*J. exp. Biol.* ***174****, 215–233 (1993)*
*Printed in Great Britain © The Company of Biologists Limited 1993*



# ODOR SENSITIVITY OF CULTURED LOBSTER OLFACTORY RECEPTOR NEURONS IS NOT DEPENDENT ON PROCESS FORMATION

BY D. A. FADOOL, W. C. MICHEL* AND B. W. ACHE

*Whitney Laboratory and Departments of Zoology and Neuroscience, University of Florida, 9505 Ocean Shore Blvd, St Augustine, FL 32086, USA*

*Accepted 24 August 1992*

## Summary

Cultured lobster olfactory receptor neurons (ORNs) were surveyed for their odor sensitivity with whole-cell, voltage-clamp recording. The nature of the adequate stimuli, the degree of tuning (response spectra) of the cells, the threshold of sensitivity and the dual polarity of the odor-evoked currents are consistent with chemosensitivity in the cultured ORNs being olfactory. The ability of odors to evoke currents in cultured ORNs that lack processes suggests that lobster ORNs can be induced *in vitro* to insert all the elements of the transduction cascade in the soma, including those that might normally be confined to processes. This should greatly facilitate analysis of olfactory transduction in these cells.

## Introduction

The small size and thin, elongated morphology of olfactory receptor neurons (ORNs) has made the study of olfactory transduction difficult. The advent of patch-clamp recording ameliorated this situation and facilitated progress towards understanding olfactory transduction (reviews: Anholt, 1991; Firestein, 1991). Central to this effort has been the ability to dissociate ORNs from their surrounding epithelium in order to study them directly or in sustained primary culture. Dissociated ORNs are not only accessible for patching, they often assume a more compact form than their counterparts *in situ* that allows a reasonable space-clamp and facilitates diffusion of membrane-impermeant probes from the electrode to the site of transduction. While transduction is thought to occur in the cilia (outer dendrites, in invertebrates) of the ORNs (Kurahashi, 1989; Firestein *et al.* 1990; Lowe and Gold, 1991), at least some elements of the transduction cascade are not confined to the cilia. Specifically, cyclic-AMP-gated cation channels that are the effectors in the transduction cascade in amphibian ORNs also occur on the dendrite and soma of the cells, although in lower densities than on the cilia (Firestein *et al.* 1991; Zufall *et al.* 1991*a*). Indeed, this variability in density was exploited to obtain favorable channel density for recording (Firestein *et al.* 1991; Zufall *et al.* 1991*a*).

*Present address: Department of Physiology, University of Utah School of Medicine, Salt Lake City, UT 84108, USA.

Key words: olfaction, receptor cell, cell culture, lobster, *Panulirus argus.*

Previously, we reported preliminary evidence that cultured lobster ORNs respond to odors regardless of whether the cells had sprouted processes (Fadool *et al.* 1991). This observation raises the interesting possibility that, *in vitro*, all elements of the transduction cascade may be expressed and inserted into the soma of ORNs that fail to sprout processes. Given the ease of patching the soma compared to the extremely thin cilia (outer dendrites) and the ability to culture ORNs from vertebrates (Calof and Chikaraishi, 1991; Coon *et al.* 1989; Ronnett *et al.* 1991; Pixley and Pun, 1990) and other invertebrates (Stengl *et al.* 1989; Zufall *et al.* 1991*b*), such a phenomenon could be of general use for studying olfactory transduction.

Without the normal polarity of the cell, however, it must be established that applied 'odors' are activating what would otherwise be ciliary (dendritic) chemoreceptors. The need to establish the adequacy of odor stimuli is particularly important when studying ORNs from aquatic animals such as fish and lobsters. Adequate olfactory stimuli for many aquatic animals are the blood-borne components of prey, compounds such as amino acids, amines and nucleotides (review: Carr, 1990). Such compounds could be expected to activate cells as neurotransmitters or neuromodulators. In order to establish the usefulness of cultured lobster ORNs for analysis of transduction mechanisms, we have attempted to obtain functional evidence that cultured lobster ORNs with no processes or varying numbers of processes are morphs of the same type of cell and that the odor-evoked properties of the cultured cells reflect those of lobster ORNs *in situ*.

## Materials and methods

### *Tissue culture*

The distinct clusters of the ORNs were dissected from the aesthetasc (olfactory) sensilla on the lateral antennular filament (olfactory organ) of adult specimens of the Caribbean spiny lobster, *Panulirus argus* Latreille. The clusters were enzymatically dissociated, and the resulting cells sustained in primary culture as described previously (Fadool *et al.* 1991). Briefly, the isolated clusters were incubated for 50min at $80\,\mathrm{revs\,min^{-1}}$ on an orbital shaker in a $0.2\,\mu\mathrm{m}$ filter-sterilized solution of 2.5mg papain and 12mg L-cysteine in 10ml of *Panulirus* saline (PS) containing 1% penicillin, streptomycin sulfate and amphotericin B (Gibco). Proteolytic digestion was stopped by replacing the enzyme solution with low-glucose L-15 medium supplemented with L-glutamine, dextrose, fetal calf serum and BME vitamins. Cells were immediately plated on poly-D-lysine-coated glass coverslips. Cells were maintained at saturation humidity in a modular incubator chamber (Billups-Rothenberg) at 24°C. Neurite outgrowth in individual cells was recorded on a TL Panasonic 6050 time-lapse video cassette recorder. Images were later captured and subsequently analyzed using Image 1 analysis software.

### *Electrophysiology*

Voltage- and odor-activated currents were recorded in the whole-cell configuration with an integrating patch-clamp amplifier (Dagan 3900). The analog signal was filtered at 5 kHz and digitally sampled every 4ms. Data acquisition and subsequent storage and analysis of the digitized records were carried out using pCLAMP software (Axon

Instruments). Cells were viewed at 40× magnification with Hoffman optics. Patch electrodes, pulled from 1.8mm o.d. borosilicate glass, were fire-polished to a tip diameter of approximately 1.0 μm (bubble number 4.8; Mittman *et al.* 1987). High-resistance seals (8.0–14 GΩ) were formed by applying gentle suction to the lumen of the pipette upon contact with the cell. In all experiments, cells were voltage-clamped at a holding membrane potential of −60mV. Membrane resistance changes were determined by injecting current sufficient to elicit 30mV, 300ms hyperpolarizing voltage steps into the cells from the holding potential. Each cell was photographed to allow correlation of soma size, length of process and cell morphology with physiological properties.

### *Odor stimulation*

Odors were 'spritzed' on the cells for 120ms from a seven-barrel (six barrels were used) glass micropipette (Frederick Haer) coupled to a pressurized valve system (Picospritzer, General Valve). In most trials, fluorescein was used as an indicator in one barrel of the pipette, which was varied from one experiment to another, to position the tip of the pipette relative to the cell and to ensure that the delivered odorant completely surrounded the cell and its associated processes. The magnitude of the response to odors was found to be independent of which of the six barrels contained the odor. Dilution of the odor between the pipette and the cell surface, an average distance of two cell diameters, was estimated to be approximately 9%, based on the calculated $K^+$ permeability method of Firestein and Werblin (1989). Odor concentrations are reported as the pipette concentration and are not corrected for this dilution.

The odors used were solutions of (1) an equimolar mixture that included ($10^{-3}$ mol $l^{-1}$) betaine, glycine, lactic acid, taurine and trimethylamine oxide, referred to as S-1; (2) an aqueous extract of TetraMarin, a commercially available fish food, prepared as described earlier (Schmiedel-Jakob *et al.* 1990) and diluted 1000-fold, referred to as TET; and (3) single substances known to be effective odors for the lobster, which included ($10^{-3}$ mol $l^{-1}$) adenosine monophosphate (AMP), arginine, ascorbic acid, betaine, cysteine, glycine, histamine, proline, taurine and trimethylamine oxide (TMAO). All odorant solutions were prepared daily in modified L15 medium and applied at the stated concentration unless otherwise noted.

