
High resolution spatio–temporal analysis of aquatic chemical signals using microelectrochemical electrodes

Paul A. Moore, Greg A. Gerhardt¹ and Jelle Atema²

Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543 and ¹Departments of Psychiatry and Pharmacology, University of Colorado Health Sciences Center, 4200 East 9th Ave., Denver, CO 80262, USA

²*To whom correspondence should be addressed*

Abstract. Detailed understanding of chemoreceptor cell transduction and filtering depends on precise control and thus measurement of the chemical stimulus. In contrast to vision and hearing, accurate stimulus measurement in chemoreception has not been possible at biologically relevant spatial and temporal scales. In this paper we introduce a new high-speed (200 Hz) electrochemical method for the direct measurement of odor signals at biologically relevant space scales (10–100 μm). We tested this system in three applications: (i) temporal and spatial features of odor plumes, (ii) stimulus calibrations in physiological recording chambers and (iii) boundary layer diffusion measurements within receptor structures.

Introduction

The environmental transport of chemical signals by advection and diffusion forms a signal which is chaotic at various biologically relevant space and time scales (Murlis and Jones, 1981; Elkinton and Cardé, 1984; Atema, 1985, 1987, 1988; Moore and Atema, 1988). Following detection by chemoreceptor cells and filtering by neural networks, these signals serve as the basis for behavioral decisions (Bell and Tobin, 1981). In contrast to vision and hearing, we still know little about the spectral and particularly the temporal response properties of receptor cells in chemoreception. This is an important measure due to the lack of knowledge of chemical signal structure in the environment and hence the lack of proper stimulus control in electrophysiological and behavioral test situations. For example, during orientation behavior even with carefully controlled stimulus introduction, the exact temporal stimulus profile arriving at the receptor structures has not been measured. This has made direct correlation of stimulus and behavior impossible. In addition to dispersal patterns, the subsequent diffusion of odor molecules through receptor boundary layers plays an important role in shaping the odor signal before it reaches the receptor cell. This process has been calculated (Adam and Delbrück, 1968; DeSimone, 1981; Futrelle, 1984; Getchell *et al.*, 1984; Kaissling, 1987) but it has not been measured. Before we can understand receptor cell filtering and its relation to animal behavior, we first must know the odor signal or input on which these behavioral outputs are based. To do this, odor signals must be sampled with a temporal and spatial resolution relevant to the animal behavior or the cell physiology under study.

A variety of detection methods have been used to characterize odor signals. Over the last 5–10 years, a spectrophotometric probe (Atema, 1985), conductivity probes (Johnson *et al.*, 1984; Moore and Atema, 1988), a fast fluorometric probe

(Zimmer-Faust *et al.*, 1988) and ion collectors for aerial signals (Murlis and Jones, 1981) have been used. Although these techniques have high temporal resolution, the spatial sampling volumes are either large or poorly defined, or both. In addition, the chemical species used for detection are tracers, not the odor chemicals themselves and the transport and dispersion of such tracers—although for general purposes sufficiently similar—may not always accurately reflect the true chemical odor dispersal processes.

Electrochemical recording microelectrodes offer two important advantages over previously used probes, besides good temporal resolution, they have both excellent spatial resolution and specificity for biological stimuli. First, microcomputer-based recording techniques permit a temporal sampling rate as high as 200 Hz. Second, over the past decade, electrochemical electrodes have been miniaturized and extensively employed for studies of monoamine neurotransmitter diffusion and release in the mammalian brain (Gerhardt *et al.*, 1987; Gratton *et al.*, 1988, 1989). The response and spatial recording characteristics of such electrodes have been well defined (Rice *et al.*, 1985; Gerhardt and Palmer, 1987). The sampling process of the electrochemical electrodes is similar to odorant detection by chemoreceptor cells in that a molecule must actually come into contact with the exposed surface of the electrode to be measured. Thus, the exact sampling volume of the electrode is accurately controlled by the dimension of the sensor. Furthermore, electrochemical electrodes can selectively detect specific molecular species against a high salt and chemical noise background. No special adaptations are necessary to use these electrodes in biological tissues or in seawater.

The present study introduces the use of electrochemical electrodes in three applications for measuring marine odor signals. First, odor plumes under laboratory flow conditions were sampled and the results compared with those of previous studies relying on other probes. Second, on-line stimulus concentrations were monitored in electrophysiological recording chambers for correlation of receptor responses and stimulus pulse shape. Finally, electrodes were placed at various locations within the boundary layer around the receptor hairs of a lobster nose (*Homarus americanus* antennule) to study the effect of boundary layers on temporal structure of odor signals.

