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Evaluation of Neuron-Based Sensing with the Neurotransmitter Serotonin

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ABSTRACT

Results are presented on the development of a novel biosensor which will use neurons or neuronal components as both the recognition elements and primary transducers for analyte quantitation. This concept is demonstrated and evaluated by exposing identified neurons from the visceral ganglia of the pond snail Limnea stagnalis *to the model analyte serotonin. Experiments reveal a reversible, concentration-dependent increase in the rate of spontaneous action potential generation, over a concentration range of four orders of magnitude. Studies with the antagonist methysergide verify that this response is mediated through serotonin-sensitive receptors. Exposure of the neurons to serotonin causes the firing frequency to rapidly increase to a maximum and then slowly diminish to a sub-optimal level. It was found that the maximum frequency provides an indication of chemical concentration that is repeatable. Data are also presented which further advance the field of neuronal biosensing by demonstrating both the effects of cell to cell variability on response reproducibility and the effects of the desensitizing response on the operation of a neuron-based sensor in both a continuous and discontinuous mode.*

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1 INTRODUCTION

A major problem for all disciplines that involve work with biological and bioprocess systems is the lack of small, fast, accurate, and reusable sensing devices. Many solutions to this problem have been devised as detailed in the recent reviews by Arnold & Meyerhoff (1988) and Janata & Bezegh (1988). Among these approaches have been the use of isolated enzymes, antibodies, antigens, neuroreceptors, or microorganisms, and plant and animal tissue, linked to devices such as field effect transistors, piezoelectric crystals, thermistors, fiber optic devices, and electrochemical sensors, yet each of these has their own limitations. For example, with enzyme-, neuroreceptor-, and antibody-based sensors is the difficulty in obtaining the isolated biological molecules in a pure, stable form. Microbial and tissue-based devices avoid the need for isolating and purifying the necessary molecules by allowing them to remain in their native environment. However, the response time of these sensors tends to be slow since they are limited by the diffusion rate of the chemical to the biocatalytic layer, metabolism of the substance, and subsequent diffusion of the metabolite to the detecting interface.

In recent years, the feasibility of using excitable tissue for chemical sensing has been demonstrated with isolated antennae dissected from the crab *Callinectes sapidus* (Belli & Rechnitz, 1988; Buch & Rechnitz, 1989). By monitoring random afferent axons of the antennae, these authors showed that there was both a concentration-dependent response to compounds such as the adenylic acids AMP and ADP, and the amino acid glutamate, and desirable sensing characteristics such as fast response time, repeatability, selectivity, and a sensitivity range spanning at least six orders of magnitude (Buch & Rechnitz, 1989). The use of intact nervous tissue also demonstrates the inherent ability of neurons to convert binding event signals into a digital form. This is important since digital signals are less prone to noise and interference than the analog signals produced by most biosensing devices (Middelhoek *et al.*, 1988). Besides, the availability of reliable digital signals has been cited by Brignell (1986) and Koelman and Regtien (1984) as an important factor required for future development of microsensors because such responses are microprocessor compatible, and can be easily applied in bus-organized data acquisition systems. The digital output of excitable tissue

is generated because as the analyte interacts with the tissue it triggers changes in the transmembrane potential, either directly or through secondary messengers, which are then amplified by voltage dependent ionic channels to produce discrete all-or-none voltage spikes or action potential (AP) events. A typical AP lasts only a few milliseconds and is tens of millivolts in amplitude. The large amplitude of the AP events makes them easy to monitor. In addition, it has been shown that the concentration of the detected chemical is encoded in the frequency at which AP events occur.

The potential applications for a neuron-based sensor are many, suggested by the large number of compounds reported to cause changes in the electrical properties of various excitable membranes. Besides the compounds studied by Rechnitz's group, this list includes compounds such as low molecular weight alcohols, ketones, aldehydes, carboxylic acids (Kashiwayanagi & Kurihara, 1984, 1985), and various pesticides (Orchard, 1986).

In this research, the development of a neurosensor to take advantage of the sensing characteristics of identified neurons is being explored. Sensors of this type may find use in in-vivo applications as well as in on-line monitoring of cell culture processes where the necessary nutrients are available to maintain viability. In addition, one long term emphasis of this kind of work will be on the development of synthetic membranes which embody the necessary functional groups to mimic neuronal responses.

Although it is well known that neuronal electrical properties can be correlated to stimulus concentration, and others have demonstrated the feasibility of neuronal biosensing, the goal of this work is to further understand these phenomena as it will relate to a practicable biosensor by systematic characterization of a simplistic system which provides good experimental repeatability. To accomplish this, two electrophysiologically similar identified neurons for the snail *Limnea stagnalis* were exposed to the neurotransmitter serotonin. Important sensing properties such as reversibility, repeatability, and specificity of the binding event were considered. In addition, response variability from cell to cell and desensitization were evaluated to provide new insight on signal reproducibility as well as the operation of a neuron-based sensor in both a continuous and discontinuous mode. Serotonin was chosen for this study since it is present in a wide range of species, and has been extensively studied in invertebrates (Walker, 1986), thus, providing insight for evaluation of results. Furthermore, a sensor for serotonin would have potential significance in both clinical medicine and physiological studies since this neurotransmitter has been linked to

disorders such as Parkinson's disease (Curzon, 1978), and some forms of depression (De Montigny, 1984).

