FIGURES AND LEGENDS

Figure 1

A schematic drawing of the cellular anatomy of the olfactory epithelium. Olfactory receptor neurons (OR) lie in the olfactory epithelium, the cilia on their dendrites bathed by mucus (MU). From the base of the cell body, each sensory neuron extemds an axon. Abbreviations: OR, olfactory receptor neuron; SC, supporting cell; BG, Bowman's gland; SP, supporting cell; MU, olfactory mucus.

MU

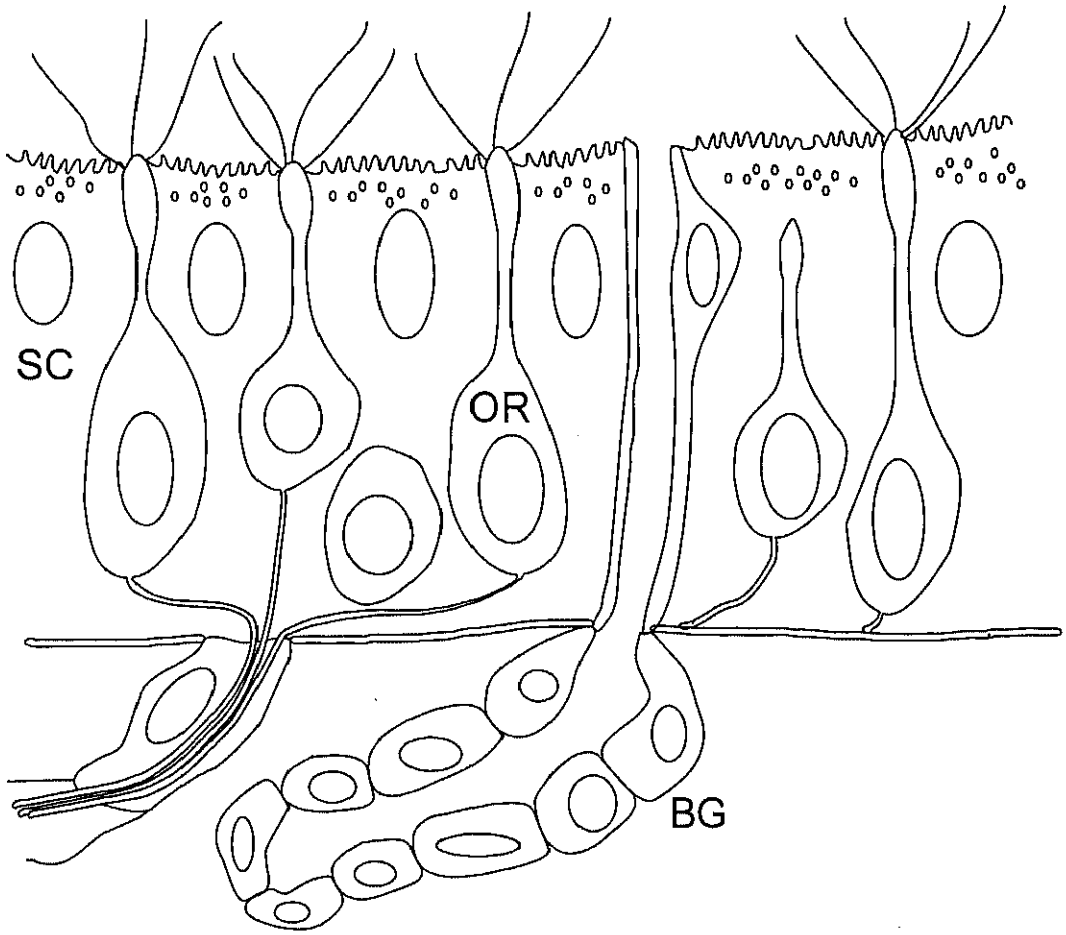
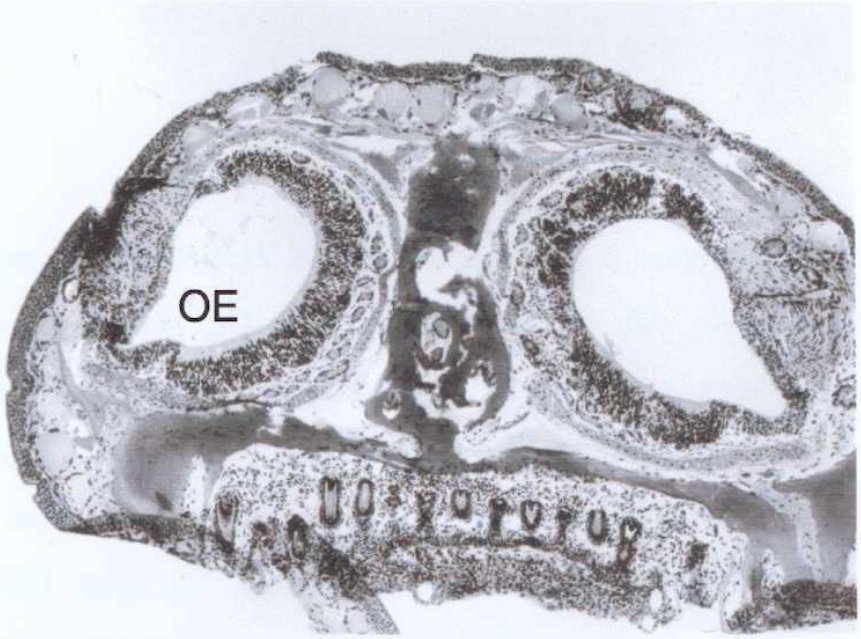


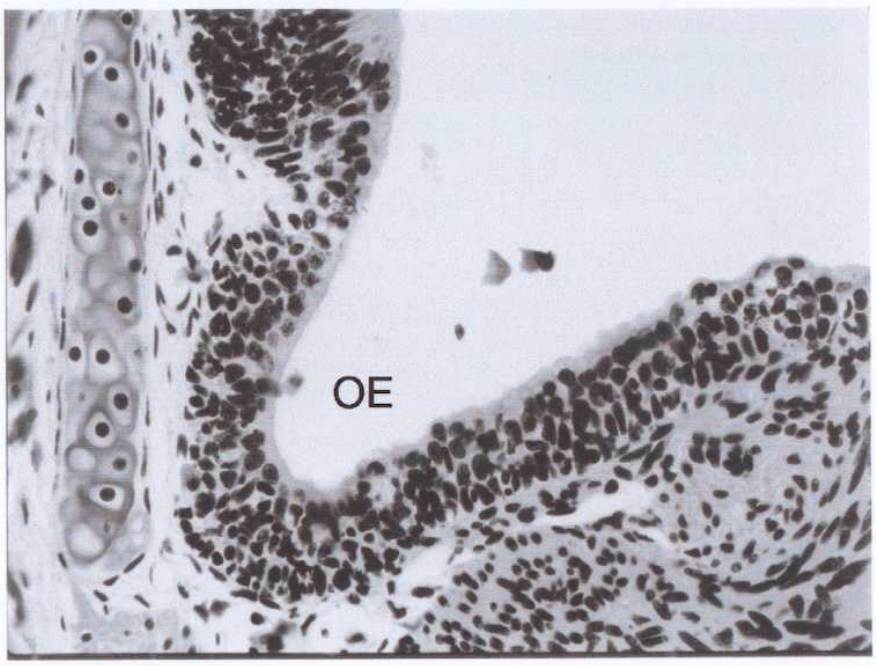
Figure 2

Olfactory epithelium and olfactory receptor neurons. (A) Coronal section of a newt head. Cells' nuclei were stained with hematoxylin-eosin. The dense cell layer was olfactory epithelium(OE). (B) An expansion of (A). (C) Phase-contrast photograph of a dissociated olfactory receptor neuron. Scale bar: 20 μm.

A



B



C

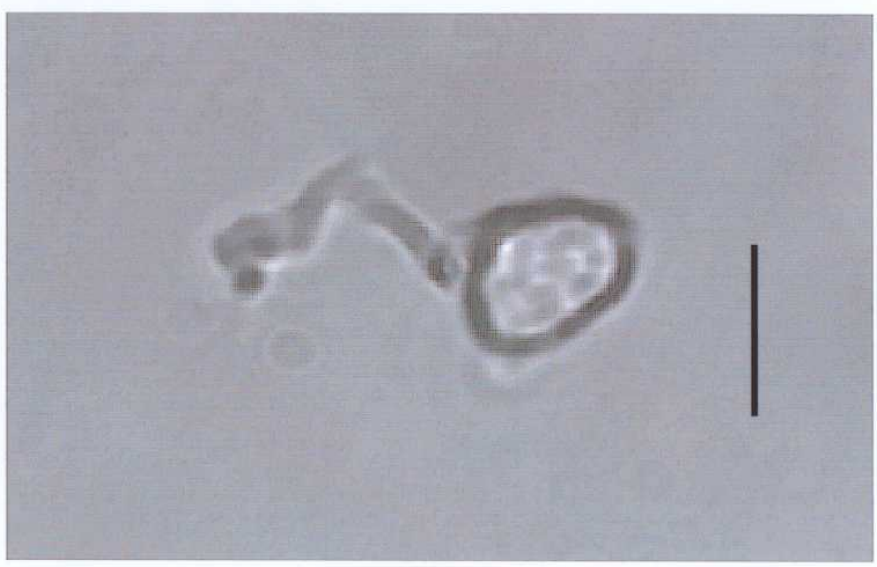


Figure 3

Models of olfactory signal transduction in vertebrate. Odorants bind to odorant receptors (R), which interact with G-proteins (G). G-proteins stimulate an adenylate cyclase (AC), causing an increase in cAMP (cA). The cAMP opens a cyclic nucleotide-gated channel (CNG-channel), leading to a change in membrane potential that culminates in action potential generation in the sensory axon. Calcium influx through the open CNG-channels activates chloride channels (Cl-Channel) that lead to an increase in the inward chloride current that further depolarizes the cell. The cAMP was then degenerated by phosphodiesterase (PDE) into 5'AMP (AMP).

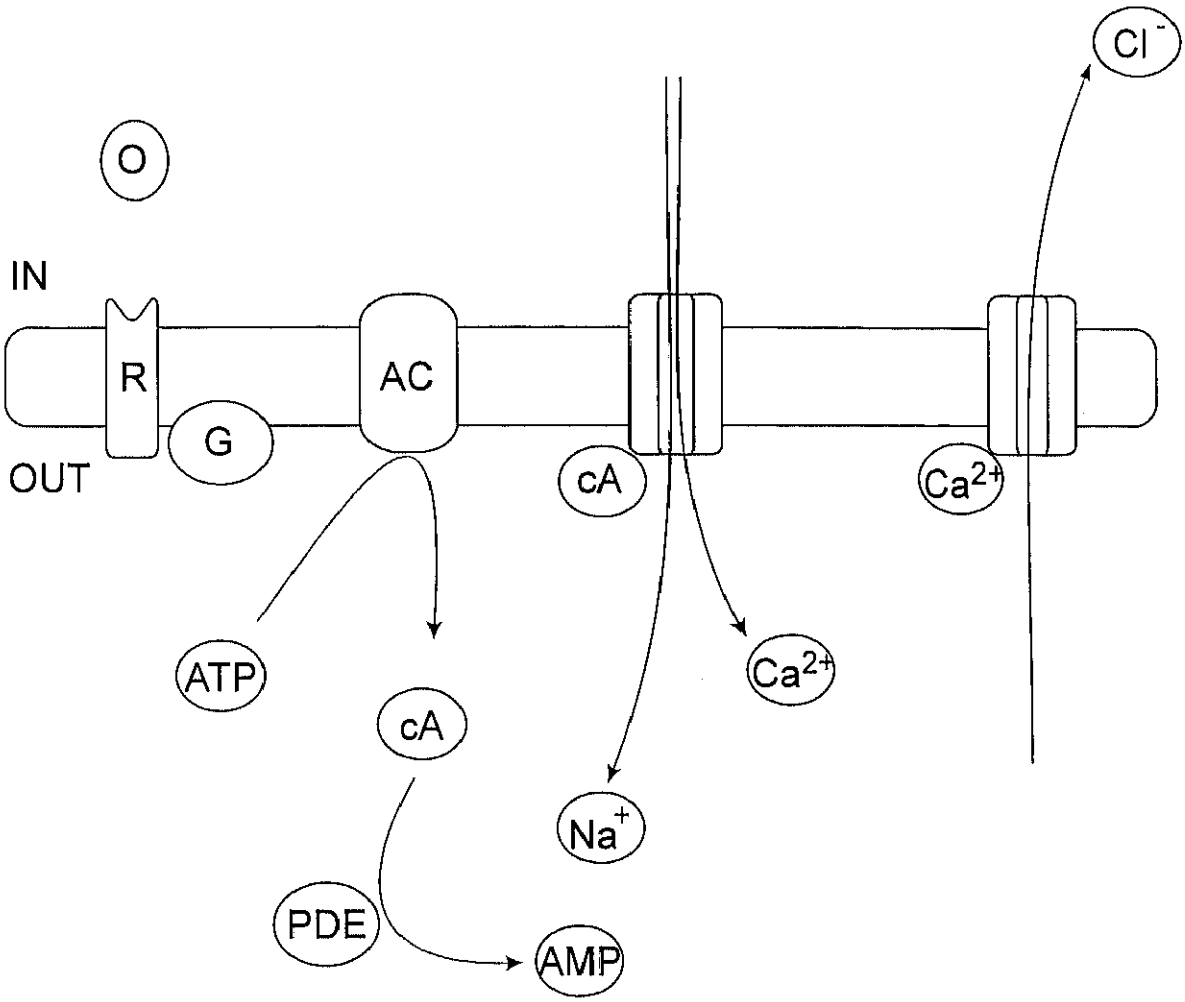

O
IN
R
G
AC
OUT
cA
Ca2+
Cl-
ATP
cA
Ca2+
Na+
PDE
AMP

Figure 4

Models of olfactory signal transduction in lobster (Ache and Zhainazarov, 1995). Two second messenger pathways have been implicated in lobster olfactory transduction. Both involve G-protein (G) coupled receptors. In one case, odorant binding to a receptor stimulates adenylate cyclase AC), enhancing the production of cAMP (cA). The cAMP activates cyclic nucleotide-gated K^+ channels. In the other case, receptor binding enhances phosphatidylinositol turnover with phospholipase C (PLC), producing inositol 1, 4, 5-trisphosphate (IP_3). The IP3 activates nonselective cation channels, causing depolarization. Each pathway is activated by different molecular components of the odor.

