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Application of Artificial Neural Networks on Mosquito Olfactory Receptor Neurons for an Olfactory Biosensor

Luqman R. Bachtiar, *Member IEEE*, Charles P. Unsworth, *Member IEEE*, Richard D. Newcomb

***Abstract*— Various odorants such as carbon dioxide (CO_2) and 1-octen-3-ol, underlie the host-seeking behaviors of the major malaria vector *Anopheles Gambiae*. Highlighted by the olfactory processing strength of the mosquito, such a powerful olfactory sense could serve as the sensors of an artificial olfactory biosensor. In this work, we use the firing rates of the *A. Gambiae* mosquito Olfactory Receptor Neurons (ORNs), to train an Artificial Neural Network (ANN) for the classification of volatile odorants into their known chemical classes and assess their suitability for an olfactory biosensor.**

With the implementation of bootstrapping, a more representative result was obtained wherein we demonstrate the training of a hybrid ANN consisting of an array of Multi-Layer Perceptrons (MLPs) with optimal number of hidden neurons. The ANN system was able to correctly class 90.1% of the previously unseen odorants, thus demonstrating very strong evidence for the use of *A. Gambiae* olfactory receptors coupled with an ANN as an olfactory biosensor.

I. Introduction

Malaria is a disease caused by a parasite called Plasmodium which is transmitted via the bites of infected mosquitoes and it afflicts hundreds of millions of people each year [3]. The *Anopheles Gambiae* mosquito is recognized as a major malaria vector, contributing to widespread transmission of the disease throughout sub-Saharan Africa [1]. Both carbon dioxide (CO2) and 1-octen-3-ol are emitted by humans and are known as universal attractants to many mosquito species [4, 5]. Thus, olfaction is known to play a major role in the behavioral aspects of vector-human interactions of the *A. Gambiae*.

An olfactory biosensor device that emulates the odor sensing behaviors of the *A. Gambiae* can be developed to detect and recognize odors. Such a product can potentially be used in olfactory testing, providing an alternative to methods such as gas chromatography [6] and gas chromatography-mass spectrometry [7] that have inadequate sensitivity and exhibit instability.

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L. R. Bachtiar is with the Department of Engineering Science, the University of Auckland, New Zealand. (phone: +64-9-373-7599 x87056; e-mail: lbac004@aucklanduni.ac.nz).

C. P. Unsworth is a Senior Lecturer at the Department of Engineering Science, The University of Auckland, New Zealand. (e-mail: c.unsworth@auckland.ac.nz).

R. D. Newcomb is a Team Leader at The New Zealand Institute for Plant & Food Research, Auckland, New Zealand and is Associate Professor in Evolutionary Genetic at the School of Biological Sciences, The University of Auckland, New Zealand. (e-mail: Richard.Newcomb@plantandfood.co.nz).

A. The Mosquito Olfactory System

Through the use of its Olfactory Receptor Neurons (ORNs), the *A. Gambiae* responds to a myriad of chemicals to perform functions such as host-seeking and nectar feeding [4, 8, 9]. The antenna and maxillary palp of the *A. Gambiae*, as illustrated in Fig. 1, are populated by sensilla that house the ORNs. Sexual dimorphism is present in the *A. Gambiae*, where a female mosquito possesses three to four times more antennal sensilla than males [10]. This reflects the importance of olfaction in the female's role of reproduction as it feeds on a host's blood as a source of protein for the development of eggs.

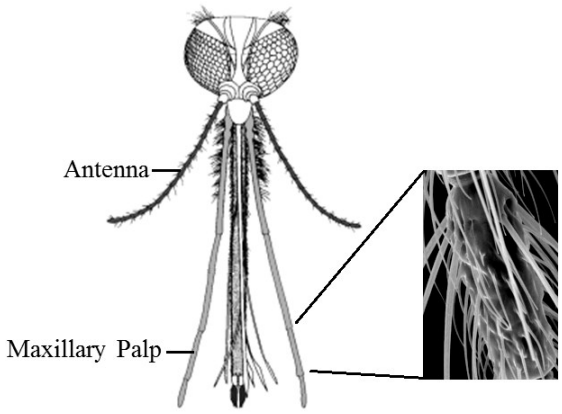

Figure 1. The *Anopheles Gambiae* utilizes its antennae and maxillary palps for odor sensing, among other things [1]. Hair-like sensilla cover the maxillary and act as sensory receptors [2].