2 METHODS

2.1 Ganglia preparation

The methods for preparing the ganglia for recording were similar to those used by Byerly and Hagiwara (1982). In brief, the circumoesophageal nerve ring was dissected out of an adult snail *Limnea stagnalis* and placed in a dish of *Limnea* saline made up of 50 mM Na^+, 2·5 mM K^+, 4 mM Ca^{2+}, 4 mM Mg^{2+}, 10 mM glucose, 68·5 mM Cl^-, and buffered to a pH of 7·4 with 10 mM HEPES (Sigma, St. Louis, MO). The visceral ganglion was then separated from the cluster and the connective tissue cleaned away. After isolating the ganglion, it was next transferred by pipette to a Sylgard 184 (Dow Corning, Midland, MI) lined 35 mm petri dish, and pinned using a microdissecting pin (Fine Science Tools, Belmont, CA). The orientation of the ganglia after pinning allowed the visual identification of the giant visceral neurons VV1 and VV2 (Winlow & Benjamin, 1976). The petri dish contained a 0·2% solution of trypsin (Sigma type III) in which the ganglion was soaked for 30 min to soften the protective fibrous sheath. Following the trypsin soak, flow lines were attached to the petri dish and fresh *Limnea* saline was continuously added and withdrawn at a constant rate.

2.2 Experimental apparatus

The recording chamber was a Sylgard lined 35 mm petri dish that was fitted with inlet and outlet flow lines and surrounded by a water jacket. Water from a temperature controlled bath (VWR Scientific, San Francisco, CA) was used to maintain the chamber at a constant temperature and to adjust the temperature of the inlet flow by circulation through an insulated water jacket around the feed line. All experiments were operated at a chamber temperature between 300 and 302 K. Before each experiment, a solution of the highest concentration to be used was freshly premixed in *Limnea* saline using serotonin creatinine sulfate complex (Research Biochemicals Inc., Natick, MA). The stock solution was then diluted in tenfold increments to concentrations ranging between 10^{-8} M and 10^{-2} M. For the antagonists' studies, methysergide solutions were prepared by mixing methysergide (Sandoz, Hanover, NJ)

in *Limnea* saline and diluting in ten-fold increments to the appropriate concentrations. Solutions that contained both methysergide and serotonin were made by adding the necessary amounts of the concentrated solutions to *Limnea* saline. For each experiment the solutions to be tested were then placed in separate burettes and introduced to the ganglia by opening the corresponding stopcock. The addition and removal of fluid from the recording chamber was accomplished using the same ten roller peristaltic pump (Rainin Instrument Co., Woburn, MA) on both the inlet and outlet lines. To provide more rapid mixing in the chamber, the inlet flow was introduced near the bottom in two locations at approximately 90° from each other. The outlet port for the chamber was suspended near the upper one-third of the dish. Manifold tubing for the outlet had a larger diameter than the inlet to avoid chamber overflow. However, this resulted in fluctuations in chamber volume as the liquid rose to the outlet port and was drawn off lowering the level to significantly below the outlet port because of surface tension interactions between the port and the liquid. The average minimum and maximum chamber volume were 2·6 ml and 4·3 ml respectively. Tracer studies showed the chamber had complete solution turnover in 4 min at the 4·0 ml/min flow rate used for all experiments. Since 4 min were required for the chamber to reach a steady concentration, all solutions were applied for at least 5 min to ensure the cells had time to respond to the final concentration. This system allowed for stable intracellular recording and impalements could be maintained for 1–4 h.

2.3 Data collection

Intracellular recordings were performed using a glass microelectrode filled with 3 M KCl. Electrode resistances ranged between 18 and 30 MΩ. The giant cells VV1 and VV2 were identified visually through a dissecting microscope and impaled with the microelectrode using a Narishige MO-203 hydraulic micromanipulator (Narishige USA, Inc., Greenvale, NY). On impalement, if a cell could not generate repetitive AP events greater than 50 mV it was assumed damaged and the impalement abandoned. Intracellular signals were conducted via a silver wire from the microelectrode to a Dagan 8700 high-input impedance amplifier (Dagan Corporation, Minneapolis, MN). Spontaneous activity from the impaled neuron was continuously monitored on an oscilloscope (Tektronix Corp., Beaverton, OR), stored on a modified VCR recorder (Vetter Co., Rebersburg, PA), and sent to an on-line data processing system.

2.4 Data analysis

On-line data analysis was accomplished using an analog-to-digital converter (Das-16F Metrabyte Corp., Taunton, MA) and an IBM-AT compatible microcomputer (Isotropic Computer Inc., Post Falls, ID). Real time voltage signals were collected by the A/D board and passed to in-house software which identified AP events based on their characteristic shape. The frequency between the previous and most recent AP was calculated and stored in the computer along with the time the recent AP occurred. To allow real time monitoring of the progress of an experiment, voltage versus time signals were continuously displayed along with the average frequency for the past 30 s. Final analysis of the frequency versus time data was performed off-line using in-house software.

2.5 Tracer studies

Tracer studies were performed in the recording chamber by step introduction of a red food coloring dye solution into the flow system which was initially filled with clear deionized water. Samples of 100 μl were taken from the chamber at 30 s intervals for 5 min. This process was repeated three times at each of three locations in the chamber. The three locations tested were a position directly in front of an inlet port where the fluid velocity was highest, a position behind the inlet ports which was visually observed to have relatively slow mixing, and a position 2 cm away from the inlet ports, which would be more representative of the bulk fluid. Each 100 μl sample was placed in a cuvet and diluted with deionized water to a final volume of 1·0 ml. The dye concentration, expressed as a percentage of the maximum concentration, was determined by comparison of the absorbance measured at 485 nm with known standards.