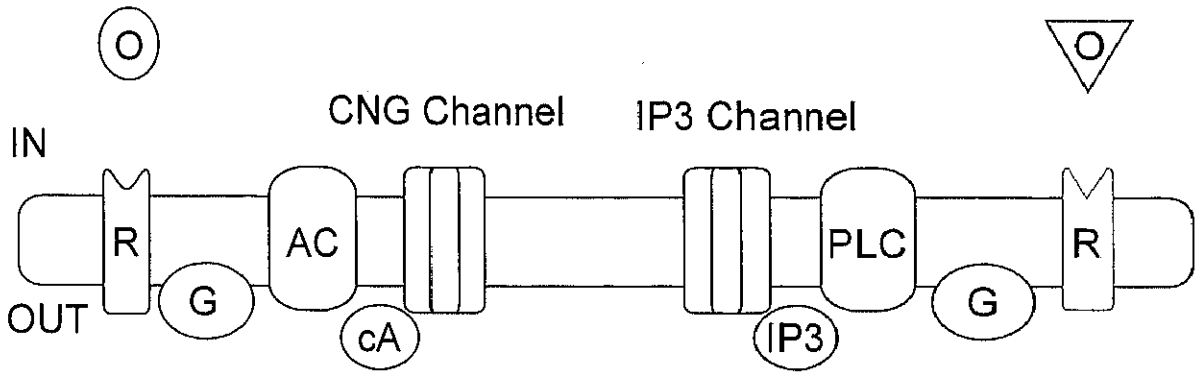
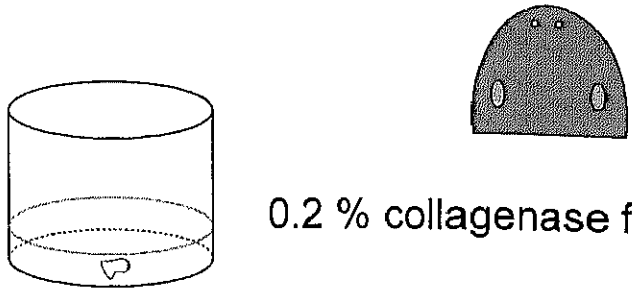
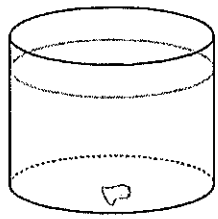

O
O
CNG Channel
IP3 Channel
IN
R
AC
PLC
R
G
G
OUT
cA
IP3
Inhibitory Pathway
Excitatory Pathway

Figure 5

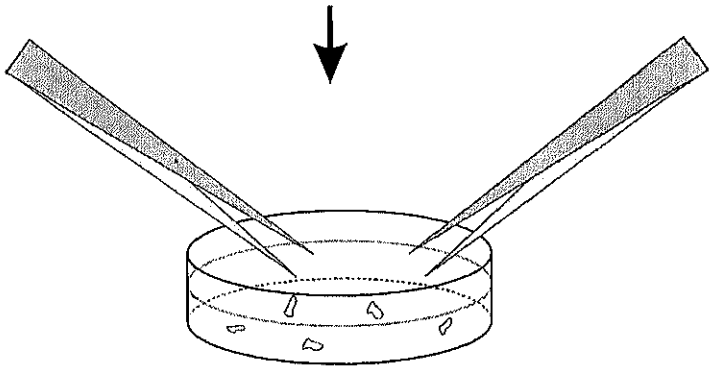
Outline of the dissociation procedures of olfactory receptor neurons. After decapitation, the nasal cavity was opened and the flat sheets of nasal epithelium on the dorsal and ventral walls were dissected free of the underlying cartilage in single pieces. The tissue was incubated for 10 min at 35 ℃ in a Ca^{2+} and Mg^{2+} free Ringer contained 0.2 % collagenase. The tissue was then rinsed with Ringer's solution and kept at 4 ℃ for 1 hr. The tissue was then teased with forceps and gently triturated by a firepolished Pasteur pipette.



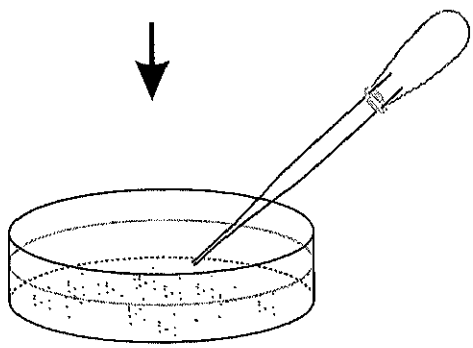
0.2 % collagenase for 10 min at 35 ℃



Rinse with Ringer's solution



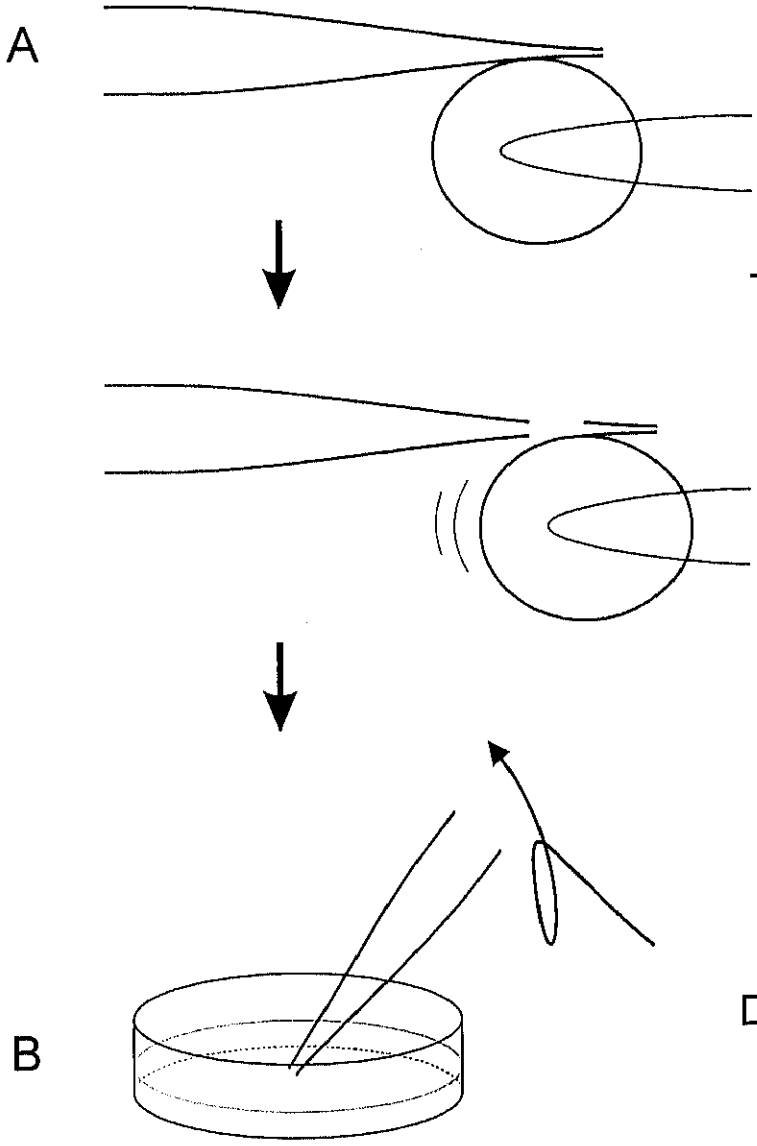
Trituration



Pipetting

Figure 6

Outline of fabrication procedure of a patch-pipette. (A) Trimming a pipette tip with a microforge. An electrodes with ~1 μm inner diameter tips were pulled from borosilicate glass pipette by a conventional double-pulled technique. The pipette with small inner tip diameter was touched to a melted glass on the microforge under a microscope. After the pipette adhered to the microforge, the power of microforge was turned off. As cooling of the microforge, a Pt wire of the microforge shrank, and the pipette tip was pulled and trimmed as a result. (B) The pipette tip was then dipped into a mixture of molten Parafilm, light mineral oil, and heavy mineral oil. (C) To clear the pipette tip, positive pressure was applied via a syringe during heating with the microforge.



Trimming a pipette tip with a microforge

Dipping a pipette tip into an oil mixture

Clearing a pipette tip

Figure 7

A photograph of a pipette tip. Scale bar: 10 μm.

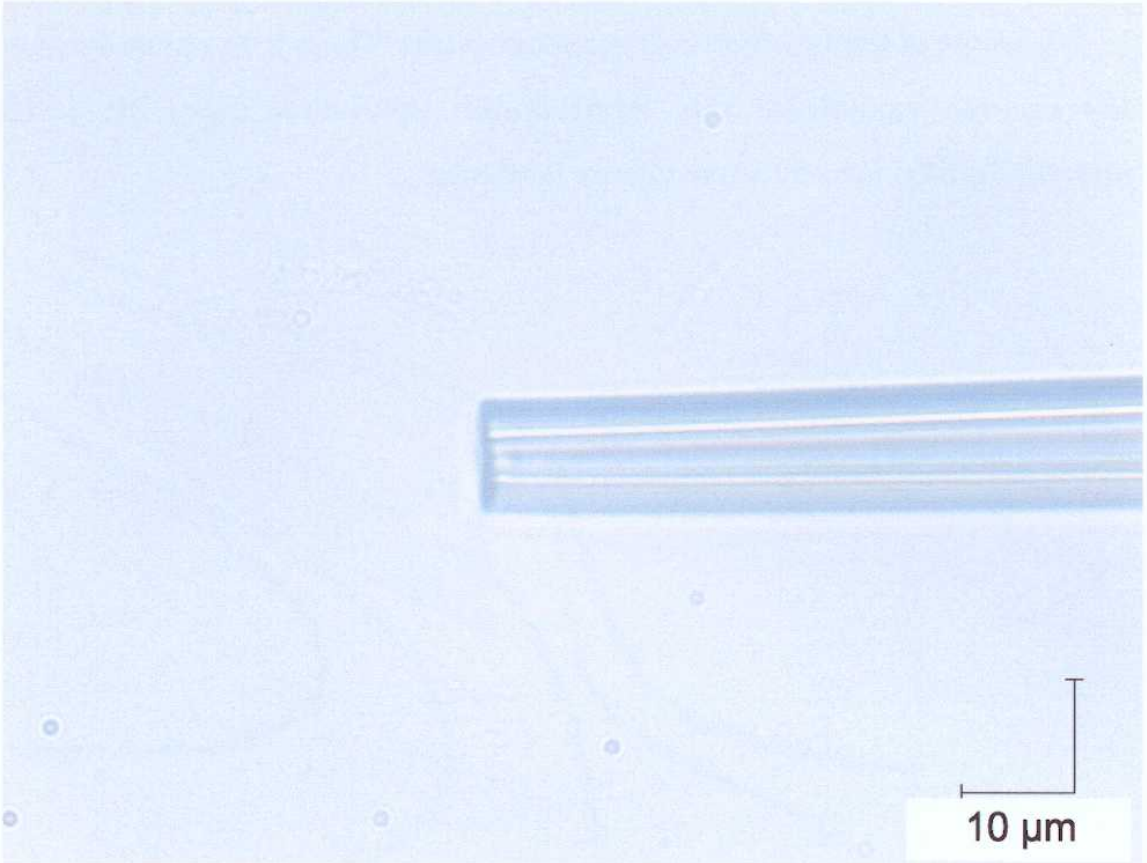

10 µm

Figure 8

Diagrams of whole cell recording configuration. The cell was placed inside the solution application pipe. Symbols: AP, application pipe; PP, patch pipette; IT, inlet tube for intra-pipette perfusion.

