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A microcomputer-controlled response measurement and analysis system for insect olfactory receptor neurons

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A microcomputer system has been built to stimulate, record, and analyze responses from peripheral insect olfactory receptor neurons. Software has been developed to sort action potentials (spikes) in extracellular records obtained from multi-innervated antennal sensilla, and perform frequency, interspike interval, distributional, and regression analyses on responses in groups of records. The olfactory stimuli can be presented in different patterns, and plots or histograms of analyses, spike records, and individual spikes can be displayed in different formats and hard-copied during a recording session. This report describes the main features of the system and discusses the methods used for sorting spikes, performing analyses, and managing records and analyses in a database. Examples are given of renewal density analyses for 4 classes of peripheral receptor neuron in records from 2 types of olfactory sensillum on the antenna of the cabbage looper moth, *Trichoplusia ni* (Hübner).

Introduction

Continuing advancements in microcomputer memory capacity and interfacing capability are encouraging developments of microcomputer-based systems for neurophysiological research. Much of the appeal of these systems is due to their adaptability. A few, generally applicable design principles can be used to develop the initial system. Then, after some experience has been gained from its operation, it can be modified easily to achieve specific objectives. Here, we describe a system developed during an investigation of specialized olfactory receptor neurons on the antennae of male moths. Such neurons detect an important odor cue, a blend of two or more chemicals called the sex pheromone, which induces mate-seeking and copulatory behaviours (Kaissling, 1971; Baker, 1985; Mayer and Mankin, 1985).

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A microcomputer system for analysis of sex pheromone detection and discrimination processes must perform a number of functions in common with any system that stimulates, records, and analyzes responses from peripheral chemoreceptors. First, the system must be able to analyze many different combinations and intensities of stimuli. This is because, in general, the stimuli are multi-component blends of chemicals that can vary considerably in the dynamic ranges over which they are stimulatory physiologically and behaviorally. Also, chemosensory receptor neurons are small in diameter and occur in groups that cannot be separated physically. The responses must be recorded extracellularly and separated after they have been recorded. Extracellular records often show considerable variability. It is difficult to separate such responses using simple time/amplitude hardware discriminators.

For example, pheromone receptor neurons of the insect, *Trichoplusia ni* (Hübner) (see example in section in System Operation), are found in groups of 2–3 in sensory hairs (sensilla) about 2 μm in diameter on the antenna. Sensillar recordings frequently contain responses from more than one neuron, and occasionally responses from adjacent sensilla can be detected. Each individual neuron in a sensillum responds differently than its neighbor to the different components of the sex pheromone blend; consequently, each response must be classified and analyzed separately. An inexpensive time/amplitude hardware discriminator fails to discriminate the responses reliably because the spike durations are nearly identical, and the spikes from two of the neurons usually have overlapping amplitude distributions, especially under strong stimulation.

Although the system was developed specifically for insect olfactory receptor neurons, the description and procedures presented here apply to microcomputer analysis of peripheral chemoreception in general. Parts of this description are generally applicable to any system that handles numerous recordings containing spikes of different types with overlapping amplitude distributions. The data acquisition and data storage procedures apply primarily to system configurations similar to the one at our laboratory, but much of the analysis software is system-independent. Also, depending on the variability of the recorded spike trains from their own preparations, other investigators may prefer to develop either simpler or more complex spike classification procedures than those presented here. Finally, the procedures we developed over a period of time to facilitate system expansion may be of interest to those who are continually developing new analyses that must be integrated into an existing system.

System description

The complete system consists of an electrophysiological work station, a calibrated odor delivery system, and a microcomputer with peripherals for digital/analog (D/A) and analog/digital (A/D) conversion, video and hard-copy graphics, ASCII communication, and signal timing, collection, display, and storage. The electrophysiological recording techniques are described in Mayer et al. (1987) and O'Connell (1975), and the odor delivery system is described in Grant et al. (in preparation).

This report deals primarily with the microcomputer software and the spike classification and analysis methodology.

Hardware

The microcomputer system is a DECLAB-11/MNC. It has a 16-bit (binary digit) LSI-11/23 microprocessor with 64KB (Note: 1K = 1024, B = 1 Byte = 8 bits, and 1 word = 2B = 16 bits) of direct-access main memory, which maps to an additional 64KB of secondary (virtual) memory via the RT-11 single-job operating system. Peripherals include a VT-125 video graphics terminal, a LA-50 graphics printer, two RL02 disk drives, each with 5.2 MB of storage, a MNCKW clock, a MNCAD 12-bit A/D converter, a MNCAA 12-bit D/A converter, DLV11-J asynchronous 4-channel RS-232 interface, and a 1200-baud modem. Newer, improved components are available from many vendors.