The numerous pores or slits along the walls of the olfactory chemosensilla allow for the site entry of odorant molecules. The high concentrations of Odorant Binding Proteins (OBP) in the aqueous lymph solubilise the odorants and provide a medium of transport across the aqueous environments of the sensillum [10]. The solubilized odorants are delivered to receptors on the dendrites of Olfactory Sensory Neurons (OSNs) that extend up into the lymph.

The clustering of OSNs in a sensillum allows for physiological analysis on the cellular level [10]. Electrophysiological recordings have revealed that different morphological classes of sensilla are functionally distinct, exhibiting characteristic spontaneous firing rates. ORNs of some single-walled sensilla responded to pheromones, others to food odours [11], while double-walled sensilla were sensitive to polar compounds such as amines and carboxylic acids [8, 12]. OSNs present an axon structure that extends to the antennal lobe, where those that express the same receptor converge to a glomerulus. Local interneurons within the lobe

modulate the signals and projection neurons transduce the information to the higher brain of the mosquito.

B. Designing an Artificial Olfactory System

The data acquisition method used by Carey, et al. [13], in which the resulting data is used by this work, is based on the experiments conducted by Hallem, et al. [14]. It involves using a mutant 'empty' sensory neuron to express each individual olfactory receptor. Recordings of firing rates in response to various volatile chemicals were subsequently documented. Of the 72 *Anopheles Gambiae* Olfactory Receptors (AgOrs) expressed in the empty neuron, 50 AgOrs were found to be functional in which they exhibit spontaneous firing rate and excitatory and/or inhibitory responses to the odorant stimuli [13].

In line with the combinatorial model of odor coding [14, 15], Carey, et al. [13] found that individual receptors responded to subsets of odorants and individual odorants activated subsets of receptors. Thus, classification of odorant molecules can be achieved by analyzing the information embedded in the neuronal firing rates of the AgOrs. Regression analysis and multivariate data analysis are statistical techniques widely used for classification [16]. However, an Artificial Neural Network (ANN) is employed for the analysis in this work. In our previous work, we have investigated odorant classification using ANNs on the firing rates of the *Drosophila Melanogaster* olfactory receptors [17, 18] and on chemical descriptor values [19].

An ANN can be seen as a mathematical interpretation and simplification of the complex temporal properties of biological neurons [20]. The wires and interconnections defined between neurons of the ANN are modeled after the axons and dendrites found in biological neural networks. An essential element of the ANN is the activation function which simulates the electrical activity experienced by the cell membrane. The change in cell membrane potential represents the transfer of data through the biological information highway. These features allow ANNs to extract essential characteristics from data sets and make use of this information to predict values. Thus, ANNs are a viable method in the field of olfactory recognition which presents highly complex relationships between the data and the classes to which they belong to.

II. Recognition of Odorants

A. Data Used and Pre-Processing

The data used in this work was obtained from the report by Carey, et al. [18] in which the firing rate from 50 ORNs of an adult mosquito to 104 different volatile odorant compounds was documented. The odorants fell into 9 distinct chemical classes (with the number of chemicals listed in brackets): Carboxylic Acid (24); Terpene (8); Aldehyde (6); Ketone(9); Aromatic (18); Heterocyclics (9); Alcohols (15); Esters (11) and 'Other' (4). The 'Other' chemical class include components of human emanations that provide a strong attractant for several species of mosquito [21]. The data originally included odorants of the Amine, Lactone and Sulphur Compound classes; however, the chemical classes listed presented limited data, where each class contained less than four odorants. These chemical classes were subsequently removed from our analysis to prevent network overtraining and overgeneralization of the data [22]. In addition, large distributions of data has been found to negatively affect a networks error learning and its prediction performance [23], hence the data was zero-meaned and normalized prior to ANN testing.

B. ANN Architecture and Training

For this work, the ANN architecture of a feed forward Multi-Layer Perceptron (MLP) with binary sigmoid activation functions is used to interpret the changes in firing rates of the *A. Gambiae* olfactory receptors. As highlighted by Baum [24], the quality of a solution relies heavily on the network size as it inherently affects the network's learning time and generalization capabilities. We employed an array of single-output MLPs in parallel as depicted in Fig. 2, called the Hybrid network system [17, 19, 25], which allowed the system to capture more of the complex relationships present in the data. This hybrid system consisted of nine MLPs, where each MLP corresponded to a unique chemical class of the odorant data.