The number of different odors that stimulated a given cell (the response spectrum) was quantified using the breadth of responsiveness metric of Smith and Travers (1979). Here, the breadth of responsiveness ($H$) is defined as:

$$H = -K \sum_{i=1}^{N} p_i \log p_i ,$$

where $K$ is a proportionality constant, $N$ is the number of odors tested, $p_i = |pA|$ the absolute current (pA) elicited from the $N$th odor and expressed as a proportion of total pA elicited from all odors.

### *Solutions*

*Panulirus* saline (PS) consisted of (in mmol $l^{-1}$) 458 NaCl, 13.4 KCl, 9.8 $MgCl_2$, 13.6 $CaCl_2$, 13.6 $Na_2SO_4$, 3 Hepes and 2 glucose; pH7.4. Modified L15 medium consisted of

50ml of Liebowitz L15 stock, 50ml of 1.6 times normal concentration of PS, 0.6g of dextrose, 0.026g of L-glutamine, and 0.01% gentamicin. The patch electrode solution consisted of (mmol $l^{-1}$) 30 NaCl, 11 EGTA, 10 Hepes, 1 $CaCl_2$, 180 potassium acetate and 696 glucose; pH7.0. All salts were obtained from Sigma.

## Results

### *Morphology: neurite outgrowth*

The cultures consisted largely of the five morphological types of small (8–16 μm diameter soma) 'neuron-like' cells, described previously (Fadool *et al.* 1991): (1) soma only, (2) soma with bud, (3) unipolar, (4) bipolar or (5) multiprocess, four of which were used in the present study (Fig. 1A). The processes ranged from 3 to 160 μm long. Each of the four morphological types was present as early as 2h post-plating. Initially, the predominant form was 'soma only', but the proportion of each morphological type changed over time; the relative proportion of cells lacking processes decreased, with a concomitant increase in the proportion of cells with processes (Fig. 1B). To distinguish whether the change in the relative proportion of the morphological types reflected selective loss of cells lacking processes or process proliferation, or both, 218 'soma only' cells were followed individually with digital time-lapse imaging for three consecutive days starting 2h after plating. 42 (20%) of the cells died within the observation period, indicating that a selective loss of 'soma only' contributed to the change in the relative proportion of the cell types. While many of the 176 cells that persisted failed to sprout processes, 61 of the cells sprouted processes throughout the observation period, becoming uni-, bi- and, eventually, multipolar. The latter finding supports our contention that the four morphological types of 'neuron-like' cells are morphs of a single type of cell.

### *Physiology*

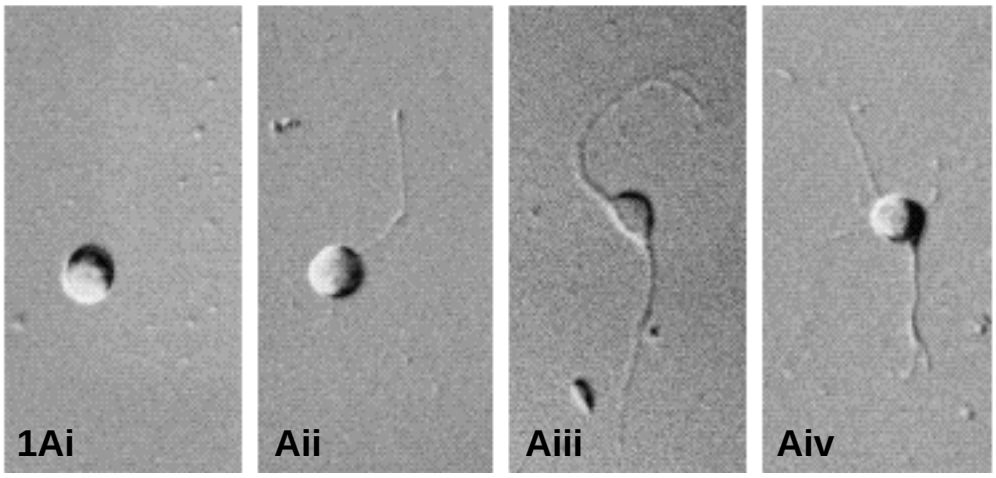
#### *Electrical properties*

That the four types of 'neuron-like' cells were morphs of a single type of cell was supported by the finding that 268 cells tested for their current–voltage relationship had similar voltage-activated properties. The total membrane current evoked in a typical cell by depolarizing voltage steps consisted of a transient inward current (≤570 μs duration) that activated around −30mV, followed by a much larger, prolonged outward current that activated around −20mV and persisted with little decay throughout the 15ms duration of the pulse (Fig. 2A). The magnitude of the inward and outward currents was independent of the number of processes on the cell (ANOVA, $P$=0.47 for inward currents and $P$=0.71 for outward currents) (Fig. 2B). A subsample of 12 cells, including at least one cell of each morphological type, had a mean input resistance ($R_N$) at rest of 1.1±0.2 GΩ and a membrane time constant ($t_0$) of 67.3±11.3ms. No detectable equalizing time constant could be measured in any of the morphs, including morphs with multiple processes.

*Physiology*

*Response to odors*

472 cells (including the cells tested for their electrical properties) were tested for their ability to generate a current in response to stimulation with up to five different odors. The odor arrays usually, but not always, included the complex mixture, TET. 64% of the cells responded when they could be tested with at least one odor. This percentage increased to



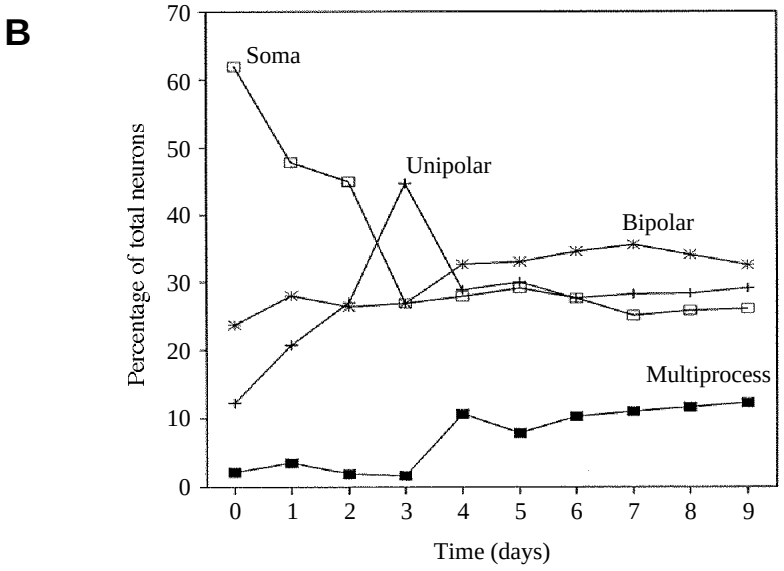




Fig. 1. (A) Light micrographs of four morphs of cultured lobster ORNs observed under Hoffman modulation contrast optics. (i) Soma only, (ii) unipolar, (iii) bipolar, (iv) multiprocess. Magnification 780×. (B) Changes in the proportion of the four morphs over 9 days in culture. Each data point represents the incidence of that morph in 10 random fields of view, expressed as a percentage of all four types.