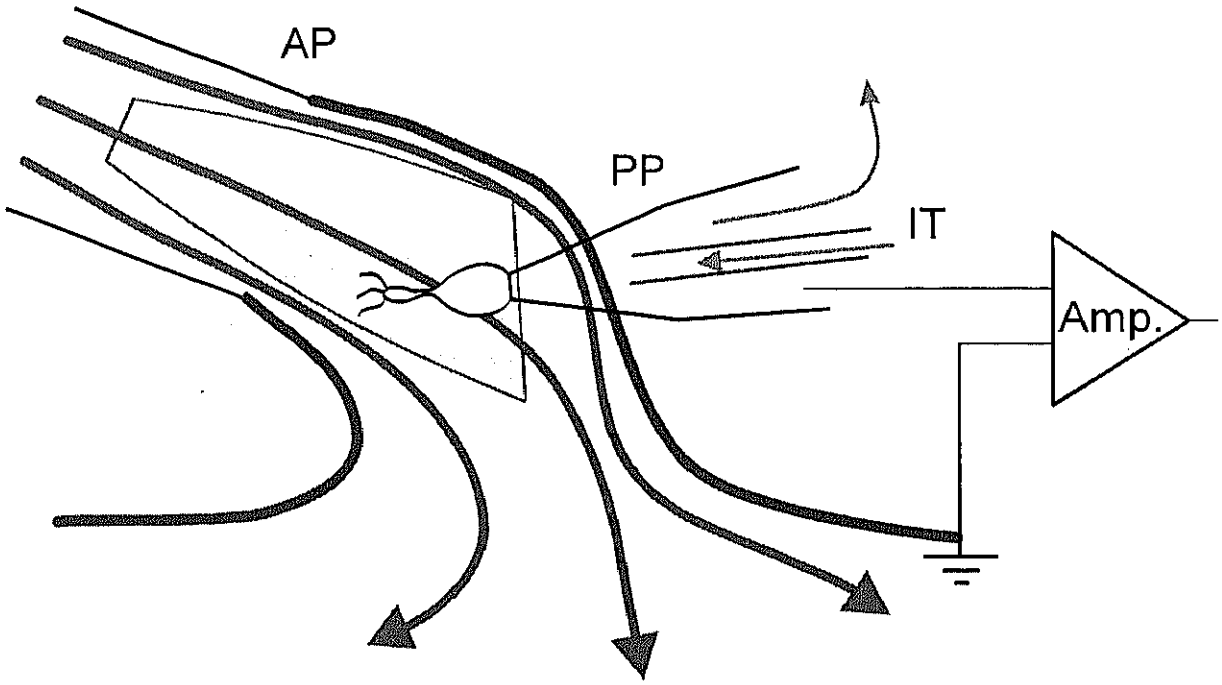
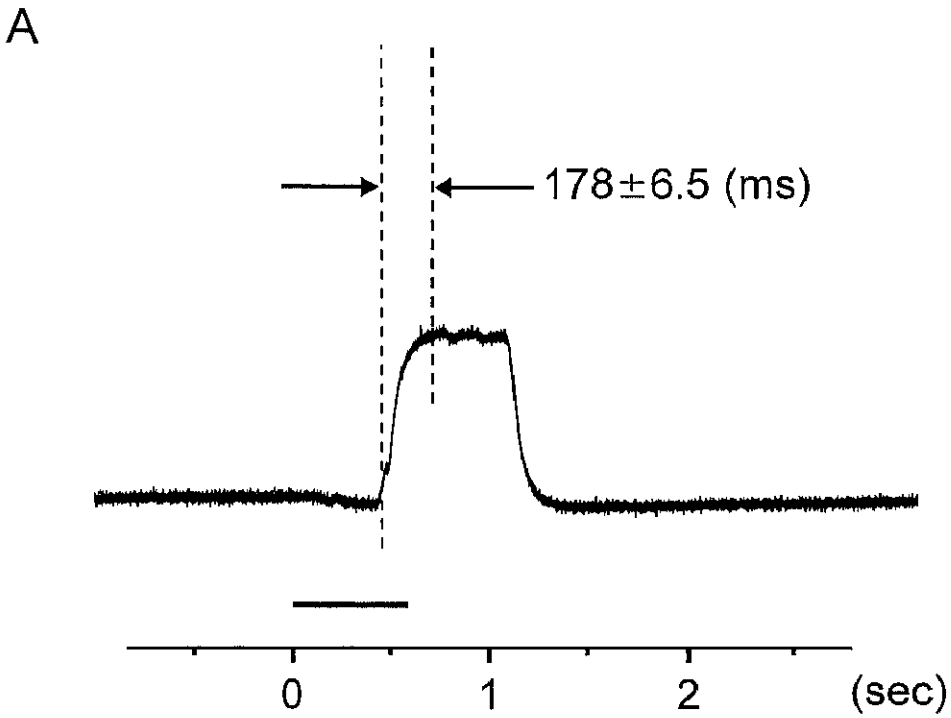
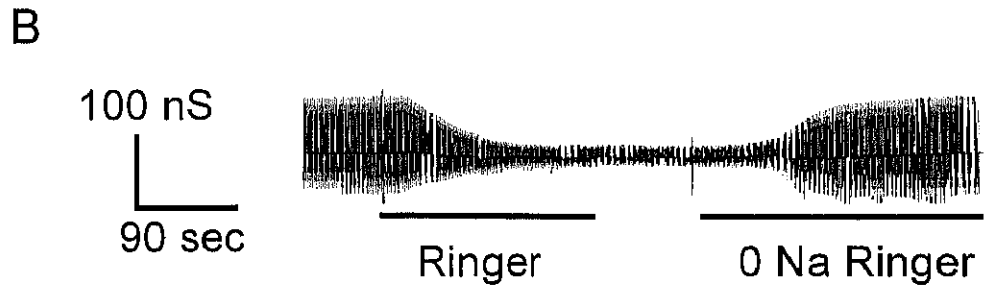

AP
PP
IT
Amp.

Figure 9

Test of solution changing. (A) Open-tip current response, solution exchange between Ringer's solution and choline-replaced Ringer. Solid bar indicates a timing of PC trigger. Values are means ± SD (n=10). (B) Open-tip responses during repetitive application of voltage pulse. Intra-pipette solution exchanged between Ringer's solution and 0 Na Ringer. Because ionic strength of the two solutions is different, conductance was varied as changing solution. (C) Current responses during repetitive application of voltage pulse (from -30 mV to +30mV) from an intact olfactory receptor neuron. Intra-pipette solution exchanged between a standard pseudo-intracellular solution and the standard pseudo-intracellular solution containing 5 mM cAMP. Solid bar indicates timing of perfusion of the 5 mM cAMP containing solution.


A
178±6.5 (ms)
0
1
2
(sec)


B
100 nS
90 sec
Ringer
0 Na Ringer

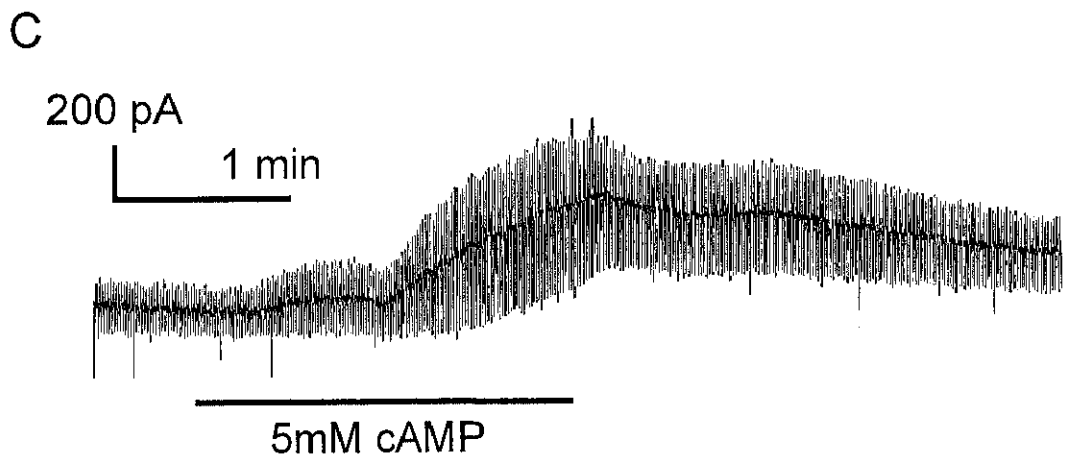

C
200 pA
1 min
5mM cAMP

Figure 10

Diagrams of EOG recording configuration. The newt was dicapitated, placed in a perfusion chamber, and positioned under olfacto-meter (OF) with exposing olfactory epithelium. Olfactory stimulation was accomplished by an application of purified moist nitrogen gas that was in equilibrium with an odorant-water solution of known concentration by passing a nitrogen gas stream over evaporation tubes (Tubes) containing the appropriate odorant solution. Nitrogen gas (N_2) from gas cylinder was purified with distilled water (D.W.) and activated charcoal, and then made bubble in one of odorant-water solutions of known concentration. Olfactory stimulation was accomplished by an application of this odorant containing-nitrogen gas thorough the olfacto-meter (OM).

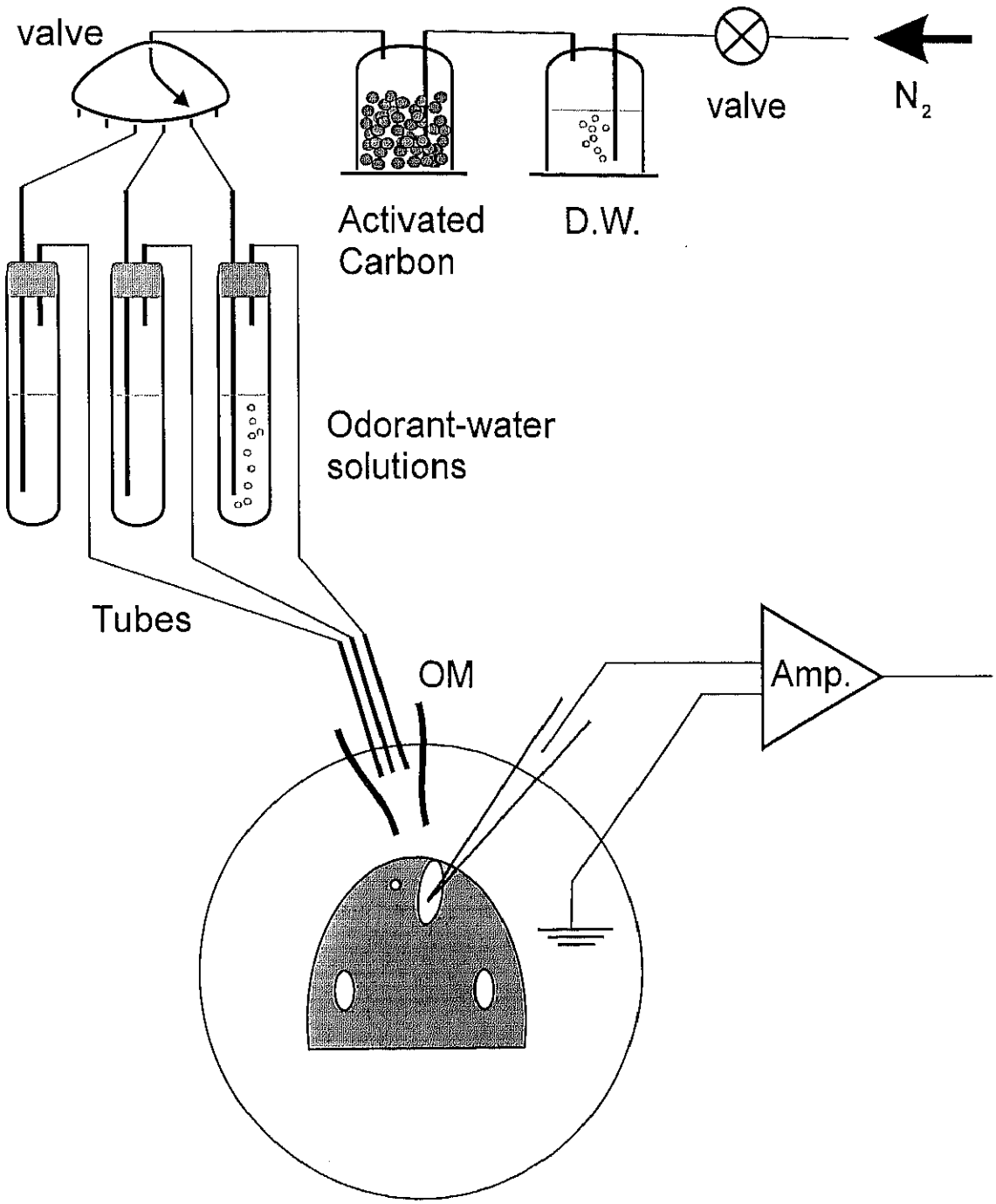
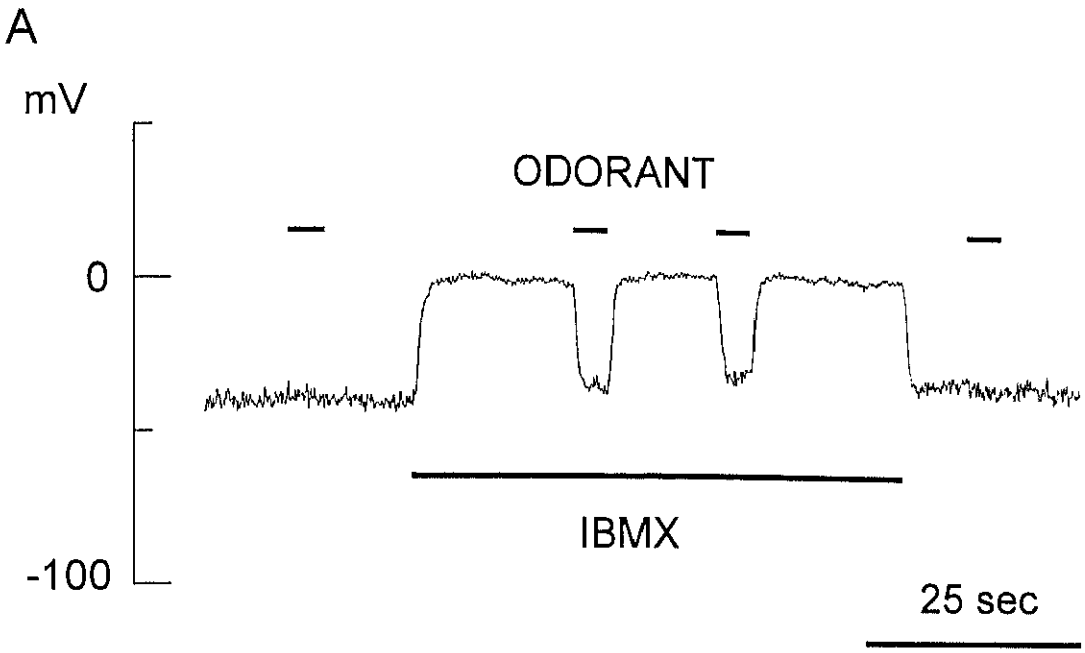

valve
valve
N_2
Activated Carbon
D.W.
Odorant-water solutions
Tubes
OM
Amp.

