# Induction of Olfactory Receptor Sensitivity in Mice

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**Repeated exposure to olfactory ligands (odorants) increased peripheral olfactory sensitivity in mice. For two unrelated ligands, androstenone and isovaleric acid, induction of olfactory sensitivity was odorant-specific and occurred only in inbred strains that initially had low sensitivity to the exposure odorant. These data demonstrate stimulus-induced plasticity in a sensory receptor cell, suggesting a form of stimulus-controlled gene expression. Induction with two unrelated odorants implies that olfactory induction is a general phenomenon that may occur in a large fraction of the human population.**

Individual variation in olfactory sensitivity is common in humans. For example, insensitivity to the odorant androstenone (5α-androst-16-en-3-one), an example of a specific anosmia, occurs in roughly 50% of populations tested (*1*). The causes of specific anosmia are not known but appear to have a genetic component (*2*). Thus, by analogy to color blindness (*3*), specific anosmia may result from defects in the genes encoding the olfactory receptor proteins (*4*). However, in contrast to color blindness, which is stable throughout life, specific anosmia to androstenone can be reversed by repeated exposure to this odorant (*5*). Induction of androstenone sensitivity was demonstrated by psychophysical measurements (*5*), so it is not known whether induction results from changes in peripheral olfactory sensitivity or from changes in central olfactory processing. Furthermore, it is not yet known whether induction can occur with odorants other than androstenone. We have addressed these issues by recording the olfactory receptor potential in inbred strains of mice that exhibit behavioral deficits for androstenone or for another odorant, isovaleric acid.

Inbred strains of mice that are relatively insensitive (C57BL/6J) and sensitive (AKR/J) to isovaleric acid were identified by their behaviors in a conditioned aversion task (*6*). These strains did not differ in their sensitivity to isoamyl acetate (*6*). Therefore, the C57BL/6J strain was chosen as an animal model for individuals with a specific anosmia for isovaleric acid, and the AKR/J strain was chosen as an animal model for individuals sensitive to isovaleric acid. The same approach was used to identify the NZB/B1NJ and CBA/J strains as animal models for individuals with a specific anosmia for, or sensitivity to, androstenone, respectively (*7*). We exposed mice to androstenone or isovaleric acid (*8*) and assessed shifts in receptor sensitivity by recording the voltage across the olfactory epithelium in response to olfactory stimuli, a signal known as the electro-olfactogram (EOG) (*9*, *10*). All responses had monophasic negative wave forms, which are typical of the receptor component of the EOG (*9*, *11*). Our measurements likely underestimated increases in response amplitude that occurred in a subset of receptor cells, because the EOG averages the responses of many cells.

In an NZB/B1NJ mouse, 23 days of exposure to androstenone caused an increase in the amplitudes of the EOG in response to all tested concentrations of this odorant (a 2.2-fold increase occurred at the lowest concentration) but had no effect on the amplitudes of the responses to isoamyl acetate (Fig. 1) (*12*). The averages of measurements from ten mice exposed for 2 to 4 weeks and from eight control NZB/B1NJ mice (Fig. 2) confirmed these observations. These values also demonstrated a 2.2-fold increase in the amplitude of the response to the lowest concentration of androstenone (*13*) [at a response amplitude of 0.2 mV, this corresponds to a 5.5-fold increase in androstenone sensitivity (*14*)]. The difference in response amplitude was significant at all concentrations ($P < 0.002$) (*15*). In contrast, androstenone exposure did not cause a significant change in the amplitudes of the responses to isoamyl acetate ($P > 0.80$). This result demonstrates that exposure to androstenone preferentially affected sensitivity to this odorant, as was observed in humans (*5*). The ability of an odorant to increase androstenone sensitivity in NZB/B1NJ mice also appeared to be specific for androstenone, because exposure to isoamyl acetate (*8*) had no significant effect on responses to either androstenone or isoamyl acetate (*16*). The EOG responses were recorded ~20 hours after the last exposure to androstenone. This persistence in increased sensitivity suggests that induction may be long-lasting; in humans, induction appears to be stable for at least 6 weeks after the end of androstenone exposure (*17*).

