Reference: *Biol. Bull.* **187**: 271–272. (October, 1994)

Three-Dimensional Calibration of the Non-Invasive Ion Probe, NVP_i, of Steady Ionic Currents

Joseph G. Kunkel and Peter J. S. Smith (Marine Biological Laboratory)

A non-invasive approach has been developed and applied to measure the flux of specific ions that enter and exit cells or tissues (1, 2). The NVP_i adds, to the ion measurement technology of specific ion electrodes, an oscillation of the electrode over a short distance, δr, typically 10 μm, which allows the estimation of the concentration gradient of the ion in the direction of oscillation. The NVP_i has been calibrated previously in the single dimension of oscillation, but the fit of the observed to the expected μV difference was poor close to the source (2). Recent addition of two parameters—distance to infinite source, r_0, and the electrode efficiency, **eff**— to the theoretical formula for predicting μV difference (2) allow the expected μV differences relative to the source to be modeled accurately over all radial distances from the source. The parameter r_0 is needed because it is clear that the point of closest approach to the source is some distance, r_0, away from an infinite source. The parameter **eff** is needed to account for the inability of the microelectrodes to fully respond to the gradients being measured within the time constraints of the oscillation cycle. At 0.3 Hz, the electrode is able to make one measurement of a calcium gradient in one direction in about 3 s with an efficiency of 50%. If the oscillation is increased to 0.5 Hz, the efficiency drops to 30%.

After the development of routines for oscillating in three dimensions and collecting three-dimensional data (Fig. 1), the advantages of a method of 3-D calibration become a consideration. The most important technical advantage of a 3-D calibration is that it removes an obligation of the probe operator to position the probe precisely on the diffusional axis of the ion source, an exacting and time-consuming task in three dimensions. The theoretical advantage of the 3-D calibration is that it should conform to the law of conservation of charge moving through concentric shells about the source. The artificial diffusional source of ions provides a physical standard from which an observed dose-response curve and the efficiency of the probe can be estimated. The concentration of the ion of interest can be predicted knowing the radial distance from the source to the measurement point. Such a source has been used to create a calibration in a single dimension. A correct 1-D calibration is only obtained when measurements are made on a diffusional axis emanating from the source. Until now the 3-D version of the NVP_i has been calibrated by using this artificial source and applying the 1-D predictive equation. However, a method incorporating all of the strengths of the 3-D approach would now be beneficial. A 3-D collection of data typically results in a $7 \times 7 \times 7$ grid of 343 individual, equispaced, 3-D observation vectors, $dV_O = O_{ijk} = \{\mu Vx_i, \mu Vy_j, \mu Vz_k; i,j,k = 0 \ldots 6\}$, of μV-differences with element O_{303} at the nominal point of closest approach to the diffusional source which has geometric location $\{x_3, y_0, z_3\} = \{0,0,0\}$ (Fig. 1). If one gradient estimate were made for each dimension at each grid position at 0.3 Hz, the time to complete each 7×7 plane of data would be $(3 \times 7 \times 7 \times 3\ s) = 7$ min 21 s. The resultant planes of 49 dV_Os can be

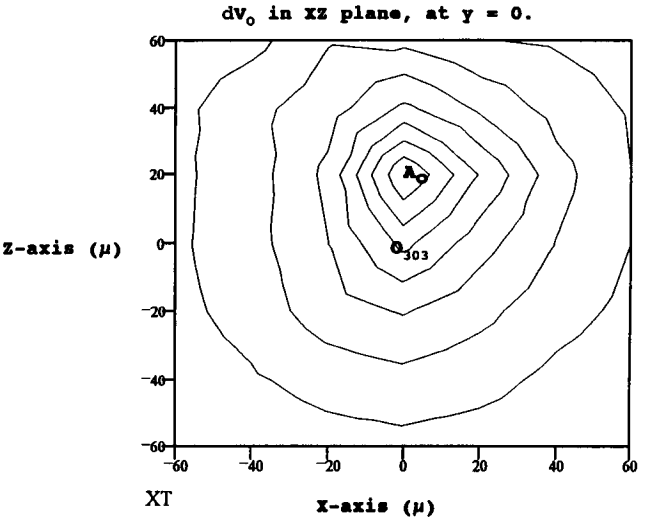

Figure 1. *Contour plot of 7×7 observed μV-differences, dV_o, at 20 μm intervals in the plane of closest approach to a 100 mM $CaCl_2$ source diffusing into a background concentration of 1 mM $CaCl_2$. The point of closest approach was at O_{303} but the estimated axis of ion diffusion in this plane is 20 μm away at A_o.*

used to fit the unknown parameters of an expected μV-difference equation. The probe need not be placed on the actual axis of diffusion but merely close to it. The position of the axis of diffusion, $A_o = \{x_A, 0, z_A\}$, in the 0-plane of closest approach {*i.e.*, at y = 0) becomes another parameter to be fit by the equation of linear concentration relation with the inverse of distance,

$$C_{x,y,z} = Cb + K/(r_{xyz} + r_0).$$

The equation for the expected value of μV-difference, $dV_E(r)$, to be measured at a radial distance, r, units along that concentration curve is now given by,

$$dV_E(r) = -10^3 \cdot S \cdot eff \cdot K \cdot e^{-1} \cdot \frac{\delta r}{r^2 \cdot (Cb + K/(r + \delta r/2))}\ (\mu V),$$

where the effective radius from an observation point {x,y,z} to the infinite source, r, is given by