Methods

Theory of electrochemical recordings

The electrochemical recordings are based on the principles of electrochemical measurements at solid electrodes (Adams, 1969; Bard and Faulkner, 1980). In general terms, the electrode is polarized in aqueous solution using a potentiostat (e.g. see Gerhardt and Adams, 1982). In solution, molecules that physically come in contact with the exposed surface can donate or receive electrons from the electrode surface, provided that the polarization potential is sufficient to cause the molecule to oxidize or reduce. The resulting current flow due to the exchange of electrons from or to the molecule at the tip of the sensor is directly related to the number of molecules that touch the surface of the probe: a simple calibration procedure is used to convert the current flow into concentration or molecular units. Such an electrochemical reaction for dopamine is seen in Figure 1. As depicted in this Figure, a positive polarization of the probe

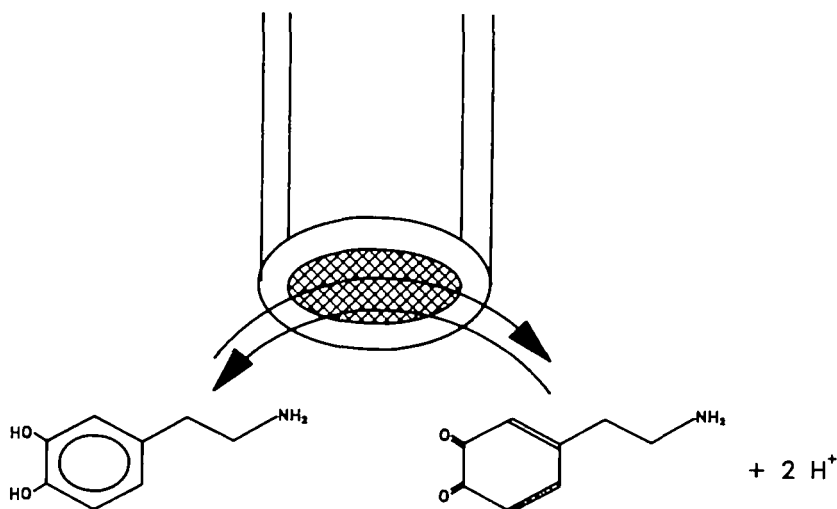


Fig. 1. Electrochemical reaction for dopamine at a carbon electrode surface.

(see below) will cause dopamine to oxidize at the electrode surface and give up two electrons per molecule to the surface. If the potential of the electrode surface is then made negative (reducing potential) some of the oxidation product, a quinone, can be reduced back to dopamine. Thus, the electrochemical measurements entail causing chemical reactions at the surfaces of the miniature recording electrodes, and measuring the amount of current flow that results from these chemical reactions in order to quantitate the number of molecules that have come in contact with the surface of the recording electrode.

Electrochemical electrodes and recording system

The electrochemical electrodes used in these experiments were of the graphite-epoxy capillary (Gerhardt *et al.*, 1984) or carbon fiber (Brazell *et al.*, 1987) type. High-speed electrochemical recordings were made using a custom-designed microcomputer system built around the IVEC-5 (Medical Systems Corp.). The electrochemical recording electrodes were sampled every 5 or 50 ms: analog-to-digital conversions of samples occurred at 1 kHz and data were averaged for the respective time epoch. The 50-ms measurements involved the recording of 50-ms duration square-wave pulses (-0.20 to $+0.50$ V versus a Ag/AgCl reference electrode), while the 5-ms measurements were recorded by holding the electrode at $+0.50$ V. The general methodology for these types of recordings has been described (Bard and Faulkner, 1980; Adams and Marsden, 1982). All electrodes were calibrated in solutions of dopamine prepared in raw seawater, pH 7.9, with a conductivity of 46 mmho/cm. All electrodes exhibited excellent linearity over concentration ranges of $0.5-500 \mu\text{M}$. The stimulus and test solution for the electrodes contained 1–2 mM dopamine, 0.1–0.2 mM ascorbic acid (as an anti-oxidant) and red food coloring as dye; blank determinations showed that the dye did not produce appreciable ($100\times$ less than dopamine) electrochemical responses itself.