3 RESULTS AND DISCUSSION

3.1 Concentration-dependent response of identified neurons

A total of 17 VV1 and VV2 neurons were studied to accumulate information on the concentration range over which response changes can be detected, the steady-state responses, the reproducibility of the data, and the rinse time needed between serotonin applications before a reproducible response can be achieved. When the data from all 17 cells are viewed collectively, a general trend of increasing spontaneous firing

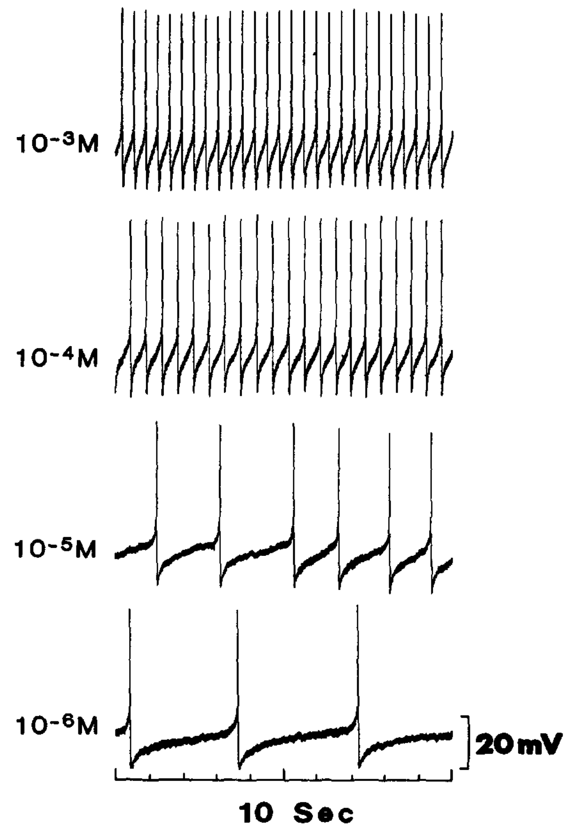

Fig. 1. An example of the effects of serotonin on the spontaneous firing rate of the VV1 and VV2 neurons in a *Limnea stagnalis* snail. As indicated, the traces are the cellular response to additions of 10^{-6} M, 10^{-5} M, 10^{-4} M, and 10^{-3} M serotonin. Reprinted from Skeen *et al.* (1990), courtesy of Marcel Dekker Inc.

frequency (FF) with serotonin concentration is seen. Figure 1 portrays an example of the graded increase in firing rate seen in both the VV1 and VV2 neurons with serotonin concentration. As indicated in the figure, the traces are the cellular response to additions of 10^{-6} M, 10^{-5} M, 10^{-4} M, and 10^{-3} M serotonin. It is not surprising that VV1 and VV2 have a similar response to serotonin since they share many common synaptic inputs (Winlow & Benjamin, 1976) and both show similar staining properties when treated with the serotonin analog 5,6- or 5,7-dihydroxytryptamine which stains serotonin-containing cells (Kemenes *et al.*, 1989). Besides showing the concentration dependency of the spontaneous FF, Fig. 1 also exemplifies the inherent digital nature of a neurosensor which arises because the chemical information is encoded in the frequency at which the AP events occur. This property is important in sensor design because

digital signals are less prone to noise and interference than analog signals. Also, a drifting background potential will not be a problem with this approach since the AP events are rapid, on the order of milliseconds, and uniform in size and shape and, thus, can be detected by wave shape recognition. In fact, in-house computer programs were developed to recognize AP events and used to generate all the FF data presented in this paper. This technique was found to be insensitive to both electrical noise and baseline drift.

To study the concentration dependency of the serotonin response, seven cells were exposed to 5 min applications of increasing levels of the neurotransmitter between 10^{-8} M and 10^{-2} M. Each application was followed by a 10 min saline rinse. Figure 2 shows typical results obtained from these experiments. During the first half hour of the experiment represented in Fig. 2 this cell fired sporadically at a rate below 0·5 Hz, and the addition of 10^{-8} M and 10^{-7} M serotonin caused no discernible change in the FF. However, the following four peaks show a graded increase in FF to serotonin levels of 10^{-6} M, 10^{-5} M, 10^{-4} M, and 10^{-3} M, respectively. Interestingly, Brunelli *et al.* (1976) have also reported that serotonin in a similar concentration range, between 10^{-6} M and 10^{-4} M,