$$r = r_{xyz} + r_0.$$

In the 3-dimensional case, r_{xyz}, is measured from the observation point to the diffusional axis, A_o, computed by

$$r_{xyz} = |O_{ijk} - A_o|.$$

The geometric coordinates of A_o are determined by finding the location of the maximum μV difference in the plane of closest approach (Fig. 1). The parameters of the above equations, other than r_0 and **eff**, are discussed in detail elsewhere (2): briefly, S is the familiar Nernst coefficient for the ion of interest; K is the

slope of the concentration equation for $C_{x,y,z}$; **e** is the base of natural logarithms. The unknown parameters are estimated by well-known methods of analytic geometry. First $\mathbf{r_0}$ is estimated by linearizing the equation for C_{xyz}, which then allows **K** to be estimated by linear regression. Finally **eff** is estimated by minimizing the Chi Square statistic on the observed *versus* the expected μV difference, Chi Square = $(dV_O - dV_E)^2/dV_E$.

These equations can then be applied to biological data collected in a few planes tangential to the source such that a point and a disc source of ions can be distinguished. Such sources are very often quite weak, so a simple one-dimensional transect away from a source does not yield much data.

Literature Cited

1. **Kühtreiber, W. M., and L. F. Jaffe. 1990.** *J. Cell Biol.* **110:** 1565–1573.
2. **Smith P. J. S., R. H. Sanger, and L. F. Jaffe. 1994.** *Meth. Cell Biol.* **40:** 115–134.

Reference: *Biol. Bull.* **187:** 272–273. (October, 1994)

Lobster Orientation in Turbulent Odor Plumes: Simultaneous Measurement of Tracking Behavior and Temporal Odor Patterns

Jennifer Basil and Jelle Atema (Boston University Marine Program, Marine Biological Laboratory)

Chemical cues play an important role in the food searching behavior of the lobster, *Homarus americanus*. Previous studies have shown that bilateral antennular chemoreception is necessary for search efficiency within a turbulent odor plume (1, 2). The manner in which lobsters orient within plumes indicates that there is directional information contained within turbulent odor plumes (3).

In addition, using chemical sensors (In Vivo Electrochemical System, IVEC) capable of measuring a tracer (dopamine) in seawater, Moore and Atema (4) measured the fine-scale, three-dimensional structure of plumes. Plumes showed spatial gradients of such features as pulse height, onset slope, and distribution, which could provide directional cues to orienting animals. The purpose of the present study was to implement techniques from which we could determine whether animals extract and use fine-scale features to successfully navigate to a distant odor source.

In the present experiment, behavioral and electrochemical measurements were made in a flow-through flume (250 × 90 × 20 cm). The food stimulus was gravity-fed through a Pasteur pipette positioned so that the nozzle released stimulus at the cross-sectional center of the tank, 9 cm off the bottom, at the upstream end of the flume. The lobster carried two IVEC electrodes mounted directly over the lateral antennules, and a submersible amplifier on its back. The backpack was connected, by a 2.5-m flexible cable, to a computer that ran the electrochemical software (IVEC). The overhead film record of the freely orienting lobster could be synchronized with the real-time concentration measurements.

Lobsters were fed twice weekly and were deprived of food for 3–10 days prior to each trial (3). Twenty-four hours before a trial, a lobster was fitted with a black plastic blindfold and allowed to habituate to the orientation arena. Food extracts (squid, clam, mussels) with tracer were used as stimuli (source tracer concentration = 40 m*M*).

The stimulus was introduced into the arena when a lobster settled into the downstream shelter. Localization was considered successful only if the lobster approached to within 15 cm of the pipette tip within 20 min after the start of stimulus introduction and did not walk along the side walls of the flume. Orientation paths were digitized at 1/s. Variables chosen to quantify tracking behavior included walking speed, turning angles, and headings.

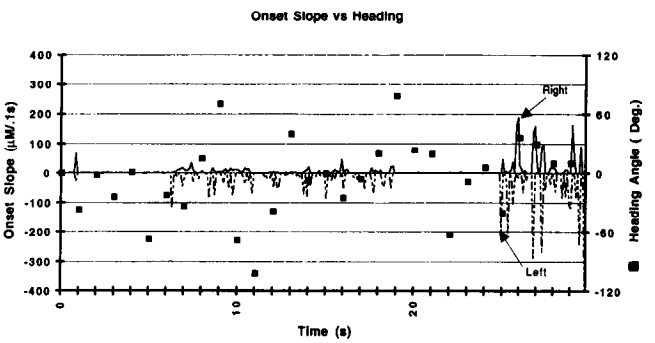

Figure 1. *Onset slope and heading angle, plotted together as a function of time (t = 0 s at start of orientation trial, t = 30 s when animal located source). Onset slope is plotted continuously at 10 Hz. Onset slope from electrode mounted over right antennule is top trace (solid line). Onset slope from left antennular area is plotted as the lower trace (dashed line). Large onset slopes are located close to the source, where heading angle improves.*

Backpacks did not affect orientation behavior. Figure 1 illustrates the onset slopes for odor tracer patches encountered by the left and right electrode (right electrode = positive values, left electrode = negative values), the slopes are plotted above the lobster's heading as it oriented towards the source (lobster hits source at 30 s). Positive headings indicate that the animal is moving to the right of the line connecting the starting point and the source. The odor patches, as encountered by the moving lobster, closely resemble those measured in previous studies (4). At points in the plume that are distant from the odor source, there were a greater number of patches of lower concentration and shallow onset slopes. Close to the source, the patches are of