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A Biophysical Analysis Of The Functional Neural Unit Of The Olfactory Bulb

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A BIOPHYSICAL ANALYSIS OF THE FUNCTIONAL NEURAL UNIT OF THE OLFACTORY BULB

by

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TO JANIS, ALWAYS

ABSTRACT

The object of this research was to determine if groups of interacting neurons could form a functional unit of the olfactory bulb.

Using glass microelectrodes, extracellular recordings were obtained from spontaneously active neurons in the olfactory bulb of the frog. These neurons were identified as secondary olfactory neurons by histological and electrophysiological controls.

Statistical analysis of the spontaneous activity indicated that in all but one neuron the activity was random in the Poisson sense, and was the result of two underlying generating processes. In the exceptional instance the spontaneous activity was extremely regular and was shown to be the result of a single underlying generating process. The spontaneous activity and its generating processes have been shown to be of bulbar origin, independent of receptor influx or input from higher brain centres. It has been argued that the two generating processes are a within-the-cell generator and a process due to interaction of neighbouring secondary neurons with the sampled secondary neuron.

The response of secondary neurons to stimuli were

investigated by means of electrical stimulation of the olfactory nerve (which was shown to result in the same response patterns in secondary neurons as natural stimulation). It has been shown that there is an extremely long-lasting and widespread inhibition generated in the secondary neurons following nerve shocks. This inhibition has been assigned to the action of entire groups of inhibitory neurons. It has been suggested further that these groups of inhibitory neurons participate in the control of the spontaneous activity as well.

In some neurons, following the period of inhibition a transient period of rapid rebound firing occurred. It has been demonstrated that during this period of rapid firing, excitatory influences on the secondary neurons swamp any inhibitory influences. Furthermore, it has been shown that the latency of this rapid firing decreases and its magnitude (measured as the number of spikes) increases with increasing stimulus magnitude. The period of rapid rebound firing has been assigned to excitatory interactions mediated to the sampled secondary neuron by the same secondary neurons responsible for the outside-the-cell generator of spontaneous activity.

In the light of the experimental evidence a model has been proposed in which the functional element of the olfactory bulb of the frog is the sampled secondary neuron plus the neighbouring secondary neurons and the inhibitory neurons which can affect it. That is, it is proposed that

such interactive groups of neurons are responsible for activity both in the spontaneous and in the post-stimulus modes. This model is a unifying concept for the function of the secondary neurons, for not only can it explain the experimental observations reported in this thesis, but also it can provide new insights into possible mechanisms by which the secondary neurons may encode receptor activity.

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CHAPTER I

GENERAL REVIEW

1.1 Statement of the Thesis

As technology has advanced, electrophysiologists interested in the nervous system have been able to investigate a given problem at the level of a single neuron, rather than being restricted to inferences derived about neurons from gross potentials. In spite of this advancement, however, many questions remain unanswered for central neurons. For example it is known, that many central neurons exhibit spontaneous activity, but the role of such activity is unclear. Furthermore, the simple frequency codes often used by first-order neurons are not necessarily used by central neurons. For example, Poggio and Viernstein (1964), in a study of neuronal impulse sequences in thalamic neurons, conclude that "it appears that thalamic neurons may generate codes more complex than a simple frequency code, which is often taken as the only parameter for the study of the activity of single units." It does not appear necessary to this author, therefore, that the single neuron, while being the anatomical unit of the nervous system, be considered the functional unit

as well.

It is the intent of this thesis to show that interacting groups of central neurons can form a functional unit. That is not to say that several neurons interact in generating the response to a stimulus. What is being proposed is that groups of neurons exist in an interactive state in both the pre- and post-stimulus conditions. The means for this study has been single-neuron recordings, since they constitute an undeniably powerful technique. It is in the analysis and interpretation of such recordings that we may demonstrate interactive neuronal groups.

The system chosen for this research was the olfactory bulb of the frog. As Shepherd (1970) has discussed, the olfactory bulb may be considered a model cortical system. Furthermore it is a brain structure rich in complex cellular interconnections. For this reason one is drawn to this system as one in which many-neuron functional groups might be demonstrated. The structure is also ideally suited for several other reasons:

a) as with many other central neurons, the output neurons of the bulb (the secondary neurons of the olfactory system) have been reported to be spontaneously active in the frog (Döving, 1964, 1966). Spontaneous activity is an important requirement of the system chosen for examination. In order to detect neuronal interactions in the pre-stimulus state, one needs some endogenous

activity in that state. By analysis of such activity via statistical techniques, one may glean a great deal of information about neuronal interactions in the pre-stimulus state.

b) the input pathways to the olfactory bulb, the olfactory nerves, are easily accessible. Consequently the interactions of the neurons in the post-stimulus state may be picked by the convenient technique of electrical stimulation of the olfactory nerve.

c) since the olfactory bulb is situated at the rostral end of the brain, transection of the forebrain just behind the bulb may be used to remove any influence of centrifugal fibres. Thus one can determine that the effects seen are due to the bulbar nervous system itself, and are not due to the rest of the brain.

Thus the olfactory bulb provides an extremely useful example of a central neuronal system. To study this system, extracellular recordings have been used. This technique is particularly amenable to the statistical analysis of the spontaneous activity, since it allows one to obtain long-term recordings without cellular damage. While intracellular recording techniques might be expected to yield additional supplementary information, obtaining such recordings is a prohibitive task. Shepherd (1970) has stated that "intracellular recordings are exceedingly difficult to

obtain from the relatively small mitral cell and, when they are obtained, the membrane potential is low and deteriorates rapidly." Since short-term recordings would have been of little use to this study, intracellular techniques were not used.

1.2 Structure of the Review

This thesis is concerned with a biophysical model of certain aspects of the olfactory bulbar neural system. However, as research reported on this system in the literature is almost exclusively neurophysiological in nature, a few words on the content of the review, as viewed biophysically, are in order.

In terms of electrophysiological research, olfaction has been a relative late-comer to sensory physiology. Nonetheless, quite a substantial literature dealing with sensory discrimination already exists, beginning notably with the pioneering work of Adrian (1942, 1953). However, this area of neurophysiology does not pertain to the mainstream of this thesis, and for this reason, no review of such studies is provided here. The excellent reviews by Ottoson (1963) and Beets (1970) provide thorough accounts of such research.

