remains unclear whether the volatile chemosignals in male mouse urine can stimulate the murine accessory olfactory bulb. A previous study showed that although cAMP levels in vomeronasal cells changed after exposure to DB and BT in a dose-dependent manner, cAMP levels in these cells were not affected by citronellal exposure (Zhou and Moss 1997). Therefore, we postulate that citronellal may mask the response of the main olfactory system to other urinary odorants; however, the mechanisms governing this masking effect remain elusive.

One recent report suggested that fennel and clove odors (which add flavor and mask fat and fish odors) activated glomeruli and suppressed mitral cell activity in fish odorant-responsive glomerular clusters via lateral inhibitory connections in the local neuronal circuits of the olfactory bulb (Takahashi et al. 2004). Citronellal is one of the major components in Japanese pepper; therefore, it is conceivable that citronellal could suppress age-related changes in male mouse urine odor in the same manner as fennel and clove odors. Alternatively, citronellal could directly suppress olfactory nerve responses (Sanhueza and Bacigalupo 1999) to urinary chemicals, acting as an olfactory receptor antagonist (Oka et al. 2004) or as an inhibitor of the cyclic nucleotide-gated channels located on olfactory nerve cells (Kurahashi et al. 1994).

In conclusion, although the results presented in this study were observed in an experimental setting, our findings clearly illustrate the following points: 1) odor from an exogenous

odorant, citronellal, was discharged into mouse urine following its ingestion; 2) citronellal in mouse urine altered the odor of the urine; and 3) age-related changes in male mouse urine odor were masked by citronellal released into the urine. The finding of odor masking by citronellal described in this report provides a strong rationale for further studies not only in mice but also in other mammals that rely strongly on chemicals to communicate social and sexual messages, including humans.

## References


Appell L. 1974. Odor intensity. Am Perfumer Cosmet. 79:43–48.

Beauchamp GK. 1976. Diet influences attractiveness of urine in guinea pigs. Nature. 263:587–588.

Beauchamp GK, Yamazaki K. 2003. Chemical signalling in mice. Biochem Soc Trans. 31:147–151.

Bishop JO, Clark AJ, Clissold PM, Hainey S, Francke U. 1982. Two main groups of mouse major urinary protein genes, both largely located on chromosome 4. Embo J. 1:615–620.

Boyse EA, Beauchamp GK, Yamazaki K. 1983. Critical review: the sensory perception of genotypic polymorphism of the major histocompatibility complex and other genes: some physiological and phylogenetic implications. Hum Immunol. 6:177–183.

Broad KD, Curley JP, Keverne EB. 2006. Mother–infant bonding and the evolution of mammalian social relationships. Philos Trans R Soc Lond B Biol Sci. 361:2199–2214.

Brooks R, Kemp DJ. 2001. Can older males deliver the good genes? Trends Ecol Evol. 16:308–313.

Brown RE, Schellinck HM, West AM. 1996. The influence of dietary and genetic cues on the ability of rats to discriminate between the urinary odors of MHC-congenic mice. Physiol Behav. 60: 365–372.

Choi HS, Sawamura M. 2002. Effects of storage conditions on the composition of Citrus tamurana Hort. Ex Tanaka (hyuganatsu) essential oil. Biosci Biotechnol Biochem. 66:439–443.

Epple G, Bryant BP, Mezine I, Lewis S. 2004. Zanthoxylum piperitum (dc), a potential feeding deterrent for mammals: studies with Microtus ochrogaster (Wagner). Pest Manag Sci. 60:624–630.

Ferkin MH. 1999. Attractiveness of opposite-sex odor and responses to it vary with age and sex in meadow voles (Microtus pennsylvanicus). J Chem Ecol. 25:757–769.

Ferkin MH, Sorokin ES, Johnston RE, Lee CJ. 1997. Attractiveness of scents varies with protein content of the diet in meadow voles. Anim Behav. 53:133–141.

Gomes-Carneiro MR, Felzenszwalb I, Paumgartten FJ. 1998. Mutagenicity testing (+/-)-camphor, 1,8-cineole, citral, citronellal, (-)-menthol and terpineol with the salmonella/microsome assay. Mutat Res. 416: 129–136.

Halliday TR. 1978. Sexual selection and mate choice. Oxford: Blackwell Scientific.

Halliday TR. 1983. The study of mate choice. Cambridge: Cambridge University Press.

