Odor Discrimination Using a Hybrid-Device Olfactory Biosensor

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Abstract- **Current trends in artificial nose research are strongly motivated by knowledge of biological olfactory systems, but are primarily confined to improving pattern recognition strategies for data derived from a relatively simple sensor array. Biological olfactory systems are able to discriminate weak, transient, broad-band signals ranging over a poorly-defined parameter space, and therefore outperform current artificial nose systems in several respects. Here, a biological olfactory sense organ, the insect antenna, has been exploited in a hybrid-device biosensor. An algorithm was developed to analyze the electrophysiological responses recorded from a sensor array comprised of antennae from different species of insects. A training period during which the array was exposed to known target odors established response signatures for those odors. Subsequent odor stimuli were then classified using a forced-choice nearest-neighbor technique. As odorants arrived in discrete packets of air in the turbulent air stream, individual sensor response events lasted less than one second, and could be classified correctly nearly 100% of the time.**

Keywords **- Olfaction, biosensor, insect**

I. INTRODUCTION

Detection and identification of chemical compounds *via* air-born volatiles (i.e. odor) have many potential applications in military, industrial, clinical and research arenas. Biological noses are capable of detecting many odors of no possible evolutionary usefulness, including explosives and drugs. Artificial nose technology takes many forms, but is universally characterized by slow response times, poor discrimination, low sensitivity, and a low number of identifiable odors. The artificial nose, as presently conceived, can be distinguished from other chemical detectors (such as pH or NO electrodes) by the promise of detecting a number of different compounds with the same device. Where single-chemical detectors often rely on a semi-permeable membrane specific to the molecule to be detected, artificial noses generally consist of an array of semi-selective, cross-reactive sensors which demonstrate distributed specificity. There are a number of different artificial nose technologies currently being developed, including metal-oxide and MOSFET, conductive polymers, piezoelectric-based (acoustic wave devices) and fiber-optic / solyatochromic fluorescent dye sensors [1-4]. An array of different classes of sensors yields a set of response vectors representing the sensor output, which must then be interpreted by a pattern recognition scheme. This has been done by using pattern recognition methods based on statistical and computational neural networks approaches [4].

However, there are three important limitations of artificial nose technology. First, the long response times (tens of seconds to minutes) of most approaches limit them to steady-state measurements, where steady-state may take impractically long times to reach. Steady-state is not often attained under many field conditions where an artificial nose might find application. Second, the number of sensor classes comprising the array is limited to about three in present designs. This limits the number of compounds which can be distinguished, and generally requires advanced knowledge of the compounds to be detected. Third, all artificial nose technologies exhibit low sensitivity.

For the past several years, a hybrid system for odor detection based on the olfactory organs of insects has been under development [5]. Important aspects of the biological olfactory system are a short response time, high sensitivity, and broad-band discrimination. It is thought that all three of these desirable qualities derive in part from the sensor-level design of the olfactory system, and a biomimetic theme has strongly influenced artificial nose development. Here we describe early odor classification results deriving from a sensor array comprised of antennae from different species of insects.

The principal arrangement of the biological olfactory system is quite well conserved across phyla, from insects to mammals. Sensory neurons exhibit a response when airborne molecules bind to metabotropic membrane receptors and activate G-protein cascades, providing amplification and eventually leading to membrane potential changes and characteristic trains of action potentials [6-9]. These sensory neurons synapse onto a variety of interneurons in the olfactory bulb, the output of which appears on mitral cells which lead to higher processing structures in the brain. Sensory cells, numbering in the 100's of thousands, have overlapping, semi-selective, yet broad response spectra. The result of the transduction-level coding and the olfactory bulb processing is a system that exhibits a remarkably high sensitivity with broad-band detection and discrimination. These are desirable features in any detector system, and represent active areas of research in many areas of information technology.

The parameter space of the system/model input (molecular properties of odorants to which the sensory neurons are sensitive) is not precisely defined. However, structure-activity studies (chain-elongation, double-bond position, functionality) performed on noctuid olfactory

neurons *in vivo* have been particularly enlightening in understanding that ligand-receptor interactions can behave according to conformational energy and electron distribution models and not merely to space-filling [10-12].

Several groups have shown the potential use of insect antennae in a hybrid-device biosensor [13-16]. However, each of these studies made use of a single antenna, which cannot provide discrimination between odors. In work recently published [5], electroantennogram (EAG) response profiles of five different insect species, *Drosophila melanogaster*, *Heliothis virescens*, *Helicoverpa zea*, *Ostrinia nubilalis* and *Microplitis croceipes*, showed different, species-specific EAG response spectra to 20 volatile compounds tested. The EAG response profiles were then re-constructed for each compound across the five insect species. Most of the compounds could be distinguished by visually comparing the response spectra. A four-antenna array, called a Quadro-probe, was then implemented to discriminate among odorants based on the relative EAG amplitudes evoked when the probe was placed in plumes in a wind tunnel and in a field. Stable EAG responses could be simultaneously and independently recorded with four different insect antennae mounted on the Quadro-probe, and different volatile compounds could be distinguished in real time by comparing relative EAG responses with a combination of differently tuned insect antennae. Regardless of insect species or EAG amplitudes, antennae on the Quadro-probe maintained their responsiveness with higher than 1 peak/s of time resolution.