The contents of the A/D, D/A, and asynchronous data transmission channels are deposited in 1-word buffer registers by the LSI-11 bus, whence they can be accessed by user-written assembly language subroutines. One subroutine controls the odor stimulus by sending a voltage pulse down the D/A channel to actuate a valve. Another subroutine, triggered by the clock at the rate of 10 kHz, transfers neuronal potentials from the A/D register to buffer storage memory and disk. The maximum rate of data transfer from memory to disk is about 7 kHz. Because the transfer rate is less than the digitization rate, the system can store uninterrupted samples on disk for no more than 2–3 s before it fills the buffer memory. Discontinuous samples, however, can be stored on disk for records of up to 420 s duration. Usually, to economize on the storage costs, the spikes are extracted from the noise before the record is stored. The permanent record is a set of 20 samples of each spike that comprise the first 2 ms of the spike's 3-ms duration. The remainder of the spike is discarded because it is of little use in classifying the spikes from the preparations we have studied to date.

Software

The complete software consists of a main program, SPIKES, the only permanent resident in memory, and 13 modules called individually from disk, selected from a menu by the user. The functional relationships and the flow of data among the different modules are shown in Fig. 1. Each module has access to about 100 user- and 50 vendor-written FORTRAN and assembly language subroutines that also are stored on disk until use. This format permits new analyses to be written quickly, incorporating sections of software written previously, and contributes to standardization of programming and error-debugging conventions. It also permits large subroutines to be implemented because only a small part of the program resides permanently in memory. The modules' operations subdivide under 4 categories: data acquisition, spike classification, statistical analysis, and graphic displays. The main features of the modules are described here, by category, and the following section discusses some detailed methodology.

Each of the 3 data acquisition modules offers a different stimulus presentation and recording period. ATODIS, the most often used data module, triggers a

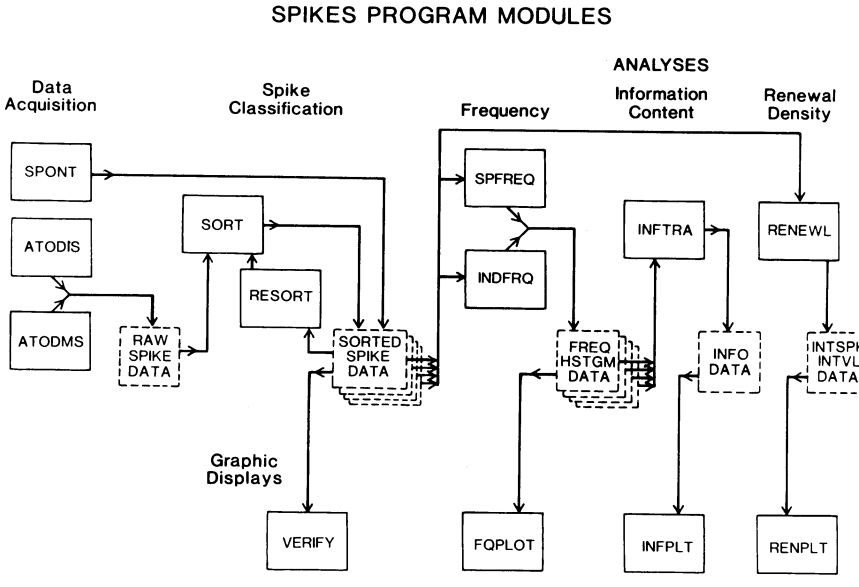

Fig. 1. Functional relationships and data flow among the microcomputer system software modules. Data files are indicated by dashed boxes, and modules by solid boxes.

continuous stimulus pulse for a user-determined period within a total recording period of 10.24 s. ATODMS triggers user-set multiple pulses, and has been useful for examining dynamic characteristics of the pheromone receptor response. SPONT monitors spontaneous (unstimulated) activity for a user-set number of spikes up to 1000, which facilitates comparisons with responses to low-level stimulation. The data modules also store and process records differently. Both ATODIS and ATODMS store neuronal responses, unprocessed, in a Raw Spike file for input to the main spike classification module, SORT. SPONT, however, operates in a recording-processing cycle. It records for 1.024-s periods, sorts the recorded spikes, and stores them in a Sorted Spike file. Then it displays the spike frequencies, and continues in the cycle until the user-set number of spikes have been collected.

SPONT uses a faster but less accurate spike classification procedure than SORT to provide a more instantaneous description of the neuronal response. (It classifies by spike amplitude, which in many cases is not sufficient to distinguish the spikes reliably), while SORT uses additional criteria described below.) After the recording session, however, the classification errors in SPONT-generated files can be corrected by passing them through a reformatting module, RESORT, and then through SORT (Fig. 1).