On the other hand, some neurophysiological studies have been directed towards an elucidation of the neuronal interactions within the olfactory bulb. An understanding of these studies is obviously necessary for an appreciation of

this thesis. To present these studies as conveniently as possible, the review has been structured as follows. In this chapter an overall view of the pertinent neurophysiological literature is provided, along with a description of the anatomy of the olfactory bulb and a review of the statistical methodology used in the present study. The second chapter of the thesis comprises a detailed review of neurophysiological studies of the olfactory bulb. We begin here with the anatomy of the olfactory bulb.

1.3 Review of the Anatomy of the Olfactory Bulb

In reviewing the comparative anatomy of the olfactory system in the vertebrates, Allison (1953) remarked that the olfactory bulb remains quite constant in morphology and microscopic structure throughout the various forms. Consequently the discussion here will be of the anatomy of the olfactory bulb of the frog, noting, where they exist, the distinctions between this structure and that of mammals.

The histological structure of the olfactory bulb of anurans has been investigated by Ramón y Cajal (1922), and is summarized in Figures 1A, B, and C. In addition to the above mentioned work by Allison, there exists a more recent review by Nieuwenhuys (1967). The latter review has been followed closely in this discussion.

The olfactory bulb is situated at the anterior end of the forebrain mass. For the purposes of discussion it is perhaps convenient to consider the olfactory bulb to be

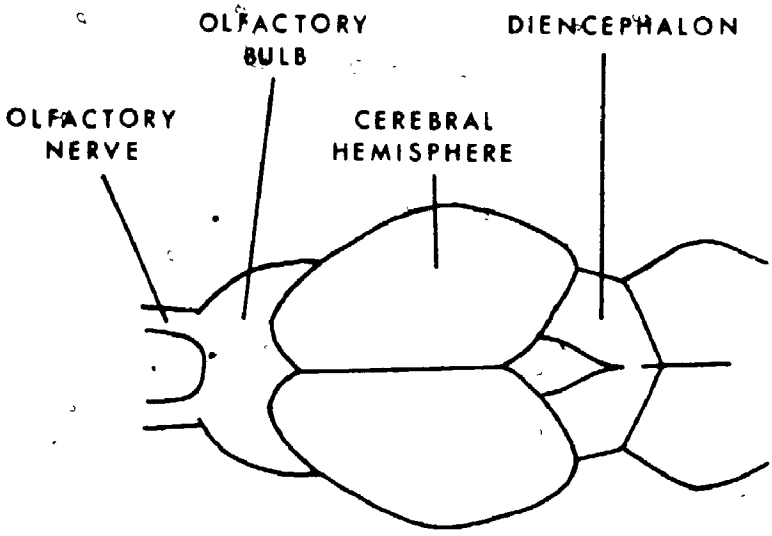
FIGURE 1A

The Forebrain of the Frog

FIGURE 1B

Cut-away View of the Olfactory Bulb

This figure illustrates the gross histological organization of the olfactory bulb. The inner, periventricular region of granule cells has been represented by dots. This region is completely enclosed, except caudally, by a sphere of secondary neurons several cells thick (this has been represented by triangles). Finally, external to the secondary neurons and the plexiform layer is the spherical distribution of basket-like glomerular synapses, indicated by the circular arrays of dots.


