

## ADVANCES IN THE DEVELOPMENT OF MICRO-RECORDING SYSTEMS AND SINGLE UNIT ANALYSIS IN TOADS (*BUFO BUFO* L.)

H.-W. BORCHERS\*

Neuroethology and Biocybernetic Laboratories, FB 19,  
University of Kassel, D-3500 Kassel, West Germany

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### Abstract

The exploration of the toad's (*Bufo bufo* L.) prey-predator recognition system is based on the analysis of the animal's behavior and single unit activity in the visual system. Recordings are performed in paralyzed as well as in freely moving animals. For the freely moving toad a special micro recording assembly has been developed to chronically record single unit activity extracellularly in the central visual pathway. Coincidence between neuronal discharge and behavioral patterns is examined by means of computer evaluation and frame-by-frame analysis. Several types of neurons were characterized. They can be classified into the following three groups, depending on the correlation between their response characteristics and the behavior of the toad. (1) Neurons which are activated by moving retinal images irrespective of whether the toad is moving or sitting still. (2) Neurons which are activated by moving visual stimuli. However, the neuronal activity is suppressed in the presence of certain movements caused by the toad. (3) Neurons exhibiting spontaneous activity which correlates with the toad's general motor activity. An activity change precedes and 'predicts' the subsequent behavioral pattern.

### 1. Introduction

Neurobiological investigations on information processing in sensory systems are based on a broad field of experimental techniques. Basic explanations of how environmental stimuli are recognized by animals can be expected from recording the activity of single neurons in corresponding parts of the nervous system. However, a prerequisite for the interpretation of the interactions among single units and in nerve nets is the ethological analysis of an animal's behavior. The method of studying the neuronal response is determined by both behavioral experiments and the recording technique. In the Neuroethology and Biocybernetic Laboratories at the University of Kassel these problems are investigated in toads (*Bufo bufo* L.) as part of a larger research project on visually guided behavior of lower vertebrates (Ewert 1974, 1982). Quantitative behavioral experiments have shown that the prey-predator recognition system of toads uses principally two

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kinds of Gestalt information: the size of an object in the direction of movement (wormlike stimulus with prey characteristic) and its extension perpendicular to the direction of movement (antiwormlike stimulus with enemy characteristic) (Ewert, 1969; Ewert *et al.*, 1979). One of the methods utilized to explore this system at a neuronal level is to record single unit activity in immobilized as well as in freely moving animals.

### 2. Microrecording technique

Our previous knowledge of information processing in neuronal networks of the toad's visual system is mainly obtained from investigations performed with paralyzed animals. In these experiments the response characteristic of single units in the visual pathway is analyzed by presenting precisely defined behaviorally relevant visual stimuli (such as little black or white cardboards on a contrasting background) to an awake immobilized animal sitting in a perimeter-like set up (Figure 1).

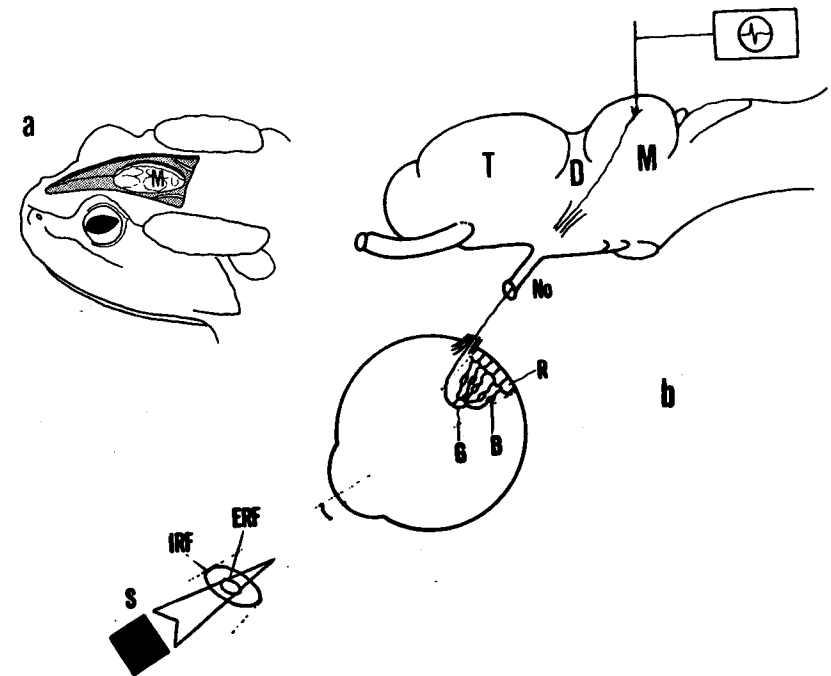


Fig. 1. Simplified scheme of the recording experiment with the toad. (a) Exposed Midbrain (M). (b) A visual stimulus (S) is moved through the receptive field which is composed of a central excitatory (ERF) and a peripheral inhibitory (IRF) area. The retina consists of receptor cells (R) and different sequentially connected neuron types: bipolar (B), amacrine, and ganglion cells (G). Among these cells there are cross connections via amacrine and horizontal cells (not illustrated).

Recording microelectrodes, either metal filled glass pipettes (Gesteland *et al.*, 1959) or electrolytically etched insulated steel needles, are advanced into the tissue of the brain close to a neuron by means of a micromanipulator. The electrode may be kept in the same position for several hours. (All preparations were performed under anesthesia to avoid stress to the animal; for surgical technique see Ewert and Borchers (1971).) The recording site can be marked at the end of the experiment by passing an anodal direct current of  $5 \mu\text{A}$  for 5 seconds through the recording steel electrode. The iron deposit is stained by the 'Prussian blue' reaction in paraplasm embedded brain sections of  $12 \mu\text{m}$ .