SORT screens Raw Spike files, identifying and sorting peaks into non-spike, spike, and double spike categories, depending on the peaks' waveforms. Double spikes occur when 2 neurons in a sensillum discharge nearly simultaneously, and the spikes overlap. When a peak is identified to be a spike, its type is assigned initially by the distribution of a span area index (Fig. 2), which is the sum of the potential

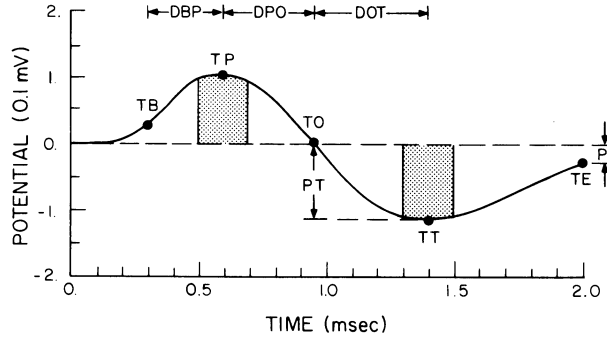

Fig. 2. Sample spike waveform recorded from a *T. ni* HS(a) neuron showing critical time points, time intervals, and span area (shaded area) used for spike classification: TB, time where potential first exceeds cutoff; TP, time of peak maximum; TO, time where potential crosses through zero; TT, time of potential minimum or trough; TE, time of last stored sample, 1.4 msec after TP and 2.0 ms after spike onset; PT, trough potential; PE, potential of last stored sample; DBP, interval TP–TB; DPO, interval TO–TP; DOT, interval TT–TO.

differences at 6 sampling points, 3 spanning the top and 3 spanning the trough of the peak. Because the 2–3 neurons in a pheromone-sensitive sensillum have spikes with distinctly different span areas, the spikes usually fall into separate groups on a span area histogram. If SORT has difficulty in classifying the spikes, the user can intervene by adjusting the span area cutoffs that determine the spike groupings. The user also can adjust the criteria that determine whether a peak is a spike.

Once the spikes are split into groups, a mean spike template is made for each group by averaging the digitized potentials at each of the 20 sampling points. Then the initial spike assignments are confirmed by matching each peak against each template. This procedure of prescreening and then template matching eliminates many of the problems frequently encountered when templates are used to sort spikes from extracellular recordings (Harding and Towe, 1976). SORT stores the spike waveforms, times, and span areas in a Sorted Spike file for input to the analysis and display modules.

The VERIFY module displays the spikes (and other peaks) in a Sorted Spike file to let the user determine if they have been identified and classified correctly It has the capability to display spikes singly or in groups. Different groups can be displayed on the basis of their beginning and ending times, their beginning and ending spike numbers, or by spike type. A sample display of a complete spike record is shown in Fig. 3.

VERIFY corrects single or multiple classification errors, produces hard copies of individual or groups of peaks upon request, and reclassifies double spikes into their 2 components. At the end of a verification session, it presents a summary of the mean spike frequencies for each type during the pre, post, and stimulus intervals.

Frequency, interspike interval, regression, information content, and distributional analyses are performed on Sorted Spike files by the SPFREQ, INDFRQ, INFTRA, and RENEWL modules. SPFREQ and INDFRQ both calculate instantaneous spike

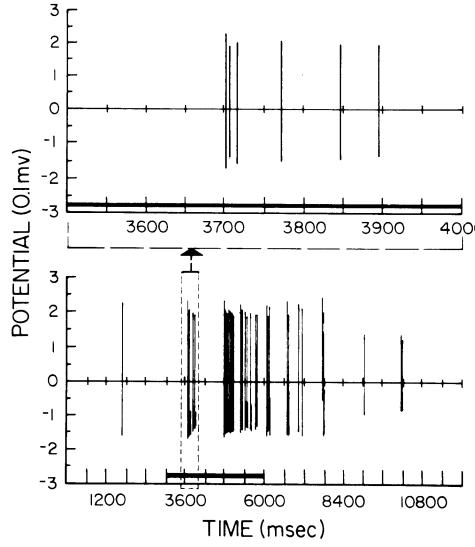

Fig. 3. A: spike train recorded from a *T. ni* HS sensillum exposed to 3 s of a low-intensity sex pheromone stimulus. Bar at bottom denotes the stimulus period. B: expansion of Fig. 2A showing onset of response about 0.7 s, after initiation of stimulus.

frequency during 0.1-s intervals. (This interval is adjustable, but we use 0.1 s as the default.) SPFREQ analyzes records from different receptor neurons to generate a histogram of response across time at a single stimulus concentration. INDFRQ examines records from a single receptor neuron to determine the response pattern of a single neuron across different stimulus concentrations. The spike frequencies are stored by type in a separate Frequency Histogram file (Fig. 1), displayed or hard-copied by the FQPLOT module. The FQPLOT histograms are particularly useful for examining the dynamics of response from individual and groups of peripheral receptor neurons. The results from the SPFREQ and INDFRQ analyses also serve as input to subroutines that calculate regressions of neuronal response on stimulus intensity.