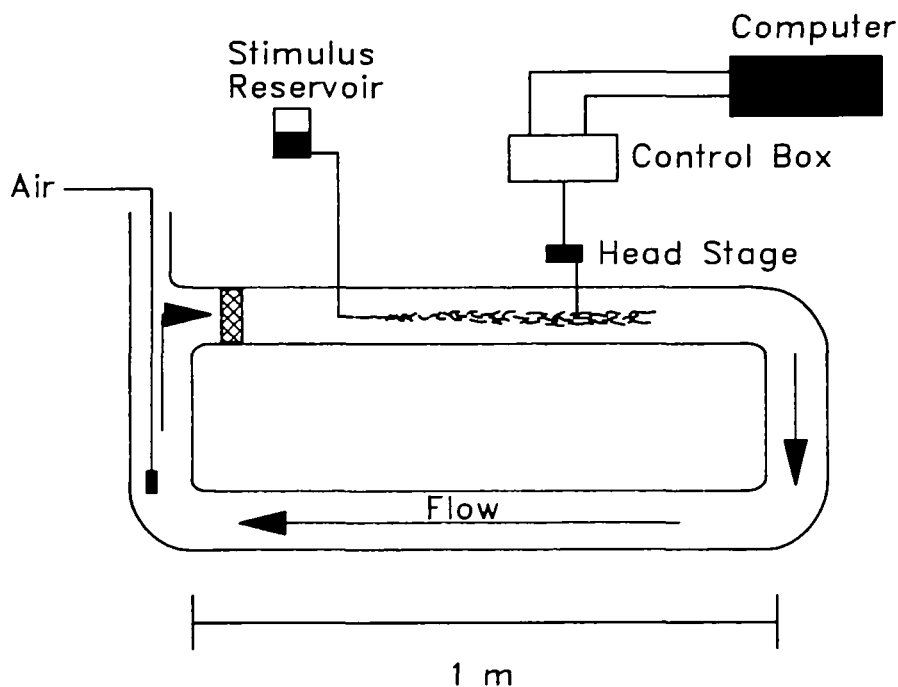


Fig. 2. Diagram of recirculating flume used to measure mildly turbulent odor signals with microelectrochemical electrodes. The head stage is an initial amplifier. The control box is composed of various filters and gain settings.

Plume measurements

Odor plume measurements were recorded in a recirculating flume with a working area of $80 \times 9.5 \times 15.5$ cm (Figure 2). A carrier flow of raw seawater was driven by an air bubbler at a flow rate of ~ 2 cm/s. This bubble method resulted in mild turbulence of the carrier flow. The stimulus (described above) was gravity-fed into the cross-sectional center of the flume at a rate of 15 ml/min through a 1-mm i.d. Pasteur pipette. The flow velocity at the tip was ~ 7.5 cm/s resulting in additional gentle turbulence due to the velocity differential between stimulus and carrier flow. Measurements were taken at three distances in the cross-sectional center of the plume: 5, 20 and 40 cm from the pipette tip to the electrochemical electrode, and at two sampling rates: 10 and 200 hz.

Electrophysiological chamber calibrations

Flow chamber studies were performed within an electrophysiological recording chamber for the lobster antennular filament (Figure 3) (c.f. Johnson *et al.*, 1984). An excised lateral antennular filament was placed in the cylindrical compartment of the chamber with the aesthetasc hairs pointing upward. A background of artificial seawater flowed through the main part of the chamber at a rate of 30 ml/min (3.9 ± 0.3 cm/s). Electrochemical electrodes were placed in the free flow around the antennule (Figure 3b). Electrode placement was controlled by micromanipulators. The stimulus (described