II. METHODOLOGY

Methods for recording electroantannogram (EAG) responses have been described in detail previously [25], and are summarized here. The excised antennae from two species of insects were recorded from simultaneously to obtain a differential response to selected volatile compounds. Species used were either *Helicoverpa zea* (corn earworm) and *Ostrinia nubilalis* (European corn borer), or *H. zea* and *Trichoplusia ni* (cabbage looper). Each antenna was fixed between a wire recording electrode near the base and a common ground wire electrode near the tip; electrical contact was made with a conductive gel. This comprised a two-species antenna array; however, four antennae were routinely recorded from (two from each species) using the custom designed Quadro-probe EAG recording system (Syntech®, The Netherlands). A four-species array has been utilized in previous work [5], but data presented here use only two species, which provided ample discrimination for the odorants used. The Quadroprobe was positioned 1.5 m downwind from the odor source in a wind tunnel; flow rate 50 cm/sec. The odor source consisted of 100 µg of a chosen compound in solvent (hexanol) applied to a piece of filter paper and placed in the tunnel after the solvent had evaporated. Compounds used represent major components in the pheromones of the insects used in this study: (*Z*)-11-hexadecenal, (*Z*)-11-tetradecenyl acetate, and (*Z*)-7-dodecenyl acetate.

A data analysis algorithm was implemented in LabView (National Instruments). Raw data consisted of four channels (from the four antennae) of voltage vs. time records from the Quadroprobe under each experimental condition (clean air, or a specific odor). Data were digitized at 55.8 Hz for storage, and smoothed offline by applying a symmetric 5-point moving average. When a filament of odor-containing air traversed the Quadroprobe, each antenna exhibited a depolarization of amplitude proportional to its sensitivity to the odorant and the concentration of the odorant in the filament. A peak-detection algorithm used a threshold of the second derivative of the voltage vs. time record, and peak times and amplitudes were stored in an array. At least one channel (antenna) had to have a response peak above an arbitrary threshold for a response event to be acknowledged. Coincident peaks (within $\pm$ 55 ms of each other, to account for different arrival times of a single odor filament at each antenna) were analyzed for response amplitude. If an event was acknowledged, only peaks above a second, smaller threshold were assigned a value above zero. These amplitudes were plotted in an n-dimensional space, where n is the number of antenna species in the array (n = 2 for data presented here).

The system was trained by presenting target odors, one at a time, for approximately 30 seconds each, yielding approximately 20 sets of n coincident response peaks (one set of peaks for each filament of odor in the turbulent air stream). The collection of response amplitudes from a training presentation formed a cluster of points representing the signature of that odor. Test odors were then presented, and the response to each filament was classified (forced choice) using a modified distance-weighted k-nearest neighbor technique, where the classification was made based on the maximum value of X, where

$$X = \sum_{k=1}^{10} \frac{1}{d_k + \alpha} \tag{1}$$

where d_k are the Euclidean distances to the 10 nearest points in each training set, and α is a constant which avoids over-weighting very close data points. X was calculated for each training odor.

III. RESULTS

Figure 1 plots representative response records recorded simultaneously from the antennae of two species in the presence of clean air and two odorants. Upper trace in each panel plots the response of *T. ni*, lower trace plots the response from *H. zea*. Characteristic depolarizations in response to individual odorant filaments in the turbulent airflow arriving at the antenna array have durations of approximately 0.5 sec. These data show an extreme case of one species being highly sensitive to a given odorant, and the other being minimally sensitive. These odors were chosen to provide highly discriminable responses to use during the development of the analysis algorithm.

The data shown in Fig. 1 were used as a training set, and the amplitudes of coincident peaks are plotted in two