The INFTRA module calculates the information content (entropy) of the responses in groups of Frequency Histogram files by a method based on information theory (Shannon and Weaver, 1949). Information content analysis is a non-metric analog of the more commonly used analysis of variance. It provides several parameters of interest in characterizing the discrimination of stimulus intensity and the rate of information flow. For example, it permits the calculation of a differential threshold determining the minimum number of different levels that a neuron can distinguish across a continuum of different stimulus intensities (see e.g. Smith et al., 1984). The output of the module is a set of values indicating the amount of information transmitted per stimulus presentation during consecutive 0.1 s intervals after the beginning of the stimulus. The values are stored in a separate Information Data file and can be displayed by INFPLT (Fig. 1).

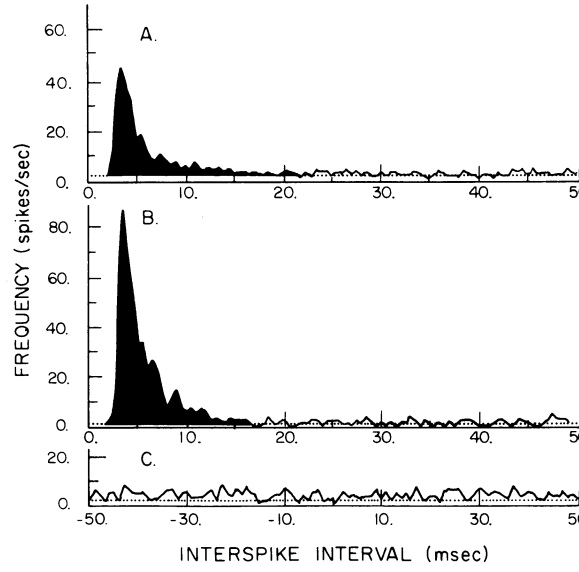

Fig. 4. Auto- and cross-renewal densities of Type (a) and (b) neurons in a 5000-spike record of spontaneous activity from a *T. ni* HS sensillum. Dashed line is mean frequency (= expectation frequency of a Poisson distribution). The shaded area indicates the burst size, the number of spikes in excess of the expected number: A: autorenewal of Type (a) spikes on (a) spikes; B: autorenewal of Type (b) spikes on (b) spikes; C: cross-renewal of Type (a) spikes on (b) spikes.

The RENEWL module calculates the interspike interval mean, variance, and coefficient of variation for each spike type in groups of Sorted Spike files, and generates a renewal density histogram, which is a measure of auto- and cross-correlation (Perkel et al., 1967). The renewal densities and interspike intervals are stored by type in a separate Interspike Interval file, displayed or hard-copied in histograms by RENPLT (Fig. 4). RENEWL has been useful for characterizing the unstimulated state of a neuron, for testing hypotheses about cross-excitation between neurons, and for identifying damaged neurons. Both damaged and undamaged pheromone-sensitive neurons tend to fire in bursts, i.e., groups of 2 or more spikes closely spaced, the size of which can be determined from the auto-renewal density histogram. The damaged neurons, however, appear to produce larger-sized bursts than the undamaged neurons. An example of renewal density analysis is presented later in the report.

Signal processing methodology

We next describe some pertinent details of the signal storage, sorting, error checking, and analysis methodology.

Signal timing and collection methods

The data acquisition modules record potentials in continuous segments of 1.024-s (10K words) duration. Each segment recorded by ATODIS and ATODMS is followed by a break of about 0.2 s, during which the data storage buffer empties to disk. The breaks between recording periods are longer for SPONT, about 3 s, because the spikes are sorted during the breaks. In ATODIS, the D/A port triggers a stimulus valve at the end of the break between the 3rd and 4th segments (3.672 s in real time, 3.072 s in recorded time). The D/A port resets the valve at the end of the 6th segment, which turns off the stimulus. ATODMS enables the stimulus trigger to be set at the beginning or end of any recording segment.

A simple record from ATODIS is shown in Fig. 3, as displayed by SPPLOT. In this example, the stimulus pulse reaches the antenna about 0.6 s after activation of the valve. The Raw Spike record thus comprises a 3.6-s prestimulus interval, a 4.1-s stimulus interval, and a 3.4-s post-stimulus interval in real time, and occupies 102 400 words on disk. Because of the large amount of disk space required, the Raw Spike records usually are not stored permanently. Instead, the interspike periods are discarded and the spikes are stored in Sorted Spike records in blocks of 24 words per spike (20 potential samples + double precision time + spike type + span area). The disk storage occupied by the record thus decreases to no more than 24 000 words for a spike train of 1000 spikes, the largest number that SORT can analyze simultaneously.