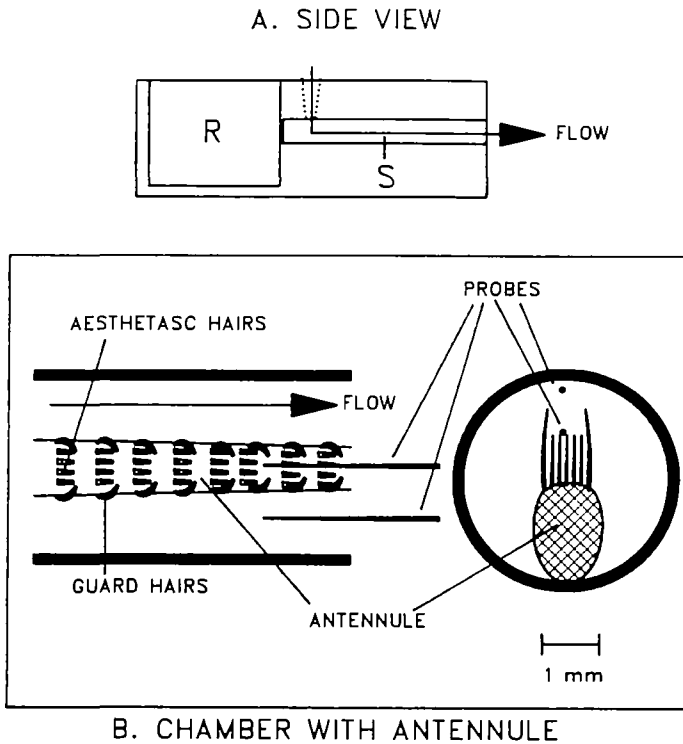


Fig. 3. Diagram of the electrophysiological recording chamber used to deliver chemical stimuli to the receptor cells of the lobster, *Homarus americanus* lateral antennular filament. **A.** side view. **B.** electrode positions in the stimulus channel of the chamber outside and within the aesthetasc hairs. R, recording bath; S, stimulus channel.

above) was introduced into the flow by one of the two side ports either by hand ($44 \mu\text{l}$) or by automatic valve injections (General Valve P/N 3-111-900). Measurements outside of the hair tuft consisted of two series: 150-ms valve openings at pulse intervals of 10, 5, 2.5 and 1-s, and 2.5-s pulse intervals with valve openings of 100, 50 and 25 ms.

Receptor boundary layer measurements

Measurements were made within the recording chamber described above. An artificial seawater background flowed at 20 ml/min ($2.6 \pm 0.3 \text{ cm/s}$) through the main channel. The stimulus (2 mM dopamine, 0.5 mM ascorbic acid) was injected by valve (180-ms opening) into one of the side ports. The electrode (carbon-fiber type) was placed at various depths within the aesthetasc hairs by a micromanipulator (Figure 3B).

Data analysis

All data were converted from counts to micromolar concentration by the calibration factor determined for each electrode. Probability distributions of two odor plume parameters (peak amplitude and onset slope) were calculated from the odor plume profiles

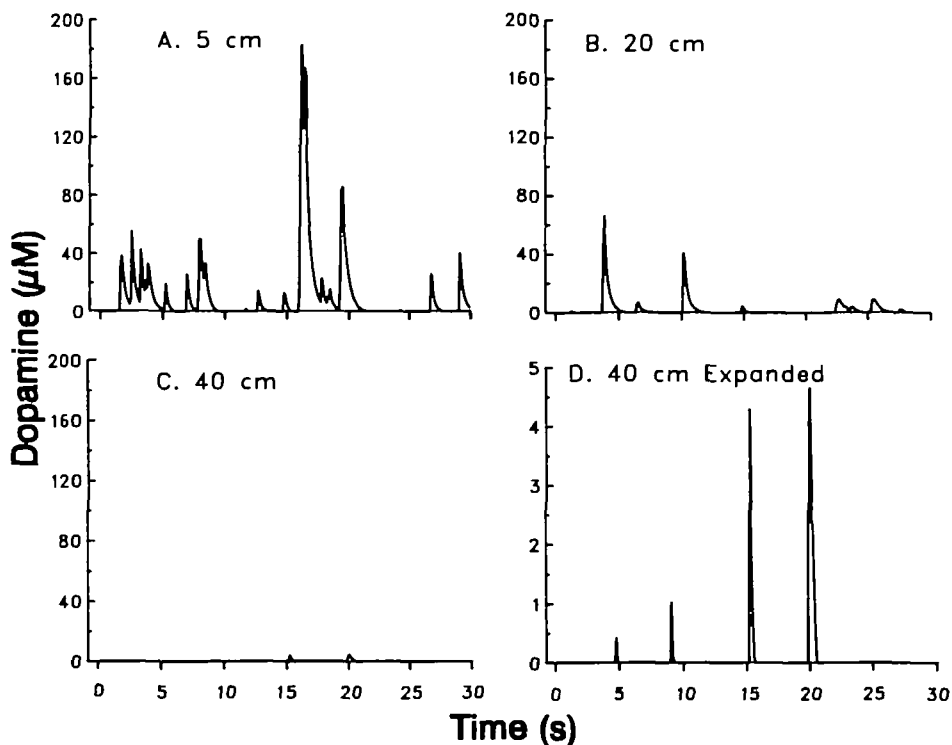


Fig. 4. Random 30-s samples of turbulent spectra from 5-min electrochemical recordings in the recirculating flume at three sites. Sample rate, 10 Hz. A, 5 cm; B, 20 cm; C, 40 cm distances from pipette mouth to electrochemical electrode. D, expanded y-axis of C 40-cm site.

(see Moore and Atema, 1988). Chamber calibration data were evaluated using Student's two-tailed t -test and Fisher's F -test.