Hurst JL, Payne CE, Nevison CM, Marie AD, Humphries RE, Robertson DH, Cavaggioni A, Beynon RJ. 2001. Individual recognition in mice mediated by major urinary proteins. Nature. 414:631–634.

Isles AR, Baum MJ, Ma D, Keverne EB, Allen ND. 2001. Urinary odour preferences in mice. Nature. 409:783–784.

Jiang L, Kubota K. 2004. Differences in the volatile components and their odor characteristics of green and ripe fruits and dried pericarp of Japanese pepper (Xanthoxylum piperitum dc.). J Agric Food Chem. 52: 4197–4203.

Kimoto H, Haga S, Sato K, Touhara K. 2005. Sex-specific peptides from exocrine glands stimulate mouse vomeronasal sensory neurons. Nature. 437:898–901.

Kokko H, Lindstrom J. 1996. Evolution of female preference for old mates. Proc R Soc Lond B Biol Sci. 263:1533–1538.

Krautwurst D, Yau KW, Reed RR. 1998. Identification of ligands for olfactory receptors by functional expression of a receptor library. Cell. 95:917–926.

Kurahashi T, Lowe G, Gold GH. 1994. Suppression of odorant responses by odorants in olfactory receptor cells. Science. 265:118–120.

Kwak J, Willse A, Matsumura K, Curran Opiekun M, Yi W, Preti G, Yamazaki K, Beauchamp GK. 2008. Genetically-based olfactory signatures persist despite dietary variation. PLoS One. 3:e3591.

Larsen GL, Bergman A, Klasson-Wehler E. 1990. A methylsulphonyl metabolite of a polychlorinated biphenyl can serve as a ligand for alpha 2mu-globulin in rat and major-urinary-protein in mice. Xenobiotica. 20: 1343–1352.

Leinders-Zufall T, Lane AP, Puche AC, Ma W, Novotny MV, Shipley MT, Zufall F. 2000. Ultrasensitive pheromone detection by mammalian vomeronasal neurons. Nature. 405:792–796.

Leon M. 1975. Dietary control of maternal pheromone in the lactating rat. Physiol Behav. 14:311–319.

Manning JT. 1985. Choosy females and correlates of male age. J Theor Biol. 116:349–354.

Mennella JA, Johnson A, Beauchamp GK. 1995. Garlic ingestion by pregnant women alters the odor of amniotic fluid. Chem Senses. 20: 207–209.

Muller MN, Thompson ME, Wrangham RW. 2006. Male chimpanzees prefer mating with old females. Curr Biol. 16:2234–2238.

Nhu-Trang TT, Casabianca H, Grenier-Loustalot MF. 2006. Authenticity control of essential oils containing citronellal and citral by chiral and stable-isotope gas-chromatographic analysis. Anal Bioanal Chem. 386: 2141–2152.

Novotny M, Harvey S, Jemiolo B, Alberts J. 1985. Synthetic pheromones that promote inter-male aggression in mice. Proc Natl Acad Sci U S A. 82: 2059–2061.

Novotny M, Jemiolo B, Harvey S, Wiesler D, Marchlewska-Koj A. 1986. Adrenal-mediated endogenous metabolites inhibit puberty in female mice. Science. 231:722–725.

Novotny MV, Ma W, Wiesler D, Zidek L. 1999. 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 Biol Sci. 266:2017–2022.

Oka Y, Nakamura A, Watanabe H, Touhara K. 2004. An odorant derivative as an antagonist for an olfactory receptor. Chem Senses. 29:815–822.

Osada K, Curran M, Kurachi M, Tsunoda K, Yamazaki K. 2008. Effect of an orally ingested mugwort and mushroom extract mixture on urine odor from aged mice. Biosci Biotechnol Biochem. 72:1249–1256.

Osada K, Kashiwayanagi M, Izumi H. 2009. Profiles of volatiles in male rat urine: the effect of puberty on the female attraction. Chem Senses. 34: 713–721.

Osada K, Tashiro T, Mori K, Izumi H. 2008. The identification of attractive volatiles in aged male mouse urine. Chem Senses. 33:815–823.

Osada K, Yamazaki K, Curran M, Bard J, Smith BP, Beauchamp GK. 2003. The scent of age. Proc R Soc Lond B Biol Sci. 270:929–933.