OLFACTORY BULB
DIENCEPHALON
OLFACTORY NERVE
CEREBRAL HEMISPHERE

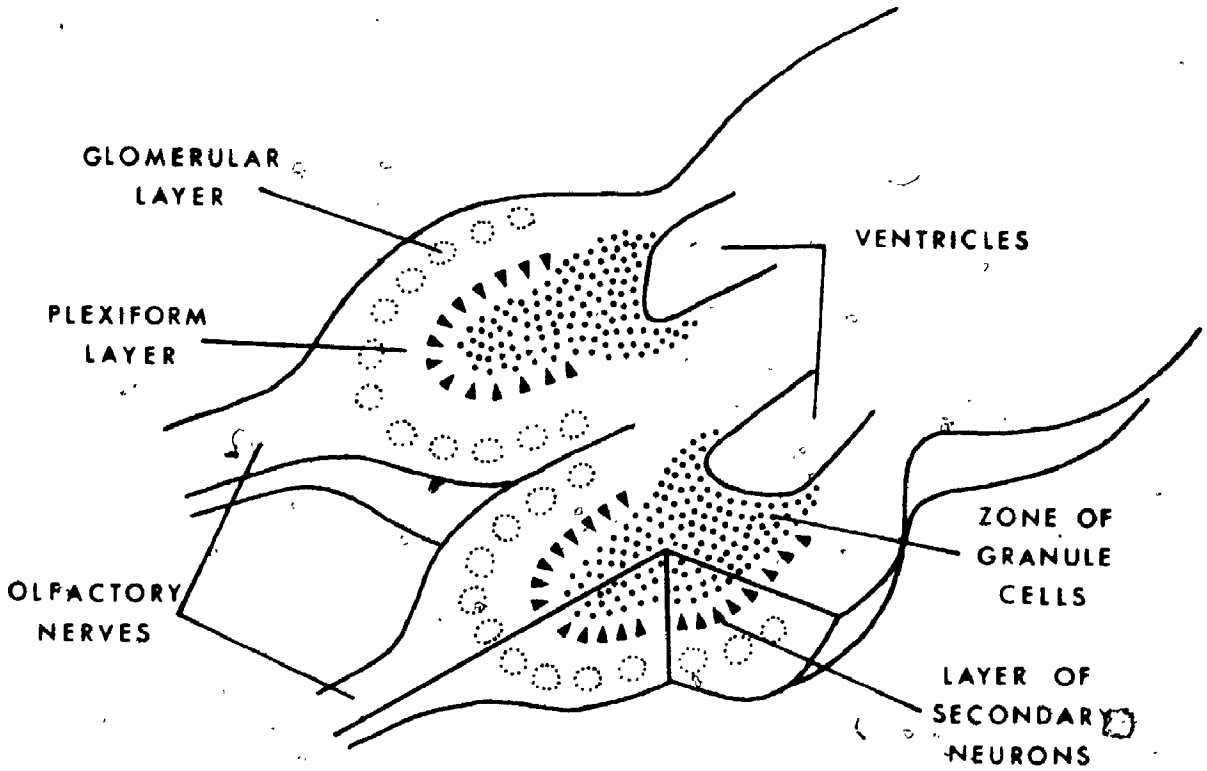

GLOMERULAR LAYER
VENTRICLES
PLEXIFORM LAYER
ZONE OF GRANULE CELLS
OLFACTORY NERVES
LAYER OF SECONDARY NEURONS

FIGURE 1C

Cellular Organization of the Olfactory Bulb

gs - glomerular synapse

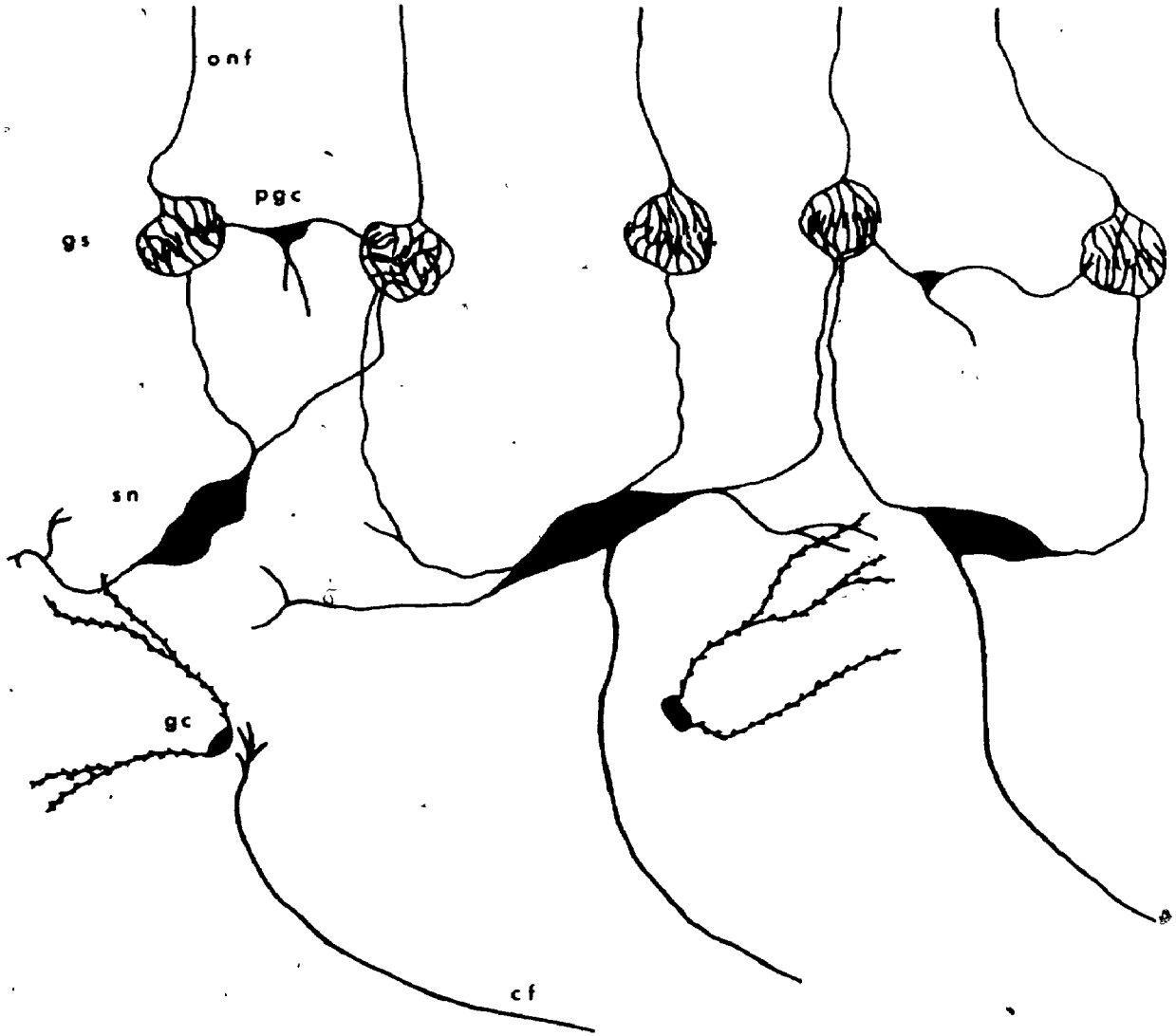
pgc - periglomerular cell

sn - secondary neuron

gc - granule cell

cf - centrifugal fibre

onf - olfactory nerve fibre



IMPLANTATION LAYER OF OLFACTORY NERVE FIBRES

GLOMERULAR LAYER

PLEXIFORM LAYER

LAYER OF SECONDARY NEURONS

LAYER OF GRANULE CELLS

divisible into layers, as follows:

a) an implantation region of olfactory nerve fibres,

b) a glomerular layer,

c) a plexiform layer,

d) a layer of secondary neurons, and,

e) a periventricular zone of closely packed granular cells.

The olfactory nerve fibres, which are the axons of the olfactory receptor cells, spread out over the surface of the bulb and terminate in spherical synaptic structures, the olfactory glomeruli. Here the olfactory nerve fibres break up into terminal fibrils.

The large secondary neurons of the bulb give off coarse spreading dendrites, most of which synapse with the incoming olfactory nerve fibres in the glomeruli. One secondary neuron may contact several glomeruli via several dendrites. Some of the dendritic branches of the secondary neurons ramify in the bulb without contacting the glomeruli. The axons of the secondary neurons cross the granular layer and form the olfactory projections to higher centres. Through the granular layer the axons of the secondary neurons give off collaterals which ramify among the granule cells and probably contribute to the neuropil of the external plexiform layer.

Periglomerular cells are found scattered throughout the glomerular layer. Their tangentially spreading dendrites

appear to interconnect with the glomeruli.

The small granule cells which comprise the inner layer of the bulb have a few widely spreading long dendrites which extend into the plexiform layer, but no axons. Synapses do occur within the granule layer where mitral cell collaterals and bulbopetal fibres of unknown origin ramify and terminate, some of them enmeshing granule cell bodies.