Spike identification and sorting methods

The SORT module passes through the full Raw Spike file once, and any peaks that it detects are rescreened twice — first to determine if a peak is a spike and then to classify the identified spikes by type. In the initial pass, a peak detector steps through consecutive digitized samples until the potential exceeds the predetermined threshold. The peak detector stores in memory the threshold time (double precision), designated as TB in Fig. 2, and places a set of 50 contiguous samples into a temporary disk file, beginning at the 6th sample before TB and ending at TB + 43. Then the peak detector continues to screen samples until the end of the file. Only 20 of the 50 samples in this set are stored later in the Sorted Spike file, beginning at the 2nd sample before TB and ending at TE. The samples after TE are of little value for classification purposes, but the longer set is stored temporarily to capture double spikes.

Next, the sets of peak samples are re-examined and compared against time and amplitude criteria (Fig. 2). The main purpose of this pass is to screen out peaks that should not be included in the calculation of a spike template and to realign the spike samples relative to the time of peak maximum, TP, instead of the time of peak threshold, TB (Fig. 2). The time of peak maximum is less affected by noise than TB; consequently, the templates generated from spikes aligned together at TP have less variance than templates from spikes aligned at TB. This pass also stores the times of the peak maximum, zero-crossover, and trough, if they exist. Then the span area (shaded area in Fig. 2) is calculated if the differences among the critical times (Fig. 2), DBP, DPO, and DOT fall within user-selectable limits and the potential at TE

does not rise above zero. Typical criterion limits that have proved useful for screening the *T. ni* pheromone receptor neurons are 1–4 for DBP, 3–7 for DPO, and 2–5 for DOT (in units of 0.1 ms). We have occasionally found it necessary to adjust these criteria to classify spikes from other insect preparations.

After the 2nd pass, SORT generates a span-area histogram, with the spikes that meet the time/amplitude criteria, divides the groups of each spike type into regions, and displays the divided histogram to the user for approval. The regions can be reset manually, if necessary. Then a mean spike, with standard deviation, is calculated for the spikes in each region. In the final pass, the peaks in the record are matched with the mean spike templates, point by point. A peak that otherwise meets the criteria above is rejected if the potential at any point is more than 2 standard deviations away from the mean potential at that point. Depending on the degree of similarity, a peak is determined to be noise, assigned a spike type, or flagged as an outlier for the user to examine in VERIFY.

The cluster-separating and template-matching procedures that we used are commonly found in the literature, so they will not be discussed here in detail (see e.g. Schmidt, 1984).

Spike-type error correction methods

Early in the system's development, it became evident that locating and correcting spike classification errors was the most time-consuming part of a recording session. We have reduced this time considerably by incorporating 3 key features into VERIFY. One is the implementation of a fast spike-ordering algorithm, based on Singleton (1969). Ordering by span area permits the correction of multiple errors with a single readjustment of a cutoff between 3 regions or spike types. This benefit is counterbalanced, however, by the time needed to order the spikes. Singleton's algorithm reduced the spike ordering time to a few seconds for files as large as 1000 spikes. A 2nd time-saving feature of VERIFY lets the user superimpose a series of spikes in order of either ascending or descending span area, one by one on the video display. The 3rd feature is an interrupt capability, implemented by an assembly language subroutine, that lets the user halt the series after any spike and branch immediately into an error-correction subroutine.

The benefit of these features derives partly from the typical distribution of classification errors made by SORT. Most of the errors occur at the extremes of the span area ranges. For example, double spikes in which the components are separated by less than 0.3 ms sometimes escape detection because they fit within the limits of one of the mean spike templates. Often, however, these spikes have span areas that are intermediate between the 2 component types, and they can be located by examining a few spikes with span areas just above and below the cutoff. Because the errors tend to be clustered at the extremes, the fastest way to verify a large file is to pass through about 15 spikes at the extreme span areas of each spike type.

Frequency, interspike interval, and information content analyses

In analyzing the response characteristics of insect olfactory receptor neurons, we have found it particularly helpful to generate frequency, interspike interval, and

information content histograms. The histograms are generated by the SPFREQ, INDFRQ, RENEWL and INFTRA modules. The first 3 modules retrieve groups of Sorted Spike files and generate frequency, interspike interval, and renewal density histograms. INFTRA retrieves groups of Frequency Histogram Data files and generates a histogram of the information contained in the response at different times after the stimulus begins.