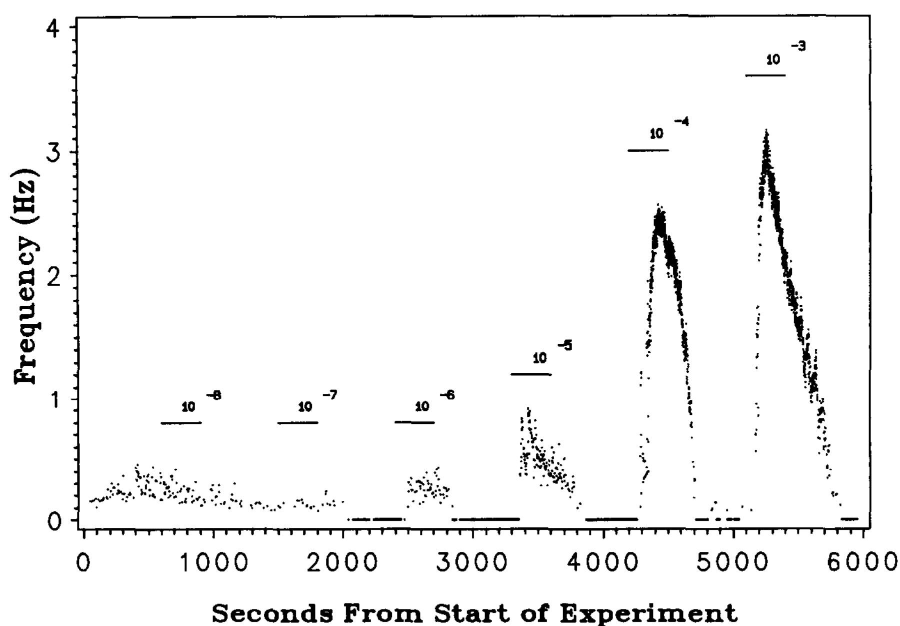

Fig. 2. Typical results obtained from experiments where identified neurons VV1 or VV2 are exposed to 5 min applications of increasing levels of serotonin. Reprinted from Skeen *et al.* (1990), courtesy of Marcel Dekker Inc.

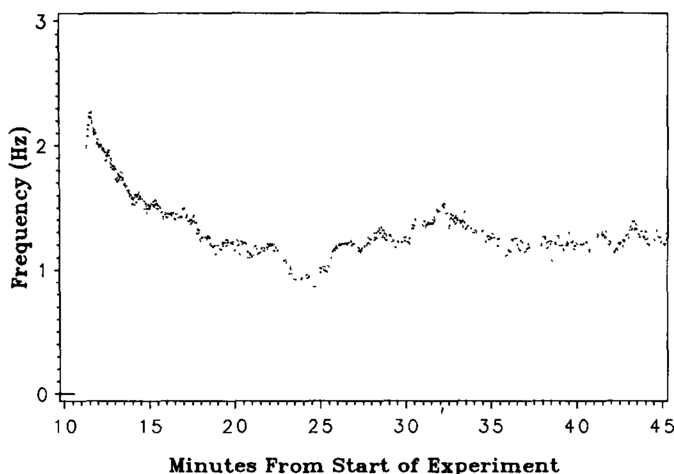

Fig. 3. Example of a neuron response during continuous exposure to 10^{-3} M serotonin. Exposure begins at 10 min and soon after the firing frequency of the cell rapidly increases to a maximum and then diminishes to a suboptimal level.

causes a graded change in the behavior of *Aplysia* sensory neurons which are involved in the gill-withdraw reflex.

It is clear from Fig. 2, particularly at the higher concentrations of serotonin, that shortly after exposure the FF of the cell rapidly increases to a maximum and then begins to diminish. A more complete representation of the effects of sustained serotonin exposure on the FF is given in Fig. 3 which represents a 35 min exposure of a VV2 neuron to 10^{-3} M serotonin. For this cell, serotonin application began at 10 min and the FF reached a maximum during the first two minutes of exposure, diminished over the next several minutes to 60% of the maximum, and then remained there. Serotonin concentration also has an effect on the rate at which the post-maximum response diminishes. This is shown qualitatively by observing that in Fig. 2 the slope of the post-maximum response is more negative for the higher concentrations. Also, there appears to be a critical concentration beyond which serotonin can inhibit firing activity, presumably due to excessive depolarization. This was observed during the application of 10^{-2} M serotonin where, as the concentration in the chamber was increasing to its final level, the FF rapidly increased and then suddenly fell to zero as all firing stopped. Upon rinsing, the neuron again began to fire rapidly, as the chamber concentration dropped, and then the FF diminished in a normal manner as the chamber concentration continued to decrease.

To determine if the effect caused by serotonin is mediated through

serotonin binding receptors, studies were done using the serotonin antagonist methysergide (Walker, 1985). For these experiments, the control response to a 5 min application of serotonin was measured and then the cell was rinsed with fresh saline for 10 min. After the rinse, the neurons were exposed to a 5 min addition of methysergide immediately succeeded by a solution containing both methysergide and serotonin. It was found that at 10^{-4} M, methysergide eliminated the increase in FF caused by additions of either 10^{-5} M or 10^{-4} M serotonin. However, lower levels of methysergide only partially blocked the response to serotonin. For example, with 10^{-4} M serotonin, 1×10^{-5} M methysergide caused only a 5% reduction in the maximum FF while 3×10^{-5} M methysergide reduced the maximum by 16%. These results suggest that both methysergide and serotonin are acting at the same site, indicating that the serotonin response is dependent on serotonin-sensitive receptors.