To investigate whether induction occurs only in strains that have low sensitivity to

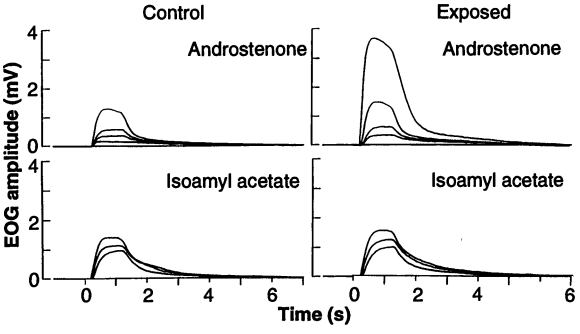


**Fig. 1.** The odorant-induced voltages across the olfactory epithelia (EOG) (*9*) of NZB/B1NJ mice in response to 1.0-s pulses, starting at zero time, of androstenone and isoamyl acetate (the odorant concentrations are the same as those for the NZB/B1NJ data in Fig. 2).

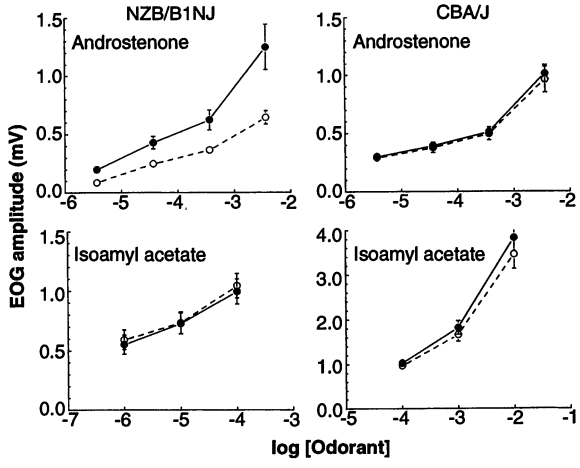


**Fig. 2.** Peak amplitudes of the EOG as a function of the log of the odorant concentration (*10*) for the indicated strains of mice (solid circles, androstenone-exposed; empty circles, control). For androstenone, the solution concentrations were absolute; for isoamyl acetate, the solution concentrations were relative to a saturated aqueous solution. Although the experiments on CBA/J mice used 100-fold higher concentrations of isoamyl acetate than did the experiments on NZB/B1NJ, the two strains exhibited similar concentration response curves, as shown by the responses to the log of the odorant concentration at −4. Error bars are shown as the mean ± 1 SE.

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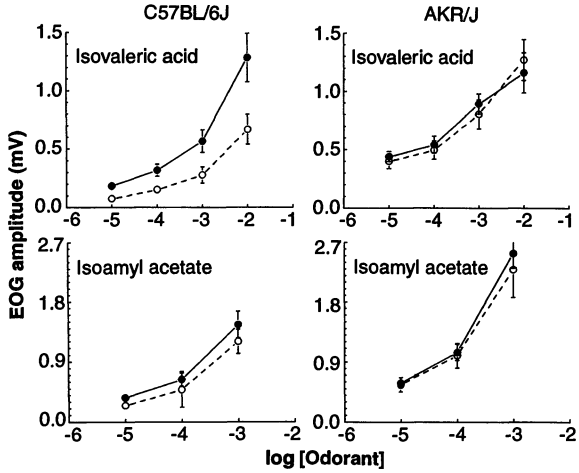


**Fig. 3.** Peak amplitudes of the EOG as a function of the log of the odorant concentration (*10*) for the indicated strains (closed circles, isovaleric acid–exposed; open circles, control).

the inducing odorant, we measured the effects of androstenone exposure in CBA/J mice, which, unlike NZB/B1NJ mice, exhibited behavioral sensitivity to both androstenone and isoamyl acetate in the conditioned aversion assay (*7*). The EOG responses to androstenone were larger in the control CBA/J mice than in the control NZB/B1NJ mice, which is consistent with the behavioral observations. In contrast to results from the NZB/B1NJ strain, responses from the CBA/J mice to androstenone or isoamyl acetate were not significantly affected by the 2 to 6 weeks of exposure to androstenone (Fig. 2) (*18*). This result suggests that induction does not occur in mice that initially have appreciable sensitivity to the inducing odorant.

