Supplemental Figure legends

Figure S1. The vapor concentration of odorants. A log-log plot of vapor (M) vs. liquid (% v/v) concentration for the various odorants. Mineral oil was used as the solvent. Saturated vapors of odorant solutions in a 4- or 5-ml glass tubes were sampled with a gas-tight syringe and loaded into GC-MS system. To measure the saturated vapor concentration, odorant solutions were used at least 3 h after dilution. Measurements were typically repeated three times for propyl acetate (triangle), benzaldehyde (reverse triangle), and EG (square), and the results from each experiment were averaged. The vapor concentration were calculated using the following equation $[C_{vap}] = \alpha\ [C_{liq}]^{\beta}$ where α is the vapor concentration of the pure substance, and exponent β is the slope from the log-log plot of vapor vs. liquid concentration (Cometto-Muniz et al., 2003). For example, α and β values for EG are 551 and 0.49 (Oka et al., 2006). Thus, approximately 100 times higher concentration in solution is required to achieve 10-fold higher vapor concentration.

Figure S2. Flow-rate effects in the synapto-pHluorin (SpH) transgenic mice. Odorant-evoked neurotransmitter release was quantified in Ea glomerulus by measuring fluorescent changes in SpH transgenic mice at two different flow-rates (25 ml/min and 100 ml/min) with different concentrations of EG (Bozza et al., 2004). Each data point was shown as a percentage of maximum response (n=3, mean ± SE). Each concentration corresponds to the vapor pressure of 0.01, 0.1, 1 and 10% dilution of EG in mineral oil. SpH signal generally decreased at above saturated concentration due to the desensitization of transmitter release machinery.

Figure S3. GC-MS analysis of head-space odorants emitted from natural flavor oils. Odorous components of peppermint and rose oil were analyzed by GC-MS. Saturated

vapors of 1% natural flavor oils in a 4- or 5-ml glass tubes were sampled with a gas-tight syringe and loaded into the GC-MS system as the same way described in Figure S1. Eight main components were numbered on the total ion chromatogram. The amount of each component was quantified and represented below as a percentage.

Figure S4. Effects of change in airflow rate and concentration on odorant responses in the OB. A. EG and PA responses at different stimulus concentrations or airflow rates. Odorant-evoked Ca^{2+} responses are represented as psudocolored images where red corresponds to the greatest response. A summary diagrams of the glomerular responses are shown in the right panel. Each glomerulus was categorized according to the same criteria as in Fig. 7A. (upper) Freely breathing mice were used to examine the concentration effect. For EG, the same data were reanalyzed from our previous data (Figure 3 in Oka et al., 2006). (lower) 0.01% EG and 0.1% PA were used to examine the flor-rate effect. The same data were reanalyzed from Fig. 4A (for EG), and Fig. 5A (for PA). B. The Number of activated glomeruli was counted and represented for each odorant. We compared responses between 0.01% vs. 1% for concentration effect, and between 25ml/min vs. 100ml/min for flow-rate effect.

References cited in Supplemental figure legends

Bozza T, McGann JP, Mombaerts P, Wachowiak M (2004) In vivo imaging of neuronal activity by targeted expression of a genetically encoded probe in the mouse. Neuron 42:9-21.

Cometto-Muniz JE, Abraham MH (2008) Human olfactory detection of homologous n-alcohols measured via concentration-response functions. Pharmacol Biochem Behav 89:279-291.

Oka Y, Katada S, Omura M, Suwa M, Yoshihara Y, Touhara K (2006) Odorant receptor

map in the mouse olfactory bulb: in vivo sensitivity and specificity of receptor-defined glomeruli. Neuron 52:857-869.