Results

Plume measurements

The profiles as sampled by the electrochemical electrodes at 10 Hz (Figure 4) were typical of a turbulent odor spectrum (Murlis and Jones, 1981; Moore and Atema, 1988). The patchy spatial distribution of odor results in pulses detected temporally. Thus, the intensity gradient across patch boundaries was detected as the onset slope of a pulse. These pulse profiles served as the initial data for further analysis.

Probability distributions of the peak amplitude (Figure 5a) showed decreasing peak height with increasing electrode-to-pipette distance. Although the distributions of all three sites overlapped, the mean amplitude at the 5-cm sampling point was significantly different from the 20- and 40-cm mean amplitudes (t -test, $P < 0.05$). This same gradient was seen in the mean values of the onset slopes (Figure 5b). As with the amplitude data there was considerable overlap in all three probability distributions, but the mean

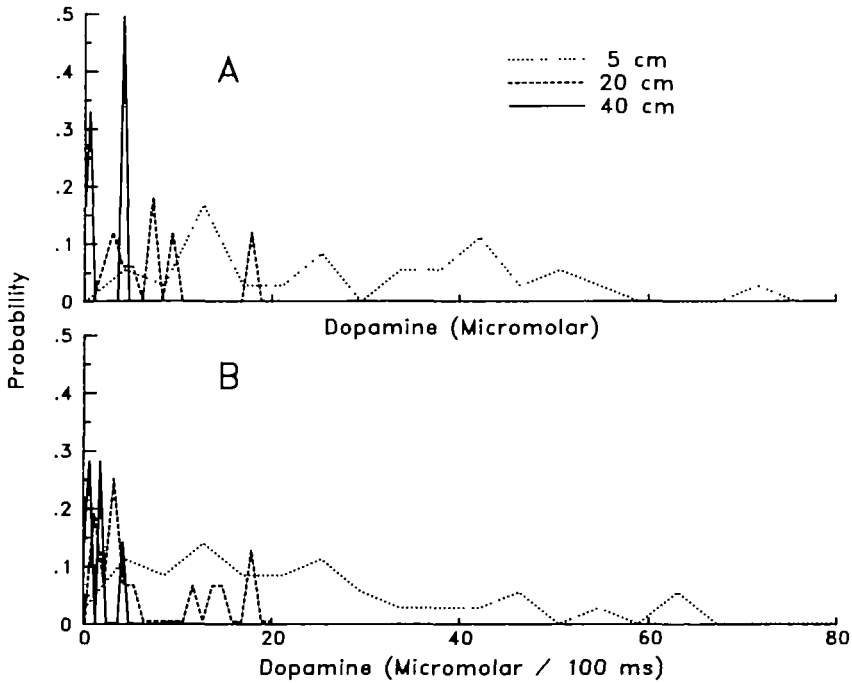


Fig. 5. Probability distributions of odor plume parameters calculated from the 5-min electrochemical recordings (segments shown in Figure 3). A, peak amplitude (μM) and B, onset slope ($\mu\text{M}/100\text{ ms}$) of odor pulses; $n = 34$ (5 cm), 16 (20 cm), 7 (40 cm).

onset slope at the 5-cm sampling site was still significantly different from the 20- and 40-cm sites (t -test, $P < 0.05$). The number of peaks encountered at a sampling site decreased from 34 (5 cm) to 16 (20 cm) and finally to 7 (40 cm). Thus, both the slope and amplitude values and number of peaks decreased with distance from the source.

Chamber calibrations

Table I shows the variability of stimulus peak concentrations measured in the chamber flow outside the aesthetasc hairs resulting from repeated stimulus presentations. Mean peak concentrations resulting from valve openings of 150 ms were not significantly different from each other regardless of pulse interval 2.0–10.0 s (columns 1–3). The mean concentration of the 1-s pulse interval was significantly higher (t -test, $P < 0.001$) as insufficient clearance of previous pulses apparently results in build-up of stimulus within the chamber. SD of the 2.5-s interval (column 3) is significantly lower (F -test, $P < 0.01$) than those for other intervals (columns 1, 2 and 4). Such an effect might occur due to flow resonance of pressure injection within the chamber. As the valve opening time was decreased (columns 5–7) both peak concentrations and the consistency of stimulus presentation (as coefficient of variance) decreased. Hand injections (column 8) were surprisingly consistent when compared with valve-controlled injections (e.g. column 7).

Table I.