To determine whether induction can occur with odorants other than androstenone, we measured the effects of 2 to 4 weeks of exposure to isovaleric acid on C57BL/6J and AKR/J mice, strains that are relatively insensitive and sensitive, respectively, to this odorant (6). The results (Fig. 3) paralleled those described above for induction of androstenone sensitivity: Exposure to isovaleric acid increased the responses to the odorant in the C57BL/6J strain but not in the AKR/J strain and did not affect the responses to isoamyl acetate in either strain (*19*).

Our data show that induction can occur within the olfactory epithelium and is odorant-specific; that is, it occurred only in strains that have low sensitivity to the exposure odorant, and sensitivity increased preferentially for that odorant. This specificity provides evidence that induction results from changes within the olfactory receptor cells. The increase in response amplitude could result from an increase in the number of receptor cells that respond to the exposure odorant, an increase in the sensitivity of cells that already respond to the exposure odorant, or both. In any case, however, the temporal stability of induction suggests that it is mediated by altered gene expression in the exposed animals, possibly reflecting increased expression of genes that encode olfactory receptor proteins (*4*) with a high affinity for the exposure odorant.

The EOG responses of the control NZB/B1NJ mice to androstenone were smaller than those of the CBA/J strain. This difference provides the second example of an olfactory receptor-cell correlate of low behavioral sensitivity in mammals; the first is the low sensitivity to isovaleric acid in the C57BL/6J strain [(*20*) and Fig. 3]. Thus, our data support the hypothesis that at least some specific anosmias are caused by genetic defects that are expressed in olfactory receptor cells.

Our results demonstrate stimulus-induced plasticity in a sensory receptor cell. The ability of the olfactory receptor cells to exhibit this plasticity may be related to their continual turnover (*21*), which suggests that the full differentiation of immature olfactory receptor cells and the determination of their odorant specificity may be controlled, in part, by olfactory stimulation. Therefore, because of the relative simplicity of the olfactory epithelium, olfactory induction may provide a useful model for the study of the control of gene expression during neuronal development and differentiation.

## REFERENCES AND NOTES


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7. In the conditioned aversion task, NZB/B1NJ mice could detect isoamyl acetate (a 1000-fold dilution of a saturated aqueous solution) with preference ratios (*6*) as low as 0.08 but could not detect androstenone ($3.67 \times 10^{-3}$ M solution in mineral oil; lowest preference ratio, 0.43). Mice of the CBA/J strain could detect the same concentrations of isoamyl acetate and androstenone with preference ratios of 0.04 and 0.02, respectively.
8. Male mice (~7 weeks old when received from Jackson Laboratory, Bar Harbor, ME) were housed individually in plastic cages. Odorant exposure was accomplished by attachment of a piece of cotton gauze, soaked with a solution of androstenone ($3.67 \times 10^{-3}$ M in mineral oil), isoamyl acetate (100-fold dilution of a saturated aqueous solution), or $10^{-2}$ M isovaleric acid [made as described in (*6*)], to the top of the cage (the mice were unable to contact the gauze directly). The cages were loosely covered with a polyethylene sheet to increase the concentration of the odorant in the cage air. The gauze was present for ~16 hours daily (starting at ~5:00 p.m.); it was removed for ~8 hours daily to reduce habituation to the odorant. Unexposed (control) mice were received at the same time as the exposed mice, were the same age as the exposed mice, and were housed in a different room exactly as were the exposed mice, except for the absence of the odorant in the cotton gauze. At the end of the exposure period, a pair of animals (control and exposed) were killed for EOG recording each day. Exposure continued until the day before each experiment, so that the exposure period for individual animals varied from 2 to 6 weeks.
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10. In accordance with institutional guidelines, mice were killed by rapid cervical dislocation and decapitated, and the medial surfaces of the left olfactory turbinates were exposed. The EOG (basal potential minus apical potential) was recorded with an agar- and saline-filled glass microelectrode that contacted the apical surface of the olfactory epithelium as described by J. Kauer [*J. Physiol. London* **243**, 695 (1974)] and S. G. Shirley, E. H. Polak, R. A. Mather, and G. H. Dodd [*Biochem. J.* **245**, 175 (1987)]. Olfactory stimulation was accomplished by a change in the airstream directed onto the tissue from clean humidified air to air that was in equilibrium with an odorant solution with the concentration indicated (flow rate of 210 ml/min through 1.5-mm internal diameter Teflon tubing, the end of which was located ~5 mm from the preparation). The concentrations of the odorant solutions are expressed as moles per liter for androstenone (in mineral oil) and isovaleric acid (in water) or as the dilution of a saturated aqueous solution of isoamyl acetate. Mineral oil alone induced a small response (mean amplitude of 0.2 mV), which did not differ in amplitude between the exposed and control animals and was subtracted from the observed androstenone responses. Stimuli were presented in the order of increasing concentration at 1-min intervals (androstenone or isovaleric acid before isoamyl acetate). Our conclusions are based on differences between the responses of animals to the same stimuli. Consequently, the absolute odorant concentration that reached the tissue was not a critical experimental parameter and so was not measured. The experiments and response amplitude measurements were conducted without knowledge of the animal's exposure condition.
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12. The exposure-induced increase in androstenone response amplitude varied from 1.5-fold to 3.6-fold among eight locations spaced approximately equally on the four exposed olfactory turbinates and was largest in the middle two turbinates. Such variation is expected from the spatial heter-