In addition to the frequency histogram, SPFREQ and INDFRQ also calculate the overall mean frequency and the expectation frequency of the Poisson distribution (Kaissling, 1971; Mankin and Mayer, 1983). The Poisson distribution is of interest in the analysis of responses at low stimulus concentrations where it is difficult to distinguish stimulus-evoked spikes from spontaneous activity. The expected Poisson frequency is calculated from the equation:

$$F_p = \ln(N/N_0) \tag{1}$$

where F_p is the frequency (number of spikes/sampling interval), ln is the natural logarithm, N is the number of intervals sampled, and N_0 is the number of intervals in which no spikes occurred. We have found 0.1 s intervals to be the most convenient to use, but the procedure is applicable for any interval greater than or equal to the 3 ms spike duration. There are some benefits to considering different sampling intervals, but their discussion is outside the scope of this report.

The instantaneous and Poisson frequencies both are calculated by distributing interspike intervals within a 3-dimensional stack (matrix) of bins or counters. The first dimension indicates the spike type. In recordings from *T. ni* preparations two types usually occur, designated (a) and (b). The 2nd dimension indicates the particular 0.1-s interval in which spikes are to be counted. There are 102 segments (bins) of 0.1 s in a typical record from ATODIS. The 3rd dimension indicates the number of spikes that occur during a given 0.1-s interval. Because the refractory period limits the maximum frequency to about 300 spikes/s, we consider only 35 possibilities for each 0.1-s interval, from 0 to 34 spikes/interval. Thus, the stack for a record which has 2 types of spike is 2 bins deep by 102 bins long by 35 bins high.

As each Sorted Spike file is examined, the number of spikes of each type occurring during each interval is counted and added to the bin corresponding to the appropriate spike type, the interval, and the number of spikes in that interval. The Poisson frequency for each interval and type is calculated by dividing the number of files examined by the number of occurrences of 0 spikes during the interval. The instantaneous frequency for a given interval is calculated by summing the number of spikes in the interval and dividing by the product of the interval period and the number of files examined.

In INFTRA, the information content of the signal transmitted by a neuron is calculated by a non-metric approach that subdivides both the stimulus and response continua into categories instead of magnitudes. The stimulus–response relationship for a given type of neuron is represented by a stack (matrix) of bins similar to that described above for calculation of the frequency histogram. One dimension designates responses, r, of different intensities (the third dimension in the frequency histogram matrix), a second subdivides the responses into different time intervals

(the second dimension in the frequency histogram matrix), and a third designates stimuli of different intensities, s. When frequency histograms are generated for each of the different stimulus levels considered in the information content analysis, the entries that INFTRA needs for the stimulus–response matrix do not have to be calculated because they already are stored in the Frequency Histogram Data files.

To calculate the information content, INFTRA performs several different sums on the entries of each time interval. One sum, N, is the total number of stimulus presentations, i.e., it is the sum of all the matrix entries, n_{sr} at a particular time interval. Another, n_s, is the sum of the matrix entries for each stimulus across all response categories, and a third, n_r, is the sum of the matrix entries for each response category across all stimulus categories. The information content is a combination of several terms of the form:

$$\log(N) - n \log(n) \tag{2}$$

where log is the logarithm in base 2 and n is either n_{sr}, n_s, or n_r. A more complete discussion of information content is found in Smith et al. (1984), Mankin et al. (in preparation), and in references therein. The final result is a set of values for the information content of the stimulus at each interval after the beginning of the stimulus, which is stored in an Information Data File (Fig. 1).

The logic for calculating the renewal density is similar to that for the frequency analysis. The interspike intervals are subdivided into periods of 1 ms (adjustable, if the periods are too broad or too narrow), and a 4-dimensional set of bins is constructed, 2 by 2 by 1500 by 2 units, to count intervals of particular kinds. The 1st and 2nd dimensions indicate, respectively, the type of spike for the 1st and 2nd spike of each spike pair. The 3rd dimension indicates the number of spike pairs with interspike intervals of a given duration (in ms) over a range of 1–1500 s. The 4th dimension is for the cross-renewal density analysis, and denotes whether an (a) spike follows or precedes a (b) spike.

System operation

This section considers some of our experiences with the microcomputer system during 4 years of operation. In particular, we assess spike classification errors and describe procedures to facilitate file identification and retrieval. An example of the system operation is provided by a renewal density analysis of the spontaneous activity of pheromone-sensitive receptor neurons in *T. ni*.

Spike classification errors

Two types of spike classification errors can occur, one where a spike is misclassified, and the other where an actual spike is miscategorized as an outlier. Analyzing *T. ni* recordings that contain spikes from two neurons, SORT misclassifies about 0.5% of the spikes examined later by VERIFY, and another 1–2% are miscategorized as outliers for the user to sort manually. Most of the miscategorized spikes deviate significantly from the template near the baseline, due to noise. However, if

the standard deviation criterion for template matching is relaxed sufficiently to keep such spikes from being rejected, double spikes begin to pass through SORT undetected. It has been our practice to use stringent criteria that ensure the detection of double spikes.

