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# PARTICIPATION OF THE PERIPHERAL TASTE SYSTEM IN AGING-DEPENDENT CHANGES IN TASTE SENSITIVITY

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**Abstract—Previous studies have shown that aging modifies taste sensitivity. However, the factors affecting the changes in taste sensitivity remain unclear. To investigate the cause of the age-related changes in taste sensitivity, we compared the peripheral taste detection systems in young and old mice. First, we examined whether taste sensitivity varied according to age using behavioral assays. We confirmed that the taste sensitivities to salty and bitter tastes decreased with aging. In other assays, the gustatory nerve responses to salty and sweet tastes increased significantly with aging, while those to bitter taste did not change. Thus, the profile of the gustatory nerve responses was inconsistent with the profile of the behavioral responses. Next, we evaluated the expressions of taste-related molecules in the taste buds. Although no apparent differences in the expressions of representative taste receptors were observed between the two age groups, the mRNA expressions of signaling effectors were slightly, but significantly, decreased in old mice. No significant differences in the turnover rates of taste bud cells were observed between the two age groups. Thus, we did not observe any large decreases in the expressions of taste-related molecules and turnover rates of taste bud cells with aging. Based on these findings, we conclude that changes in taste sensitivity with aging were not caused by aging-related degradation of peripheral taste organs. Meanwhile, the concentrations of several serum components that modify taste responses changed with age. Thus, taste signal-modifying factors such as serum components may have a contributing role in aging-related changes in taste sensitivity.** 

**Key words: aging, taste, taste detection, peripheral taste system.**

## INTRODUCTION

In advanced countries, the general population is getting older. Taste is a vital sense during nutritional intake and consequently in the maintenance of health and longevity. In general, the pleasure derived from eating food diminishes with age, partly through deterioration of smell and taste sensations. Elucidation of the age-dependent changes in taste cognition is important for better understanding of the factors that govern the changes in taste perception during an individual's lifespan.

Taste is classified into five basic categories: salty, sweet, bitter, sour, and umami. The taste of food is detected by taste cells that transmit information to the gustatory nerves and subsequently to the central nervous system (Yarmolinsky et al., 2009). Recent studies have identified taste receptors and taste-related molecules in taste bud cells. Tas1R2/Tas1R3, Tas1R1/Tas1R3, and Tas2Rs play as the receptors for sweet, umami, and bitter tastes, respectively. Transient receptor potential channel type M5 and phospholipase Cβ2 (PLCβ2) are known as the downstream signaling effectors of these taste receptors. Polycystic kidney disease (PKD) 2L1/PKD1L3 and epithelial sodium channel (ENaC) are candidates for the sour and salty taste receptors, respectively (Yarmolinsky et al., 2009).

Several studies have investigated the correlation between aging and taste sensation in rodents. Thaw (1996) reported that 28-month-old Sprague–Dawley rats showed higher thresholds for detection of sucrose and NaCl than rats younger than 23 months (Thaw, 1996). Tordoff (2007) described that old C57BL/6J mice aged 125 weeks showed a higher preference for NaCl than young mice aged 8 weeks, but found no age-related changes in the preferences for saccharin, quinine hydrochloride, and citric acid (Tordoff, 2007). Shin et al. (2012) reported that although 18-month-old B6C3F1/J mice showed a lower sweet response than 10-month-old mice, there were no changes in other taste qualities with aging (Shin et al., 2012). Although these reports produced inconsistent results, they all suggested that taste sensitivity is modified by aging. However, the factors affecting the changes in taste sensitivity remain poorly understood.

To clarify the participation of the peripheral taste system in the aging-dependent changes in taste sensitivity, in this study, we compared the peripheral taste detection systems in young and old mice. First, we investigated whether taste sensitivities changed with aging under our experimental conditions. Next, to

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*Abbreviations:* BrdU, 5-bromo-2′-deoxyuridine; CT, chorda tympani; CvP, circumvallate papillae; ENaC, epithelium sodium channel; FuP, fungiform papillae; GL, glossopharyngeal; IMP, inosine 5′-monophosphate; MSG, monosodium glutamate; PKD, polycystic kidney disease; PLCβ2, phospholipase Cβ2.

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identify the cause of the changes in taste sensitivities, we analyzed the expressions of taste-associated molecules, turnover rates of taste bud cells, and levels of serum components known to modify taste responses. As differences in experimental conditions such as species, age, and concentration range of tested taste solutions were considered the main reasons for the inconsistent results among previous studies, we designed our study under the following conditions: (1) C57BL/6J (B6) mice aged > 120 weeks were used as the old group; (2) mice were not exposed to strong taste stimuli until the behavioral experiments in both the young and old groups; and (3) a broad concentration range from weak to strong taste was used for taste stimulation in behavioral assays.