Ramm SA, Cheetham SA, Hurst JL. 2008. Encoding choosiness: female attraction requires prior physical contact with individual male scents in mice. Proc R Soc Lond B Biol Sci. 275:1727–1735.

Roberts ES, Vaz AD, Coon MJ. 1991. Catalysis by cytochrome p-450 of an oxidative reaction in xenobiotic aldehyde metabolism: deformylation with olefin formation. Proc Natl Acad Sci U S A. 88:8963–8966.

Robertson DHL, Hurst JL, Hubbard S, Gaskell SJ, Beynon RJ. 1998. Ligands of urinary lipocalins from the mouse: update of environmentally derived chemicals. J Chem Ecol. 24:1127–1140.

Sanhueza M, Bacigalupo J. 1999. Odor suppression of voltage-gated currents contributes to the odor-induced response in olfactory neurons. Am J Physiol. 277:C1086–C99.

Schaefer ML, Yamazaki K, Osada K, Restrepo D, Beauchamp GK. 2002. Olfactory fingerprints for major histocompatibility complex-determined body odors II: relationship among odor maps, genetics, odor composition, and behavior. J Neurosci. 22:9513–9521.

Suarez F, Springfield J, Furne J, Levitt M. 1999. Differentiation of mouth versus gut as site of origin of odoriferous breath gases after garlic ingestion. Am J Physiol. 276:G425–G430.

Swaney WT, Curley JP, Champagne FA, Keverne EB. 2007. Genomic imprinting mediates sexual experience-dependent olfactory learning in male mice. Proc Natl Acad Sci U S A. 104:6084–6089.

Takahashi YK, Nagayama S, Mori K. 2004. Detection and masking of spoiled food smells by odor maps in the olfactory bulb. J Neurosci. 24:8690–8694.

Tashiro T, Mori K. 1999. Pheromone synthesis, CXCVII synthesis of enantiomers of 2-sec-butyl-4,5-dihydrothiazole and (1r,5s,7r)-3,4-dehydro-exo-brevicomin, pheromone components of the male mouse, Mus musculus. Eur J Org Chem. 2167–2173.

Tashiro T, Osada K, Mori K. 2008. Syntheses of 2-isopropyl-4,5-dihydrothiazole and 6-hydroxy-6-methyl-3-heptanone, pheromone components of the male mouse, Mus musculus. Biosci Biotechnol Biochem. 72: 2398–2402.

Trivers RL. 1972. Paternal investment and sexual selection. Chicago: Aldine Publishing Company.

Yamaguchi M, Yamazaki K, Beauchamp GK, Bard J, Thomas L, Boyse EA. 1981. Distinctive urinary odors governed by the major histocompatibility locus of the mouse. Proc Natl Acad Sci U S A. 78:5817–5820.

Yamazaki K, Beauchamp GK, Curran M, Bard J, Boyse EA. 2000. Parent-progeny recognition as a function of MHC odortype identity. Proc Natl Acad Sci U S A. 97:10500–10502.

Yamazaki K, Beauchamp GK, Shen FW, Bard J, Boyse EA. 1994. Discrimination of odortypes determined by the major histocompatibility complex among outbred mice. Proc Natl Acad Sci U S A. 91: 3735–3738.

Yamazaki K, Boyse EA, Bard J, Curran M, Kim D, Ross SR, Beauchamp GK. 2002. Presence of mouse mammary tumor virus specifically alters the body odor of mice. Proc Natl Acad Sci U S A. 99:5612–5615.

Yamazaki K, Boyse EA, Mike V, Thaler HT, Mathieson BJ, Abbott J, Boyse J, Zayas ZA, Thomas L. 1976. Control of mating preferences in mice by genes in the major histocompatibility complex. J Exp Med. 144: 1324–1335.

Yamazaki K, Yamaguchi M, Baranoski L, Bard J, Boyse EA, Thomas L. 1979. Recognition among mice. Evidence from the use of a y-maze differentially scented by congenic mice of different major histocompatibility types. J Exp Med. 150:755–760.

Zhang JX, Sun L, Zhang JH, Feng ZY. 2008. Sex- and gonad-affecting scent compounds and 3 male pheromones in the rat. Chem Senses. 33: 611–621.

Zhou A, Moss RL. 1997. Effect of urine-derived compounds on camp accumulation in mouse vomeronasal cells. Neuroreport. 8:2173–2177.