3.2 Trends in maximum firing frequency

An important consideration in sensor development is how to achieve the maximum response for a given analyte. For a neuron-based sensor the diminished steady-state response demonstrated in Fig. 3 suggests that the stable output of the neuron will not give as sensitive an indication of serotonin concentration over the same concentration range as the peak response. Thus, maximum FF is a more logical choice to use as an indication of chemical concentration. In addition, the use of maximum FF reduces the time required to analyze the serotonin concentration in a sample. This is because the maximum FF appears rapidly after an application, typically 2–3 min for the system used in these experiments, and the steady-state develops more slowly as suggested by Fig. 3 where the cell was exposed to serotonin for 10 min before a stable output was obtained. Also, Fig. 3 indicates that the steady-state level fluctuates and several minutes of data would need to be averaged before an accurate indication of concentration could be determined. The use of maximum FF has been shown by Buch and Rechnitz (1989) to give good concentration-dependent responses to other analytes.

Shown in Fig. 4 is the maximum FF data for all seven cells exposed to increasing levels of serotonin between 10^{-8} M and 10^{-2} M plotted against the logarithm of the serotonin concentration. VV1 neurons are indicated by solid lines while dashed lines represent the response of VV2 cells. Comparison of the responses of VV1 and VV2 cells shows that although there is greater scatter in the data for the VV1 neurons when considered collectively, the two cell types show similar trends. All cells showed an excitatory response, and all but one were sensitive in the range between

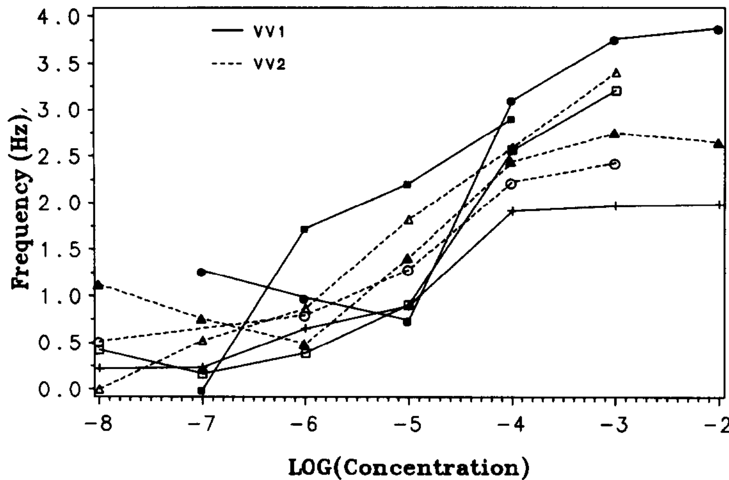

Fig. 4. Maximum firing frequency data for all seven cells exposed to 5 min applications of increasing levels of serotonin between 10^{-8} M and 10^{-2} M. Each cell is represented by a different symbol. Solid lines indicate VV1 neurons while a dashed line is used for VV2 cells.

10^{-6} M and 10^{-3} M. The one exception was only sensitive up to 10^{-4} M. It is important to point out that a sensor incorporating these neurons as the biological components of the device could have applications in detecting both the low concentration of serotonin in human body fluids such as blood serum and amniotic fluid (10^{-6}–10^{-5} M) and the higher serotonin levels found in rabbit serum and spleen (10^{-5}–10^{-4} M) (Essman, 1978). It should also be noted that rinse time between applications has an effect on the magnitude of the maximum FF. As will be discussed in a later section, our data suggest that a 10 min rinse after 5 min of exposure to 10^{-3} M serotonin is not adequate for the neuron to completely recover and give its true maximum response to the next application. Thus, the maximum FF response to 10^{-2} M serotonin seen in these experiments could be lower than if there was no prior application of 10^{-3} M serotonin. If this is true then the analytical limit of this preparation, suggested by Fig. 4, may be an underestimate. However, the sudden loss of spontaneous AP generation with exposure to 10^{-2} M serotonin suggests that the upper detection limit of this tissue is not much greater than 10^{-2} M.

3.3 Effect of the mixing environment on the maximum response

It was not uncommon for the FF to reach its peak value sooner than the 4 min required for the surrounding medium to reach its final concen-

tration. So, to correlate maximum FF with the actual chamber concentration it was necessary to establish a means to estimate the concentration in the chamber at the time the maximum response occurred. Also, for each experiment the ganglia were pinned in slightly different locations in the chamber. Hence, the uniformity of mixing for the system needed to be studied to determine if there was variability in the concentration history of each ganglion since this could affect the magnitude of the response. To accomplish these tasks, tracer studies were done at the three locations in the chamber as described in the methods section. The results of these tests are shown in Fig. 5 with different symbols representing the different locations. In this figure a time of zero corresponds to the time the dye solution first reached the chamber. It was found that the differences in the concentration versus time behavior of the three positions tested were no greater than the error in the individual measurements. Therefore, the ganglion location was not a variable affecting the response of the neurons.

To estimate the actual concentration in the chamber at any time, the tracer data was fitted to eqn (1), below, which describes the time dependency of mixing after a step change in feed concentration in an ideal stirred vessel with constant volume and flow rate.