Figure 11

Odorant-inhibited the membrane depolarization caused by application of IBMX. (A) Responses to 1 mM anisole before, during, and after application of 0.25 mM IBMX under whole cell current clamp mode. The application of chemicals (ODORANT, 1 mM anisole; IBMX, 0.25 mM) are indicated by solid bars (see METHODS). (B) Responses to 1 mM anisole with superimposed hyperpolarizing voltage pulses. The recording was obtained after the experiment in (A) from the same cell. The hyperpolarizing voltage pulses were caused by repetitive injection of a 20 pA current pulse.


A
mV
ODORANT
0
IBMX
-100
25 sec

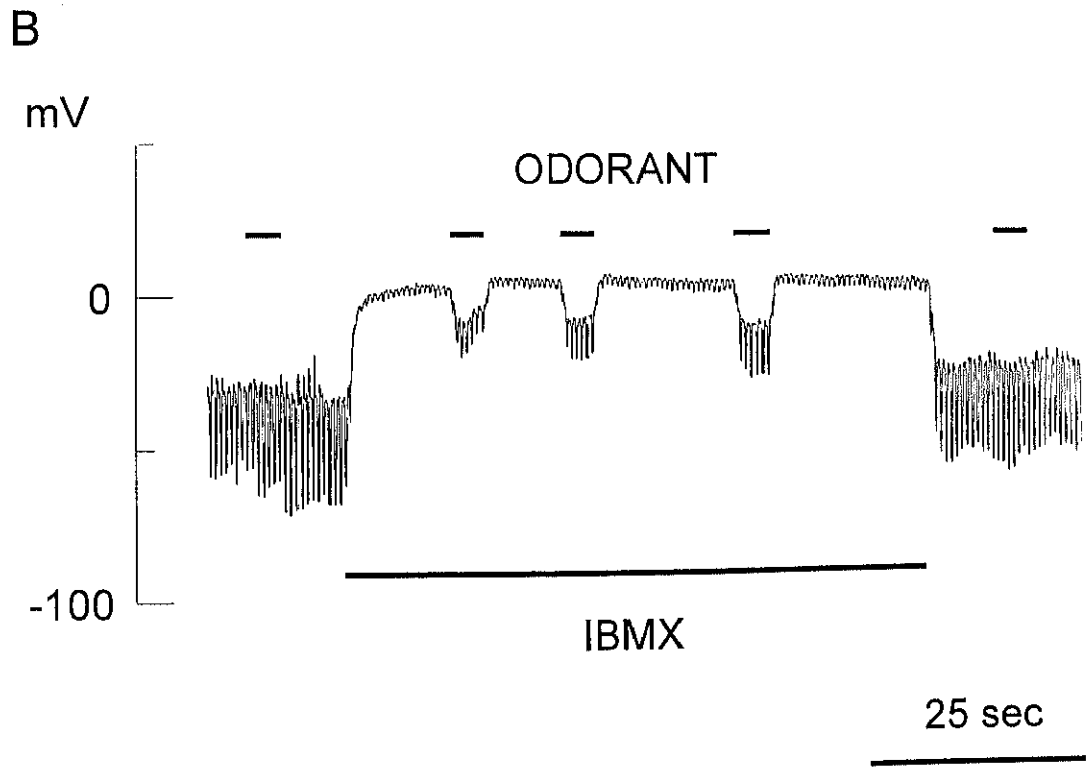

B
mV
ODORANT
0
IBMX
-100
25 sec

Figure 12

Suppression of IBMX-induced current by odor stimulation. (A) Current recordings under whole cell voltage clamp at the holding potential +25 mV. Solid bars indicate the application of chemicals (IBMX, 0.25 mM; ODORANT, 1 mM anisole; TEA, 10 mM). Spikes in the trace represent the application of voltage ramps (35 to -45 mV). Outward membrane current is plotted upward. (B) Current-voltage (I-V) relationship obtained from the experiment in (A), in the absence (b1, solid line) or presence (b2, dotted line) of 1 mM anisole before the application of 0.25 mM IBMX. (C) I-V relationship obtained from the experiment in (A), in the absence (c1, solid line) or presence (c2, dotted line) of 1 mM anisole during application of 0.25 mM IBMX. (D) I-V relationship of IBMX-induced current (c1-b1, solid line), which is the difference between c1 in (C) and b1 in (B), and I-V relationship of odor suppressed current (c1-c2, dotted line), which is the difference between c1 and c2 in Fig. 2C.

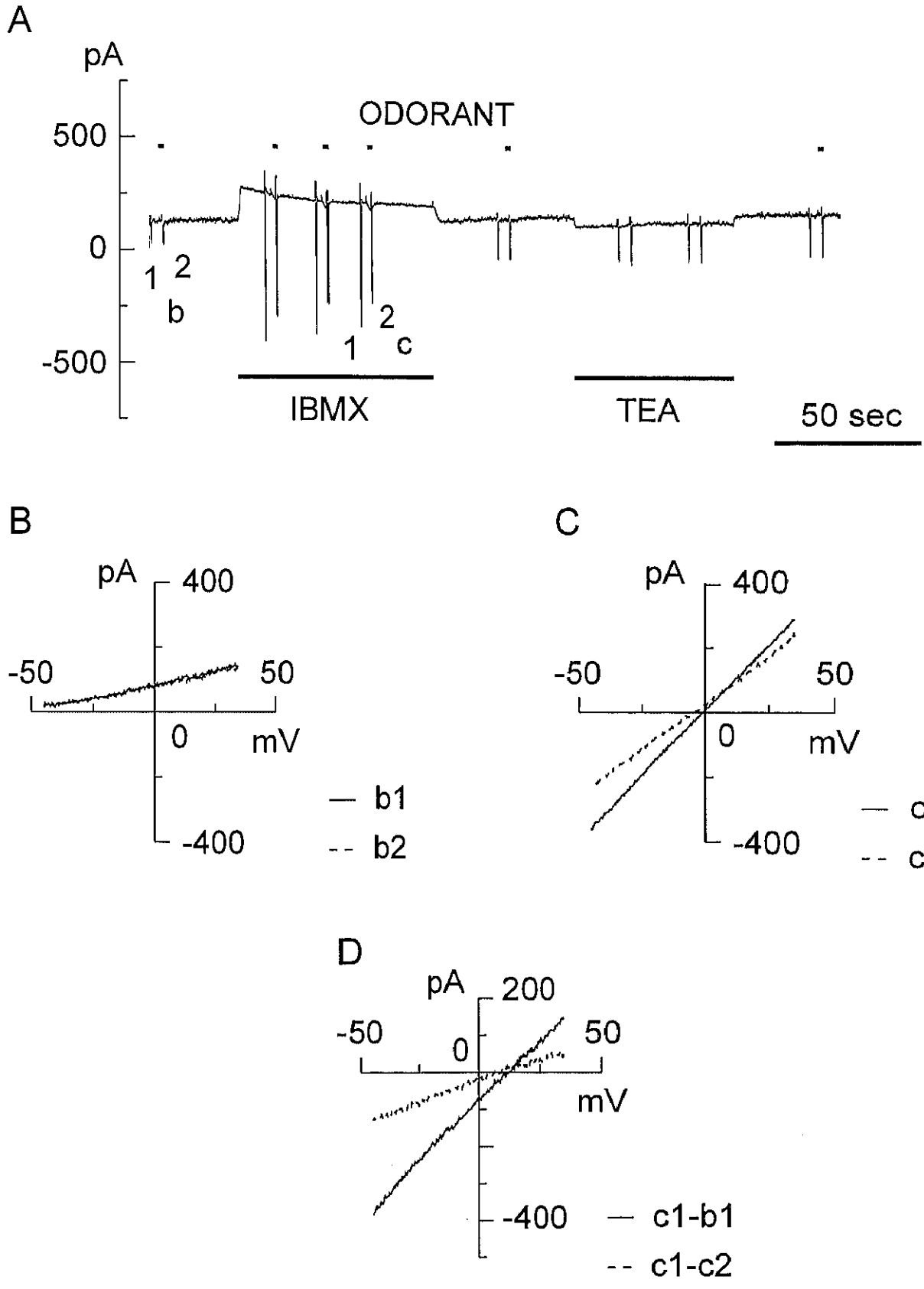
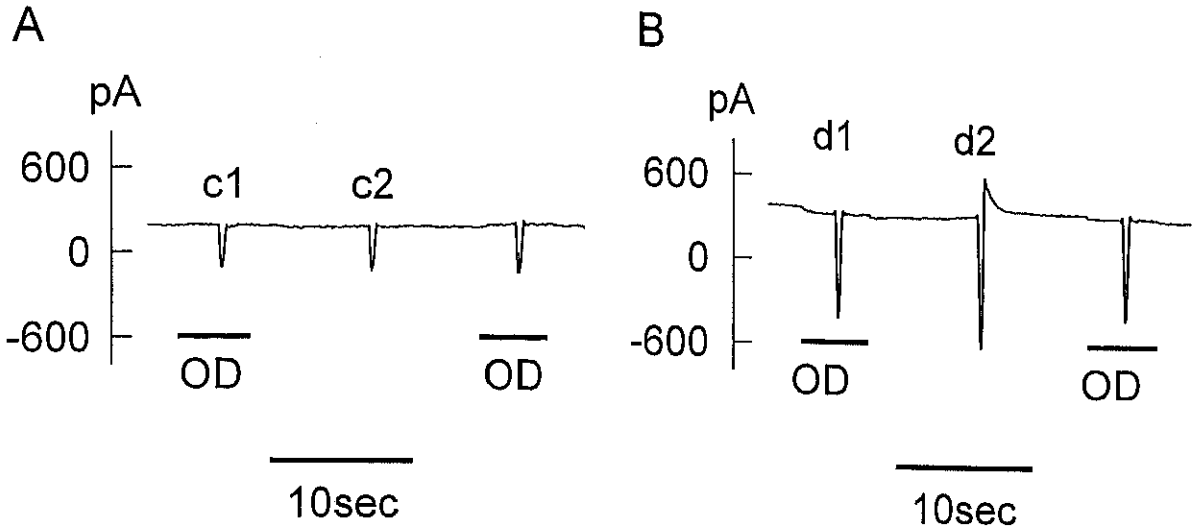
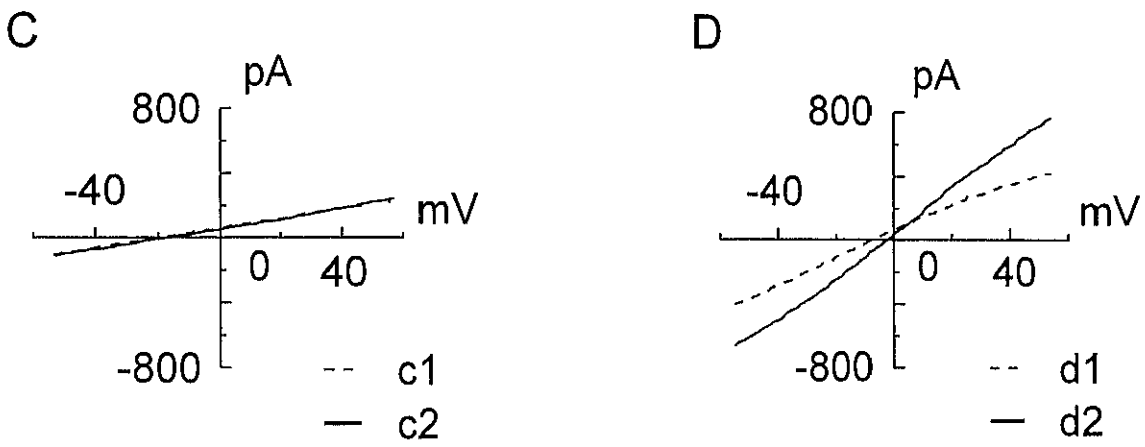

A
pA
500
0
-500
ODORANT
1
2
b
2
1
c
IBMX
TEA
50 sec
B
pA
400
-50
50
0
mV
-400
b1
b2
C
pA
400
-50
50
0
mV
-400
c1
c2
D
pA
200
-50
0
50
mV
-400
c1-b1
c1-c2

Figure 13

Suppression of the cAMP-induced current by odorant. Current recordings at +25 mV were performed before (A) and during (B) intracellular perfusion with 0.5 mM cAMP. Spikes in the trace represent the application of voltage ramps (-55 to +55 mV). Bars below the trace indicate odorant stimulation (a mixture of 1.3 mM n-amylacetate, 1.3 mM isoamylacetate, and 0.25 mM limonene). The recording pipette was filled with the standard pseudo-intracellular solution before the perfusion (A). (C) I-V relationship obtained from the results of the experiment in (A), in the presence (c1) and absence (c2) of odorant mixture. (D) I-V relationship obtained from the experiment in (B), in the presence (d1) and absence (d2) of odorant mixture during intracellular cAMP perfusion. (E) I-V relation of the current suppressed by odorants (dotted line), which is the difference between d2 and d1 in (D), and I-V relation of the cAMP-induced current (solid line), which is the difference between the I-V relations d2 in (D) and c2 in (C).