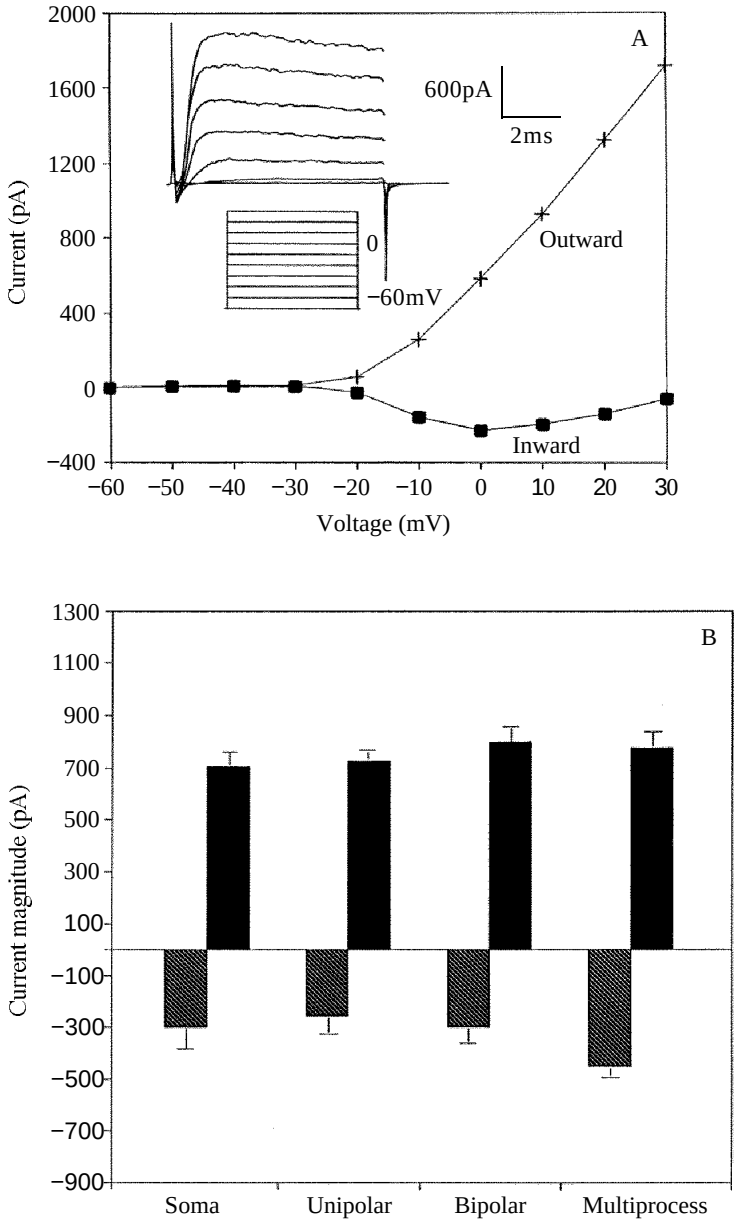



Fig. 2. Total voltage-activated currents of cultured lobster ORNs. (A) Representative current–voltage relationship of one cell. Inset: macroscopic currents (upper traces) evoked by depolarizing voltage-steps (lower traces) when the cell was held at −60mV and stepped to +30mV in 10mV episodes. Records are not leak-subtracted. (B) Plot of the maximum amplitude (mean ± S.E.M.) of the inward (hatched bars) and outward (filled bars) currents of 268 cells grouped according to number of processes (*N*=68 soma only, 76 unipolar, 69 bipolar, 55 multiprocess).

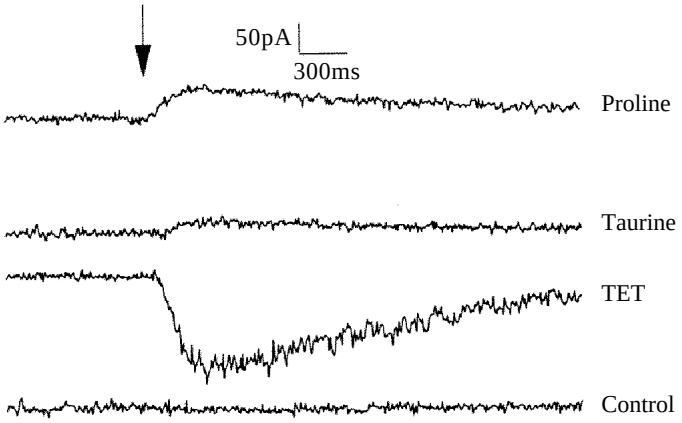



Fig. 3. Whole-cell, voltage-clamp recording from a cultured lobster ORN in response to spritzing the cell (arrow) with $10^{-3}$ mol $l^{-1}$ proline (top trace), $10^{-3}$ mol $l^{-1}$ taurine (second trace), TET (third trace) and culture medium only (bottom trace). Holding potential, −60mV for all traces.

89% when the cells could be tested with at least three different odors ($N$=182). Odors evoked a transient current that rose to a maximum over several hundred milliseconds and subsequently declined more slowly to rest (Fig. 3). The current could be of either polarity, depending on the cell and the odor tested and different odors could evoke currents of opposite polarity in the same cell (Fig. 3). In cells that could be tested with at least three different odors ($N$=182), the odors that were tested evoked only inward currents in 48 cells (26%), only outward currents in 58 cells (32%) and currents of both polarities in 56 (31%) cells. The remaining 11% of the cells did not respond to any of the odors tested.

Odor-evoked currents of both polarities were associated with an increase in membrane conductance, as indicated by a decrease in input resistance, when hyperpolarizing voltage steps were injected into the cells prior to and during odor-stimulation (Fig. 4). The input resistance ($R_N$) decreased significantly under odor stimulation from a mean of 1.1±0.1 to 0.7±0.1 GΩ for the inward current ($N$=8) and from a mean of 1.3±0.6 to 0.7±0.1 GΩ for the outward current ($N$=4) (paired $t$-test, $P$<0.05). Concomitantly, the membrane time constant ($t_0$), decreased significantly during odor stimulation from 59.6±12.6 to 21.6±4.1ms for the inward current ($N$=14) and from 82.5±20.7 to 34.6±9.5ms for the outward current ($N$=7) (paired $t$-test, $P$<0.05).

The latency to the onset of the odor-evoked currents, measured from the activation of the spritzer, ranged from less than 20ms to more than 1s, but typically was less than 100ms (Fig. 5). Overall, the mean latency to onset for the inward current, 186.2±31.3ms ($N$=100), was significantly longer than that for the outward current, 81.0±10.6ms ($N$=121) ($t'$ statistic, $P$<0.05). To determine whether this difference was caused by variations between cells, we performed a paired comparison of the latency in 19 cells that supported odor-evoked currents of both polarities. The mean latency in these cells for the inward current was not significantly longer than that for the outward current (paired $t$-test, $P$<0.05).

The peak amplitude of odor-evoked currents of both polarities increased with the concentration of the odor and saturated over 3–4 orders of magnitude (Fig. 6). The mean slope of the concentration–response function in the steepest region of the curve was 8.3±1.7pA per decade ($N$=3) for the inward current and 1.3±0.2pA per decade ($N$=7) for the outward current. Thresholds were less than $10^{-8}$ mol $l^{-1}$, the lowest concentration tested, for currents of either polarity.