$$\frac{C(t)}{C_0} = 1 - \exp(\frac{\dot{V}t}{V}) \tag{1}$$

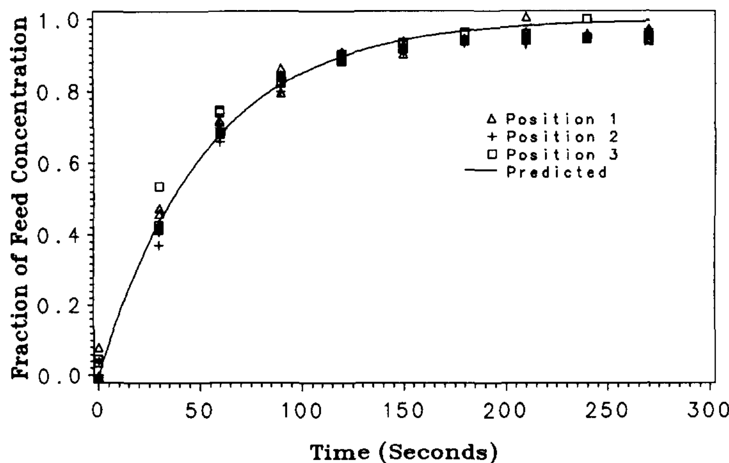

Fig. 5. Results from the recording chamber tracer study. Three locations in the chamber were sampled and each is shown by a different symbol. The solid line is the result of the least squares fit of the data with the mixing equation for an ideal stirred vessel with constant volume and flow rate. This prediction was used to estimate the concentration in the chamber at the time the maximum firing frequencies occurred.

where: $C(t)$ = concentration at time t;
C_0 = feed concentration;
$\dot{V}$ = volumetric flow rate;
V = volume of system.

A nonlinear least squares technique was used to fit the data to eqn (1) by allowing the volume term to vary. Since the system used in these experiments did not have a constant volume the choice to use eqn (1) to fit the data is somewhat arbitrary. However, if the volume term is thought of as a time averaged value, then the choice of this equation is justified provided the least squares routine returns a value for the volume that is close to the calculated time average of 3·3 ml. This was the case since the tracer data best fit the equation with a volume term of 3·5 ml. The resulting prediction is shown by the solid line on Fig. 5. Using this equation and the time each of the peak FF values occurred, the concentration in the chamber at the maximum response may be estimated.

Figure 6 shows the maximum FF data for all 17 cells exposed to serotonin plotted against the chamber concentration estimated using eqn (1). The outer lines on Fig. 6 represent the 95% confidence limits on the mean and the center line corresponds to the mean. Both VV1 and VV2 neurons are grouped in the same data set because of their similar response patterns as indicated in Fig. 4. It is evident from comparing the

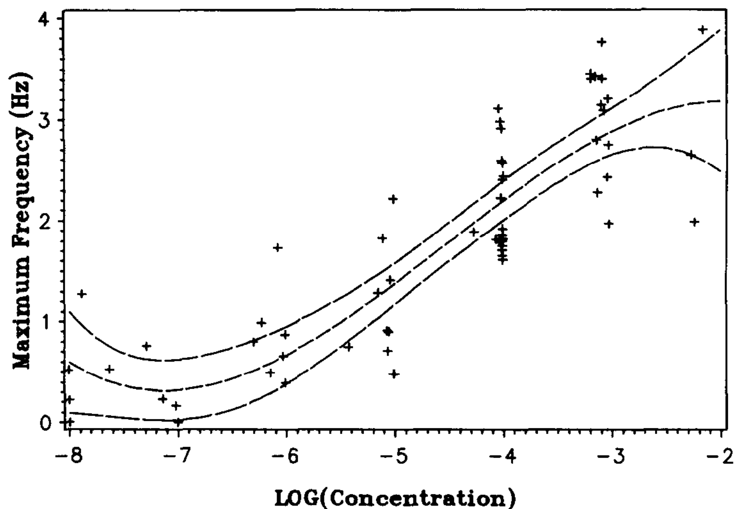

Fig. 6. Combined maximum frequency response data plotted against the logarithm of the serotonin concentration. The data represents 17 VV1 and VV2 neurons. Outer lines are the 95% confidence limits on the mean values while the center line corresponds to the mean.

uncorrected data on Fig. 4 with Fig. 6 that the concentration correction has little effect.

The comparison of the responses from the different cells shown in Fig. 4 with the average response of Fig. 6 raises an important question in sensor design; is it better to monitor the response of one cell to indicate concentration, or a population of cells and use an averaged response? It is evident from these figures that both the average response as well as the individual responses give an indication of chemical concentration. However, the scatter in Fig. 4 suggests that if a single neuron response is used, each sensor will need to be individually calibrated. Furthermore, if the differences in the cellular responses are an age dependent phenomena then frequent recalibration will also be required to account for drift. Using an averaged response for a population of cells could circumvent the need for frequent calibration since the average may remain stable even though individual responses are drifting. Future experiments that reveal the long time drift of these neurons are needed to determine if this is true.

3.4 Considerations for sensor design

To address the question of whether a chemical sensor that uses signals from the identified neurons VV1 and VV2 would give a repeatable response, four experiments were performed where multiple 5 min applications of the same concentration of serotonin, each followed by a rinse, were applied to a ganglion. The duration of the rinse time was also varied in several of these experiments. It was found that the response to a single concentration was reproducible if the length of the rinse between applications was long enough. This is illustrated in Fig. 7 which shows the results of multiple 5 min applications of 10^{-4} M serotonin to a VV2 neuron. The maximum FF values for the first, third, fourth, and fifth additions, where the rinse time was 10 min or greater, were all within 8% of each other. However, there was less agreement for the shorter rinse time of 5 min between the first and second peak where maximum FF values differ by 18%.