A
pA
600
0
-600
c1
c2
OD
OD
10sec
B
pA
d1
d2
600
0
-600
OD
OD
10sec


C
pA
800
-40
mV
0
40
-800
c1
c2
D
pA
800
-40
mV
0
40
-800
d1
d2

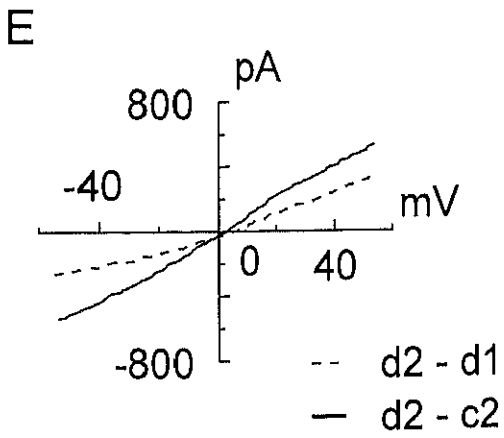

E
pA
800
-40
mV
0
40
-800
d2 - d1
d2 - c2

Figure 14

Suppression of the 8-Br-cGMP induced current by odorant. Current recording was performed under whole cell voltage clamp at +25 mV. Solid bars indicate the application of chemicals (8-Br-cGMP, 1 mM; ODORANT, 1 mM anisole). Spikes in the trace represent application of voltage ramps (45 to -55 mV).

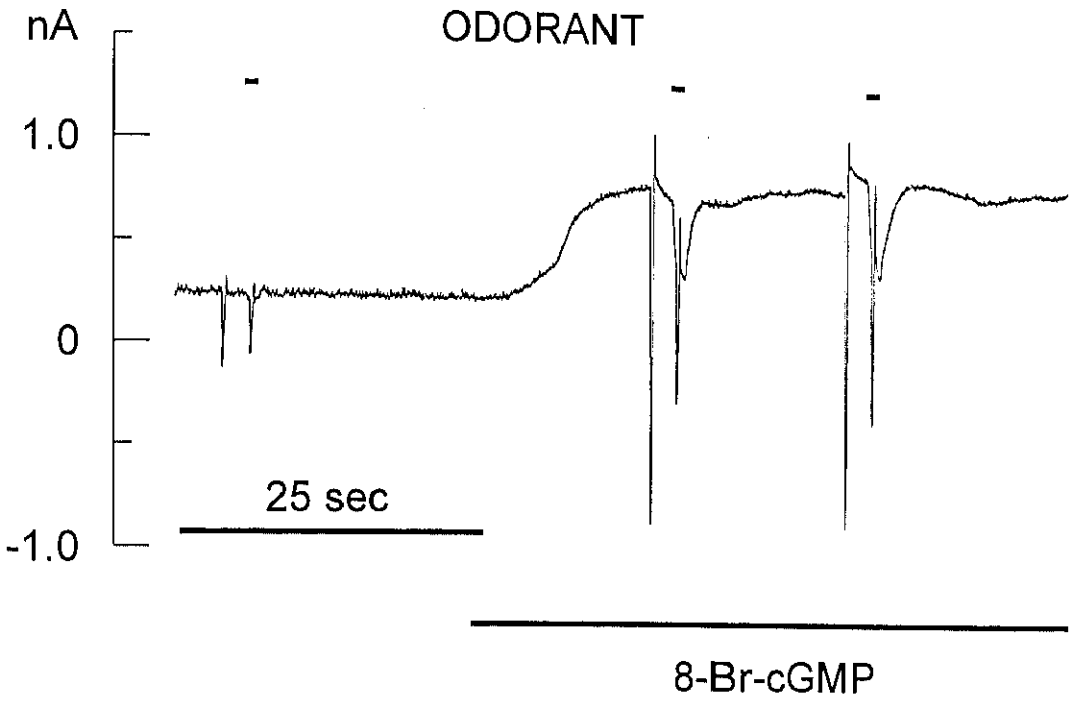

nA
ODORANT
1.0
0
-1.0
25 sec
8-Br-cGMP

Figure 15

Comparison of odor inhibitory effects on the IBMX-, the cAMP-, and the 8-Br-cGMP-induced current. Shown data are collections of the recordings that were performed under the same conditions as for Figure 12, 13, and 14 respectively. Bar graphs show the average percentage suppression of the IBMX-, the cAMP-, and the 8-Br-cGMP-induced current during odor stimulation relative to those without odorant in each cell. Short vertical bars represent the SE; the numbers of cells are shown in each bar. The percentages of the suppression are almost the same ($p>0.25$).

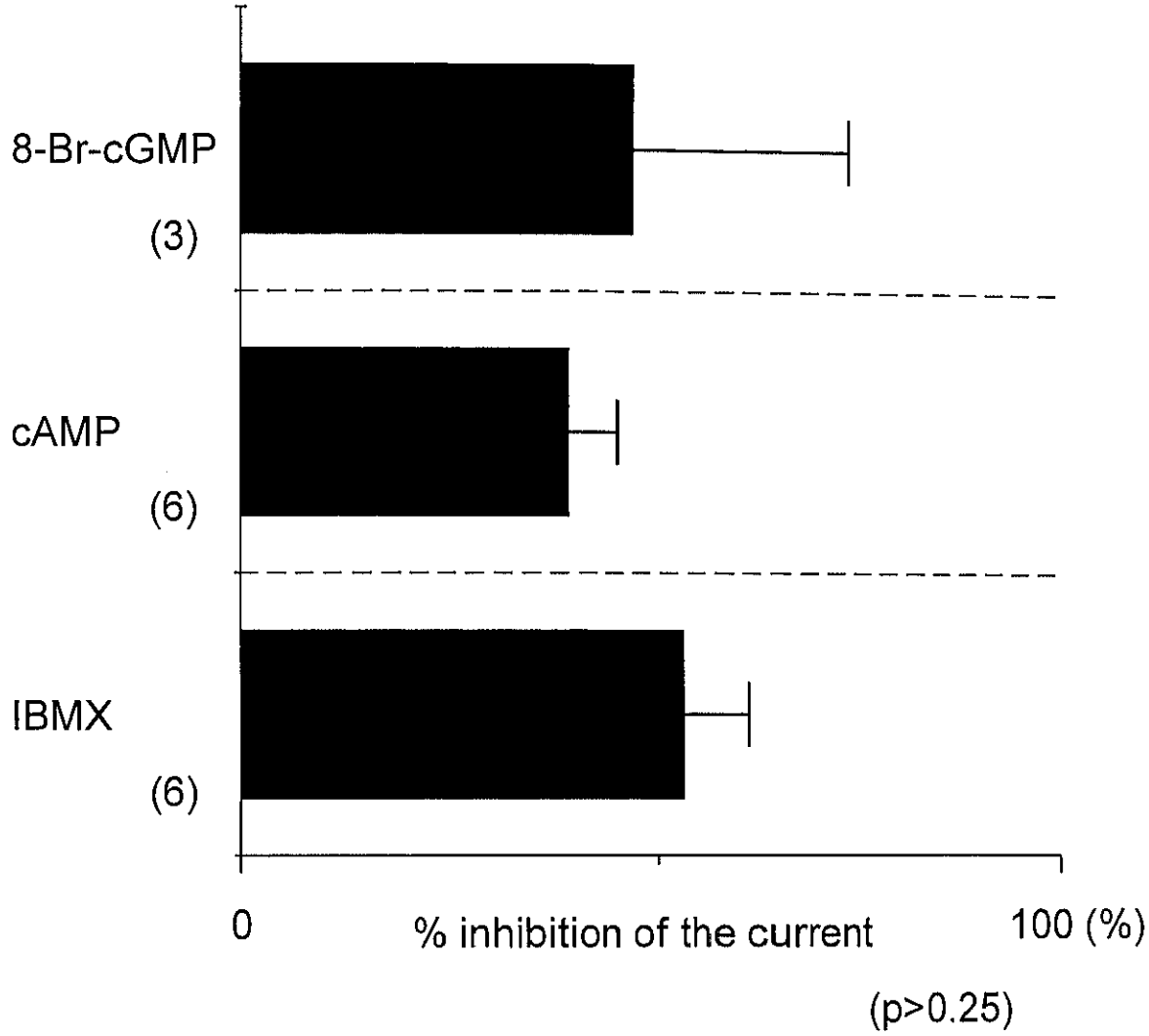
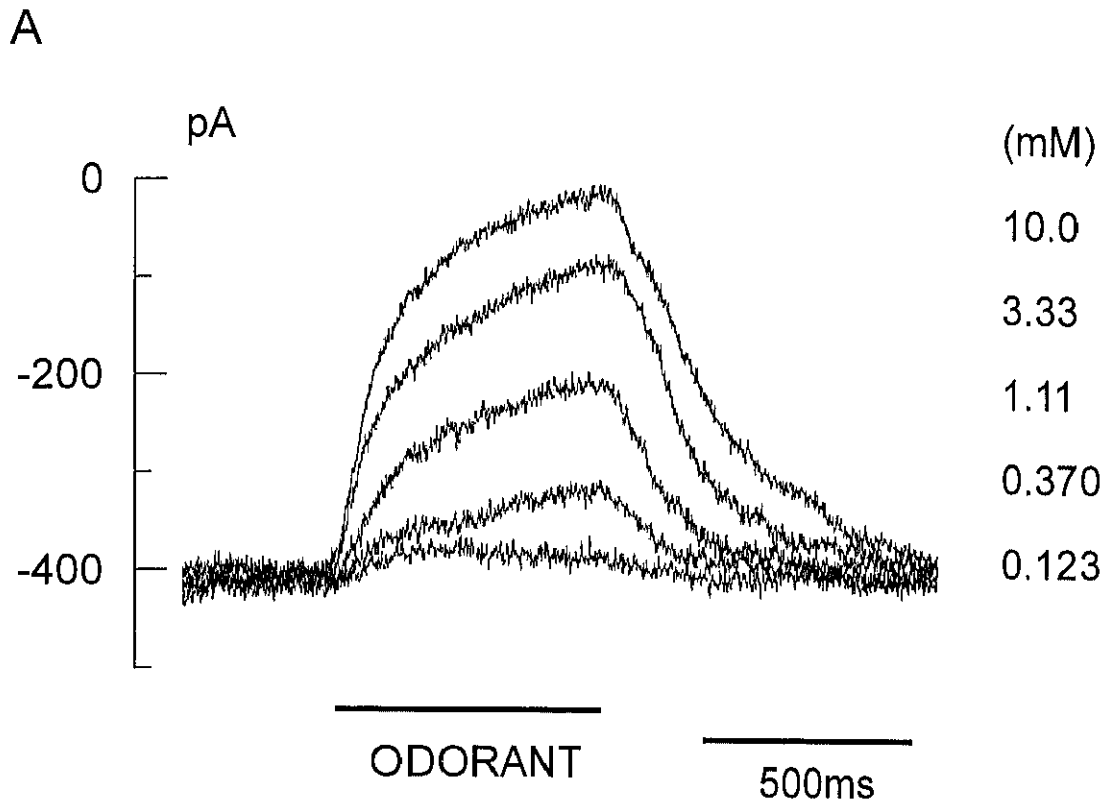

8-Br-cGMP
(3)
cAMP
(6)
IBMX
(6)
0
% inhibition of the current
100 (%)
(p>0.25)

Figure 16

Dose-dependent suppression of the IBMX-induced current by odorant. (A) Suppression of 0.25 mM IBMX-induced current by 10, 3.33, 1.11, 0.370, and 0.123 mM anisole. Recording was performed under whole cell voltage clamp at -50 mV from the cells that did not respond to the tested odorants alone. After eliciting inward current by the application of 0.25 mM IBMX, the cell was placed sequentially in one of a series of solutions with decreasing concentration of odorant for 600 ms. (B) Collected dose-response curves from five cells. The vertical axis shows the percentage of current suppression during odor stimulation relative to the IBMX-induced current in each cell; the horizontal axis shows the odorant concentration. Dotted curves is the best fit of the Hill equation; $y=E_{max}[x^n/(x^n+IC_{50}{}^n)]$, where E_{max} is the maximal percentage of inhibition, which was normalized to 100%. Values of n and IC_{50} were as follows: (+) n=1.2, IC_{50}=0.84 mM; (○) n=2.2, IC_{50}=1.4 mM; (△) n=1.0, IC_{50}=1.6 mM; (□) n= 0.93, IC_{50}=2.0 mM; (×) n=1.2, IC_{50}=1.0 mM.