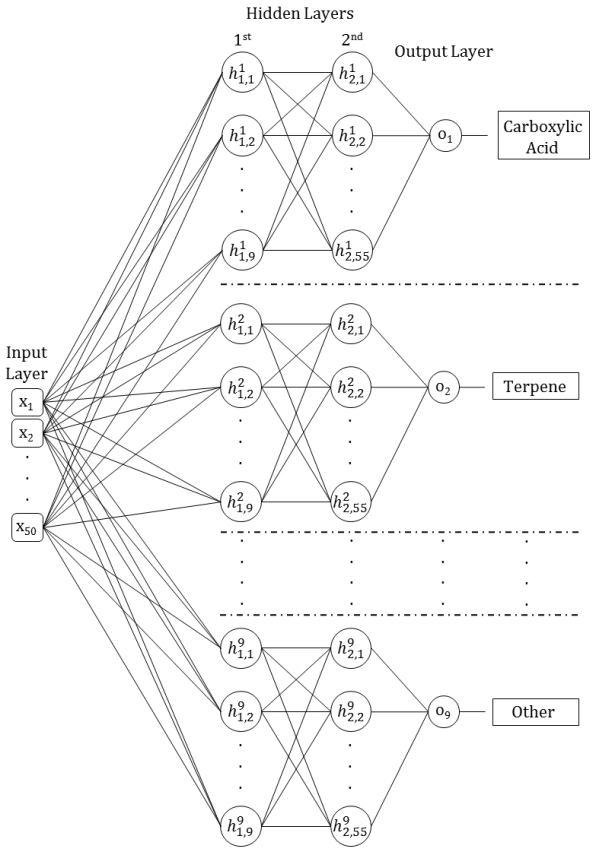

Figure 2. Schematic of the Hybrid Multi-Layer Perceptron used classify the odorants into chemical classes.

Hidden layers have been found to have the ability to extract higher-order information [26] from a given data set, hence a double hidden layer structure was integrated into the hybrid system. Furthermore, by altering the number of neurons of the hidden layer(s), an optimum number of

hidden neurons that produce the best network performance can be found [18]. An input length of 50 neurons corresponding to the olfactory receptor sensors of the *A. Gambiae* was defined. A maximum layer length of 100 neurons for the first and second hidden layer was established as it has been found that hidden layers with neurons more than double that of the input layer are ineffective as they require more training time and training samples to achieve adequate generalization [20, 27-29]. The size of the hidden layer(s) was incrementally altered and the network size which produced the best prediction performance was used.

Network learning in this work is achieved by updating the network architecture via its weighting functions to produce an increasingly effective network. Back-propagation is the learning scheme employed. However, it is weak against the presence of local minima [20], thus a momentum function was applied to the system [30]. Supervised learning is employed in which each MLP of the hybrid system is assigned to a chemical class, creating a true output for the desired class and a false value for all other classes. Measurement of training time is based on an epoch, where a single epoch is defined when the complete, albeit randomly shuffled, training set has passed through the network.

A symmetric Gaussian distribution with a zero mean and variance was used to obtain the weighting functions of the network, giving values between -1 and 1. Small weighting values were chosen to allow optimization of the 'weight decay' regularizer, which improves network generalization and prevents over-fitting the MLPs [31]. The stopping criterion chosen was based on reducing computational time [31], hence a series of initial simulations were performed which involved ending network learning based on a number of fixed iterations or if the prediction error fell below a defined limit. It was found that 200 epochs provided suitable CPU run times, wherein bootstrapping simulations lasted ~2 weeks. Furthermore, prediction error cut-off was set to 0.01 which occurred around 200 epochs for the optimal network.

Bootstrapping methods are used to capture variability of simulations and to provide more accurate estimates of predicted values [32]. It involves repeatedly sampling B training and validation sets from a raw data set for refitting (retraining) of the system [32-34]. Though B typically ranges from $20 \leq B \leq 200$, we have opted for 10,000 samples to ensure validity of the network's error estimation. The application of bootstrapping will be presented as a bar graph of the mean and standard error of the network prediction.