Recordings are carried out against a grounded platinized metal plate serving as reference electrode in contact with the body of the toad (monopolar recording). The recorded action potentials are conducted via a preamplifier with impedance matching and frequency filters to an oscilloscope. Neuronal activity is rendered audible as short clicks in a loudspeaker. Stimulus parameters are recorded simultaneously after conversion to electrical signals on other traces of the oscilloscope. The displayed spike sequences can be photographed or filmed by a cine camera or recorded on magnetic tape for later evaluation (Figure 2).

Coincidence between neuronal activity and corresponding behavioral patterns as well as the effect of the animal's behavior through possible feedback mechan-

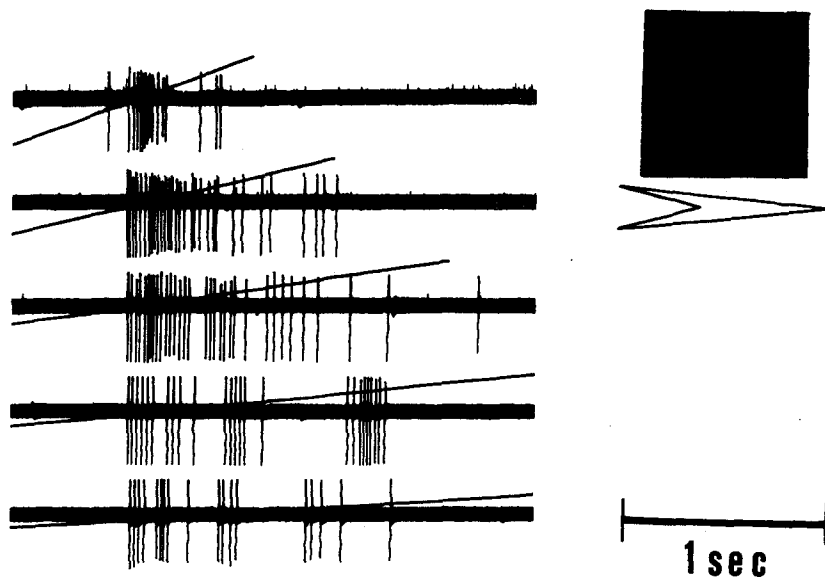


Fig. 2. Neuronal responses of a tectal T1 neuron to a black square of  $8^\circ$  by  $8^\circ$  angular size moved through the receptive field with different angular velocities on a white background. The slope of the diagonal line on each trace represents the stimulus velocity. (From top to bottom  $23^\circ/\text{s}$ ,  $13^\circ/\text{s}$ ,  $9^\circ/\text{s}$ ,  $5^\circ/\text{s}$ ,  $3^\circ/\text{s}$ .)

isms on the ongoing neuronal activity is analyzed in the freely moving toad. For these experiments a special micromanipulator has been devised to chronically record single unit activity extracellularly in the central visual pathway (Figure 3).

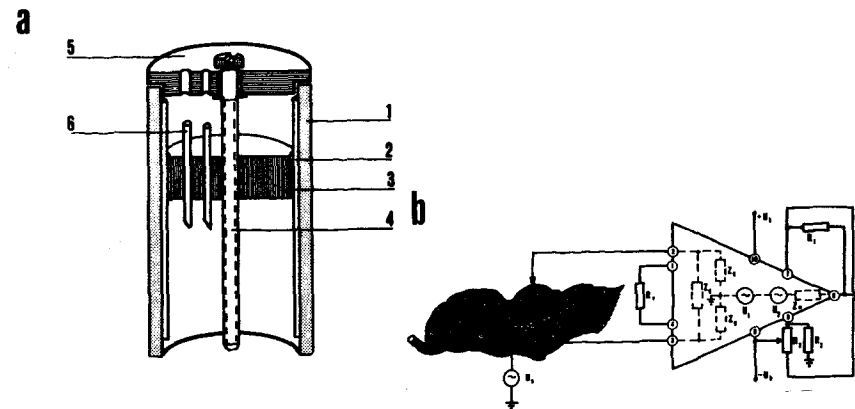


Fig. 3. Microdrive assembly for single unit recording in freely moving toads. (a) A piston (3) with two electrode holders (6) can be moved up and down within the cylinder (1) by turning a screw (4). The piston is guided by two ridges (2). The screw axle is journaled within the cap (5). (b) Schematic of differential recording by means of an instrumental amplifier (Burr & Brown) from the toad's midbrain (M). (D—diencephalon, T—telencephalon.)