A
pA
0
-200
-400
(mM)
10.0
3.33
1.11
0.370
0.123
ODORANT
500ms

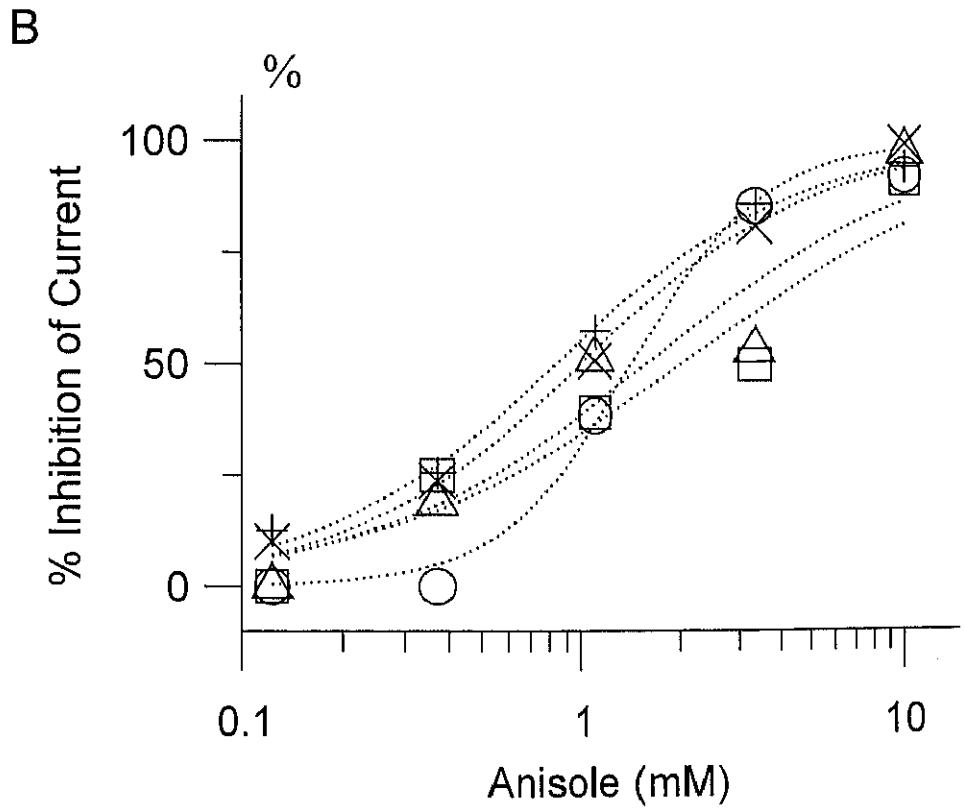
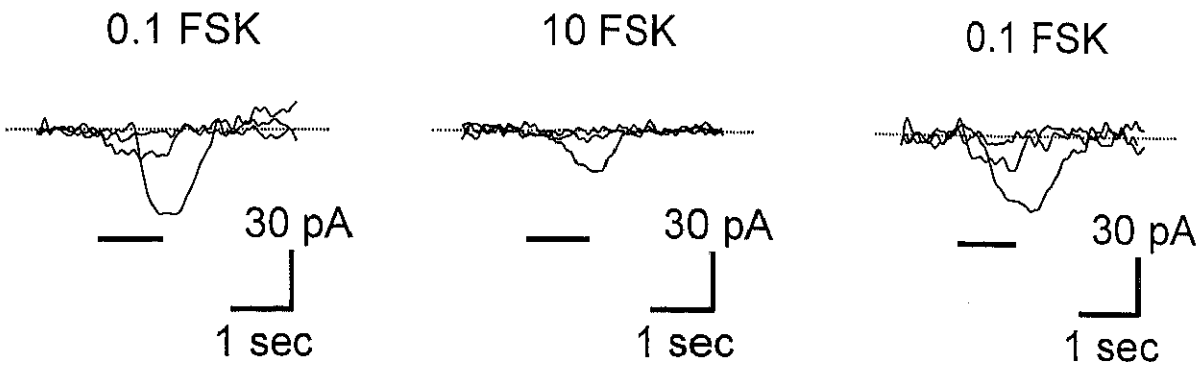

B
%
100
50
0
% Inhibition of Current
0.1
1
10
Anisole (mM)

Figure 17

Antagonizing odor suppression with forskolin. (A) Current suppressions by 0.2, 1, and 5 mM isoamyl acetate during bath application of 0.1 μM forskolin (left panel), 10 μM forskolin (middle panel), and reapplication of 0.1 μM forskolin (right panel). This recording was obtained from a cell voltage clamped at +25mV. A pipette was filled with standard pseudo-intracellular solution containing 1 mM ATP. (B) Peak amplitudes of the current suppression as a function of the odorant concentration during the application of 0.1 (open circle, cross) and 10 (solid circle) μM forskolin. The data points were plotted from (A) (open circle, left traces; solid circle, middle traces; cross, right traces in (A)).

A


0.1 FSK
10 FSK
0.1 FSK
30 pA
1 sec
30 pA
1 sec
30 pA
1 sec

B

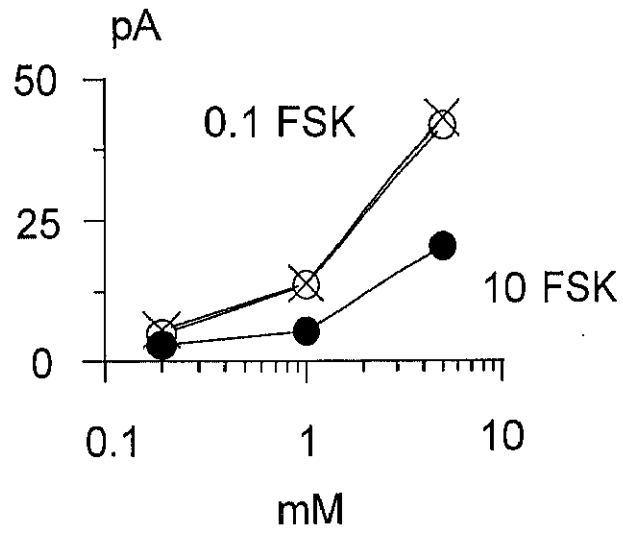
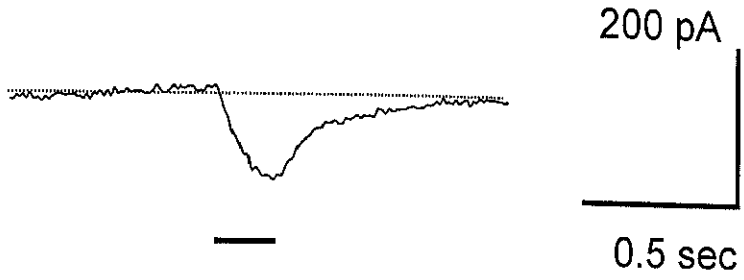

pA
50
25
0
0.1 FSK
10 FSK
0.1
1
10
mM

Figure 18

Antagonizing odor suppression by cAMP. (A) Current suppression on 0.1 mM cAMP introduced cell by an odorant (a mixture of 1.1 mM cineole, isoamyl acetate, anisole, and limonen). A recording pipette was filled with standard pseudo-intracellular solution containing 0.1 mM cAMP. After rupturing patch membrane, the introduction of cAMP into a cell developed outward current at +25 mV. After developing outward current, an application of odorant suppressed the outward current. The timing of odor application is indicated with bars at the bottom. (B) Comparison between amplitudes of current suppression on 0.1 mM cAMP introduced cells (0.1 cAMP) and those on 10 mM cAMP introduced cells (10 cAMP). Recording pipettes were filled with standard pseudo-intracellular solution with 0.1 mM cAMP (0.1 cAMP) or 10 mM cAMP (10 cAMP). Amplitudes of current suppression in each condition were recorded in the same procedure shown in (A). Bars indicates mean peak amplitudes of current suppressed by the odorant mixture. Short vertical bars represent the SE; the numbers of cells are shown in the Figure. The difference in the amplitude between responses in 0.1 mM cAMP introduced cells and those in 10 mM cAMP introduced cells was statistically significant ($p<0.01$).

A



B

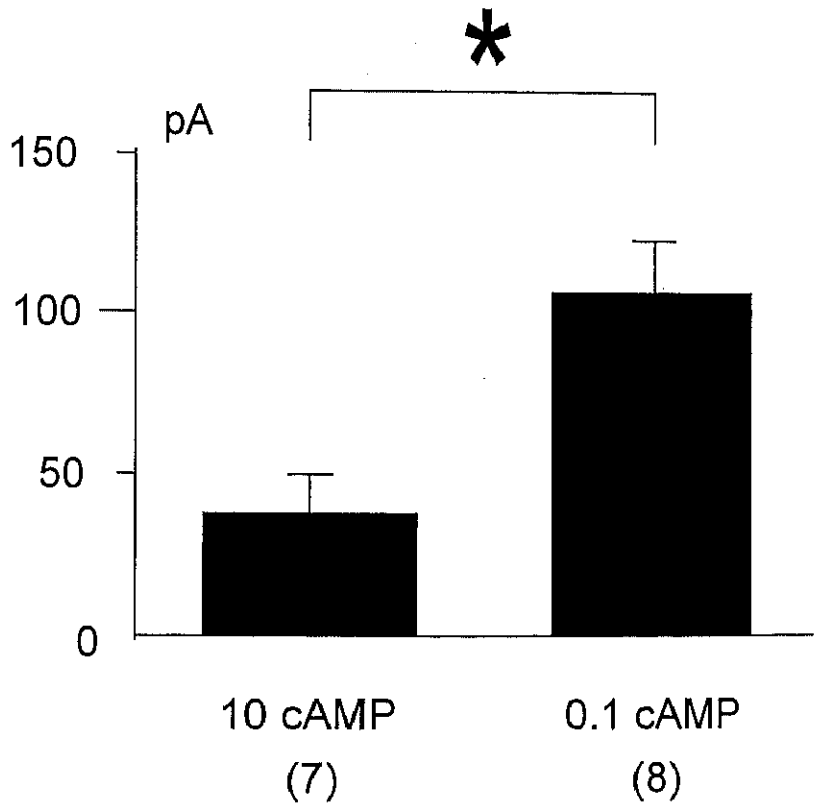


Figure 19

Inhibitory effect of various odorants on the IBMX-induced current. Recordings were performed under the same conditions as for Fig. 16. Bar graphs show the average percentage suppression of IBMX-induced current during odor stimulation (CIN, 1 mM cineole; ISO, 1 mM isoamyl acetate; LIM, 1 mM (+)-limonene; IVA, 1 mM isovaleric acid). Short vertical bars represent the SE; the numbers of cells are shown under the graphs.

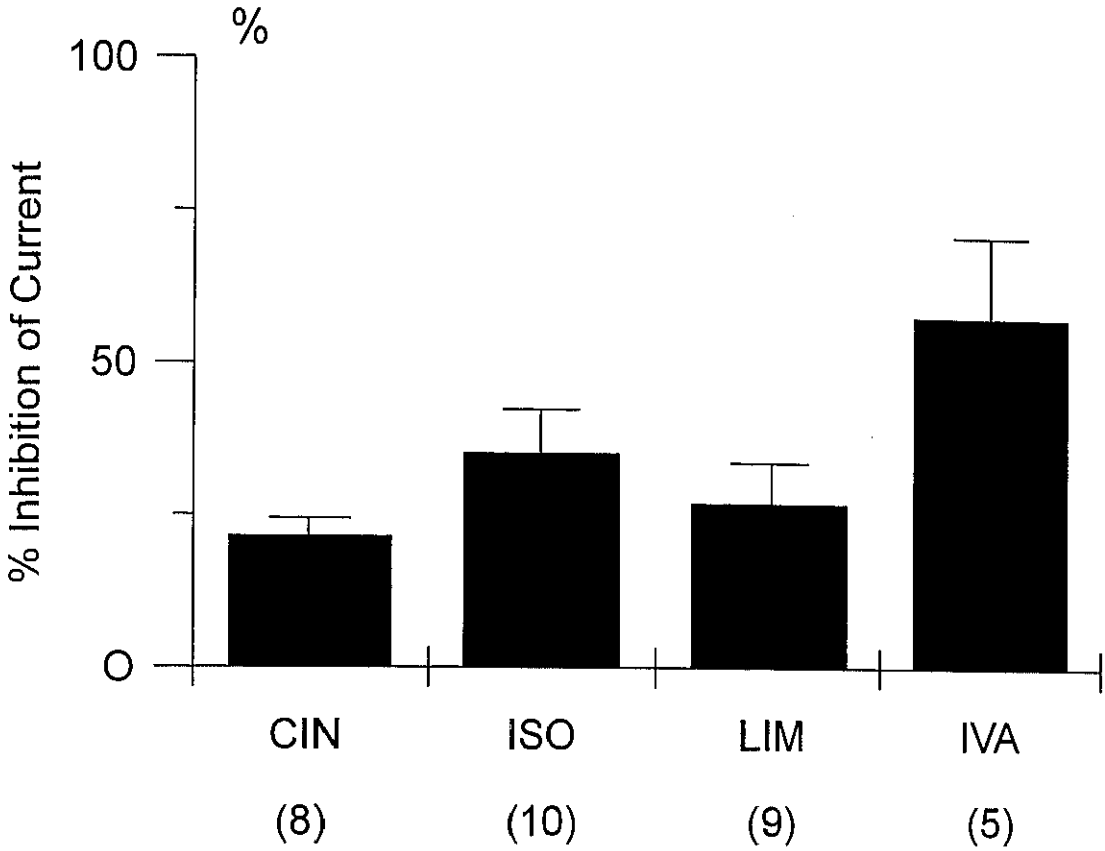

%
100
50
O
% Inhibition of Current
CIN
ISO
LIM
IVA
(8)
(10)
(9)
(5)

Figure 20

Comparison of odor response profiles between inhibition and excitation. (A) Profiles of cell's inhibitory responses to odorants (1mM cineole, isoamyl acetate, anisole, and (+)-limonene). Recordings from eight cells were performed under the same conditions as for Fig. 16. These profiles recorded from eight cells are a subset of collection data in Figure 21. Bar graphs show the amplitude of current suppression of IBMX-induced current during each odorant. (B) Profiles of cell's excitatory responses to odorants (1mM cineole, isoamyl acetate, anisole, and (+)-limonene). Bar graphs show amplitudes of excitatory potential responses to the odorants, which were recorded by current-clamp mode, obtained from another subset of eight cells.