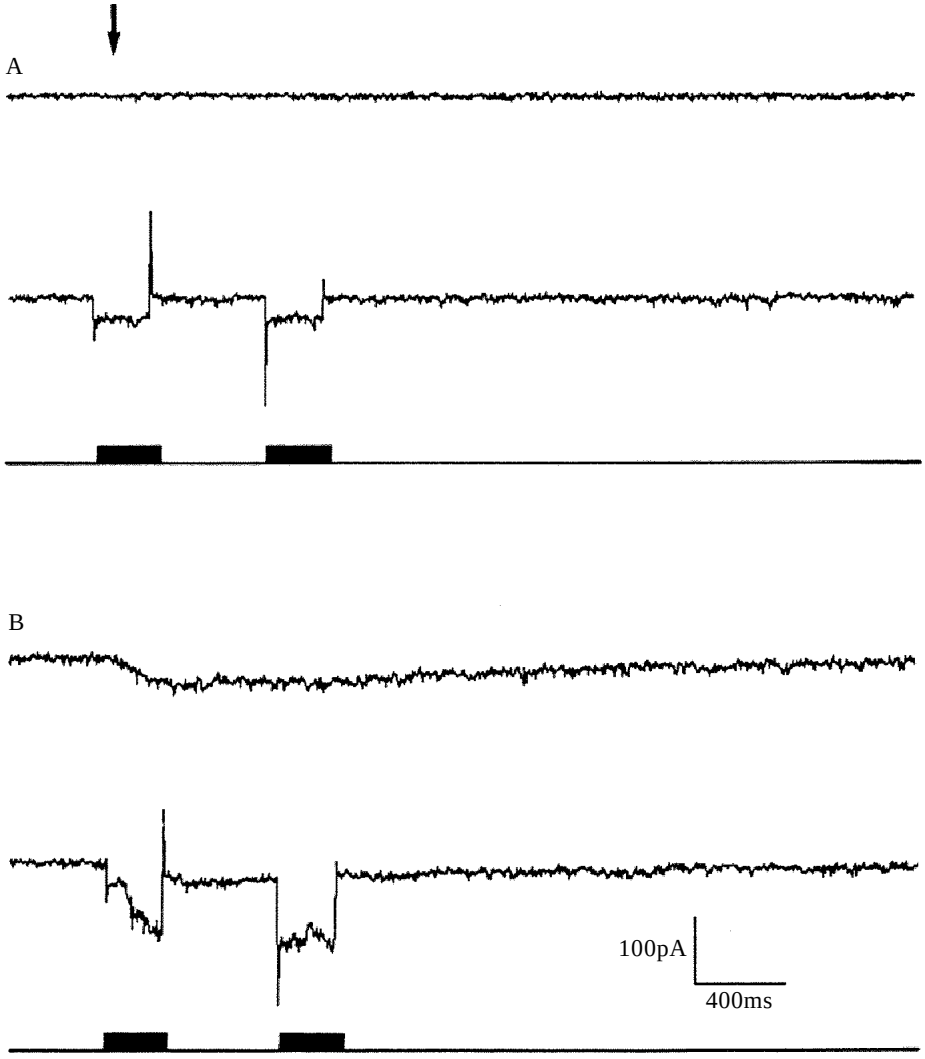



Fig. 4. Whole-cell, voltage-clamp recordings from a cultured lobster ORN showing increased conductance associated with the odor-stimulated inward current. (A) Neither current (upper trace) nor change in conductance (lower trace) was evoked by stimulating (arrow) with culture medium control. (B) Inward current (upper trace) associated with an increase in conductance (lower trace) was evoked by stimulating with TET. Holding potential, −60mV. Solid bars denote 30mV, 300ms hyperpolarizing voltage pulses used to monitor membrane conductance.

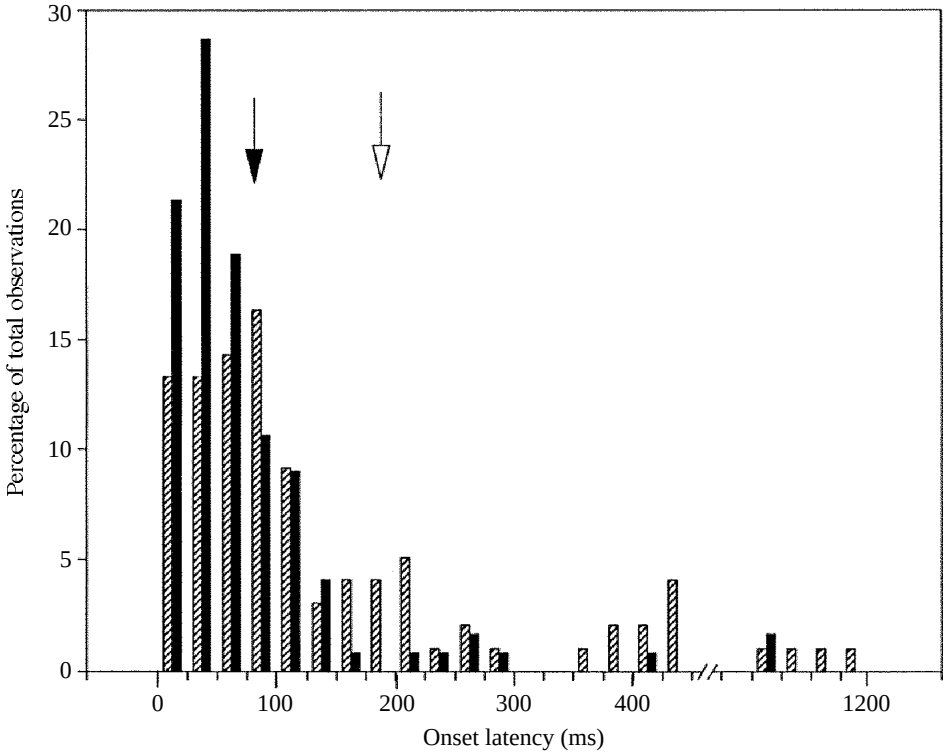



Fig. 5. Plot of the distribution of the latency of odor-evoked currents in cultured lobster ORNs. Hatched bars, inward currents ($N$=100); filled bars, outward currents ($N$=121). Arrows denote the mean latency to onset of the inward (open arrow) and outward (filled arrow) currents, respectively.

The peak amplitude of currents of both polarities evoked by a single concentration ($10^{-3}$ mol $l^{-1}$) of odor ranged, typically, from 5 to 85pA (Fig. 7A). The average magnitude of the inward current (39.2±3.0pA) was significantly greater than that of the outward current (20.1±1.4pA) measured across all cells and odors ($N$=386; $t'$ statistic, $P \leq 0.05$). The polarity and the magnitude of the currents evoked by odors were independent of the cell morphology for six different odors. The results for two of the six odors, proline ($N$=111 cells) and taurine ($N$=102 cells), are shown in Fig. 7B. The peak amplitude of the odor-evoked currents of either polarity was also independent of the length of the process in cells bearing processes ($N$=55, correlation analysis, $r$>3.86) (Fig. 8A) and the diameter of the soma in cells lacking processes ($N$=60, correlation analysis, $r$>3.86) (Fig. 8B).

Single odors activated 14–77% of the cells (Table 1). The stimulatory effectiveness was betaine > histamine > glycine > proline > taurine > AMP > TMAO > ascorbate > arginine > cysteine. An equimolar mixture of five compounds (S-1: betaine, taurine, glycine, TMAO, lactate) ranked intermediate in stimulatory effectiveness, as did the complex mixture (TET), although the concentration of TET relative to that of the pure compounds is unknown. Culture medium itself, tested as a control, stimulated none of

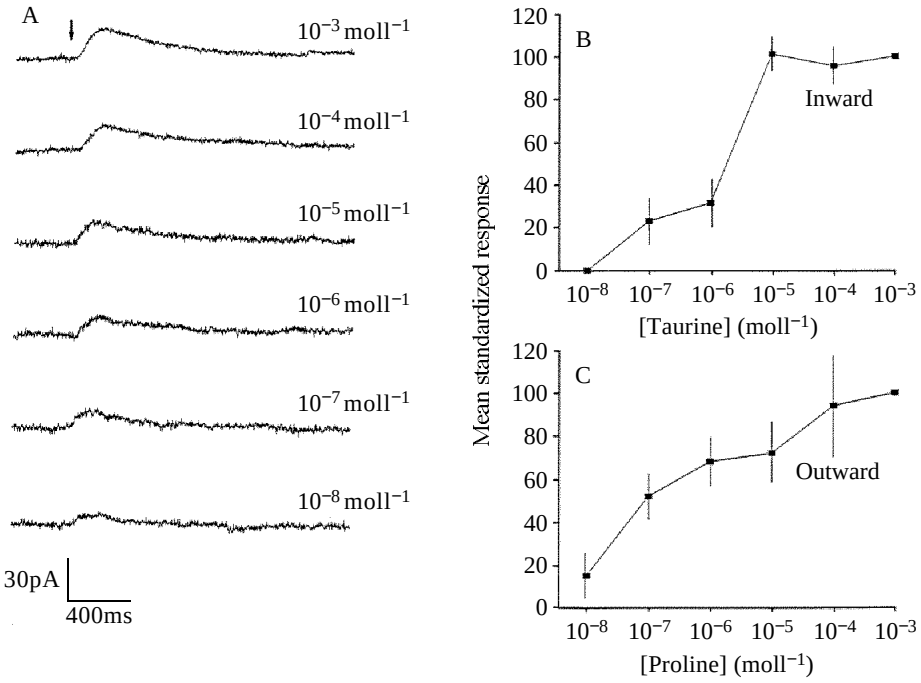



Fig. 6. (A) Whole-cell, voltage-clamp recordings of outward currents in a cultured lobster ORN evoked by stimulating the cell (arrow) with decreasing concentrations of proline. Holding potential, −60mV. (B) Plot of the peak inward current (mean ± S.E.M.) elicited in three cells by taurine at the concentrations shown. (C) Plot of the peak outward current (mean ± S.E.M.) elicited in three other cells by proline at the concentrations shown. Current magnitudes in B and C are normalized to that evoked by $10^{-3}$ mol l$^{-1}$ odor.

the cells tested, even though it contained some of the same amino acids as above as well as L-glutamine. Some odors selectively activated either inward or outward currents (Fig. 9). Taurine elicited inward currents twice as frequently as outward currents, while proline showed the opposite tendency. Betaine and ascorbic acid elicited strictly outward currents, although the number of cells tested in each of these instances was not large.