The microdrive has been designed according to the hydraulic master-slave system or to mechanical screw-axle technique (Figure 3). Within the piston, etched twin stainless steel or tungsten microelectrodes (INSL-X-coated, with an exposed tip diameter of  $2\text{--}3 \mu\text{m}$ ) are vertically fixed. The depth of the electrode tips in the tissue of the brain can be adjusted in dorso-ventral direction by turning the screw, which moves the piston up and down. As the tip of the recording electrode must not move relative to the site of recording when the toad moves, the holder of the electrode positioner is firmly attached by means of dental cement to the dorsal surface of the skull above the exposed midbrain. It is possible to keep the electrode close to a fibre even when the toad jumps. The potential ( $U_n$ ) at the active electrode is led into the positive input and the potential ( $U_i$ ) of the indifferent electrode is led into the inverting input of an instrumentation amplifier with differential FET input stages. Thus the differential recording set up permits one to reject common spurious muscle potentials ( $U_m$ ) and part of background noise by subtraction. The resulting output voltage is

$$U_{\text{out}} = (U_n + U_m) - (U_i + U_m) = U_n - U_i.$$

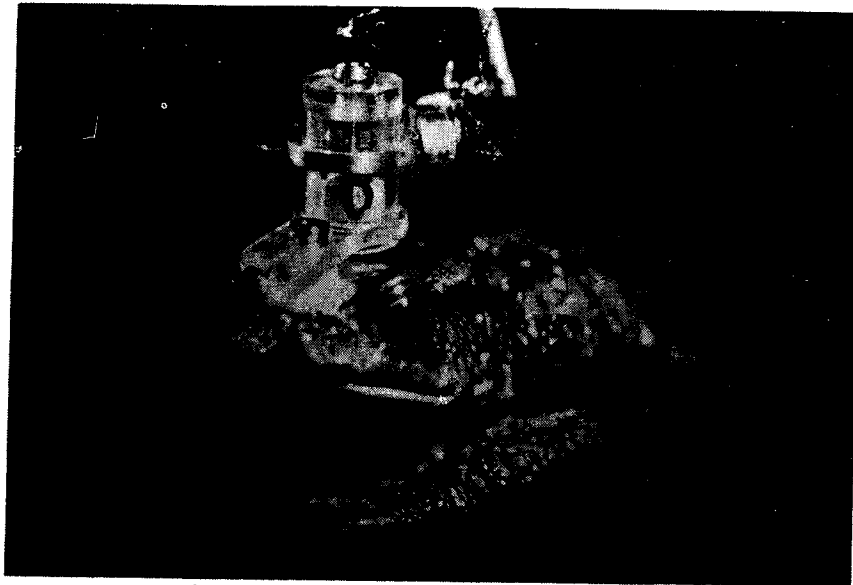


Fig. 3 (cont.)(c). The micromanipulator assembly with the amplifier is mounted on the toad's skull.

The electronic device is directly fixed to the headpiece (Figure 3). The action potentials are conducted by thin copper wires to a storage oscilloscope with stepper. Four traces are subsequently displayed and automatically erased after the fourth sweep. The action potentials recorded are transformed by a pulse generator into unitary rectangular impulses and fed into the z-axis input (intensity) of the oscilloscope. By adjusting the threshold of the pulse generator in such a way that it responds only to the potentials with highest amplitude, these will be enhanced in their luminance, outshining all others. Only these spikes are included in the analysis by film, tape or computer (Figure 4).

A video system consisting of camera, mixer and scan converter is designed to simultaneously record the following three kinds of events. (i) The toad's movement. (ii) A visual stimulus. (iii) The neuronal discharge. The spike train which is in the form of an analog signal is transformed by the scan converter into video signals and superimposed in the picture, obtained by the video camera. The composite video signal can be stored on magnetic tape for later analysis (Figure 4). Correlation between neuronal event, behavior and stimulus can be investigated in space and time by means of frame-by-frame analysis (Figure 5).