Injection	Valve								Hand
Pulse intervals (s)	10.0	5.0	2.5	1.0	2.5	2.5	2.5	2.5	—
Valve opening (ms)	150.0	150.0	150.0	150.0	100.0	50.0	25.0		
Number	34	20	21	42	17	28	30	22	
Mean ($\times 10^{-6}$ M dopamine)	194.8	193.8	198.7	217.5*	174.2	96.5	62.7	65.0	
SD	22.3	17.9	9.3**	19.6	21.6	15.3	12.9	11.3	
CV*	11.5	9.2	4.7	9.0	12.4	15.9	20.6	17.3	

*Coefficient of variance.

*Significantly different (*t*-test, $P < 0.001$).

**Significantly different (*F*-test, $P < 0.01$).

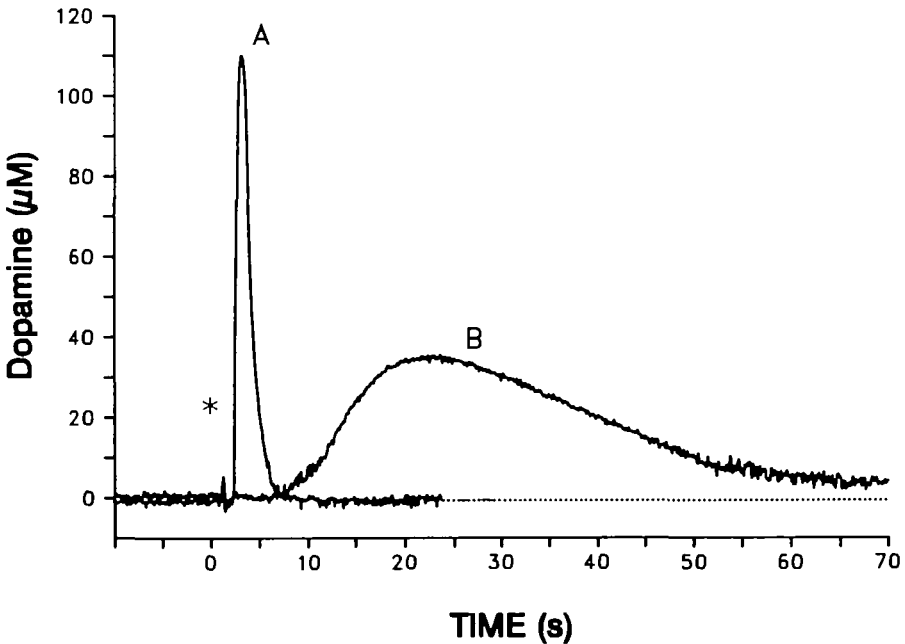


Fig. 6. Temporal profile of odor pulse in the free flow (A) of the stimulus chamber (shown in Figure 2) and within the boundary layer (B) of the aesthetasc hairs both resulting from an identical 180 ms valve injection of dopamine as detected by a carbon-fiber microelectrode. *Injection point at time = 0.

Receptor boundary layer measurements

As expected, the concentration profiles obtained within the electrophysiological recording chamber were quite different inside (Figure 6b) and outside (Figure 6a) of the aesthetasc hairs. Outside the hairs the pulse amplitude was 106.5 μM with a rise time of 2.6 s and a total time course from injection to blending into the background of 7.1 s. The diffusion barrier created by the boundary layer at the base of the aesthetasc hairs changed

the pulse amplitude to $35.2 \mu\text{M}$, the rise time to 23.7 s and the total time course to 71.5 s. The small initial peaks appearing on both curves right after injection and preceding the main peaks may have been due to stirring of the boundary layer by the initial pressure wave. Small scale eddies within the hair tuft may have caused the small fluctuations of signal visible on the initial and – more clearly – on the falling stages of the odor pulse (see ‘jitter’ on shallow part of slopes).

Discussion

Any sensor placed within a flow field is surrounded by a boundary layer and thus the sensor does not measure the true dispersion of odor as it would exist in an unobstructed flow situation. This is equally true for chemoreceptor structures. However, for biologists attempting to study odor signals as they appear to receptor structures, a problem arises when probe size and receptor structure are significantly different. However, since electrochemical electrodes can be manufactured in sizes and configurations that closely resemble sensillar structures of lobsters and other animals, boundary layers around electrodes and sensilla are similar and thus odor transport and access are similar. Application of such electrodes to vertebrate and molluscan receptor surfaces needs to be tested, but the small size of the electrodes is critical to their success in applications such as olfactory mucosa and tongue surfaces as well. At the present time, the electrodes used here measured dopamine as a tracer species; it is not a known stimulant for lobster chemosensory behavior or chemoreceptor cells. Electrochemical recording techniques can now be developed to measure known chemical signals such as amino acids.