The frequency of misclassification errors depends primarily on the spike amplitude-to-noise ratio and the range of span areas for each spike type in a record. If the fraction of errors is large, the cause is usually that the span area histograms have subdivided indistinctly. For example, the amplitudes of the type (a) and (b) spikes may overlap so much that there is no discernible minimum in the span area histogram. At other times, a high noise level may lead to the generation of a group of peaks whose span areas overlap with those of the smallest type (b) spikes. In some preparations, spikes of unusually small amplitude appear in addition to types (a) and (b). Spikes of such amplitude usually are considered to be from neurons in adjacent sensilla. Here, however, the span area histograms typically divide into three separate regions. A sensillum preparation is discarded if the histograms do not divide cleanly into regions and the user cannot reclassify them easily in VERIFY. This kind of problem occurs only rarely in recordings from *T. ni* and usually is discovered early in a recording session.

Two special cases should be considered briefly. First, numerous errors occur if the time/amplitude criterion parameters have been set incorrectly, but this type of problem usually is identified by SORT, which informs the user when it discards an excessive fraction of the peaks. Second, a problem with decrementing amplitude occurs when the antenna is stimulated with high concentrations of some pheromone components. The *T. ni* antenna bears two types of pheromone-sensitive sensillum, HS and LS, each with two neurons (a) and (b). At high concentrations of the pheromone component that stimulates the HS(a) neuron, the amplitudes of HS(a) spikes often decrement down to about the size of the HS(b) spikes so that it is impossible to distinguish between them either visually or by computer. However, at still higher concentrations, the amplitudes of the HS(a) neurons decrement below the amplitudes of the HS(b) neurons and the spikes can again be distinguished.

It should be noted that there are many indexes in addition to span area that can be used to distinguish spikes (e.g. O'Connell et al., 1973; Harding and Towe, 1976; Van der Molen et al., 1978; Hanson et al., 1986; Frazier and Hanson, 1986). Some systems use several parameters in concert (Piesch and Wieczorek, 1982; see also Schmidt, 1984). We tested standard deviation and total spike area indexes before settling on span area as the criterion index, and found that all 3 indexes had similar error rates for *T. ni* pheromone-sensitive receptor neurons.

File retrieval

The procedure for naming and retrieving data files is an important feature of the system, not only because the data base is extensive, but also because a large number of files must be processed for statistical analysis. The SPFREQ, INDFRQ, and RENEWL modules, in particular, cannot rely on time-consuming manual input of file names. To solve this problem and expedite file identification, we have coded the file name with the information most important for retrieval. By convention, each file

name consists of several codes that are concatenated in a fixed order to describe the neuron and the stimulus conditions. Files in a particular analysis group are retrieved easily by a fast directory utility with a wild-card feature or by a user-written, file-lookup program.

Example of system operation — renewal density analysis

Several questions about the characteristics of *T. ni* pheromone receptor neurons led us to develop a renewal density analysis. First, because the (a) and (b) neurons are packed tightly together in both the HS and LS sensilla, we wanted to test for electrical cross-excitation or cross-inhibition. The cross-renewal density histogram tests for these processes (Abeles, 1982; see also discussion below) by showing any tendency of an (a) or (b) neuron to discharge immediately after its partner discharges. Second, we were interested in whether the response of each individual neuron is an independent Poisson point process. If so, the distribution of interspike intervals is predictable (Cox and Lewis, 1966). Knowledge of the interspike interval distribution simplifies the interpretation of responses at low pheromonal stimulus levels where the rate of discharge approaches the level of spontaneous activity (Mankin and Mayer, 1983). The autorenewal density provides a test of independence for spikes from the same neuron. Finally, we needed an objective method for determining whether a neuron was discharging abnormally. Autorenewal density analysis bears also on this problem because the spontaneous activity of a damaged neuron is probably non-random.

The applicability of renewal density analysis to such problems derives from several well known characteristics of Poisson point processes. In a random Poisson process, the renewal density is a constant equal to the mean frequency. Departures from the Poisson distribution appear as bulges or dips on the mean frequency baseline in the renewal density histogram. An example is Fig. 4, a sample display from RENPLT, which shows the auto- and cross-renewal densities of the (a) and (b) neurons in a 5000-spike record of spontaneous activity from an HS sensillum. The histograms in Fig. 4A, B have bulges above the baseline. The bulges indicate that these two neurons tend to fire in bursts, i.e., that the occurrence of one spike tends to facilitate the occurrence of another spike soon afterwards.