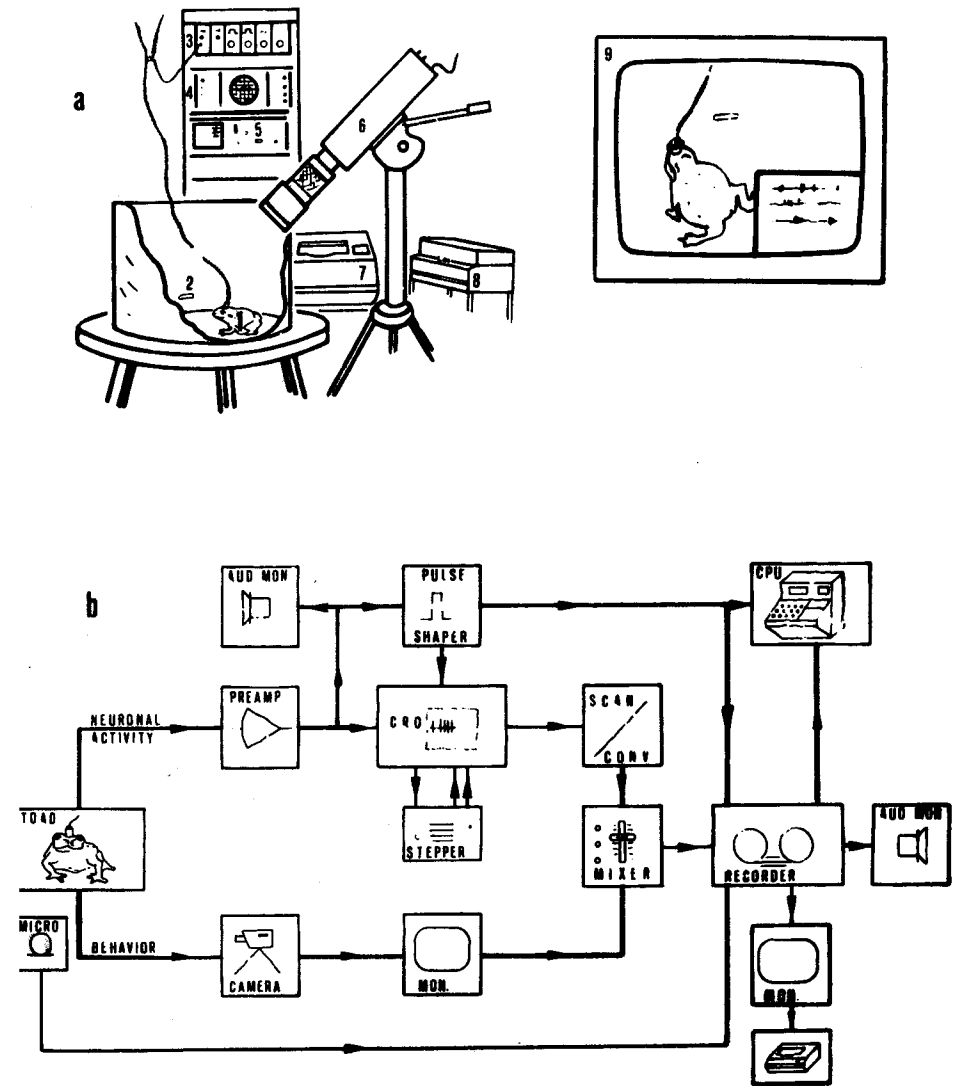


Fig. 4. Arrangement of the experimental set-up. (a) (1) Toad inside the observation vessel, (2) visual stimulus, (3) amplifier and pulse generator, (4) oscilloscope, (5) scan converter, (6) camera, (7) hard copy unit, (8) tape recorder, (9) monitor with the composite video picture. (b) Flow chart for video recording and data processing in single unit recording experiments with freely moving toads.

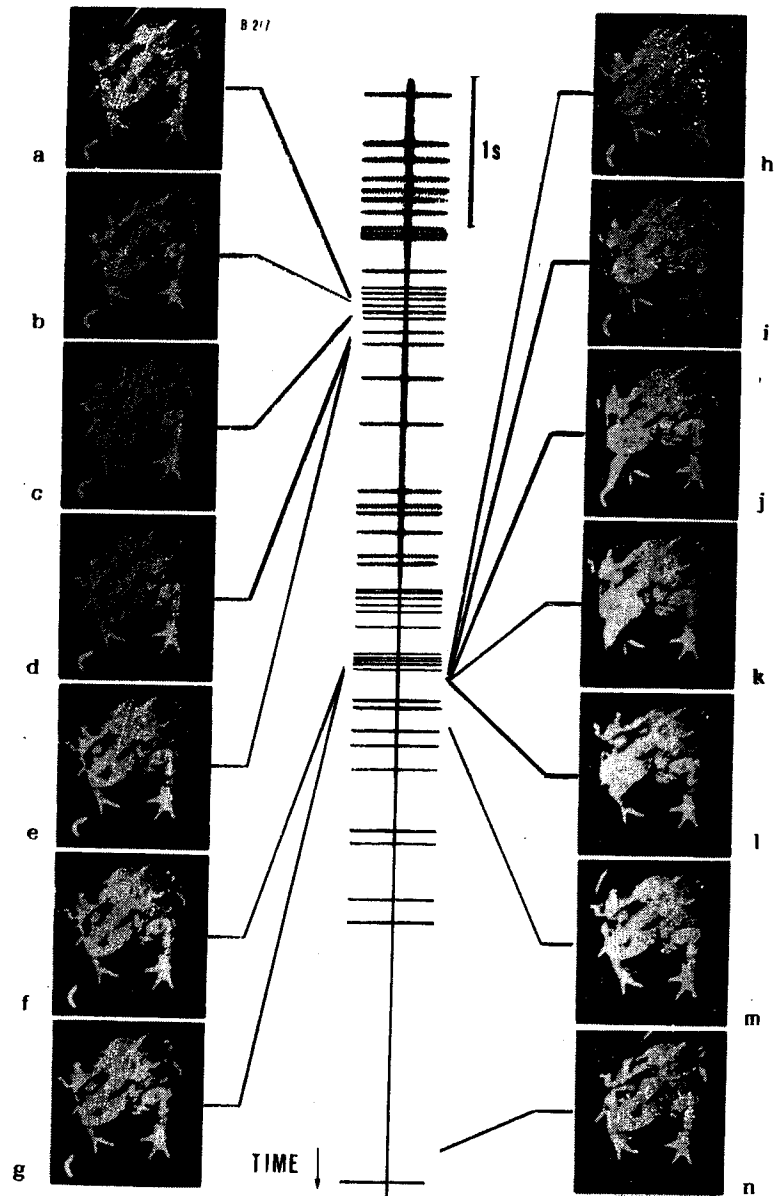


Fig. 5. Frame-by-frame analysis of capture of a mealworm by the toad and corresponding discharge pattern of a behaviorally correlated neuron (group 3). For detailed description see text.

### 3. Spike train analysis

Neuronal data are acquired and processed by a Nicolet Med 812 computer. A special program supplement based on the interrupt structure of the processor serves to detect and measure the interval durations between successive pulses of a spike train. The values of the interval times are stored in the memory in the order in which they occur. As the language of a neuron appears to be coded in the number of spikes per unit of time, the essential information of the neuronal discharge is preserved. Thus a reconstruction and analysis of any part of the spike train is possible at a later time.

A common method of obtaining information of the input-output relationship in sensory systems is the calculation of the neuronal mean discharge rate  $\bar{R}$  as a function of the stimulus parameters ( $S_p$ );

$$\bar{R} = N / \sum_{i=1}^N \tau_i,$$

where  $\tau_i$  is the length of the  $i$ th interval between two successive spikes and  $N$  is the total number of spikes of the neuronal response. Systematic variation of only one parameter at a time yields the (static) characteristic  $\bar{R} = f(S_p)$  (Figure 6).