ogeneity of odorant sensitivity [see P. F. Kent and M. M. Mozell, *J. Neurophysiol.* **68**, 1804 (1992) and references therein]. The responses shown in Fig. 1 were recorded from the middle of the third most posterior turbinate. The averages of the measurements at all recording locations were used for statistical analyses.

13. The exposure-induced increase was largest after 2 to 4 weeks of exposure and declined ~30% after 4 to 6 weeks of exposure. This decline may have resulted from the much longer exposure periods used with mice (16 hours per day) than those used with humans [3 min, three times daily (*5*)].
14. Sensitivity is defined as the inverse of the stimulus concentration required to elicit a 0.2-mV response.
15. Probability values were determined with simple effects tests within a repeated-measures analysis of covariance, where the average response to the highest concentration of isoamyl acetate served as the covariate.
16. For 10 exposed and 11 control mice, analyses of EOG responses to androstenone and isoamyl acetate revealed $F_{(1,19)} = 0.72$ ($P > 0.40$) and $F_{(1,19)} = 0.00$ ($P > 0.99$), respectively.
17. C. J. Wysocki, unpublished data.
18. Analyses of variance of EOG responses produced the following results: control CBA/J ($n = 20$) responses versus control NZB/B1NJ ($n = 18$) responses to androstenone, $F_{(1,36)} = 10.39$ ($P < 0.005$); exposed CBA/J ($n = 20$) responses versus control CBA/J ($n = 20$) responses to androstenone and isoamyl acetate, $F_{(1,38)} = 0.01$ ($P > 0.90$) and $F_{(1,38)} = 0.06$ ($P > 0.80$), respectively.
19. The EOG responses to isovaleric acid from the exposed ($n = 9$) and control ($n = 10$) C57BL/6J mice were significantly different [$F_{(1,17)} = 7.90$ ($P < 0.02$)], and the interaction between treatment condition and the isovaleric acid concentration was significant [$F_{(3,51)} = 6.12$ ($P < 0.001$)]. However, no significant differences were detected between exposed ($n = 11$) and control ($n = 10$) AKR/J mice [$F_{(1,19)} = 0.42$ ($P > 0.50$)]. The EOG responses to isoamyl acetate after exposure to isovaleric acid did not differ: exposed versus control C57BL/6J and AKR/J mice, $F_{(1,17)} = 1.47$ ($P > 0.20$) and $F_{(1,19)} = 0.18$ ($P > 0.60$), respectively.
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25 November 1992; accepted 22 February 1993