There are several distinctions worth noting between the olfactory bulb of the frog and that of mammals. In the mammalian bulb there are two distinct types of secondary neurons, the mitral and the tufted cells, each of which relates synaptically, via a single primary dendrite, to a single glomerulus (although one glomerulus may relate to more than one secondary neuron). These secondary neurons, of course, still have accessory dendrites not terminating in glomeruli, as in anurans. There is a differentiation between the mitral and the tufted cells in terms of their projections to higher centres, the axons of mitral cells gathering deep in the bulb and proceeding to the surface together to form the lateral olfactory tract on the surface of the prepyriform cortex, while tufted cell axons may end in the anterior olfactory nucleus (Powell, Cowan, and Raisman, 1965). The mammalian olfactory bulb is known to receive centrifugal fibres of two types - the thick and thin fibre systems (Ramón y Cajal, 1955). The thin fibres travel in the anterior limb of the anterior commissure, while many of the thick fibres reportedly run in the lateral olfactory tract (Powell and

Cowan, 1963).

1.4 Brief Review of Neurophysiological Studies

In this section a brief review of the neurophysiological studies which have led to the present understanding of the bulbar nervous system is presented. While the following chapter contains an in-depth discussion of these studies, the reader contented with the detail presented here may omit the following chapter without any real loss of understanding.

1.4.1 Gross Potential Recordings

Gerard and Young (1937) first reported that a preparation consisting of isolated olfactory bulb and cerebral hemispheres generated spontaneous electric potentials, and these workers concluded that the bulbar neurons were spontaneously active. From studies of the slow potential generated at the bulbar surface in response to afferent inflow Ottoson (1959a, b, c) showed that afferent inflow caused a slow potential change in the glomeruli with superimposed regular waves. These regular waves were assigned to synchronous activation of the secondary neurons.

1.4.2 Microelectrode Recordings in the Mammalian Olfactory Bulb

While the foundations were laid by such early studies of surface potentials, more complete understanding awaited singe-neuron recordings with microelectrodes. Many of the workers who first accomplished such recordings

reported that the mitral cells were inhibited following natural stimulation of the mucosa (Yamamoto, 1961; Mancia, von Baumgarten, and Green, 1962) or following electrical stimulation of the lateral olfactory tract (Green, Mancia, and von Baumgarten, 1962). However, a clear exposition of the inhibitory phenomenon awaited the work of Phillips, Powell, and Shepherd (1963). These workers investigated the inhibition generated in mitral cells following lateral olfactory tract shocks. They assigned the inhibition to pathways involving interneurons activated by mitral cell recurrent axon collaterals. Subsequently, however, on the basis of field potential analysis and electron microscopic evidence, Shepherd and co-workers assigned the inhibition to dendrodendritic synapses between mitral and granule cells (Rall, Shepherd, Reese, and Brightman, 1966; Rall and Shepherd, 1968). Nicoll (1969) substantiated this assignment with further electrophysiological studies.

There is little agreement in the literature as to the source of spontaneous activity in the mammalian bulb. Thus Yamamoto (1961) reported spontaneously active mitral cells in the rabbit, Mancia et al. (1962) and Green et al. (1962) reported all neuron types to be spontaneously active in the rabbit, and Shepherd (1963b) claimed that spontaneous activity in the rabbit bulb was most evident where periglomerular cells predominate. Yamamoto and Iwama (1962) have reported intracellular recordings from mitral cells of the rabbit olfactory bulb which show a fluctuating membrane

potential with spike generation occurring whenever a critical level of depolarization was attained.

The results of recordings from granule cells are also unclear. Green et al. (1962) report lateral olfactory tract shocks produced an inhibition of all cell types. Shepherd (1963b), however, reported that orthodromic and antidromic volleys could excite cells in the granule layer.

Nicoll (1971) studied the secondary neurons of the rabbit bulb. From these studies he has implicated the secondary neurons in mediating recurrent excitation to other secondary neurons following orthodromic and antidromic electrical stimulation.

1.4.3 Microelectrode Recordings in the Frog Olfactory Bulb

While there is no agreement as to the source of spontaneous activity in the mammalian bulb, studies in the frog have shown that the secondary neurons are spontaneously active (Döving, 1964, 1966). There has been no investigation of this activity. Studies of these neurons, as cited above, have been concerned with the response to natural stimulation. Such studies have shown that natural stimulation can enhance or inhibit the spontaneous activity, with the latter being the most usual response.

1.4.4 Centrifugal Control of the Bulbar Nervous System

In gross potential recordings, Kerr and Hagbarth (1955) in the cat and Takagi (1962) in the frog and toad have

demonstrated that the centrifugal system can suppress afferent induced activity in the bulb. However, with microelectrode recordings of single neurons, Mancia, Green, and von Baumgarten (1962) found that reticular stimulation could influence the response of bulbar neurons to the point of converting inhibition to excitation, and vice versa. Döving (1966a) and Döving and Hyvärinen (1969) both demonstrated that the efferent system in the burbot could exert both excitatory and inhibitory influences upon the secondary neurons.

1.5 Review of Statistical Analysis of Neuronal Spike Data

While the subject of statistical analysis may seem to be one which is foreign to a review chapter, the argument for its exclusion is mitigated, if not countered, by the fact that statistical analysis has become an area of neurobiology in its own right. Associated with it is the adaptation of certain techniques to neuronal spike trains, and an extensive literature encompassing the application of these techniques to the elucidation of various neuronal processes. A survey of fundamental work in this area thus seems justified. Much of what we shall discuss is due to the efforts of Gerstein and his co-workers (Gerstein, 1960; Gerstein and Kiang, 1960; Rodieck, Kiang, and Gerstein, 1962).

We begin by considering a single neuron whose electrical activity has been monitored continuously for some period of time. If we assume that the action potentials are

instantaneous and indistinguishable events, then the information contained in the record of the electrical activity is embodied solely in the set of times of spike occurrences t_0, t_1, t_2, ..., t_n, or, equivalently, in the set of intervals representing the times between the spikes, t_1-t_0, t_2-t_1, t_3-t_2, ..., t_n-t_{n-1}. As with any raw data, the analysis performed on this data is based on the assumption that the values comprise a random sample from an ensemble of theoretically possible samples. For spike trains in particular the implication is that the observed train is a single representation of a particular stochastic point process; the process is stochastic by virtue of the random variations in the interspike intervals and it is a point process since we have assumed instantaneity and indistinguishability for the individual spike events. Stochastic processes may be stationary or nonstationary. In a stationary process the underlying probability distributions, which have been sampled by the observed intervals, do not depend on the time of observation. In this review we shall discuss the techniques for the analysis of single neuron spike trains under stationary conditions. Such techniques, then, would apply to a treatment of spontaneous activity or of activity evoked uniformly in response to a constant stimulus.