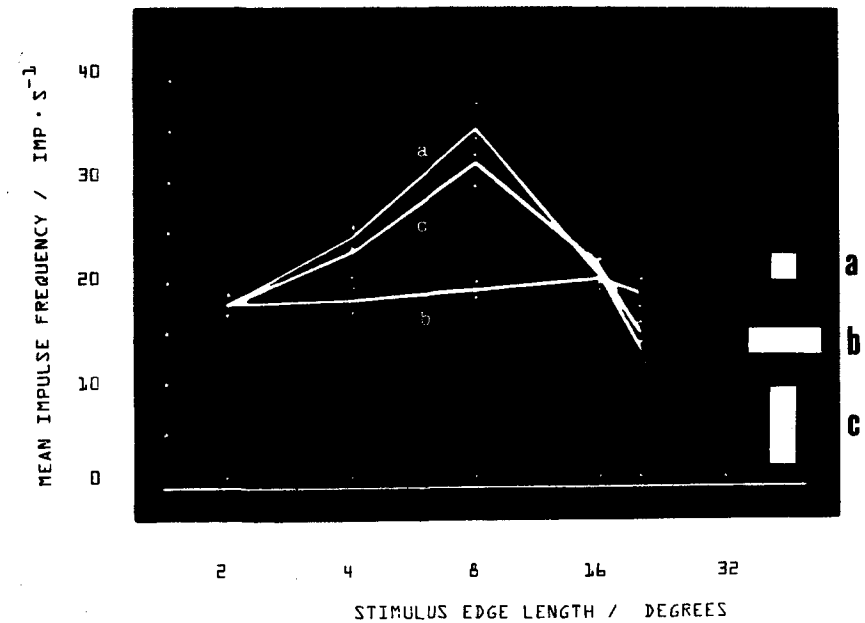


Fig. 6. Responses of retinal ganglion cells (class R3) to moving configurational stimuli a-c, visual angular velocity 7.6°/s. The curves show the mean discharge rate as a function of the stimulus edglength (visual angular size).

This method is particularly useful for driven single unit activity when the number of spikes is limited and the time intervals between successive spikes do not excessively deviate from each other. A prerequisite is that the relevant stimulus parameters are controllable and are not arbitrarily changed by the animal. This is generally the case in experiments with immobilized animals.

For events showing very irregular time intervals between spikes, histogram plots seem to be more convenient and informative since the *interval histogram* shows the distribution of interval times sorted according to their durations without regard to the order in which they occur. Therefore, any information of time dependent properties is lost (this is also valid for the neuronal mean discharge rate, mentioned above). The interval histogram allows one to employ a number of tests designed to reveal some of the details of the process generating the intervals. Analogous to the interval histogram is the *frequency histogram* which represents an impulse frequency distribution.

The *frequency time histogram* in contrast to the interval histogram reflects the approximate time course of neuronal activity. In this histogram the abscissa indicates time, divided into  $m$  discrete uniform units (bins) and the ordinate represents the number of spikes  $k_i$  in each corresponding bin. The total number of spikes is

$$K = \sum_{i=1}^m k_i,$$

and the mean frequency over the  $i$ th bin is,

$$f_i = k_i/b,$$

where  $m$  is the total number of bins and  $b$  is the bin width. The mean frequency of the response is represented by

$$\bar{f} = \frac{1}{mb} \sum_{i=1}^m k_i.$$

A reduction of data is given by the relation of bins and intervals,

$$r = \frac{m}{K-1}.$$

Transient changes of the spike train cannot be detected unless the bin width is smaller than the smallest interspike interval (Figure 7).

To demonstrate the temporal relationship between behavioral events and neuronal activity the *interspike frequency time histogram* can be utilized. The instantaneous interspike frequency  $f_i$  represents the reciprocal value of the interval duration  $\tau_i$  between two successive action potentials. This frequency is plotted on the ordinate as a function of time  $t$  and is assumed to be constant during the corresponding interval  $\tau_i$ ,

$$f_i = \frac{1}{\tau_i}, \text{ constant for } \sum_1^{i-1} \tau_j < t \leq \sum_1^i \tau_j.$$

The width of a column in the histogram is directly proportional to the interval duration. The area of each column is of constant size. Thus shorter intervals have larger values on the ordinate and vice versa. By this method variations of the discharge rate are emphasized by changes of the two parameters in the histogram.

#### 4. Relationship between single unit activity and behavior

By means of single unit recording experiments in immobilized toads, several types of neurons in the central visual pathway have been characterized and localized. They can be related to some behavioral observations.

In the toad's midbrain (optic tectum, subtectum and tori semicirculari) activity from units can be elicited by visual, tactile and vibratory stimulation (Ewert and Borchers, 1971). Detailed quantitative investigations by Ewert and coworkers (Ewert and Hock, 1972; Ewert 1974; Ewert and v. Wietersheim, 1974; Ewert, 1982) supported by correlation analysis (Borchers and Ewert, 1979) show that already at the retinal level, the ganglion cells (classes R2, R3, R4) perform important preliminary operations on the visual input. Their properties consist of a modulation of the neuronal firing rate as a function of angular velocity, angular size (e.g., Figure 6), and contrast. In the retina there are, however, no specific prey or predator detectors. Behaviorally relevant Gestalt parameters are evaluated in subsequent circuits of the brain. Thus, elongation in the movement direction (wormlike configuration) is analyzed by certain neurons in the optic tectum (neurons classes T5(1), T5(2)). Whereas elongation perpendicular to the movement direction (antiwormlike configuration) is analyzed by neuronal systems in the thalamic pretectal region (neuron class TH3). The decoding and decision making process 'prey or predator' of those neuron populations is based on additive and subtractive interactions, which work as Gestalt filters (Ewert, 1974, 1982).