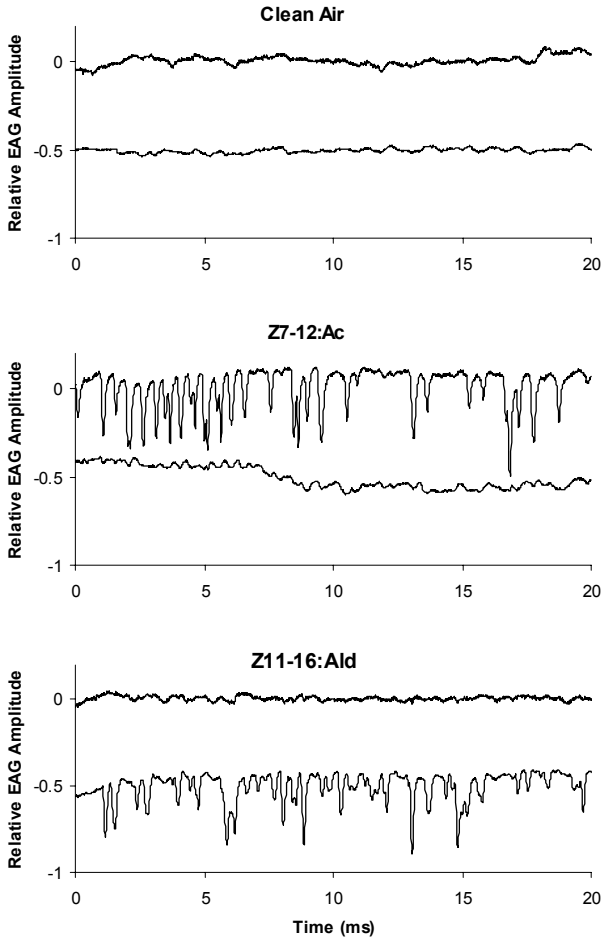

Fig. 1. Simultaneous EAG responses in two species. Upper trace in each panel from *T. ni*, lower trace from *H. zea*. Top panel, responses to clean air. Middle panel, responses to Z7-12:Ac, a major component in *T. ni* pheromone. Bottom panel, responses to Z11-16:Ald, a major component in *H. zea* pheromone.

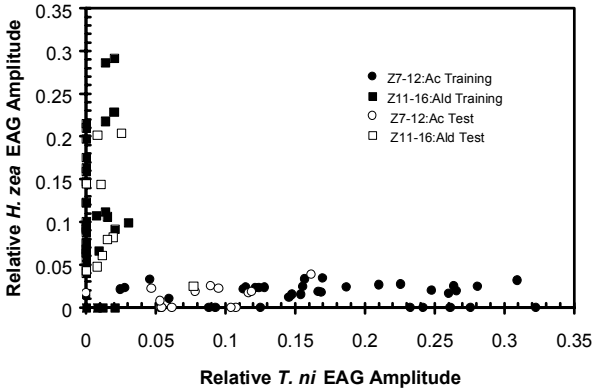

Fig. 2. EAG response amplitudes from presentation of odorants for training (filled symbols, data of Fig. 1), and response amplitudes from presentations of the same odorants for testing (open symbols). Open symbols were classified using the k-nearest neighbor technique described in the Methods.

dimensions in Fig. 2 (filled circles and squares), where each axis corresponds to the relative amplitude of one antenna. If only one antenna in the array were sensitive to a given odor, all the responses of that odor would fall along one of the axes. If the antennae were differentially sensitive to an odor, all points would lie along a line, the slope of which would represent the relative sensitivity of one antenna vs. the other; distance from the origin along this line would correspond to the strength of the stimulus (concentration of the odor). Distance from the best-fit line through a group of data results from noise in the response.

Following training, subsequent exposure to each of these odors served as a test; amplitudes from these responses are also plotted in Fig. 2 as open circles and squares. Classification of these test data using the k-nearest neighbor technique described above (Eq. 1) was nearly 100% accurate for this data set. Similar results were obtained using a second array comprised of antennae from *H. zea* and *Ostrinia nubilalis* with odorants Z11-14:Ac and Z11-16:Ald (not shown).

IV. DISCUSSION

The results presented here demonstrate the ability to discriminate odorants by analyzing an electrophysiological signal derived from an array of differentially-tuned insect olfactory organs. Using a relatively simple nearest-neighbor classification technique, odors were distinguished with near-100% accuracy. The odorants chosen represented compounds of great biological importance to one or the other insect used in the array, and are admittedly best-case scenarios. However, it is known that insect olfactory systems are sensitive to a wide range of odorants, including many anthropogenic compounds. In addition, this sensitivity is variable across species. This suggests that a hybrid olfactory biosensor could be used to detect many volatile compounds of interest in a variety of applications,

including explosives, drugs, chemical warfare agents, environmental hazards, or indicators of clinically-relevant physiological states (e.g. diabetes).

Like chemical differentiators, olfactory sensory neurons respond most strongly to the arrival of an odor, which is intuitive if one considers the turbulent airflow that carries odors under field conditions. This leading-edge sensitivity results in a rapid response time of the olfactory system, where the response is fully-developed in less than one second. The high single-peak accuracy attained with the two-species array used in this study demonstrates that under ideal conditions, sub-one-second odor classifications can be made. This can be contrasted with artificial noses, which can detect only mean concentrations over tens of seconds or minutes.

While the algorithm described here is straightforward, it appears to be robust in cases of less clear differential sensitivity between antennae (results not presented here). In addition, the algorithm is easily scalable to at least four channels while maintaining insignificant computational time for this time scale. While implemented off-line for the results presented here, a real-time version of the software is under development for field application. The use of four or five species of antenna has been shown to provide good discrimination of odorants of less evolutionary interest to insects than the pheromone components used in the present study [5]. This high discrimination, combined with rapid response times and high sensitivity, suggest that hybrid biosensors comprised of insect antennae may prove useful in several artificial nose applications.

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