A graphical representation of the effect of rinse time between a 5 min conditioning application of serotonin and a subsequent 5 min stimulus application, at the same concentration, on the magnitude of the maximum FF for the stimulus application is shown in Fig. 8. Represented are the stimulus responses for 10^{-3} M and 10^{-4} M applications, expressed as a percentage of the conditioning maximum response, as a function of the rinse time between the two applications. The data for 10^{-4} M serotonin were obtained from a single cell, while that for 10^{-3} M are a

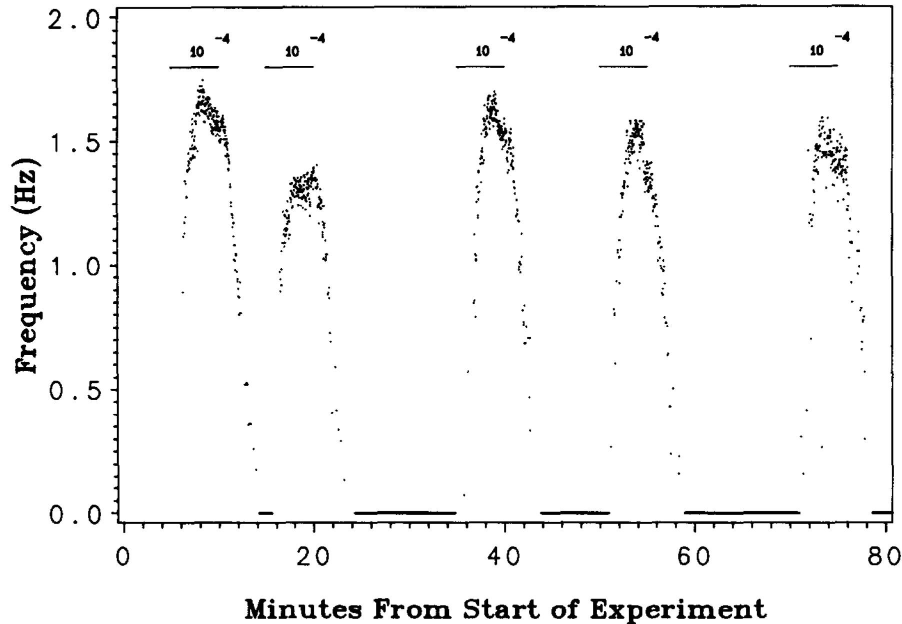

Fig. 7. Results from applying multiple 5 min applications of 10^{-4} M serotonin to a *Limnea* VV2 neuron. The maximum frequencies for the first, third, fourth, and fifth additions were all within 8% of each other, indicating the repeatability of the response. The second addition had a maximum frequency value 18% lower than the first addition because there was inadequate time for the neuron to fully recover between the first and second addition.

collective set from two cells, represented by different symbols. This figure shows that not only is an adequate rinse time required for a neuron to give its maximum response, but the length of time increases with serotonin concentration.

Further consideration must be given to the effects of application duration on successive responses as well as the reliability of data on introduction of a new concentration when the rinsing step is eliminated. These effects will have important ramifications on the potential use of such a devise in a continuous operation mode. Figure 9 shows the effects of a pre-exposure, of different durations, of 10^{-4} M serotin followed immediately by a 10^{-3} M application. From this figure it can be seen that prior exposure to serotonin diminishes the response to a latter addition and a longer pre-exposure amplifies this effect. This result along with the rinse time data from Figs 7 and 8 suggest that a neuron-based sensor would operate best if solutions to be tested were introduced to the sensor in short pulses and the time between pulses adjusted to compensate for varying serotonin concentrations. The benefit of using short pulses will

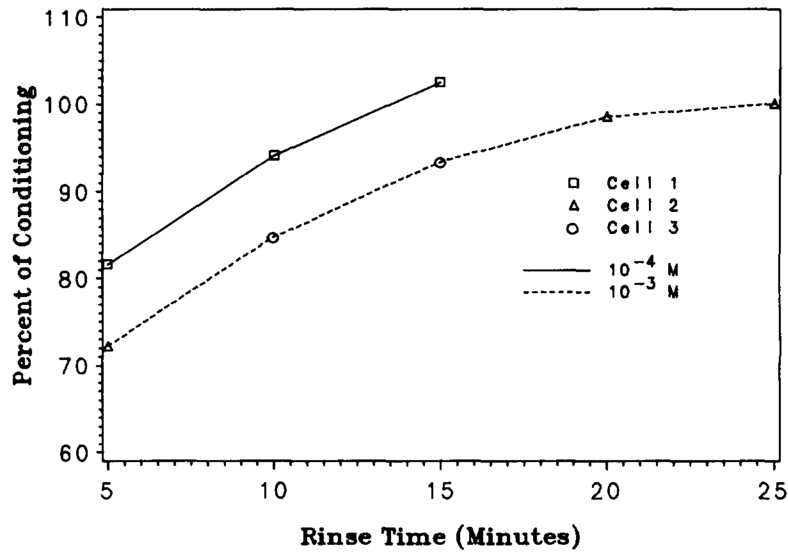

Fig. 8. Percent of pre-conditioning response as a function of the rinse time between a conditioning and stimulus pulse. Stimulus and conditioning concentrations were equal and both were applied for 5 min. As indicated, adequate rinse time between additions must be given for the cell to repetitively give its maximum response. The length of the required rinse time increases with serotonin concentration.