Obviously, since the assumption of a stationary process lies at the basis of the statistical measures to be discussed, some criterion of stationarity must be used to accept or reject records for analysis. Unfortunately, a

completely satisfactory criterion does not exist for finite spike trains. For example, constancy of the mean rate is a necessary but not sufficient requirement. Happily the statistical measurements are not too sensitive to small nonstationarities, at least for reasonable sample sizes, and large nonstationarities are obvious. Simple visual inspection of the spike train is, then, a not unreasonable check (Moore, Perkel, and Segundo, 1966).

The lowest levels of interspike interval analysis do not depend upon the ordering of the observed intervals. Thus as a first consideration we have the various parameters of the probability distribution of the intervals, as estimated by the sample. The most important parameter is the mean interval. The reciprocal of the mean interval, the mean rate, has been used as a measure of neuronal response, as for example, in the work of Burns, Heron, and Pritchard (1962) on the visual cortex, but it does not represent a very powerful index.

Considerably more information regarding neuronal events may be gleaned from the interspike interval histogram, which plots the number of times an interval occurs versus the length of that interval. Thus it serves as statistical estimator of the underlying probability distribution of the intervals. Such histograms were first used by Brink, Bronk, and Larrabee (1946) in describing the activity in nerve fibres, and subsequently by Buller, Nichols, and Strom (1953) and by Hagiwara (1954) in describing the behaviour of peripheral

sensory nerves. They have seen use as a test for randomness or unpredictability of firing time for miniature end plate potentials (Fatt and Katz, 1952), for spinal interneurons (Hunt and Kuno, 1959), and for spontaneously active Betz cells in the cerebral cortex (Martin and Branch, 1958). Smith and Smith (1965) used the interspike interval histogram to demonstrate the presence of two processes generating the spontaneous activity in cortical neurons. The main value of the interval histogram lies in the information it provides concerning randomness of firing and the recovery of excitability.

To extend the analysis of interspike intervals to account for serial dependence among the intervals, two approaches are useful, one involving the use of higher order intervals, the other using the joint distribution of pairs of intervals.

If one defines, as above, the times between consecutive spikes as first order intervals, the times between a spike and the second following spike as second order intervals, and so on, then one may define a density which specifies the probability of encountering a spike as a function of time after a given spike, irrespective of the number of intervening spikes, if any. This density is then the histogram of all intervals of all orders and is frequently called the autocorrelation. It is not necessary to deal explicitly with these higher order intervals, and the autocorrelation is perhaps more easily understood if one considers the spike train to be a signal of zero amplitude

everywhere except where a spike occurs, where it is unity. The autocorrelation for a spike train thus corresponds to that defined for continuous signals. The autocorrelation function has been used by various workers in examining neural activity, sometimes under different names. Thus Poggio and Viernstein (1964), in their examination of thalamic somatic sensory neurons, call it the expectation density, Lamarre and Raynauld (1965), in a study of cortical neurons, call it the post-firing interval distribution, while Heino, Hyvärinen, and Sovijarvi (1970), in a study of rate changes in spontaneous neural discharge, use the term intensity function. Similarities between the autocorrelogram for the neural point process and that for model cases can provide information about the neural process.

As mentioned above, serial dependence among intervals may also be treated by considering the joint probability distributions of pairs of intervals, either adjacent pairs or pairs separated by intervening intervals. Rodieck, Kiang, and Gerstein (1962) introduced the scatter diagram representation of the joint interval density for adjacent intervals.

When two spike trains are observed within a single record, one frequently wishes to determine whether the two trains are independent. A convenient measure for this is the cross correlation density, given by

$$\chi_{AB}(t)dt =$$

$$\text{prob}\{\text{ spike in B in } (t,t+dt) \mid \text{ spike in A at 0 }\},$$

where A and B refer to the two spike trains. The flatness of this measure is a test of independence.

When a single neuron is subject to stimulation, the measure of stimulus effect can be established from a histogram of the times of spike occurrence following the presentation of a stimulus. This measure, known as a post-stimulus-time histogram, is essentially a cross-correlation between the train of stimulus presentations and the train of spikes.

The use of statistical methods to analyze neural data has seen increasing prominence in neurobiological literature. A more complete account of the use of statistical analysis, and of the various models of neuronal activity to which it has led, is contained in the review by Moore, Perkel, and Segundo (1966). A somewhat more mathematical presentation of the statistical techniques is to be found in Appendix 1.

CHAPTER II

DETAILED REVIEW OF NEUROPHYSIOLOGICAL STUDIES OF THE OLFACTORY BULB

2.1 Review of Electrophysiological Studies of the Olfactory Bulb

For the purposes of this review we have chosen to partition the discussion of electrophysiological studies, somewhat artificially, into two sections. The first section deals with investigations of the bulbar nervous system itself, its background activity and its response to orthodromic and antidromic activation, while the discussion of centrifugal control of bulbar neurons has been restricted, as far as possible, to the second section.

2.1.1 Gross Potential Recordings

An interest in the electrical activity of the olfactory bulb was kindled by the report of Gerard and Young (1937), who obtained gross recordings from various parts of the brain of the frog. They reported that spontaneous electrical potentials recorded from the olfactory bulb were dramatic in terms of their amplitude as compared to those recorded from other parts of the brain. The potentials were found to persist in isolated hemisphere-olfactory bulb

preparations. Gerard and Young attributed their potential waves to the action in concert of the cell mass, and concluded that the neurons must manifest a rhythmic electrical beat in the absence of afferent nerve input.