The results are integrated in a comprehensive computer model on prey/predator interaction in the visuomotor system of frogs and toads by Arbib and Lara (1981) and Lara and Arbib (1981).

The recording experiments in the freely moving toad show different neuron types in the retino-tectal projection system. They can be classified into three groups depending on the correlation between their response characteristics and the behavior of the toad (Borchers, 1980).

Neurons of group 1 are activated by moving retinal images irrespective of whether the toad or the visual stimulus is moving. The neuronal activity is strongly influenced by the size, velocity, form and contrast of the stimulus. The neurons maintain their activity as long as the stimulus moves within their excitatory receptive field (ERF). If the toad does not move, stationary objects within the ERF do not elicit any neuronal response. Even normal respiratory movements of the buccal cavity, as they could be observed during the investigations are insufficient to activate the recorded neurons. Deeper respiration, as indicated by stronger movements of the flanks, produce corresponding rhythmic bursts.

Neurons of group 2 show activation in response to moving visual stimuli. However, activity is suppressed in the presence of certain movements initiated by the toad itself. Examples are as follows. (i) A retinal class R3 neuron responds to sudden changes in the diffuse light level—such as switching room lights on and off—with bursts of spikes at ‘off’ and ‘on’. The off-response, however, fails to occur if the animal closes the eye (Ewert and Borchers, 1974; Borchers and Ewert, 1978). The off-activation is suppressed by the toad’s behavior. (ii) Some of the neurons of the optic tectum which are strongly active in response to prey objects, are silent during any movement of the toad. For example, a tectal large field unit is activated by a moving white disk (15 mm diameter) within its excitatory receptive field. However, the neuronal activity ceases, when the toad moves towards the disk even though the stimulus movement continues within the ERF. When the toad stops moving, the firing rate increases at least to its former level.

Neurons of group 3 in contrast to those of other groups exhibit spontaneous activity. The mean spike rate is about  $6\text{ s}^{-1}$ . However, the neuronal activity is strongly correlated with the toad’s behavior. Two types have been identified. In both, a change in the discharge rate precedes a subsequent behavioral pattern. For example turning and prey-catching is preceded either by an increase (class T8(1) neuron) or by decrease of the firing rate (class T8(2) neuron). Each time the toad initiates a movement the frequency of a T8(1) unit increases, and decreases later on at the end of the movement. Peaks of about  $50\text{--}70\text{ s}^{-1}$  are reached just before the movements. These neurons have no ‘primary’ receptive fields. Visual, tactile, vibratory or olfactory stimulation is unnecessary for their activation.

The correlation between prey-catching behavior—a sequence of motor pattern which is typical for the toad—and the corresponding discharge of a class T8(1) neuron is demonstrated by a frame-by-frame analysis in Figure 5. In this episode

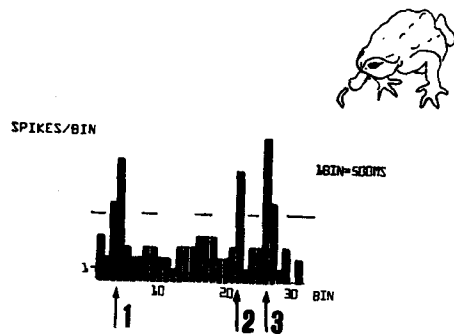


Fig. 7. Frequency time histogram. Spontaneous activity of a behaviorally correlated neuron (group 3) during capture of a mealworm. The frequency increases ( $\uparrow 1$ ,  $\uparrow 2$ ,  $\uparrow 3$ ) in connection with a movement of the toad. Precise temporal correlation between neuronal discharge and behavior is represented only by the interspike frequency time histogram (Fig. 8).

the toad responds to a small prey object, a mealworm, which appears in the visual field with the following sequence of behavioral reactions. Figure 5a,b, the toad sits motionless, the mealworm moves within the visual field; the spike rate increases up to a maximum (*c,d,e*); the toad orients towards the worm, the neuronal activity decreases; *f,g,h,i*, just before the toad thrusts out its tongue, *j*, and snaps at the worm, the spike rate increases again strongly to a maximum; *k,l,m,n*, the toad swallows the prey, the spike rate decreases. A quantitative analysis of a spike train recorded during prey-catching is shown in the frequency time histogram (Figure 7) and in the interspike frequency time histogram (Figure 8). In the same toad the prey catching behavior was elicited several times within a few minutes.