A validation set is applied on a trained MLP system to quantify the network's learning and performance. It is composed of a random set of 11 chemical odorant vectors collected from the 104 available odorants. For each B sample, the validation set was obtained by randomly selecting 10% of the odorants from each chemical class. The number of odorants of each chemical class used in the validation set is: Carboxylic Acid (2); Terpene (1); Aldehyde (1); Ketone (1); Aromatic (2); Heterocyclics (1); Alcohols (1); Esters (1) and 'Other' (1). The remaining 93 odorants were used for the corresponding B training set.

III. Results

The optimal MLP network size which produced the best performance is presented in Fig. 2: an MLP with 9 neurons in the first hidden layer and 55 neurons in the second hidden layer. Fig. 3 depicts the network performance of this Hybrid MLP system, which shows a spread in odorant prediction.

Quantification of network learning is obtained by applying a prediction threshold on the performance of the validation set. This was determined as follows: the largest chemical class present in the data is the Carboxylic Acid and Aromatic group, they represent the most chemicals in the validation set i.e. out of the 11 validating odorants, 2 were known to be Carboxylic Acids. The probability of randomly choosing a Carboxylic Acid from the validation set was 2/11 or 18.2%. This value was used to identify the minimum threshold level of detection and a ~5% margin was included as an added safeguard, producing a 23% threshold level for network prediction results, as illustrated by the horizontal broken lines in Fig. 3. Thus, prediction values exceeding the threshold value signified the classification of the odorant.

As presented in Fig. 3, the MLP system on average classed 10 odorants with a range of $8 \leq n$ odorants superseding threshold ≤ 10. The low performance of the 'Other' class may be due to the odorants not having chemical similarities; they are classed together merely due to the *A. Gambiae's* affinity toward them [21].

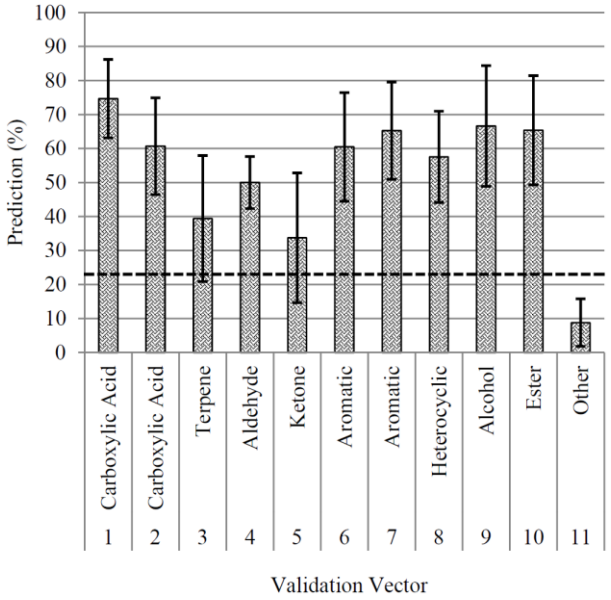

Figure 3. Performance of the MLP system across the validation set. The horizontal broken lines represent the 23% threshold value that defines correct classification of an odorant of the validation set.

Extremely high and low performing odorants can potentially provide an undesirable effect of skewing the data and results; implementing bootstrapping successfully removes such unwanted characteristics. Furthermore, the effectiveness of implementing bootstrapping is seen when comparing previous works which did not utilize bootstrapping [17, 19]. By effectively removing the outliers,

bootstrapping of the odorant data has given a more representative approximation of the network performance.

IV. Conclusion

In this work, we investigated the possibility of using the firing rates of *Anopheles Gambiae* mosquito Olfactory Receptor Neurons (ORNs) to train a Hybrid system of Multi-Layer Perceptrons (MLPs) for the classification of unknown chemical odorants into their known chemical classes. By alternating the number of neurons of the hidden layers, an optimal size MLP was found. Using 93 chemical odorants to train the Hybrid system of Optimal Sized MLPs, we were able to correctly classify 90.1% of previously unseen odorants (i.e. 10 out of the 11 unseen chemicals of the validation set). Bootstrapping was used to provide an accurate estimate of prediction accuracy; however, the performance of the large MLP system could be further improved with a larger data set. The results of this work provide very strong evidence to suggest that ORNs from the *A. Gambiae* mosquito coupled with an Artificial Neural Network (ANN) could be used as an effective signal processing backend to an olfactory biosensor.

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