Similarly Adrian (1950) reported that the mammalian olfactory bulb generated a spontaneous or intrinsic wave activity accompanied by persistent irregular discharge of axon spikes in deeper layers of the bulb. Adrian found that this activity remained after destruction of the olfactory epithelium and after sectioning of nervous connections between the bulb and forebrain. He supposed this activity originated in cells other than mitral cells, possibly the granule cells. Adrian further reported that stimulation resulted in the intrinsic waves being replaced by what he called induced waves, which were assigned to the synchronization of a number of units by stimulation.

Ottoson (1954) demonstrated that in addition to periodic oscillatory potentials (the induced waves of Adrian), the olfactory bulb also generated a sustained surface potential in response to olfactory stimuli. Further investigation in the rabbit (Ottoson, 1959a) demonstrated that the waves could be dissociated from the slow potential, indicating that these potentials probably arose from different structures. Studies in the frog using natural stimulation (Ottoson, 1959b) showed that the slow potential was negative and had its largest amplitude at the entrance zone of the olfactory nerve fibres, while close to the cerebral hemisphere it was small and

positive. This finding suggested the slow potential was synaptic in nature, occurring in the glomeruli. Further support for this localization came from the observation that shocks to the brain in the area just rostral to the optic lobe blocked the induced waves during olfactory stimulation but left the slow potential unaltered. Whether these shocks gave rise to antidromic activation of secondary olfactory pathways or to orthodromic activation of centrifugal fibres, the results suggested that the induced waves were due to activity in secondary neurons while the slow potential was probably pre-synaptic. Using electrical stimulation applied to the olfactory epithelium of the frog, Ottoson (1959c) again found that a resolution of the bulbar response into two components was possible, the first component most probably being a synaptic potential arising in the glomeruli, the second being ascribed to propagated activity in secondary olfactory neurons. While in both studies in the frog (that is, using natural and electrical stimulation) the post-synaptic component could be safely identified as such, there was no definite evidence that the pre-synaptic component of the bulbar response did not have some contribution from post-synaptic neurons (for example, as from dendrites of secondary neurons which are not invaded by antidromic impulses). Nonetheless, these results enabled Ottoson to suggest that the following events occurred in the bulb on stimulation. The afferent inflow to the bulb was said to result in the production of a slow potential change in the glomeruli. As this slow potential became larger, an increasing number of

secondary neurons were activated synchronously, this synchronous activity manifesting itself in gross recordings as regular waves superimposed upon the slow potential. Iwase, Uruha, and Ochi (1961) assigned the components of the potential recorded from the rabbit bulb to the action potential of olfactory nerve fibres and the dendrites of mitral and tufted cells.

Yamamoto (1961) found that electrical stimulation of the olfactory mucosa in the rabbit evoked a monophasic surface-negative potential in the bulb which, however, could be seen to have a second deflection in it at higher stimulus strengths. Repetitive stimulation of the anterior commissure suppressed not only this evoked potential but also the intrinsic wave activity of the bulb. On this basis it was concluded that the evoked potential due to olfactory mucosa stimulation occurred post-synaptically in the bulb. By monitoring this evoked potential at various depths in the bulb, Yamamoto concluded that it was due to depolarization in the distal portion of the dendrites of the secondary olfactory neurons.

Orrego (1961) found, in the turtle, that a single shock to the olfactory nerve evoked a complex bulbar surface potential consisting of four waves. The first three components of this slow potential were assigned to activity in olfactory nerve fibres, to activity in intra-glomerular presynaptic fibres and to synaptic potentials at the tips of the apical dendrites of the mitral cells, and to depolariza-

tion of the basal dendrites and cell bodies of the mitral cells, respectively. This potential wave complex was followed by a quiet period with a subsequent rebirth of activity responsible for the fourth wave. Spontaneous activity which was observed was assigned to the granule cells.

Orrego also observed a long lasting depression (10-15 sec.) in the piriform cortex following a response to maximal stimulation of the olfactory nerve. Two loci in the pathway were found to be responsible for this depression. The first, which is incidental to this discussion, was post-activity depression in the glomeruli. The second, however, was ascribed to a true inhibitory pathway occurring via recurrent collaterals from the mitral cell axon feeding back to granule cells, these latter cells contacting the basal dendrites of mitral cells. This recurrent pathway was said to occur in mitral cells other than the cell of origin, thereby functioning as a means to restrict activity in mitral cells surrounding the one activated by incoming olfactory nerve impulses.

Obviously the foundations for an understanding of neuronal mechanisms in the bulb have been laid by these early studies. The limited resolution available in gross electrical recordings none the less allowed inferences to be drawn concerning the spontaneous activity observed in the bulb and the pathways for excitation of the secondary neurons by olfactory nerve input. There was also the demonstration of an inhibition occurring in the bulb following the response to

stimulation. Further clarification of bulbar mechanisms was to come from studies at the level of the single neuron, as made possible by microelectrode techniques.

2.1.2 Microelectrode Recordings in the Mammalian Olfactory Bulb

Yamamoto (1961) recorded unitary activity, extracellularly, from cells in the olfactory bulb of the rabbit. He identified these cells as mitral cells on the basis of antidromic activation following electrical stimulation of the lateral olfactory tract. Among the unitary response patterns seen with mucosal stimulation was the inhibition of spontaneous discharges. Yamamoto suggested that this inhibition could occur in two ways,

a) via inhibitory synapses between olfactory nerve fibres and mitral cell dendrites (although he acknowledged that no histological evidence indicated such synapses), or,

b) by some inhibitory interaction between the secondary olfactory neurons through a neuronal network in the bulb.

Mancia, von Baumgarten, and Green (1962) recorded unitary activity extracellularly from neurons in the olfactory bulb of the rabbit. They reported that spontaneously active neurons could be recorded in all layers of the bulb. Mitral, tufted, and granule cells could be identified histologically and physiologically, and it was found that each of these cell

types could exhibit a variety of responses when the olfactory mucosa was stimulated with various odourous substances. Thus cells of each type were found which exhibited facilitation, or inhibition, or combinations of these two, on stimulation.

Green, Mancia, and von Baumgarten (1962) obtained extracellular recordings from neurons in the olfactory bulb of the rabbit with electrical stimulation of the lateral olfactory tract. To assure that the effects they saw were solely due to antidromic activation of mitral cell axons, they carried out similar experiments on chronic preparations, in which the olfactory peduncle had been transected and time allowed for any fibres running to the bulb in the lateral olfactory tract to degenerate. They identified the neurons as mitral cells, tufted cells, or granule cells by carrying out histological controls on a portion of the neurons studied.