## MATERIALS AND METHODS

### Materials

Citric acid, NaCl, and sucrose were purchased from Kanto Chemical (Tokyo, Japan). Denatonium benzoate (denatonium), monosodium glutamate (MSG), inosine 5′-monophosphate (IMP), and amiloride were purchased from Sigma (St. Louis, MO). All other reagents were of analytical grade and obtained from standard suppliers.

### Animals

The study population comprised male B6 mice (CLEA Japan, Tokyo, Japan). The animals were housed at The University of Tokyo Animal Care Facility and had *ad libitum* access to a standard laboratory chow and distilled water. A total of 30 mice were used in this study. The surrounding temperature and humidity were maintained at 23 °C and 55%, respectively, with a 12-h/12-h light/dark cycle (lights switched on at 0800 h). We divided the mice into two age groups: young group aged 8–24 weeks (body weight: 24.6 ± 0.6 g at 12 weeks) and old group aged 120–139 weeks (body weight: 35.2 ± 0.8 g at 120 weeks). Mice with normal shapes and feeding behaviors were used. All experiments were performed in accordance with protocols approved by The University of Tokyo Animal Care Committee (Approval Number: P10-457).

### Experiment schedule

Initially, the mice performed a brief access test, followed by a 48-h two-bottle preference test. After the behavioral assays, the gustatory nerve activities were measured. Subsequently, taste bud and blood samples were collected. For immunohistochemistry, we used another group of mice which did not perform the behavioral assays.

### Brief access test

Ten mice from each age group were used for the brief access test, which was performed for 2 weeks. The numbers of licks to aversive and attractive taste substances were measured in the first and second weeks, respectively.

*Evaluation of aversive taste substances in the first week.* Each animal with 23-h water deprivation was placed in a test cage on day 1 of training and given free access to distilled water during a 1-h session. The number of licks per 5 s was measured by a custom-built gustometer (Neuroscience, Tokyo, Japan). Days 2–3 were the training session. During this period, the animal was trained to drink distilled water on an interval schedule, consisting of 5-s periods of presentation of distilled water with 30-s intervals. Days 4–5 were the test session. The numbers of licks for denatonium, citric acid, and distilled water by each animal were counted during the first 5 s after the animal's first lick. After the test session, the mice were rested.

*Evaluation of attractive taste substances in the second week.* Each animal's water intake was limited to the average water intake recorded just before the brief access test. Days 8–10 were the training session. On days 11–13, the numbers of licks for sucrose, MSG + IMP, and NaCl by each animal were counted during the first 5 s after the animal's first lick.

As young and old mice had different motivations for water (average lick numbers for water after 23-h water deprivation: young mice, 32.4 ± 0.9; old mice, 27.8 ± 1.2), the taste sensitivities to tastants were expressed as lick ratios. The lick ratios of the tastants were calculated as follows: number of licks of tastant/number of licks of water. When the lick ratios to attractive taste substances were calculated, the average lick numbers for water during aversive taste test sessions were utilized. To avoid restriction effects, data for mice whose body weight fell below 80% of the *ad libitum* normal free-feeding value were excluded from the analyses. Tastant solutions for the brief access test were (in mM): 0.3–100 citric acid, 0.1–10 denatonium, 10–1000 NaCl, 1–300 sucrose, and 1–300 MSG + 0.5 IMP.

### Forty-eight-hour two-bottle preference test

Young mice ($n$ = 10) and old mice ($n$ = 6–10) were caged individually and given 48 h of access to two bottles, one containing deionized water and the other containing a tastant solution. After 24 h the bottle positions were switched to avoid positional effects. The ratio of tastant volume to total liquid consumed was recorded. The preference ratios of the tastants were calculated as follows: tastant intake/total fluid intake (tastant intake + water intake). The tastant solutions for the two-bottle preference test were (in mM): 1–10 citric acid, 0.03–3 denatonium, 10–500 NaCl, 10–100 sucrose, and 0.1–10 MSG + 0.5 IMP.

### Gustatory nerve recordings

Whole-nerve responses to lingual application of tastants were recorded from the chorda tympani (CT) nerve. After behavioral experiments, the CT nerve responses were measured in young mice ($n$ = 5) and old mice ($n$ = 4–6) under anesthesia by intraperitoneal injection of sodium pentobarbital (50 mg/kg) and urethane (500 mg/kg). A tracheal cannula was implanted in each animal, and the animal was secured with a headholder.