The shaded areas in Fig. 4A, B measure the number of spikes above the level that would occur if the neuron discharged each spike independently rather than firing in bursts. The average number of spikes in a burst, m, can be calculated from these shaded areas, s, as $m = 2s + 1$ (see Abeles, 1982; Protter and Morrey, 1963, p. 205). By this measure, the HS(a) neuron in Fig. 4A fires in bursts of 1.55, and the HS(b) neuron in Fig. 4B fires in bursts of 1.96. This indicates that the spikes produced by each individual (a) or (b) neuron are not completely independent of each other and only approximate an independent Poisson point process.

The cross-renewal density histogram in Fig. 4C, in contrast, is reflective of a true Poisson-point process. There are no bursts of (a) spikes following a (b) spike, and vice versa. Neither is there a significant reduction in spike discharge from the baseline. Apparently, the two neurons discharge independently of each other.

Several properties of the spontaneous activities of the (a) and (b) receptor

TABLE I

INTERSPIKE INTERVAL STATISTICS AND BURST SIZES IN SPIKE TRAINS OF SPONTANEOUS ACTIVITY RECORDED FROM 4 TYPES OF RECEPTOR NEURON IN TWO TYPES OF OLFACTORY SENSILLUM ON *T. ni* ANTENNA

The two sensillum types are designated HS and LS, each with two neurons: (a) and (b). The notes at the bottom indicate why the neurons listed as abnormal were discarded. The asterisk indicates that a response is different from the mean response of the normal neurons at the 0.01 confidence level according to Dunnet's test (see text).

Sens. type	Response condition	Sens. no.	Total no. spikes in train	Mean of interspike interval (s)		Variance of interspike interval		Mean no. of spikes per burst	
				(a)	(b)	(a)	(b)	(a)	(b)
HS	Normal	1	3405	0.50	1.60	0.77	7.74	1.52	2.19
HS	Normal	2	5057	0.42	1.17	0.38	4.37	1.77	2.06
Hs	Normal	3	5007	0.69	2.03	1.23	7.71	1.63	1.49
HS	Normal	4	5000	0.51	0.91	0.79	14.52	1.55	1.96
HS	Abnormal	5	4500	1.99	0.78	12.00 *	2.27	2.65 *	2.42
HS	Abnormal	6	5030	1.03	1.02	4.97	3.42	3.96 *	2.05
HS	Abnormal	7	4995	0.64	3.13	0.87	48.52 *	1.68	2.39
LS	Normal	8	315	0.76	0.41	1.55	0.74	1.99	1.44
LS	Normal	9	7378	1.47	1.29	13.02	1.21	3.06	6.65
LS	Normal	10	6437	2.72	1.63	88.65	10.44	1.78	1.47
LS	Abnormal	11	7000	18.01 *	0.71	79.66	1.21	1.52	1.89

Notes: Sensillum 5—HS(a) neuron amplitude decremented and frequency increased at 7 h after sensillum was impaled. Sensillum 6—HS(a) neuron fired rapidly just after impalement, then slowed. Sensillum 7—HS(b) neuron fired slowly just after impalement. Activity increased over 5-h period. Sensillum 11—LS(a) neuron firing frequency decreased continuously for 3 h after impalement, then stabilized.

neurons in 11 *T. ni* HS and LS sensilla are listed in Table I. (The example in Fig. 4 is sensillum 4.) The abnormal preparations usually would have been discarded after the events noted at the bottom of the table. We continued monitoring these particular preparations, however, to determine whether periods of bursting would appear in the renewal density histogram as bulges above the mean frequency baseline.

The results in Table I follow the pattern in Fig. 4, indicating that the (a) and (b) neurons in a *T. ni* pheromone-sensitive HS or LS sensillum discharge independently of their partners, but the spontaneous activity of each individual neuron is not a completely independent Poisson process. The HS(b) and LS(a) neurons, in particular, had a tendency toward bursting, with a mean burst size of 1.9 and 2.5 spikes, respectively. The mean spontaneous activities of the normal preparations, calculated as the inverse of the interspike intervals, are similar to those reported by Grant and O'Connell (1986).

To determine if the burst size could be used as a test of abnormality, we applied the Dunnet's least significant difference test (Stell and Torrie, 1960) to the abnormal neurons in Table I. Two of the 4 burst numbers from the abnormal neurons were significantly different from the normal means at the 0.01 confidence level (asterisk). We conclude that, although the burst size is a good indicator of abnormality, it is not a fail-safe test. However, each of the abnormal neurons was significantly different when the mean and variance of the interspike interval were included in the comparisons with normal receptor neurons. Consequently, it still may be possible to develop an objective test of neuronal damage to complement the subjective methods now used in insect olfactory studies.

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