Recording in the layer of mitral cell bodies, they found that single shocks to the lateral olfactory tract resulted in invasion of the mitral cells by antidromic impulses with a constant latency between 0.5 and 2 msec. The antidromic action potential usually was seen to follow the first shock to the lateral olfactory tract but sometimes failed to follow every shock. As the rate of stimulation was increased, however, the ability to follow each shock generally improved, cells often following rates over 100/sec. and occasionally up to 400/sec. Beyond rates of 400/sec. the cells did not follow. As the rate of stimulation was increased, it was also observed that cells could exhibit alternating period of following and failing. Such following and failing also was seen after

reduction of stimulus frequency following a period of lateral olfactory tract tetanization (which the cell could follow more regularly).

In the external plexiform layer Green et al. observed cells which could be driven by lateral olfactory tract stimulation. These cells usually required several repeated shocks before they fired and then their response was apparently a single firing. Their latency of firing was reported to be longer (4-10 msec.) and less constant than that for the antidromically driven mitral cells, and they did not follow rates of stimulation above 50/sec. These cells were presumed to be tufted cells activated by recurrent collaterals from mitral cells.

Single shocks to the lateral olfactory tract were seen to cause a pause of 35-150 msec. (usually about 100 msec.) in the spontaneous firing pattern of many cells, including mitral, tufted, and granule cells. In cells which were normally activated by lateral olfactory tract stimulation, this pause could be seen even when the cell failed to follow the lateral olfactory tract shock. Repetitive stimulation at low frequency was also seen to cause an inhibition. High frequency tetanization of the lateral olfactory tract at rates from 50/sec. to 700/sec. blocked the firing of the majority of spontaneously active neurons in the olfactory bulb. Since the mitral cells did not follow antidromic stimulation beyond about 400/sec., the inhibitory effect did not require that the mitral cell body be discharged. Again in the case of the

mitral cells, the inhibition sometimes affected cells not antidromically invaded by the stimulus used. Repeated tetanizations could produce a cumulative effect, with the inhibition lasting seconds.

In accounting for the inhibition observed in the mitral cells, these workers eliminated the possibility that the refractory period plays a role on the grounds that mitral cells could follow stimulation frequencies up to 400/sec. Also in many cases a pause occurred even when antidromic invasion of these cells failed to occur. They argued further that the latency of onset of the inhibition was too short to allow for the two synapses required were an interneuron involved, at least in the early phases of the inhibition. That the inhibition might be sustained by the discharge of an interneuron, in analogy to Renshaw inhibition of the motoneuron (Renshaw, 1941; 1946; Eccles, Fatt, and Koketsu, 1954), was considered, but they report that they were unable to find such interneurons. The only synaptically driven cells they found were the presumed tufted cells which seldom fired repetitively in response to lateral olfactory tract shocks. Furthermore, these synaptically fired cells could not follow stimulation at frequencies greater than 50/sec., while the inhibitory effect on spontaneous firing was usually more pronounced at higher frequencies. These facts militated against the synaptically activated cells acting as interneurons in general. These workers allow, however, that the presumed tufted cells may contribute to mitral cell inhibition at low

frequencies of lateral olfactory tract stimulation.

To account for the inhibition Green et al. favour the view that axon collaterals of mitral cells produce a direct inhibition without an interneuron. As evidence supporting this concept they cited the short latency of onset of the inhibition, the occurrence of the inhibition at frequencies of lateral olfactory tract stimulation so high that it would be unexpected for an interneuron to follow for any length of time, the massive nature of the inhibition involving essentially all cell types in the bulb, thereby tending to preclude the existence of a suitable interneuron, and the prolonged time of inhibition which suggested the accumulation of an inhibitory substance rather than the sustained discharge of an interneuron.

Yamamoto and Iwama (1962) report obtaining some intracellular recordings from mitral cells in the rabbit olfactory bulb. They observed that the membrane potential of the mitral cells, identified by their response to lateral olfactory tract stimulation, showed a continuous fluctuation of several millivolts, with a spike occurring when the fluctuation reached a critical level of depolarization. While in some mitral cells the response to lateral olfactory tract stimulation was observed to be an antidromic spike followed simply by a small after-hyperpolarization, in others the antidromic spike was seen to be succeeded by a slow hyperpolarization. This potential was assigned as an inhibitory post-synaptic potential (IPSP); Yamamoto and Iwama

proposed that this IPSP was generated by a direct connection between recurrent axon collaterals and secondary dendrites of the mitral cells.

Quite a complete study of the mitral cells in the olfactory bulb of the rabbit was undertaken by Phillips, Powell, and Shepherd (1963) and by Shepherd (1963a,b). By correlating the potential wave response in the bulb to lateral olfactory tract shocks with the position of the tip of the recording microelectrode in the histological laminae, these workers were able to localize the unitary activity they encountered (Phillips et al., 1961, 1963). Thus single unitary spikes obtained at or near the mitral cell body layer in response to a shock to the lateral olfactory tract were assigned to mitral cells. Such extracellular spikes were reported to be similar to the giant extracellular spikes encountered in other neurons of the central nervous system, as, for example, those in cerebellar Purkinje cells (Granit and Phillips, 1956) and in Betz cells (Phillips, 1959), and were considered to register the invasion of mitral cells by antidromic impulses, in concurrence with previous workers. Some of the spikes exhibited an inflexion on the positive-going phase which was seen to be accentuated in the response to a testing shock delivered shortly after a conditioning shock. This fragmentation into two components was considered as arising from the successive invasion by the antidromic impulse of two regions of excitable membrane in the mitral cell, the A and B membranes of Fuortes, Frank, and Becker (1957).