In a separate experiment, 53 cells that were determined to be odor-responsive by their ability to respond to TET were presented sequentially with the same five odors in order to determine their response spectra or ‘tuning’. The cells varied in the number of odors they

Fig. 7. Plots of the peak amplitude of odor-evoked currents in cultured lobster ORNs as a function of cell morphology. (A) Distribution of the peak amplitude of the currents evoked in 386 cells by single odors tested at a common concentration ($10^{-3}$ mol l$^{-1}$). Hatched bars, inward currents; filled bars, outward currents. Arrows denote the mean amplitude of the inward (open arrow) and outward (filled arrow) currents. (B) Plots of the peak amplitude of currents of both polarities evoked by stimulation with $10^{-3}$ mol l$^{-1}$ proline ($N$=111) and $10^{-3}$ mol l$^{-1}$ taurine ($N$=102) grouped according to morphology. Inward currents are denoted as negative, outward as positive.

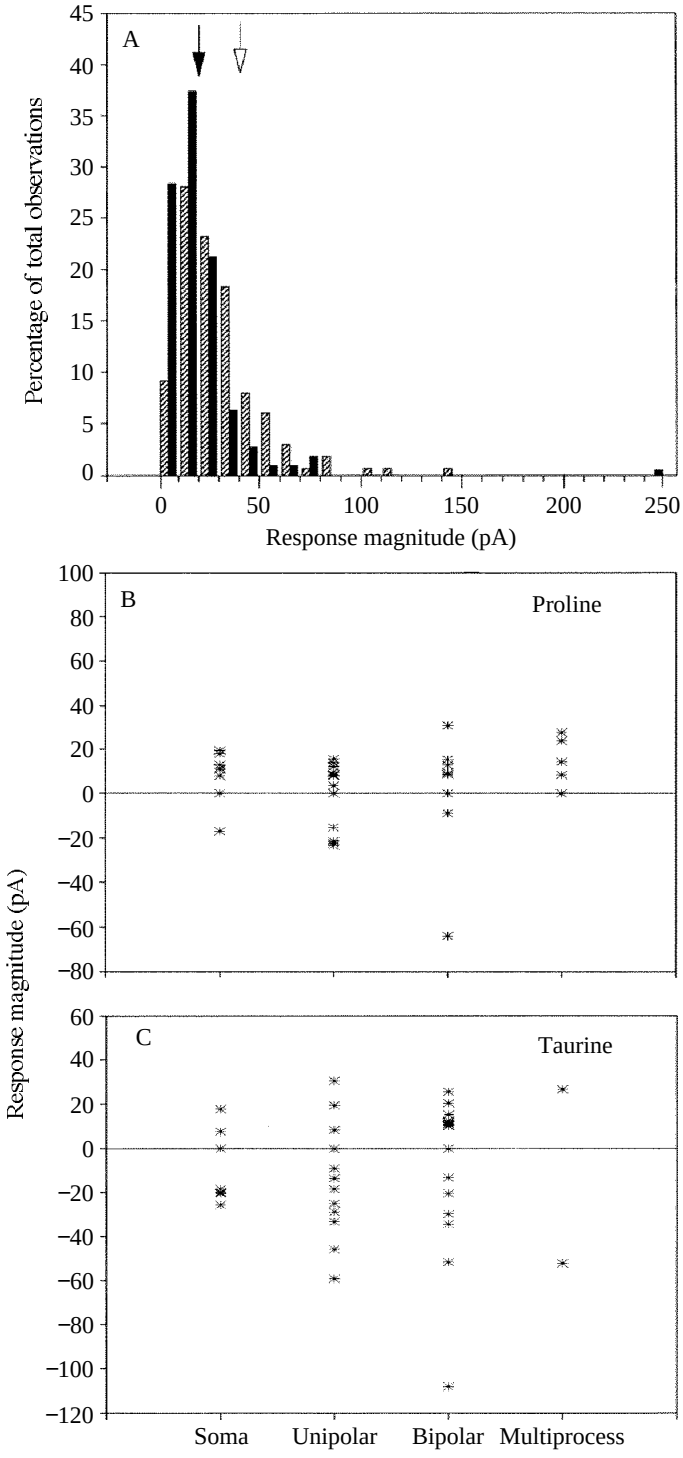

A
Percentage of total observations
Response magnitude (pA)
B
Proline
C
Taurine
Response magnitude (pA)
Soma
Unipolar
Bipolar
Multiprocess

Fig. 7

responded to, as well as in the magnitude and the polarity of the response to any one odor (Fig. 10A). The mean breadth of responsiveness (*H*) for the sample population was 0.31±0.04 (Fig. 10B). Repeating this analysis using fewer odors or the same number of different odors yielded measures of *H* between 0.2 and 0.3 (data not shown).

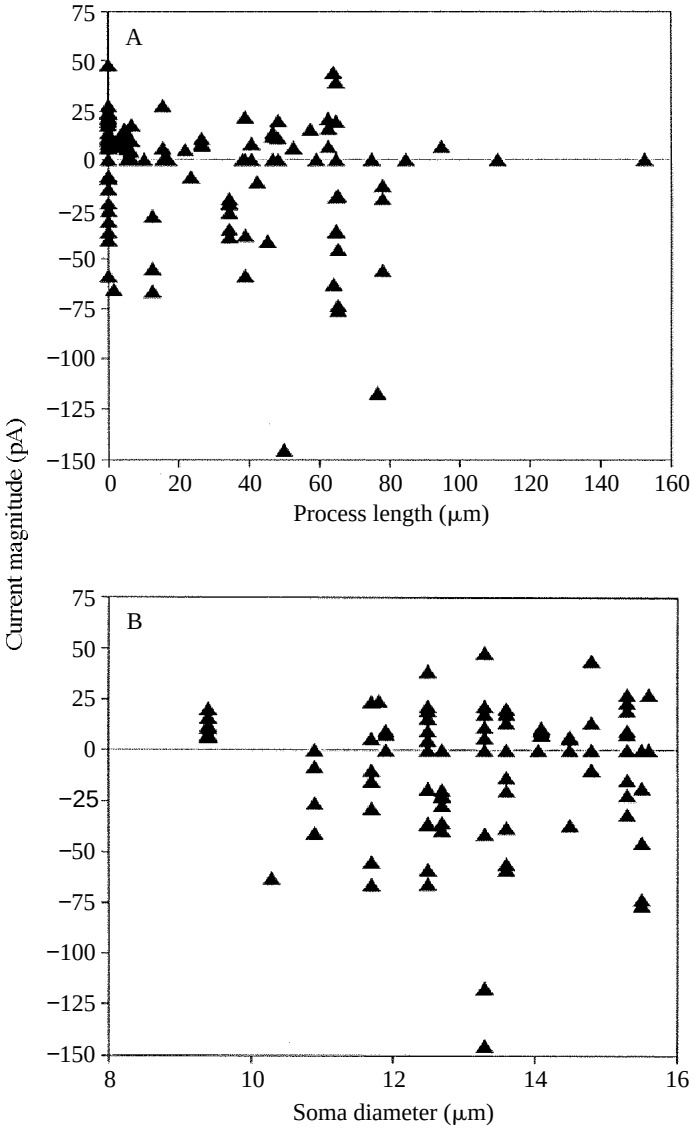



Fig. 8. Plots of the peak amplitude of odor-evoked currents in cultured lobster ORNs as a function of length of the longest process (if more than one) for 55 cells (A) or the diameter of the soma in 60 cells lacking processes (B). The data set combines currents evoked by $10^{-3}$ mol $l^{-1}$ proline, arginine, taurine, TMAO and betaine as odors (1 cell, 1 odor). Inward currents in both plots are denoted as negative, outward as positive.