In contrast to the close similarity in odor sampling between electrochemical electrodes and receptor sensilla, the electrical field of conductivity electrodes is ill-defined and results in a spatial averaging of salt pulses as they approach, pass by and leave the sampling space around the electrodes (Figure 7). The sampling volume of spectrophotometric and fluorometric probes are defined by the attenuation of the trigger light and detectors. Zimmer-Faust *et al.* (1988) estimate that, with a homogeneous solution of dye, 90% of the fluorescence is received from a distance < 1 mm from the light source, but even this estimate depends upon the concentration of dye and suspended particles in the water. For aerial measurements, ion collectors measure molecular impact at electrode surfaces. However, they are much larger than the sampling area of insect antennae and they measure only the ionized fraction of a tracer, not odor itself.

While the temporal characteristics of odor plume data collected in the present study are not directly comparable with other investigations due to different flow conditions and different tracer units, the qualitative pulse shapes seen in this investigation (Figure 4) are similar to profiles seen in previous studies (Murlis and Jones, 1981; Moore and Atema, 1988). However, unlike previous reports (Atema, 1985; Moore and Atema, 1988), the present recordings contained large epochs of time where no signals were detected. In addition, clear exponential decays of the electrochemical signals are observed. This is likely due to diffusion of the detected species away from the electrode surface. In large part the response differences between these and our earlier studies are thought to be due to the smaller sampling volume of the electrochemical electrodes. At all sites the pulses had fast rise times (> 100 ms). Thus, accurate sampling of slopes in odor plumes must take place at rates > 10 hz.

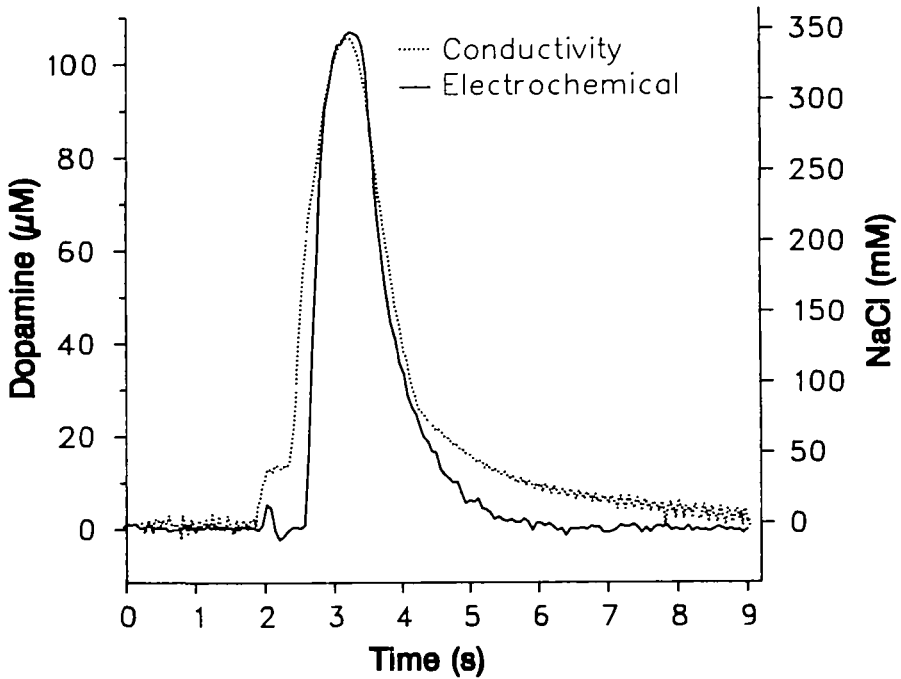


Fig. 7. Comparison of pulse profiles as measured in the free flow by electrochemical electrodes and conductivity electrodes in the antennular recording chamber in Figure 2. Salt stimulus was a 1 M NaCl solution injected into a flowing deionized carrier flow. Results of both profiles obtained under identical flow conditions.

Control and knowledge of temporal and spatial stimulus characteristics is needed when attempting to understand temporal filtering and feature extraction by receptor cells. Stimulus variability is dependent on sampling volume and rate—small volumes and low rates increase variability. This is confirmed by results presented statistically in Table I. Receptor response variability is an inherent part of any receptor system and must be included in the sampling and decision making processes of animals. On-line monitoring of stimulus variability is one of the critical steps in determining directly rather than statistically the causes of variability of receptor cell performance. The present study shows that direct on-line measurements of temporal features and variability of the stimulus can be made while recording responses and their variability (Table I, Figures 3 and 6).