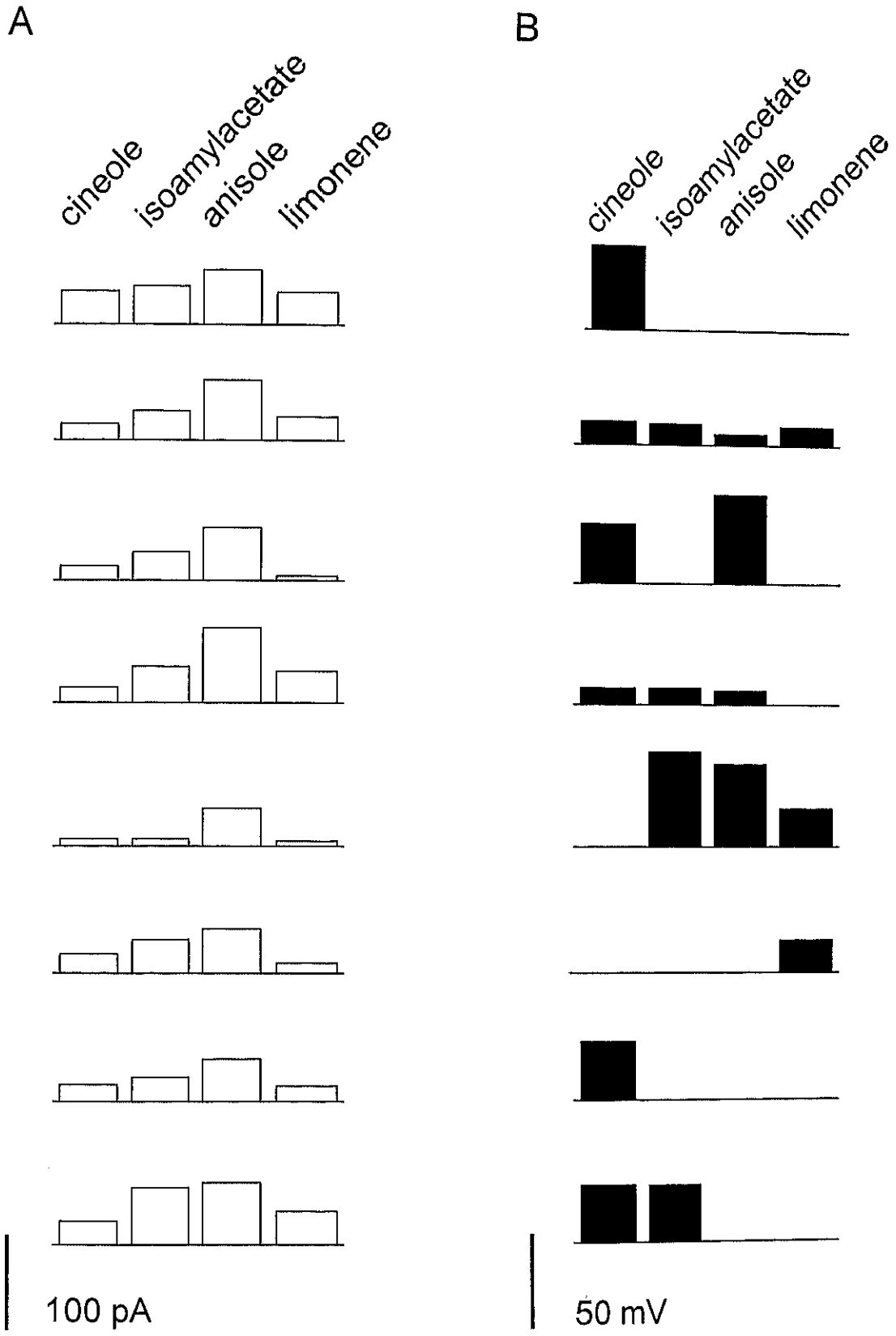
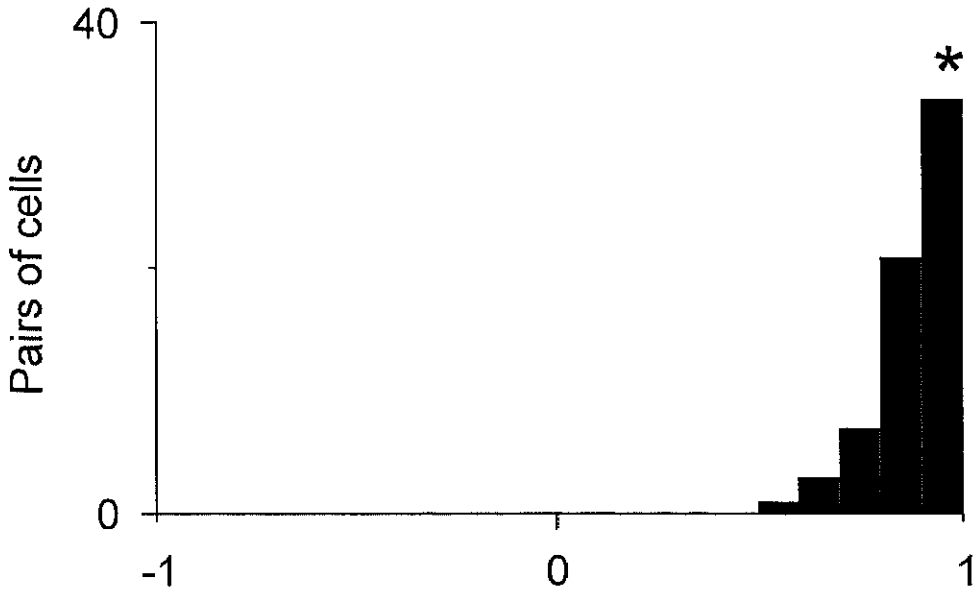

A
cineole
isoamylacetate
anisole
limonene
100 pA
B
cineole
isoamylacetate
anisole
limonene
50 mV

Figure 21

Pearson's 'r' correlation coefficient values for the pair of cells and the pair of odorants. Profiles of current suppression by 1mM cineole (CIN), isoamyl acetate (ISO), anisole (ANI), and (+)-limonene (LIM) were recorded from twelve cells under the same conditions as for Figure 16. Profiles shown in Figure 20 are a sample subset of profiles recorded from these twelve cells. (A) Distribution of Pearson's 'r' correlation coefficient values for the pair of cells. The column with an asterisk is above the threshold ($p<0.05$). (B) Pearson's 'r' correlation coefficient values for the pair of odorants. The 'r' values for all the six pairs of odorants were over 0.85, which is well above the highly significant level of $p<0.001$.

A



B

	CIN	ISO	ANI	LIM
CIN	1			
ISO	0.930	1		
ANI	0.935	0.978	1	
LIM	0.922	0.856	0.865	1

Figure 22

Odor inhibition of odor excitation, under whole cell current clamp. Solid bars indicate the application of odorants (isoamyl acetate, 1 mM; anisole, 1 mM).

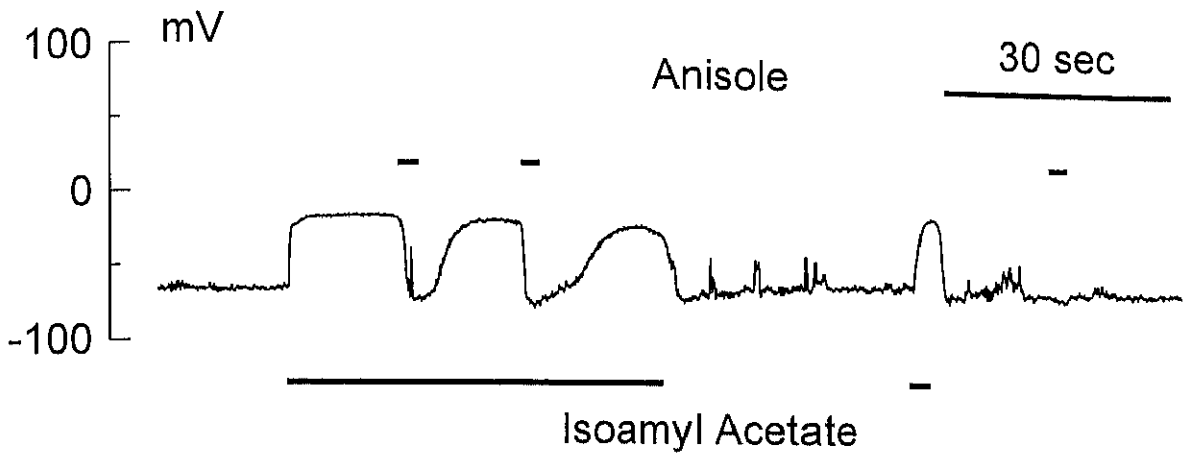

100
mV
0
-100
Anisole
30 sec
Isoamyl Acetate

Figure 23

Typical relations between amplitude of responses to individual odorants and those to mixture odorant. (Left column): Current recordings from four cells. Traces are current responses to 0.5 mM isoamyl acetate (blue line), anisole (red line), and a mixture of both odorants (dashed line). Vertical axes show normalized value. The normalized responses for each odorant was computed by dividing the test odorant response amplitude by the amplitude of the isoamyl acetate response in each cells. Cells were voltage clamped at +25 mV, so that excitatory responses to odorants exhibited outward current response. (Middle column): Normalized amplitudes of anisole responses in each cell. The value in each cell was computed by dividing the anisole response amplitude by the amplitude of the isoamyl acetate response. (Right column): % inhibition of isomamyl acetate responses. The value in each cell shows a percentage of response decrease when the mixture odorant was applied, compared to isoamyl acetate response.