Table 1. *The percentage of cultured lobster olfactory receptor cells responding to individual odors, a mixture of five odors, and a complex odor*

| Compound | Concentration (mmol $l^{-1}$) | Total number of cells tested | Number of cells responding | | Percentage responding to odor |
|---|---|---|---|---|---|
| | | | Inward | Outward | |
| L15 | – | 237 | 0 | 0 | 0 |
| Betaine | 1 | 13 | 0 | 10 | 77 |
| Histamine | 1 | 4 | 1 | 2 | 75 |
| Glycine | 1 | 14 | 5 | 5 | 71 |
| Proline | 1 | 68 | 13 | 30 | 63 |
| Taurine | 1 | 58 | 24 | 12 | 62 |
| TET | 0.001* | 52 | 19 | 12 | 60 |
| AMP | 1 | 10 | 1 | 4 | 50 |
| S-1 mix | 5 | 16 | 4 | 3 | 44 |
| TMAO | 1 | 34 | 7 | 7 | 41 |
| Ascorbate | 1 | 8 | 0 | 2 | 25 |
| Arginine | 1 | 17 | 3 | 1 | 24 |
| Cysteine | 1 | 14 | 0 | 2 | 14 |

*Concentration relative to stock concentration of Tetra Marin.

TMAO, trimethylamine oxide; S-1 mix, betaine, TMAO, glycine, taurine, lactate; L15, Liebowitz L15 medium blank control; AMP, adenosine monophosphate.

## Discussion

The fact that the cells could be observed to sprout processes in culture, together with their common active electrical properties, strengthens our earlier contention that the various types of 'neuron-like' cells in the culture are morphs of one type of cell, the ORN (Fadool *et al.* 1991). This finding is consistent with the highly enriched source of the cultured cells; the somata were harvested from the lumen of the olfactory organ, which is literally filled with grape-like clusters of the somata of the ORNs (Grünert and Ache, 1988).

Although most neurons, as well as non-neuronal cells, are chemically irritable (Lerner *et al.* 1990), it is reasonable to interpret the responses of the cultured ORNs as olfactory. Most importantly, the cultured ORNs showed a diversity of response profiles (Fig. 10). Postsynaptic or neuromodulatory receptors, in contrast, would be expected to be much more homogeneously distributed across the population of cells and show either the same or, if possessing multiple receptors, a restricted set of response profiles across cells. For example, a histamine-gated chloride channel implicated in modulating the output of lobster ORNs *in situ* occurs on the soma of more than 95% of the cells (McClintock and Ache, 1989*a*). Second, the average breadth of responsiveness of the cultured ORNs ($H$=0.31) approximated that of lobster ORNs determined *in situ* with a similar, but not identical, array of compounds ($H$=0.2, Derby *et al.* 1984) and did not differ significantly from that of lobster ORNs determined *in situ* with the same array of compounds ($H$=0.35, Student's $t$-test, $P<0.05$, pooled S.D.; M. Wachowiak, unpublished data). Third, as in their counterparts *in situ*, odors evoked both inward and outward currents in the cultured

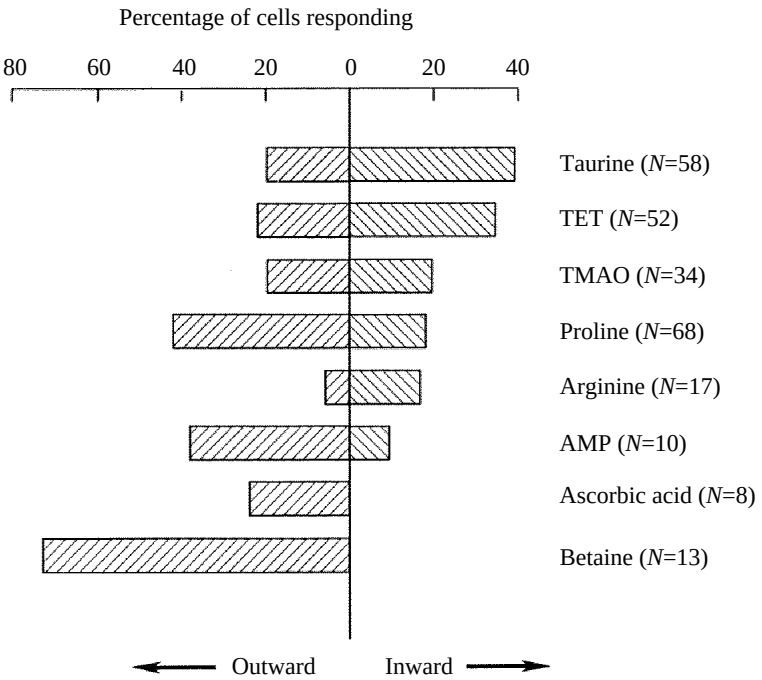



Fig. 9. Plot of the percentage of cultured lobster ORNs that respond to various odors with inward and outward currents. All odors tested at $10^{-3}$ mol $l^{-1}$, with the exception of TET, which was tested at 1000-fold dilution of stock. TMAO, trimethylamine oxide. AMP, adenosine monophosphate.

ORNs, and different compounds could evoke currents of opposite polarity in the same cell (McClintock and Ache, 1989*b*). Odors that tended frequently to elicit currents of one polarity in the cultured cells, e.g. proline and outward currents, also did so in lobster ORNs *in situ* (Schmiedel-Jakob *et al.* 1990; Michel *et al.* 1991). That currents of opposite polarity were associated with an increase in membrane permeability argues, as occurs *in situ* (Schmiedel-Jakob *et al.* 1990; Michel *et al.* 1991), that the odors were not up- and down-regulating a single conductance but, rather, were regulating two distinct conductances through two parallel transduction pathways. Finally, the threshold and dynamic range of the cultured ORNs matched those of lobster ORNs *in situ* in response to the same or similar types of odor molecules (Schmiedel-Jakob *et al.* 1989; W. C. Michel, unpublished data). We conclude that most and possibly all, of the chemosensitivity expressed by cultured lobster ORNs is sensory, i.e. is mediated by the same receptors and transduction pathways found in the outer dendrites of lobster ORNs *in situ*, and is not the

Fig. 10. (A) Graph of the response profiles of 10 cultured lobster ORNs to five odors tested at $10^{-3}$ mol $l^{-1}$. Each horizontal line depicts the responses of a different cell; the height and direction of the bars on each line indicate the amplitude and the polarity, respectively, of the odor-evoked current (pA). Outward currents are denoted as positive (upward bars); inward currents as negative (downward bars). (B) Plot of the breadth of responsiveness of 53 cells. Arrow denotes the mean *H* value. TMAO, trimethylamine oxide.