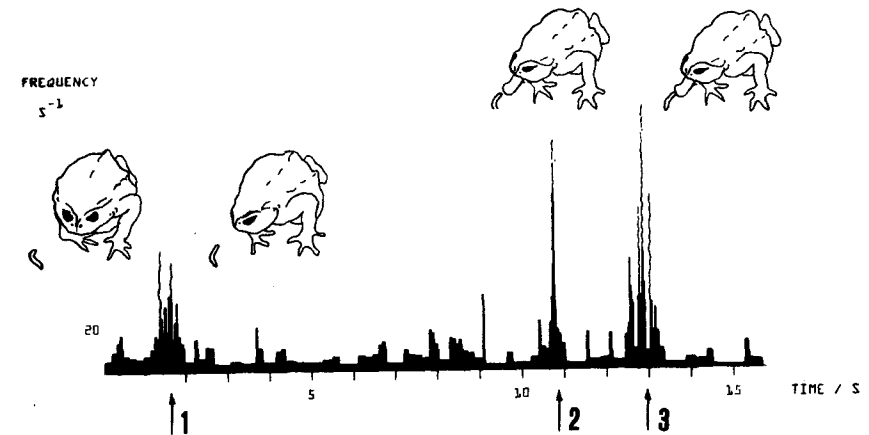


Fig. 8. Interspike frequency time histogram. Spontaneous activity of a behaviorally correlated neuron (group 3) during capture of a mealworm. Just before the toad starts to move, the discharge rate increases ( $\uparrow 1$ ,  $\uparrow 2$ ,  $\uparrow 3$ ). The toad orients (1), snaps unsuccessfully (2) and then successfully (3).

## 5. Overview and discussion

Recording in the immobilized animal allows the identification of neuronal types and their localization in certain brain areas with respect to a list of criteria (Ewert and Borchers, 1971). According to the different response characteristics of the neuron types, preliminary clues on information processing steps such as coding, decision making or decoding are derived in accordance with the toad’s behavior. Systematical quantitative experiments in which static characteristics were measured lead to the hypothesis of additive interactions in neuronal networks as the base of prey catching and avoiding behavior (Ewert, 1974). The collected data are used for computer simulation of the prey-enemy recognition system by means of model

nerve nets (Ewert and von Seelen, 1974; Arbib and Lara, 1981; Lara and Arbib, 1981). The results support the hypothesis.

The investigated neurons in the freely moving toad correspond to those of the following neuronal classes in the retina (ganglion cells, R) and optic tectum (T): R2, R3, R4, T2(1), T4 and T8 are described earlier for the immobilized preparation (Ewert, 1982; Ewert and Borchers, 1971; Grüsser and Grüsser-Cornhels, 1976). The experiments demonstrate that neurons of the retino-tectal projection system have characteristic discharge patterns which can be correlated with the behavior of the animal.

Neurons of group 1 generally exhibit response characteristics comparable to those of corresponding neuron classes in immobilized animals under similar stimulus condition (Ewert and Borchers, 1971). Since toads do not seem to have involuntary saccadic eye movements (for details in frogs see Autrum, 1959) to produce moving retinal images from the static visual environment, the question as to whether there are eye movements by which a stationary stimulus pattern can be transformed into a moving pattern (Schipperheyn, 1973) is of special interest. The normal respiratory movements of the buccal cavity are insufficient to elicit an activation of the investigated neurons. However, stronger eye movements correlated with deeper breathing or general motor activity produce displacements of retinal images, which are sufficient to activate these neurons.

In some of the neurons—those of group 2—the response to a visual stimulus is absent during certain behavioral patterns, such as eyelid closure. In this case the absence of spikes might be caused by efferent commands to the retina (v. Holst and Mittelstaedt, 1950; Maturana, 1958; Johnstone and Mark, 1971; Byzov and Utina, 1971; Miles and Rogers, 1972; Ewert and Borchers, 1974; Borchers and Ewert, 1978; Tasaki *et al.*, 1978). Together with the command for the movement (eyelid closure) possibly an 'efference copy' is transmitted to inhibit the neuronal response.

In the third group of neurons, showing spontaneous activity, a change in the discharge rate generally precedes the motor activity. We do not know the precise location of these neurons in the neural substrate of sensori-motor interfacing or whether they are a part of it at all. Their function is as yet unknown. It is possible that they receive the information about each imminent movement and then serve to converge the inputs back to an appropriate locus in the sensory system (Johnstone and Mark, 1971; Wurtz and Goldberg, 1972). Since the response of the neurons precedes and so to say predicts the movement, it may be that these neurons precedes and so to say predicts the movement, it may be that these neurons are related to elements of a command system (Larimer, 1976; Pearson, 1976; Kupferman and Weiss, 1978; Miles and Evarts, 1979).

The analysis shows, that the neurons respond with greater increase of spike frequencies in connection with sudden and fast movements like snapping. Smaller increases of frequency are correlated with walking, stalking and turning. The question of whether there are certain motor patterns tightly coupled with corresponding 'fixed' discharge patterns as is shown in Figure 9 for a phase of a turning