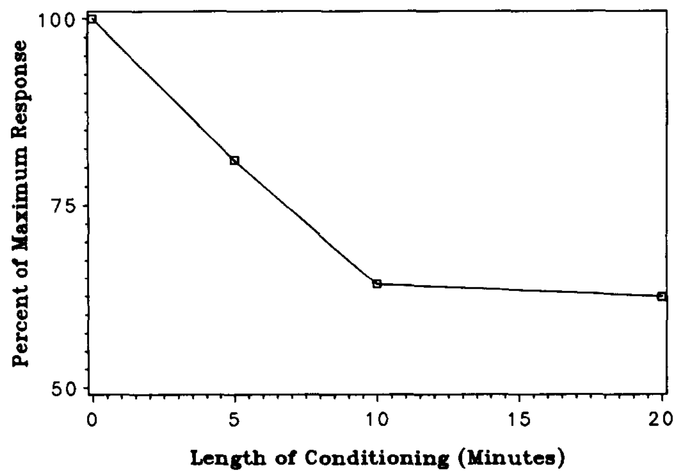

Fig. 9. Plot of the percent of maximum response to a 5 min addition of 10^{-3} M serotonin as a function of the duration of a 10^{-4} M conditioning application. Clearly, the magnitude of the cell response is diminished by the conditioning pulse.

be an increase in the achievable sampling rate since the time for each application will be reduced. To achieve short application pulses, devices should be designed to accommodate either a plug flow or a small volume well mixed flow environment.

Another positive feature in using the *Limnea* preparation to detect serotonin is the reversible nature of the response. This is demonstrated in both Figs 2 and 7 by the return to the baseline firing rate after rinsing.

An additional advantage of using nervous tissue in chemical sensing is that the cell membrane contains chemoreceptors which are specific to the binding analyte causing a change in the excitable state of the cell. Hence, the necessary components for very specific molecular recognition are inherently available. At the same time, however, a single neuron may contain chemoreceptors for many other compounds, the binding of such would also cause a change in cell excitation thereby interfering with the desired response. This loss of specificity could be overcome in several ways. First, antagonists for the interfering compounds can be added to the test solution to inhibit their effects. This technique, however, will not be desirable for continuous monitoring applications since samples would need to be pretreated. Also, cost of these reagents may be prohibitive. Second, one could identify neurons which respond to different combinations of the interfering compounds, then the response pattern from an array of neurons could be used for detection. Alternatively, one could use genetically engineered cells where only selected receptors were expressed on the membrane surface. Finally, an artificial membrane containing only one chemoreceptor and the necessary ionic channels to generate AP events could be constructed.

3.5 Future problems to address

There are still several problems to consider before a neuron-based chemical sensor can be constructed; these should be the topic of future research. First, because AP events are generated by the flow of ions along concentration gradients, an alteration in those gradients will change the FF behavior of the cell. This means that either all samples tested with a neuron-based sensor will need to have the same ionic concentrations as the solution used to calibrate the response or a means of correcting for changes in ionic concentration will need to be devised. Second, this type of sensor will be difficult to construct and, thus, ought to have a reasonably long lifetime. This will require that the neurons maintain viability by being constantly exposed to the proper nutrients. Because transport of nutrients to, and waste products from, the cell will be important, a whole tissue preparation will not be as suitable as using

single isolated cells where each cell will have good contact with the bathing medium. Isolated cells may provide a more standardized preparation since it will be free from synaptic noise and interference. Third, extracellular recording techniques are more suitable for a neuron-based sensor because they will not require the impalement of a neuron with a microelectrode, and hence will greatly decrease the chance of irreversibly damaging a cell. This requires a suitable electrical connection to be constructed which can interface to isolated neurons for a long period of time. Such devices, however, are being developed (Regehr *et al.*, 1988). Fourth, since one possible application for these kinds of devices will be in-vivo monitoring where temperature may vary from system to system, the effects of temperature on the neuronal response needs to be understood. Finally, the question of specificity will need to be addressed by either multiplexing the response of several different types of neurons in a fingerprint pattern, using blockers, genetically altering a cell to express selected receptors, or constructing an artificial membrane which contains only one type of receptor.

4 CONCLUSIONS

Serotonin causes a reversible increase in the firing frequency of visceral ventral neurons VV1 and VV2 from the snail *Limnea stagnalis*. The response to serotonin can be either completely blocked by methysergide, a serotonin antagonist, at 10^{-4} M or partially blocked with lower levels of the antagonist. This suggests that the changes are mediated through serotonin-sensitive receptors. Extended exposure of these cells to serotonin causes the FF to reach a maximum and then diminish to a sub-optimal level. The maximum FF induced by application of serotonin was found in all but one case to reversibly increase with serotonin concentration over the range of 10^{-6} M to 10^{-3} M. The one exception gave a reversible increase in firing frequency between 10^{-6} M and 10^{-4} M. Comparison of the magnitude of individual maximum firing frequency responses demonstrated that there is cell to cell variability which suggests the need for individual calibration for each neuron. However, averaging the responses of several cells may provide a more reliable indication of concentration. Repeatability of the maximum firing frequency response could be achieved provided a long enough rinse time was given between applications. The length of the required rinse increased with an increase in the concentration of the previous application. Pre-exposure of a neuron to a low level of serotonin followed immediately by a higher level diminished the response to the latter

addition. Furthermore, longer pre-exposures amplified this effect. These results suggest that a neuron-based sensor would operate best if test solutions were introduced to the sensor in short pulses and the time between applications varied depending on the concentration in the sample.

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