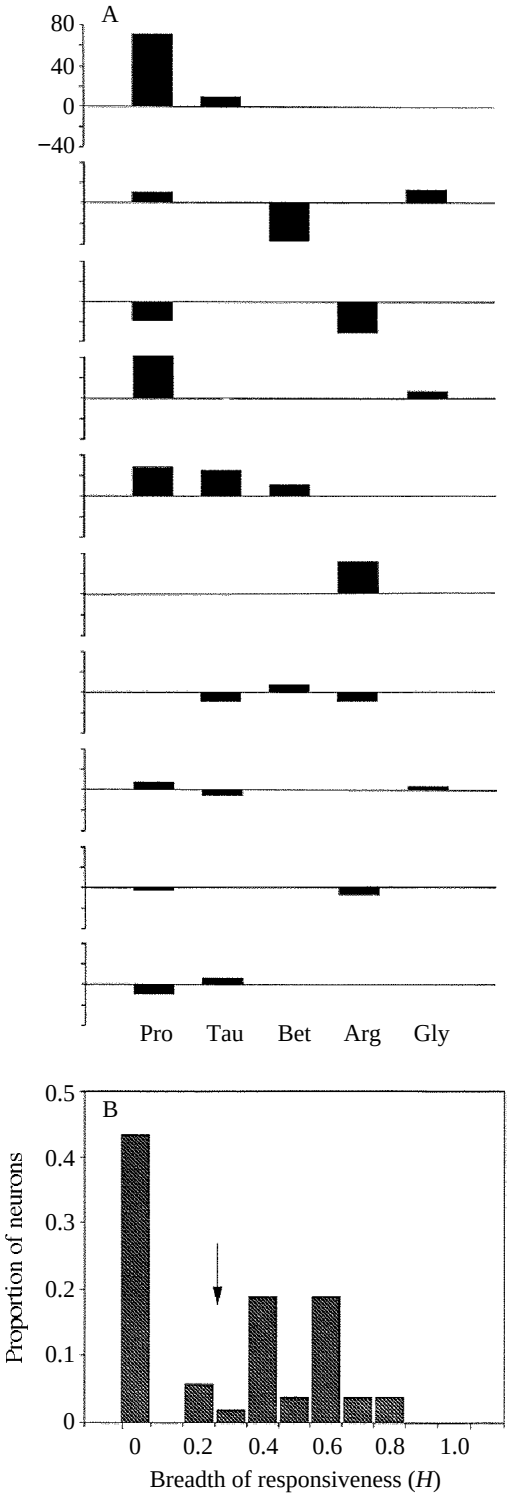

A
80
40
0
−40
Pro
Tau
Bet
Arg
Gly
B
0.5
0.4
0.3
0.2
0.1
0
Proportion of neurons
0
0.2
0.4
0.6
0.8
1.0
Breadth of responsiveness (*H*)

Fig. 10

result of activating synaptic, neuromodulatory or other receptors that may occur on the soma of lobster ORNs.

The inability of the culture medium to elicit responses from the cells when applied as a control stimulus, even though it contains amino acids shown to be odors (cysteine, glycine, arginine) and others (e.g. glutamine) that are potential odors, is not necessarily surprising. The cells were maintained and tested in the same medium and would be expected to adapt to continuous background stimulation, as do their counterparts *in vivo* (Borroni and Atema, 1988). However, adaptation to continuous background stimulation does shift the apparent threshold of the cells *in vivo* so that recording in medium might have lowered the overall sensitivity to those test stimuli present in the medium (cysteine, glycine, arginine). Indeed, the smallest percentage of cells tested responded to cysteine and arginine; in contrast, glycine was among the more effective odors tested (Table 1). The remaining test odors, including those used for threshold determinations, were not components of the culture medium.

The overall magnitudes of the current evoked by odors in cultured cells could differ appreciably from those *in situ* as a result of possible differences in the number of channels expressed in culture. It is interesting, therefore, that the average magnitudes of the odor-evoked currents in the cultured ORNs (39.2±3.0pA, inward; 20.1±1.4pA outward) were within an order of magnitude of those evoked in lobster ORNs *in situ*. A 10-fold greater concentration of TET than that used in the present study elicited an average inward current of 25.4±5.1pA, while the same concentration of proline as that used in the present study elicited an average outward current of 4.5±0.6pA in lobster ORNs *in situ* (Michel and Ache, 1992). Odor-evoked currents of dendritic origin *in situ* would be electrotonically attenuated in the soma (where they were recorded), so the actual magnitude of the current in the dendrite would presumably be closer to that found in the cultured cells. Our results are consistent with the possibility that the cultured cells express a full complement of odor-activated channels.

The average amplitude of the odor-evoked inward currents in lobster ORNs is an order of magnitude smaller than those reported for salamander or rat ORNs (Firestein *et al.* 1991; Kurahashi, 1989; Lowe and Gold, 1991; Pixley and Pun, 1990), but is consistent with our still limited understanding of unitary currents that may underlie the macroscopic current. Inositol 1,4,5-trisphosphate, Ins(1,4,5)$P_3$, the suspected excitatory second messenger, activated 1–3 channels in most (49/56) cell-free patches taken from the plasma membrane of the cultured cells (Fadool and Ache, 1992). On the assumption that these Ins(1,4,5)$P_3$-activated channels are transductory, there are an estimated 330–1990 channels per cell, given a pipette tip diameter of 0.5–1.0 μm and a total cell surface area of 380 $\mu m^2$ (Fadool *et al.* 1991); or 0.9–5.3 channels $\mu m^{-2}$ membrane. Taking the smaller of the two Ins(1,4,5)$P_3$-activated unitary conductances (2pA, Fadool and Ache, 1992), a macroscopic current of average magnitude (39pA; Fig. 7) would require activation of as few as 20 channels, or only 1–6% of the available channels. Such a small fraction of recruited channels is consistent with the presumed sensory function of the cells (e.g. Firestein *et al.* 1991).

The ability of the cultured cells to respond to odors did not correlate with the number or even the presence of processes, implying that the receptor sites for odors were not

necessarily confined to the processes. At least in vertebrate ORNs, odor receptors are thought to be selectively localized to the cilia. Deciliating the frog olfactory epithelium with Triton X-100, for example, completely abolishes the electro-olfactogram (Adamek *et al.* 1984). Lowe and Gold (1991) confirmed this hypothesis by focally stimulating the cilia of dissociated salamander ORNs. Whether somatic sensitivity to odors in cultured lobster ORNs is induced or is typical of mature lobster ORNs *in situ* is unclear. Previous attempts to record responses to odors in freshly isolated somata have been consistently unsuccessful (e.g. Anderson and Ache, 1985), suggesting that somatic sensitivity to odors in cultured ORNs may have been induced by placing the cells in culture. This conclusion would be consistent with the observation that molluscan neurons raised in suspension culture to prevent process formation insert postsynaptic receptors otherwise normally confined to processes in the soma (Wong *et al.* 1981). Nevertheless, Hatt (1990) found odor-gated channels on the soma of freshly dissociated crayfish chemosensory neurons that had the same structure–activity profiles and sensitivity as the intact cells. Ligand-gated histamine receptors on the soma of lobster ORNs resist the same enzymatic protocol used in the present study (McClintock and Ache, 1989*a*). Therefore, we cannot eliminate the possibility that the receptors in question normally occur on the somata of mature lobster ORNs, but are destroyed by the enzymatic treatment required to dissociate the cells and fail to recover in the subsequent 4–6h over which *in situ* recordings are usually made. It is known that enzymatic digestion used to prepare cells for patch-clamping alters neurotransmitter responsiveness in invertebrate neurons (Oyama *et al.* 1990) and eliminates odor-responsiveness in amphibian ORNs (Firestein and Werblin, 1989).

The latency to response was extremely brief, as short as 20ms, with the majority of the responses occurring within 125ms of valve (spritzer) activation. These values fall below the lower limit of the range of latencies reported for dissociated salamander ORNs, the other system for which comparable measurements are available (175–600ms; Firestein *et al.* 1990). Both systems lack a mucus barrier that would impede stimulus access to the receptor sites, so the latencies presumably reflect the actual time course of transduction. Breer *et al.* (1990) recently reported that levels of two second messengers in rat and insect ORNs, cyclic adenosine monophosphate and inositol 1,4,5-trisphosphate, respectively, peak in response to odor stimulation within 25–50ms. Thus, while the latency of the response of cultured lobster ORNs is considerably shorter than that of salamander ORNs, it is consistent with second-messenger-mediated transduction.

Thus, we show that lobster ORNs not only survive in culture but express the odor sensitivity and selectivity of their counterparts *in situ*, including the ability of odors to excite as well as to inhibit the cells. As the cultured cells are morphologically and, presumably, electrotonically (as shown by the absence of a measurable equalizing time constant) more compact than their counterparts *in situ*, often consisting of only a spherical soma, the ability to characterize the transduction pathways and analyze potential interactions between them should be greatly facilitated *in vitro*.