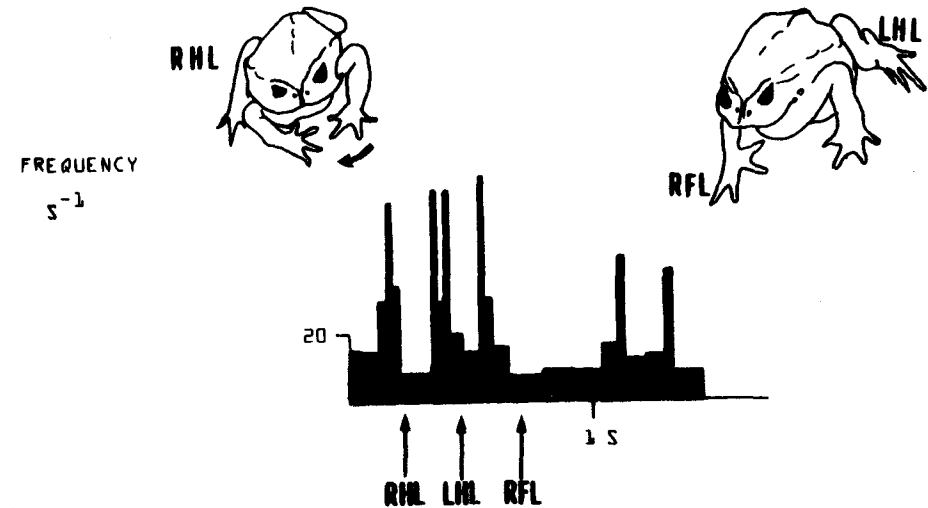


Fig. 9. Interspike frequency time histogram. Spontaneous activity of a behaviorally correlated neuron (group 3) during turning. The discharge rate increases just before the toad moves its right hind-leg (RHL), left hind-leg (LHL), and right fore-leg (RFL).

movement, cannot be answered at this time. Repeated prey-catching experiments show common features in the discharge patterns (Figure 10). However, since each experiment reflects a new situation one cannot speak of exact reproducibility.

There are many approaches for analyzing neuronal spike trains (Gerstein and Ridley, 1960; Rodieck *et al.*, 1962; Poggio and Viernstein, 1964; Hyvärinen, 1966; Moore *et al.*, 1966; Segundo *et al.*, 1966; Grey, 1967; Holmes and Houching, 1967; Perkel *et al.*, 1967a,b; Gerstein and Perkel, 1962; Eckhorn *et al.*, 1967; Glaser and Ruchkin, 1976; Holden, 1976; MacPherson and Aldridge, 1976; Aertsen *et al.*, 1979). The question of correlation between stimulus, neuronal discharge and behavior of the toad forces one to consider temporal relationship of each spike rather than the average frequency of that particular neuron. Mean values of spike rates or averaged activity of several sweeps mask the information. Each recording occurs in a different experimental situation in which stimulus parameters may have changed, e.g. by previous movements of the animal. It is nearly impossible to create exactly reproducible situations which allow a direct comparison among neuronal discharges in relation to corresponding behavioral

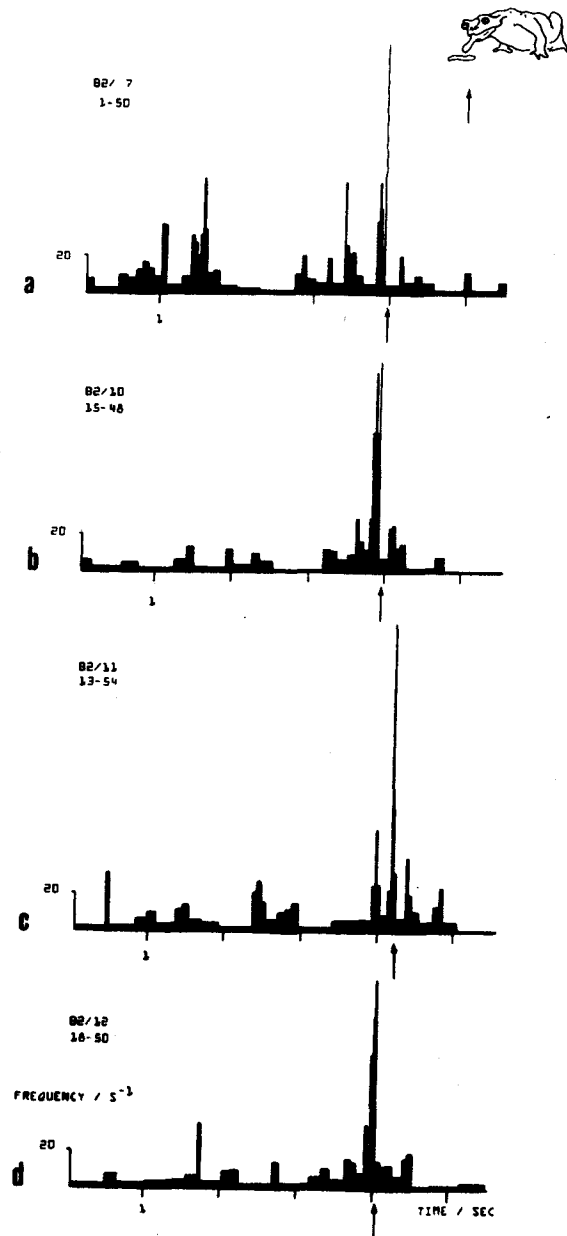


Fig. 10. Interspike frequency time histogram. Spontaneous activity of a behaviorally correlated neuron (group 3). The toad snaps successfully at a mealworm, four times (a, b, c, d) within a few minutes. Each time before the toad thrusts out its tongue (↑) the discharge rate increases to a maximum.

processes, such as snapping at a worm (Figure 10). Neither is it possible to check neuronal responses for 'identity'. Especially important is the neuronal activity occurring immediately before and during the behavioral response. A preliminary convenient method is one that reflects the time course without reducing the information and emphasizes transient changes as is with the interspike frequency time histogram.

The progress of investigations on neuronal information processing is closely coupled to the advances of recording technique. There are some points in the above described recording arrangement that could be improved for certain experiments. The electrical signals are conducted by wires between the animal and the oscilloscope. In order to avoid the encumbrance caused by the cables such as twisting, which results from continuous turning movements and to enlarge the range within which the toad is able to move, e.g. to pursue natural prey, a wireless radiotelemetric system is desirable. Preliminary steps in this direction are encouraging (Borchers and Pinkwart, 1982).

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