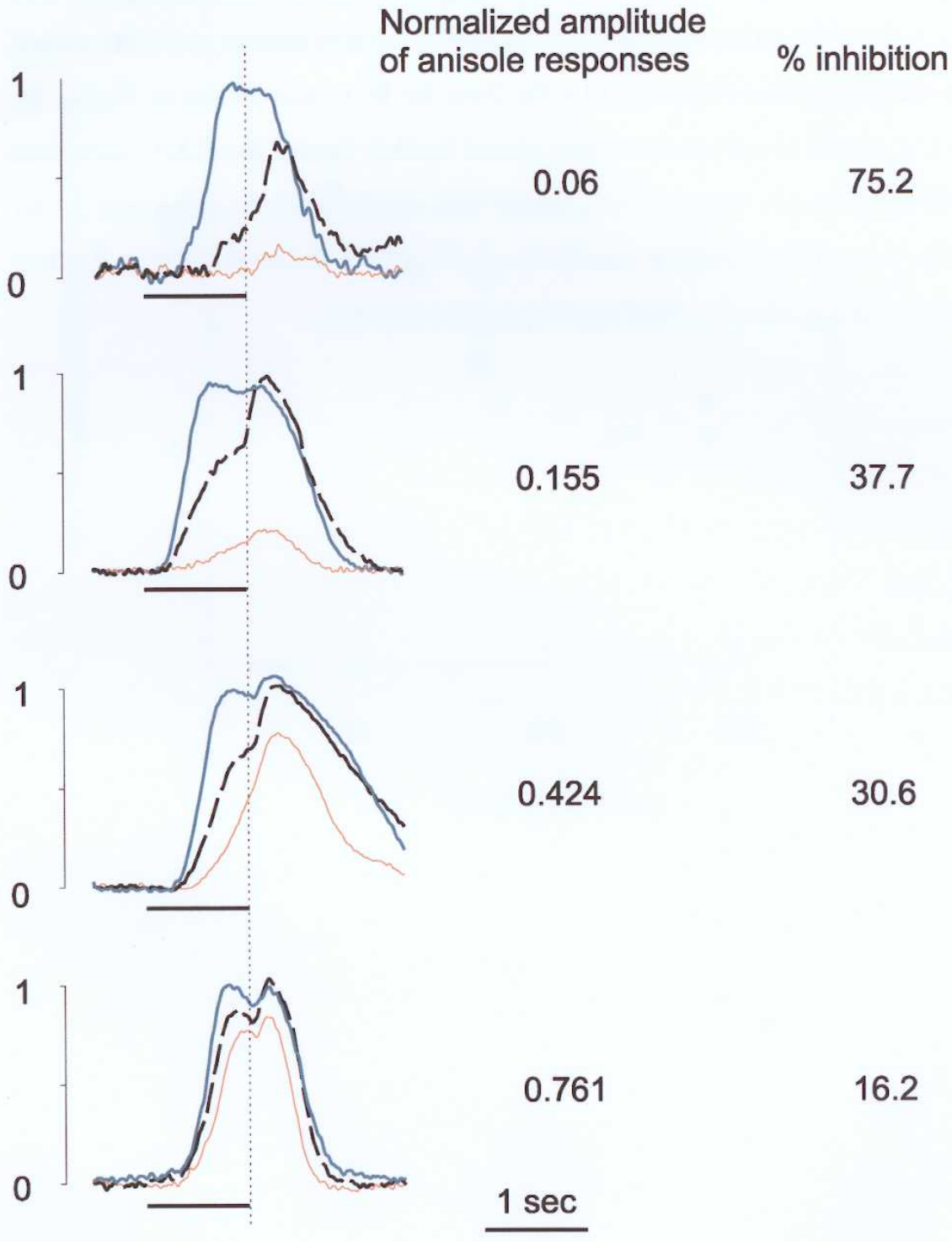
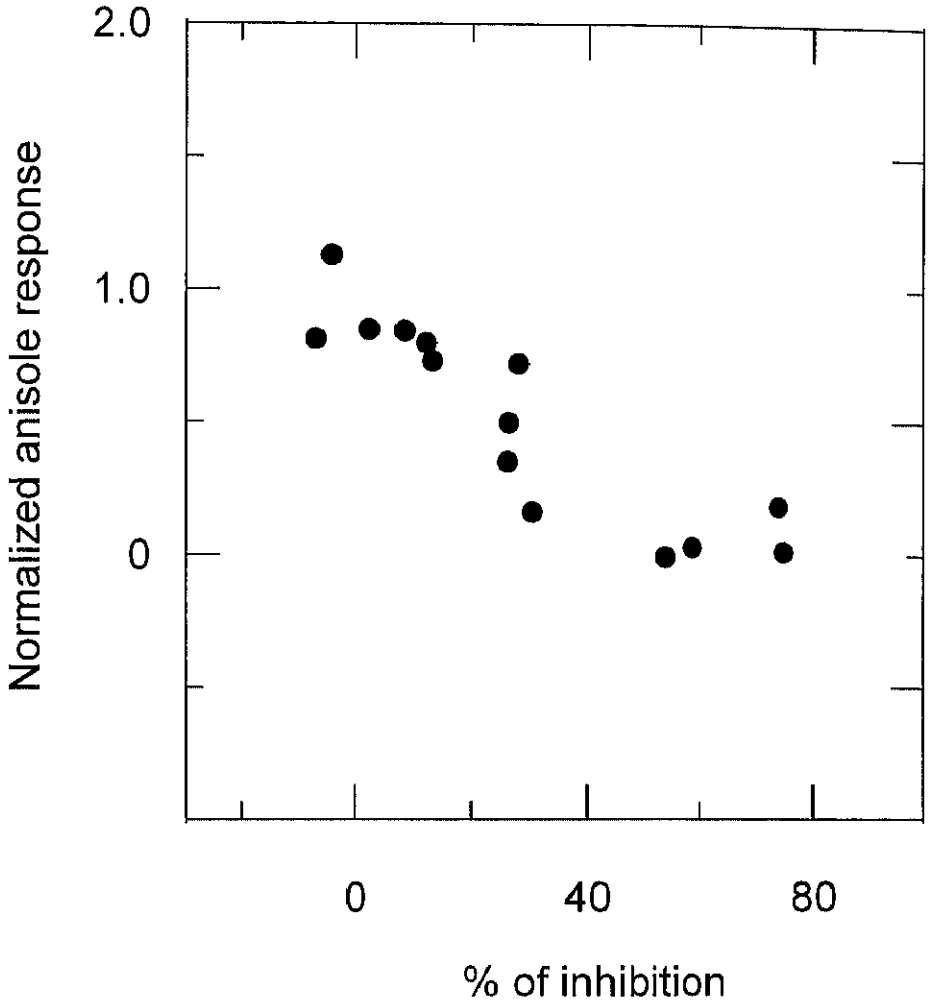

Normalized amplitude
of anisole responses
% inhibition
1
0
0.06
75.2
1
0
0.155
37.7
1
0
0.424
30.6
1
0
0.761
16.2
1 sec

Figure 24

Normalized amplitude of anisole responses versus % of inhibition. The data were derived from current responses to 0.5 mM anisole, 0.5 mM isoamyl acetate, and the mixture in 14 cells. Data for four cells, shown in Figure 23, are a subset of collection of data shown in this figure. Each dot represents calculated value from one cell, which was obtained in the same way as for Figure 23. The calculated correlation coefficient is -0.89, which is well above the threshold for statistical significance ($p<0.00002$).



r= -0.89 P<0.00002

Figure 25

Mixture suppression in EOG recordings. (A) EOG response to individual odorants and mixtures. The trace shows EOG responses to odorants (in mM): 5 benzene (B), 5 isoamyl acetate (I), 5 isoamyl acetate + 5 anisole (I+A), 5 benzene + 5 anisole (B+A), 5 anisole (A), and 20 isoamyl acetate (20 I). The amplitude of the response to a mixture of anisole and isoamyl acetate was 75% smaller than that to anisole alone. (B) Comparisons of the extent of suppression by adding 5 mM anisole to 5 mM benzene ([B+A]/[B]) or isoamyl acetate ([I+A]/[I]). The magnitude of the response to 5 mM isoamyl acetate was reduced by adding 5 mM anisole ($p<0.002$), while the effect of adding 5 mM anisole to 5 mM benzene was not significant ($p>0.6$). The extent of suppression by adding 5 mM anisole to 5 mM benzene was significantly smaller than that by adding 5 mM anisole to 5 mM isoamyl acetate ($p<0.03$). (C) Comparison of the extent of suppression by adding 5 mM isoamyl acetate to 5 mM amyl acetate ([M+I]/[M]) or cineole ([C+I]/[C]). An addition of 5 mM isoamyl acetate to 5 mM cineole reduced the magnitude of the response ($p<0.04$), while the effect of adding 5 mM isoamyl acetate to 5 mM amyl acetate was not significant ($p>0.07$). The extent of suppression by adding 5 mM isoamyl acetate to 5 mM amyl acetate was smaller than that by adding 5 mM isoamyl acetate to 5 mM cineole statically ($p<0.01$).

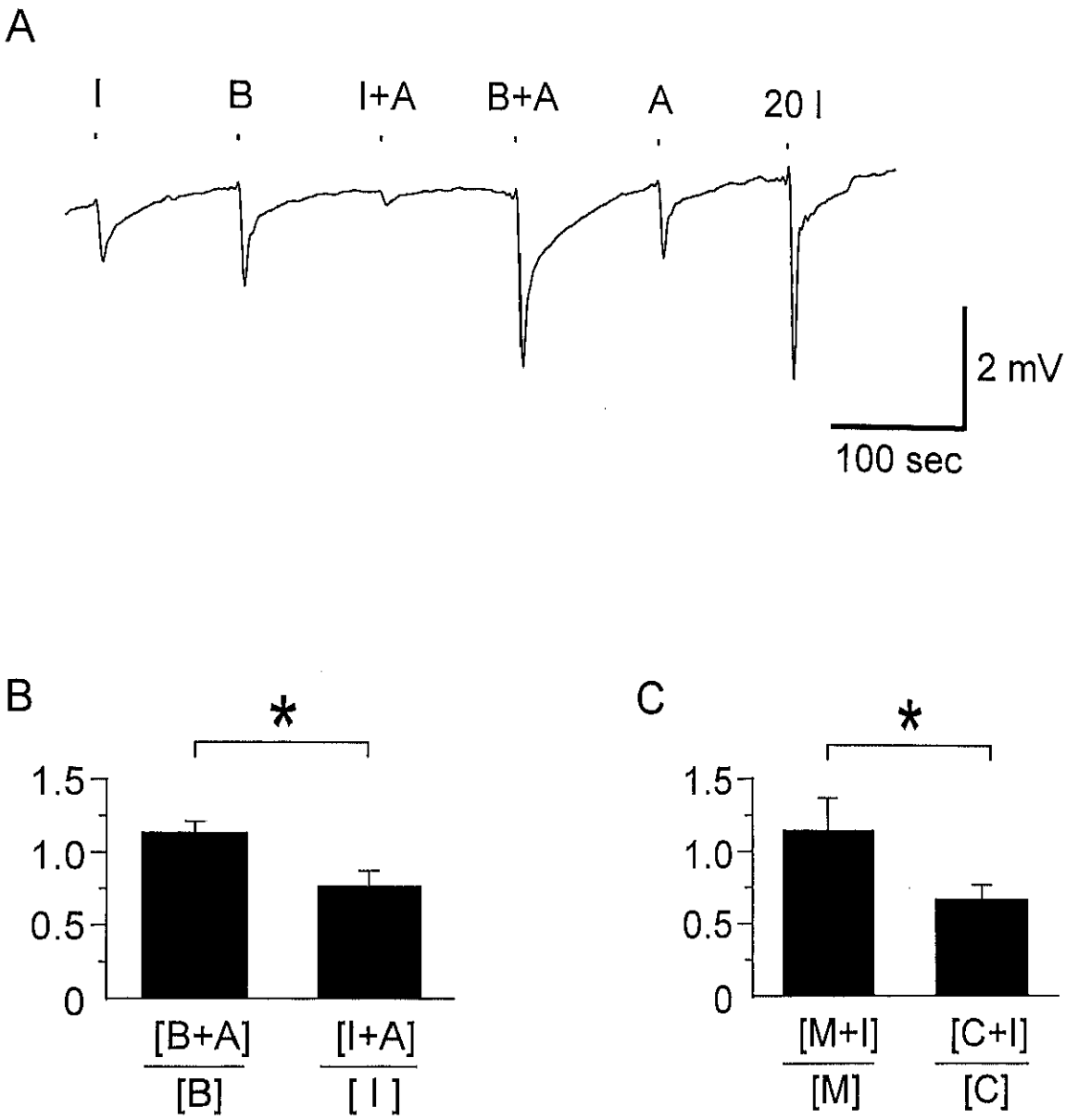
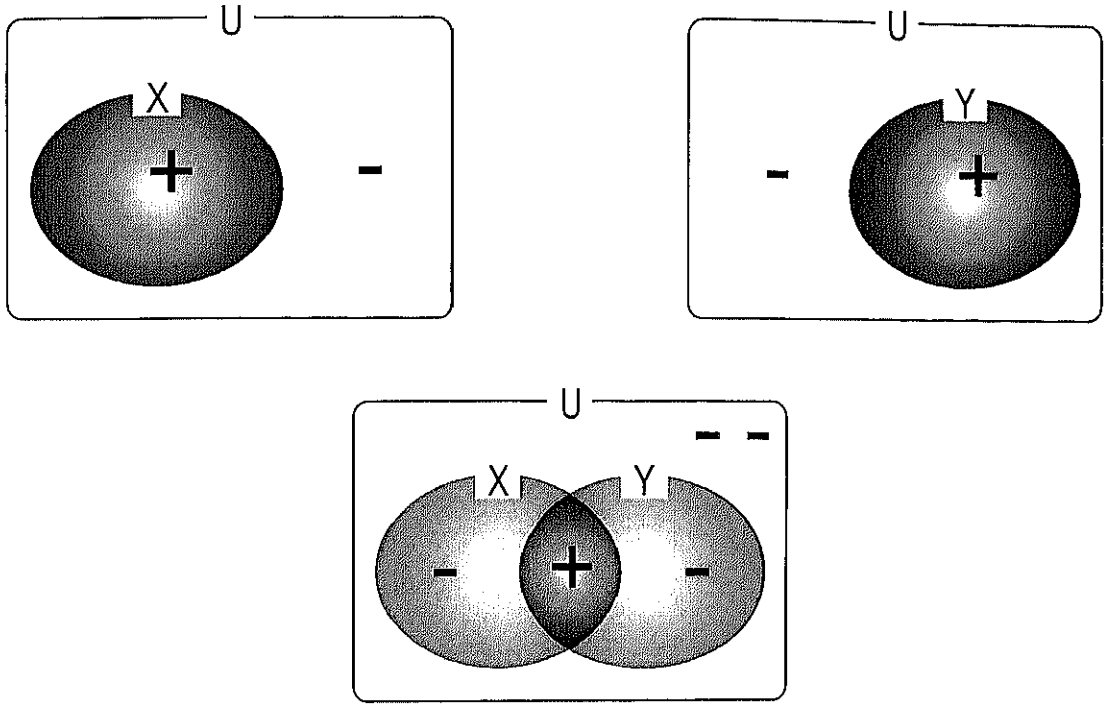

A
I
B
I+A
B+A
A
20 I
2 mV
100 sec
B
*
1.5
1.0
0.5
0
[B+A]
[B]
[I+A]
[I]
C
*
1.5
1.0
0.5
0
[M+I]
[M]
[C+I]
[C]

Figure 26

Schematic model of representation of mixture odorant. An application of an odorant elicits excitation in a particular subset of ORNs (shown as X or Y). Whereas an odorant elicits excitation in cells that have excitatory specificity to this odorant, this odorant have an inhibitory effect in cells that have no excitatory specificity to this odorant. Therefore, when two odorants are applied simultaneously, the two odorants would suppress the responses each other. Overlapping part of the two subset means a population of ORNs that responds to both odorant excitatory. When the two odorants are similar, the overlap forms a large part.


U
X
+
-
U
Y
-
+
U
- -
X
Y
-
+
-