This investigation was supported by ONR N0014-90-J-1566, NIMH NRSA 1F31MH10124-01A1. The authors would like to thank Ms Leslie Van Ekeris for

technical assistance, Ms Lynn Milstead and Mr James Netherton for assistance with the illustrations and Drs Paul J. Linser and James M. Fadool for technical discussions.

## References

ADAMEK, G. D., GESTELAND, R. C., MAIR, R. G. AND OAKLEY, B. (1984). Transduction physiology of olfactory receptor cilia. *Brain. Res.* **310**, 87–97.

ANDERSON, P. A. V. AND ACHE, B. W. (1985). Voltage- and current-clamp recordings of the receptor potential in olfactory receptor cells *in situ*. *Brain Res.* **338**, 273–280.

ANHOLT, R. R. H.(1991). Odor recognition and olfactory transduction: the new frontier. *Chem. Sens.* **16**, 421–427.

BORRONI, P. F. AND ATEMA, J. (1988). Adaptation in chemoreceptor cells. I. Self-adapting backgrounds determine threshold and cause parallel shift of response function. *J. comp. Physiol.* A **164**, 67–74.

BREER, H., BOEKHOFF, I. AND TAREILUS, E. (1990). Rapid kinetics of second messenger formation in olfactory transduction. *Nature* **344**, 65–68.

CALOF, A. L. AND CHIKARAISHI, D. M. (1991). Analysis of neurogenesis in a mammalian neuroepithelium: proliferation and differentiation of an olfactory neuron precursor *in vitro*. *Neuron* **3**, 115–127.

CARR, W. E. S. (1990). Chemical signaling systems in lower organisms: A prelude to the evolution of chemical communication in the nervous system. In *Evolution of the First Nervous Systems* (ed. P. A. V. Anderson), pp. 81–94. New York: Plenum Press.

COON, H. G., CURCIO, F., SAKAGUCHI, K., BRAND, M. L. AND SWERDLOW, R. D. (1989). Cell cultures of neuroblasts from rat olfactory epithelium that show odorant responses. *Proc. natn. Acad. Sci. U.S.A.* **86**, 1703–1707.

DERBY, C. D., HAMILTON, K. A. AND ACHE, B. W. (1984). Processing of olfactory information at three neuronal levels in the spiny lobster. *Brain. Res.* **300**, 311–319.

FADOOL, D. A. AND ACHE, B. W. (1992). Plasma membrane inositol 1,4,5-trisphosphate-activated channels mediate signal transduction in lobster olfactory receptor neurons. *Neuron* **9**, 1–20.

FADOOL, D. F., MICHEL, W. C. AND ACHE, B. W.(1991). Sustained primary culture of lobster (*Panulirus argus*) olfactory receptor neurons. *Tissue &Cell* **23**, 719–732.

FIRESTEIN, S.(1991). A noseful of odor receptors. *Trends Neurosci.* **14**, 270–272.

FIRESTEIN, S., SHEPHERD, G. M. AND WERBLIN, S. F. (1990). Time course of the membrane current underlying sensory transduction in salamander olfactory receptor neurones. *J. Physiol., Lond.* **430**, 135–158.

FIRESTEIN, S. AND WERBLIN, F. (1989). Odor-induced membrane currents in vertebrate-olfactory receptor neurons. *Science* **244**, 79–82.

FIRESTEIN, S., ZUFALL, F. AND SHEPHERD, G. M. (1991). Single odor-sensitive channels in olfactory receptor neurons are also gated by cyclic nucleotides. *J. Neurosci.* **11**, 3565–3572.

GRÜNERT, U. AND ACHE, B. W. (1988). Ultrastructure of the aesthetasc (olfactory) sensilla of the spiny lobster, *Panulirus argus. Cell Tissue Res.* **251**, 95–103.

HATT, H. (1990). Electrophysiological studies on chemoreceptive neurons in crustacea. In *Frontiers in Crustacean Neurobiology* (ed. K. Wiese, W.-D. Krenz, J. Tautz, H. Reichert and B. Mulloney), pp. 41–45. Switzerland: Birkhäuser Verlag.

KURAHASHI, T. (1989). Activation by odorants of cation-selective conductance in the olfactory receptor cell isolated from the newt. *J. Physiol., Lond.* **419**, 177–192.

LERNER, M. R., GYORGYI, T. K., REAGAN, J., ROBY-SHEMKOVITZ, A., RYBCZYNSKI, R. AND VOGT, R. G. (1990). Peripheral events in moth olfaction. *Chem. Senses* **15**, 191–198.

LOWE, G. AND GOLD, G. H. (1991). The spatial distributions of odorant sensitivity and odorant-induced currents in salamander olfactory receptor cells. *J. Physiol., Lond.* **442**, 147–168.

MCCLINTOCK, T. S. AND ACHE, B. W. (1989*a*). Histamine directly gates a chloride channel in lobster olfactory receptor neurons. *Proc. natn. Acad. Sci. U.S.A.* **86**, 8137–8141.

MCCLINTOCK, T. S. AND ACHE, B. W. (1989*b*). Hyperpolarizing receptor potentials in lobster olfactory receptor cells: implications for transduction and mixture suppression. *Chem. Senses* **14**, 637–647.

MICHEL, W. C. AND ACHE, B. W. (1992). Cyclic nucleotides mediate an odor-evoked potassium conductance in lobster olfactory receptor cells. *J. Neurosci.* **12**, 3979–3984.

MICHEL, W. C., MCCLINTOCK, T. S. AND ACHE, B. W. (1991). Inhibition of lobster olfactory receptor cells by an odor-activated potassium conductance. *J. Neurophysiol.* **65**, 446–453.

MITTMAN, S. C., FLAMING, D. G., COPENHAGEN, D. R. AND BELGUM, J. H. (1987). Bubble pressure measurement of micropipet tip outer diameter. *J. Neurosci. Meth.* **22**, 161–166.

OYAMA, Y., HORI, N., ALLEN, C. N. AND CARPENTER, D. O. (1990). Influences of trypsin and collagenase on acetylcholine responses of physically isolated single neurons of *Aplysia californica*. *Cell. molec. Neurobiol.* **10**, 193–205.

PIXLEY, S. K. AND PUN, R. Y. K. (1990). Cultured rat olfactory neurons are excitable and respond to odors. *Devl Brain. Res.* **53**, 125–130.

RONNETT, G. V., HESTER, L. D. AND SYNDER, S. H. (1991). Primary culture of neonatal rat olfactory neurons. *J. Neurosci.* **11**, 1243–1255.

SCHMIEDEL-JAKOB, I., MICHEL, W. C., ANDERSON, P. A. V. AND ACHE, B. W. (1990). Whole cell recording from lobster olfactory receptor cells: multiple ionic bases for the receptor potential. *Chem. Senses* **15**, 397–405.

SMITH, D. V. AND TRAVERS, J. B.(1979). A metric for the breadth of tuning of gustatory neurons. *Chem. Sens. Flavour* **4**, 215–229.

STENGL, M., ZUFALL, F., DUDEL, J. AND HILDEBRAND, J. G. (1989). Patch clamp analysis of male *Manduca sexta* olfactory receptor neurons in primary cell culture. *Soc. Neurosci. Abstr.* **15**, 751.

WONG, R. G., HADLEY, R. D., KATER, S. B. AND HAUSER, G. C.(1981). Neurite outgrowth in molluscan organ and cell cultures: The role of conditioning factor(s). *J. Neurosci.* **1**, 1008–1021.

ZUFALL, F., FIRESTEIN, S. AND SHEPHERD, G. M. (1991*a*). Analysis of single cyclic nucleotide-gated channels in olfactory receptor cells. *J. Neurosci.* **11**, 3573–3580.

ZUFALL, F., STENGL, M., FRANKE, C., HILDEBRAND, J. G. AND HATT, H. (1991*b*). Ionic currents of cultured olfactory receptor neurons from antennae of male *Manducasexta*. *J. Neurosci.* **11**, 956–965.