Supplemental materials

Supplemental Materials and Methods

In Experiment 2, the sizes of 11 body parts and the lengths of eight brain parts were also measured before perfusion (Supplemental Figs. 1A, 1B).

In addition to the sections described in the main text, sagittal sections of the olfactory bulb were prepared by collecting every 4 accessory olfactory bulb (AOB)-containing sections from the lateral to medial areas.

In addition to the staining procedure provided in the main text, sections of the olfactory bulb were further incubated with primary antibody for G protein Giα-2 (MAB3077; Merck Millipore) overnight, incubated with biotinylated anti-mouse secondary antibody (BA-2000; Vector Laboratories) for 2 h, processed with the VECTASTAIN Elite ABC kit (Vector Laboratories), and developed using a diaminobenzidine solution.

To compare the size of the glomerulus of the main olfactory bulb (MOB), we measured the area of 12 to 40 glomeruli in the anterodorsal region of the MOB bilaterally. To compare the areas of the rostral and caudal AOB, the glomerulus layer of the AOB was divided into a Giα-2 expressing rostral region and the remaining caudal regions. The rostral area, caudal area, and total area of the AOB was measured in 3 to 8 sections bilaterally.

All of these data were analyzed by Student’s t-test.

Supplemental Results

When we compared body parts of experimental Sj and Og rats (Supplemental Table 1), Sj rats had shorter ear length ($t_{30} = -2.19, P < 0.05$) and tended to have a
larger breadth of head ($t_{30} = 2.03, P = 0.052$) compared with Og rats.

When we compared brains of experimental Sj and Og rats (Supplemental Table 2), the length of the rostral-caudal axis of the olfactory bulb was not significantly different. In contrast, the greatest breadth of olfactory bulbs was larger in Sj rats than in Og rats ($t_{30}=2.19, P < 0.05$). In addition, experimental Sj rats had a shorter cortex length ($t_{30} = −2.82, P < 0.01$), longer cerebellum length ($t_{30} = 3.53, P < 0.01$), and larger breadth of cortex ($t_{30} = −2.42, P < 0.05$) compared with experimental Og rats.

Analyses on brain sections revealed that the area of the glomerulus was not significantly different between the experimental Sj and Og rats in the MOB. In the AOB, the rostral, caudal, and total areas were not significantly different between the experimental Sj and Og rats.

**Supplemental Discussion**

In the present study, experimental Sj rats had a broader olfactory bulb than experimental Og rats whereas the area of the glomerulus was similar between the experimental Sj and Og rats. These results suggest that the number of olfactory receptors (OR) is larger in the Sj strain than in the Og strain. It is known that the olfactory bulb is enveloped by glomeruli that each receive projections from multiple sensory neurons but which express a single OR at the main olfactory epithelium [1]. Therefore, the numbers of glomeruli in each olfactory bulb and OR should be similar. Indeed, mice have about 900 glomeruli [3] and about 1000 OR [2]. In the present study, experimental Sj rats had a broader olfactory bulb than experimental Og rats, suggesting that the surface area covered with glomeruli is larger in the Sj strain than in the Og strain. However, the area of glomerulus was similar between the experimental Sj and Og rats. To cover a larger surface area with the similar size of the glomeruli, the Sj
strain must logically have a larger number of glomeruli compared with the Og strain. This assumption suggests that the number of OR is larger in the Sj strain than in the Og strain. Further genetic studies are required to assess this possibility.

Supplemental References

1. Mori, K., von Campenhause, H. and Yoshihara, Y. 2000. Zonal organization of the mammalian main and accessory olfactory systems. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **355**: 1801-1812.

2. Niimura, Y. and Nei, M. 2005. Comparative evolutionary analysis of olfactory receptor gene clusters between humans and mice. *Gene* **346**: 13-21.

3. Royet, J. P., Souchier, C., Jourdan, F. and Ploye, H. 1988. Morphometric study of the glomerular population in the mouse olfactory bulb: numerical density and size distribution along the rostrocaudal axis. *J. Comp. Neurol.* **270**: 559-568.
**Supplemental Table 1**
Comparison body part sizes in experimental Sj and Og rats

|                      | Sj rats    | Og rats    |
|----------------------|------------|------------|
| 1. Length of body    | 308 ± 3    | 304 ± 7    |
| 2. Length from mouse to anus | 157 ± 3    | 159 ± 3    |
| 3. Length of tail    | 151 ± 1    | 145 ± 4    |
| 4. Length of head    | 31.9 ± 0.3 | 32.9 ± 0.9 |
| 5. Width of head     | 22.4 ± 0.4#| 21.2 ± 0.4 |
| 6. Length of eye     | 6.8 ± 0.1  | 7.0 ± 0.2  |
| 7. Length of ear     | 19.4 ± 0.3*| 20.5 ± 0.4 |
| 8. Length of front paw| 13.5 ± 0.2 | 13.2 ± 0.4 |
| 9. Length of hind paw| 30.8 ± 0.3 | 31.1 ± 0.4 |
| 10. Circumference of head | 75.5 ± 1.2 | 74.7 ± 1.2 |
| 11. Circumference of body | 108 ± 2    | 115 ± 3    |

All the data are expressed in cm.

See the corresponding numbers in Supplemental Figure 1.

*P < 0.05 and #P = 0.052 by Student’s t-test.
### Supplemental Table 2
Comparison in the size of brain from experimental Sj and Og rats

|                         | Sj rats | Og rats |
|-------------------------|---------|---------|
| a. Length of the brain  | 21.2 ± 0.2 | 21.2 ± 0.2 |
| b. Length of the olfactory bulb | 4.1 ± 0.1 | 3.8 ± 0.2 |
| c. Greatest breadth of the olfactory bulb | 4.6 ± 0.1* | 4.3 ± 0.1 |
| d. Length of cortex     | 10.7 ± 0.3* | 11.8 ± 0.3 |
| e. Length of cerebellum | 6.4 ± 0.2* | 5.5 ± 0.2 |
| f. Greatest breadth of cortex | 13.0 ± 0.1* | 13.5 ± 0.1 |
| g. Greatest breadth of cerebellum | 9.7 ± 0.1 | 9.9 ± 0.2 |
| h. Height of the brain  | 8.0 ± 0.1 | 8.3 ± 0.2 |

Main olfactory bulb

- area of the glomerulus | 0.21 ± 0.01 | 0.21 ± 0.01 |

Accessory olfactory bulb

- rostral area         | 0.13 ± 0.01 | 0.15 ± 0.01 |
- caudal area          | 0.21 ± 0.01 | 0.21 ± 0.01 |
- total area           | 0.34 ± 0.02 | 0.36 ± 0.01 |

Length, breadth, and height is expressed in mm.

Areas were measured in the tissue section and expressed in µm².

See the corresponding letters in Supplemental Figure 1.

*P < 0.05 by Student’s *t*-test.
Supplemental Figure 1
Schematic diagram showing the measured parts of the (A) body and (B) brain.

1. Length of body
2. Length from mouse to anus
3. Length of tail
4. Length of head
5. Width of head
6. Length of eye
7. Length of ear
8. Length of front paw
9. Length of hind paw
10. Circumference of head
11. Circumference of body

a. Length of the brain
b. Length of the olfactory bulb
c. Greatest breadth of the olfactory bulb
d. Length of cortex
e. Length of cerebellum
f. Greatest breadth of cortex
g. Greatest breadth of cerebellum
h. Height of the brain