The fluid flow around and within the receptor structures determines the access of odors to the receptor cell surfaces. Most crustaceans flick their antennules to sample odor plumes (Snow, 1973; Schmitt and Ache, 1979). Vertebrate sniffing probably accomplished the same purpose (Macrides and Chorover, 1972). Flicking decreases the size of the boundary layer (a diffusion barrier) and increases the fluid flow (and therefore odor access) to the receptor hairs (video analysis, P.A.Moore and J.Atema, unpublished). When the antennule is not flicking, a significant diffusion barrier acts as a low-pass filter. When the antennule is flicked, the diffusion barrier is lowered

and high frequency signals can reach the receptor cell. The effect of the diffusion barrier is seen in the shape of the pulses measured by electrochemical electrodes inside the tuft of aesthetasc sensilla (Figure 6).

These initial experiments have demonstrated a promising new method for studies of chemical signals in the small temporal and spatial domains characteristic of chemoreceptor structures. Electrochemical electrodes offer the ability to measure odor signals in real-time both in marine odor environments utilized for animal behavior and in liquid microenvironments from which chemoreceptor structures of all animals sample. These recording capabilities will likely facilitate studies of aquatic odor distribution patterns, odor access to receptor cells and temporal tuning and filtering of receptor cells, including receptor cell reliability.

Acknowledgements

We would like to thank Carl Merrill and Drs Rainer Voigt and Hortense Gerardo for assistance during experiments and Geoff Trager and Dr J.Rudi Strickler for use of the Flume. Special thanks to Mr Mike Parrish for the development of the software for the high-speed electrochemical recording system. The authors are indebted to Dr Thomas Finger who suggested the collaboration between the Woods Hole and Denver Laboratories. Supported by grants from NSF no. BNS-8812952 to J.A. and USPHS no. AG-06434 and AG-00441 to G.G.

References

- Adam, G. and Delbrück, M. (1968) Reduction of dimensionality in biological diffusion processes. In Rich, A. and Davidson, N. (eds), *Structural Chemistry and Molecular Biology*. W.H. Freeman, San Francisco, CA, pp. 198–215.
- Adams, R.N. (1969) *Electrochemistry at Solid Electrodes*. Marcel Dekker, New York.
- Adams, R.N. and Marsden, C.A. (1982) Electrochemical detection methods for monamine measurements *in vitro* and *in vivo*. In Iversen, L.L., Iversen, S.D. and Snyder, S.H. (eds), *Handbook of Psychopharmacology*. Vol. 15. Plenum Press, New York, pp. 1–74.
- Atema, J. (1985) Chemoreception in the sea: adaptation of chemoreceptors and behavior to aquatic stimulus conditions. *Soc. Exp. Biol. Symp.*, **39**, 387–423.
- Atema, J. (1987) Aquatic and terrestrial chemoreceptor organs: morphological and physiological designs for interfacing with chemical stimuli. In Dejours, P., Bolis, L., Taylor, C.R. and Weibel, E.R. (eds), *Terrestrial Versus Aquatic Life: Contrasts in Design and Function*. *Fidia Res. Ser.* Liviana Press, pp. 303–316.
- Atema, J. (1988) Distribution of chemical stimuli. In Atema, J., Popper, A.N., Fay, R.R. and Tavalga, W.N. (eds), *Sensory Biology of Aquatic Animals*. Springer Verlag, New York, pp. 29–56.
- Bard, A.J. and Faulkner, L.R. (1980) *Electrochemical Methods: Fundamentals and Applications*. Wiley, New York.
- Bell, W.J. and Tobin, T.R. (1981) Orientation to sex pheromone in the American cockroach: analysis of chemoreception mechanisms. *J. Insect Physiol.*, **27**, 501–508.
- Brazzell, M.P., Kasser, R.J., Renner, K.J., Feng, J., Maghaddam, B. and Adams, R.N. (1987) Electrocoating carbon fiber microelectrodes with Nafion improves selectivity for electroactive neurotransmitters. *J. Neurosci. Methods*, **22**, 167–172.
- De Simone, J.A. (1981) Physiochemical principles in taste and olfaction. In Cagan, R.H. and Kane, M.R. (eds), *Biochemistry of Taste and Olfaction*. Academic Press, New York, pp. 213–229.
- Elkinton, J.S. and Cardé, R.T. (1984) Odor Dispersion. In Bell, W.J. and Cardé, R.T. (eds), *Chemical Ecology of Insects*. Sinauer, Sunderland, MA, pp. 73–91.
- Futrelle, R.P. (1984) How molecules get to their detectors: the physics of diffusion of insect pheromones. *TINS*, **116**–120.