Using such paired conditioning and testing shocks to the lateral olfactory tract Phillips et al. (1963) found that blockage of the antidromic invasion of the B membrane occurred if the testing shock was applied early after the conditioning shock. This blockage was assigned to the refractoriness of the B membrane. Any later impairment of A-B transmission, however, was considered as possibly due to synaptic inhibition of the mitral cell due to impulses in other "neighbouring" axons that were stimulated concurrently in the lateral olfactory tract. It was found that conditioning shocks that were too weak to excite the sampled mitral cell could produce a blockage of the order of a hundred milliseconds of antidromic invasion following suprathreshold testing shocks. Furthermore, the latency of onset, the duration, and the intensity of the blockage depended on the strength of the conditioning stimulus, in other words, on the number of "neighbouring" axons which were recruited by the conditioning stimulus. On the basis of the above evidence and some brief intracellular studies, Phillips et al. assigned the suppression of mitral cells to an inhibitory post-synaptic potential produced across the mitral cell membrane. The relatively long latency of onset of the suppression, from a minimum of about 3 msec. to over 50 msec., suggested to these authors that the effect was mediated over synaptic pathways involving interneurons activated by mitral cell recurrent collaterals, in analogy with the hyperpolarization observed in Betz cells following stimulation of the pyramidal tract

(Phillips, 1959). This is in contrast to the direct connection between recurrent axon collaterals and secondary dendrites of mitral cells, as was inferred by Yamamoto and Iwama (1962).

In studying orthodromic activation of mitral cells by olfactory nerve volleys, Shepherd (1963a) reported that the mitral cells usually discharged a single impulse in response to each nerve volley. Using an antidromic impulse excited by a shock to the lateral olfactory tract to test the state of the mitral cell, Shepherd observed that in many mitral cells shocks to the olfactory nerve filaments caused a suppression of excitability. This was true for shocks both supra- and subthreshold for the sampled cells, and in the case where these conditioning shocks were suprathreshold the membrane had recovered from its refractoriness before the onset of the long-lasting suppression of excitability. The properties and time course of this suppression were similar to those of the suppression induced by antidromic volleys in the lateral olfactory tract. An enhancement of the amplitude of testing antidromic impulses was also seen. These features of the extracellular recordings from mitral cells supported the view that the suppression of excitability following either orthodromic or antidromic volleys was due to a hyperpolarization of the mitral cells.

Shepherd (1963b) subsequently recorded a variety of discharges with extracellular micropipettes from cells which he identified, again, on the basis of localization of the recording tip within the histological laminae, as

periglomerular cells, granule cells, and tufted cells.

In units tentatively identified as periglomerular cells the response to olfactory nerve volleys tended to be either one or two impulses or a burst of impulses. Following the first spike in each burst there was a diminution of amplitude in succeeding spikes which was graded in intensity with increasing shock strengths. Similar burst responses had been seen in cerebellar Purkinje cells (Granit and Phillips, 1956) and in hippocampal pyramidal cells (Kandel, Spencer, and Brinley, 1961) and in both these cases intracellular recordings showed that the diminution of spike amplitude coincided with a wave of depolarization of the cell membrane so intense that it could ultimately cause an inactivation of the impulse generating mechanisms. Such a depolarization had been seen by Yamamoto and Iwama (1962) in cells of the olfactory bulb, and is consistent with Shepherd's findings.

Orthodromic volleys in the olfactory nerves and antidromic volleys in the lateral olfactory tract could both excite cells in the plexiform layer and in the granule layer. Those in the plexiform layer were identified as tufted cells, those in the granule layer as granule cells. Presumed tufted cells were seen to fire singly while granule cells were more diverse, some firing singly, some repetitively to each volley.

According to this work, spontaneous activity was most evident in the region where periglomerular cells predominate. Apparently the activity was usually of variable frequency and could be interrupted by either orthodromic or

antidromic volleys. The interruption was reported to last up to several hundred milliseconds and often to be followed by rebound firing at higher frequencies for about another hundred milliseconds. Usually mitral cells were found to be silent, and granule cells exhibiting a resting activity were apparently infrequently encountered.

The results indicated no definite relation between the latencies of presumed periglomerular cells and the latencies of mitral cells. However, this could be expected since the latencies of mitral cells themselves varied considerably. Since the suppression of mitral cell excitability following an olfactory nerve volley was very similar to that caused by an antidromic volley in the lateral olfactory tract, it seemed unlikely that periglomerular cells participate in the generation of this depression. Furthermore, the apparent spontaneous activity in periglomerular cells was seen to be suppressed by antidromic volleys; periglomerular cells could not be responsible for the suppression of mitral cell activity when they themselves were suppressed by antidromic activation.

Since both presumed granule cells and tufted cells were activated by both orthodromic and antidromic volleys, it seemed that they could be implicated in the suppression of mitral cell activity. The average latency of response of the granule cells to lateral olfactory tract stimulation was longer than the latencies observed for antidromic responses in mitral cells and comparable to the minimum latency for the

suppression of mitral cell excitability produced by such stimuli. This was support for the hypothesis that granule cells are excited by impulses in axon collaterals of mitral cells and in turn cause a suppression of the discharge of mitral cells by their connections with the mitral cell secondary dendrites. Shepherd pointed out, however, that trains of impulses in the granule layer never lasted longer than 30 msec., not long enough to account for the observed length of suppression of mitral cell excitability.

Yamamoto, Yamamoto, and Iwama (1963) obtained intracellular recordings from mitral cells and from deep layer cells (granule cells and various short-axon cells). They, too, identified the mitral cells by their response to lateral olfactory tract stimulation and by localization of the recording tip on the basis of the field potential generated in the bulb in response to a single shock to the lateral olfactory tract.

They found that a single shock to the lateral olfactory tract generated an action potential which sometimes could be seen to be separable into two components (the A and B spikes), as reported by Phillips et al. (1963) and Shepherd (1963a, b). These antidromic spikes were seen to be followed by a prolonged hyperpolarization. In experiments in which the lateral olfactory tract shock was subthreshold for the sampled axon, a hyperpolarization was still observed, indicating that it was not an after hyperpolarization but an IPSP induced, according to these workers, via axon collaterals of

mitral cells neighbouring to the sampled mitral cell. The authors suggest that the generation of the IPSP could be due to interneurons which are excited rhythmically by a single shock to the lateral olfactory tract and whose processes make inhibitory synapses with the mitral cells.

The deep layer cells responded to lateral olfactory tract stimulation with a single spike or a train of spikes superimposed on a prolonged depolarization. This observation, plus the observation that the response to such a shock could occur at latencies shorter than that for the antidromically-induced IPSP in the mitral cells, led Yamamoto et al. to conclude, as had other workers, that at least some of the deep layer cells participate in the generation of the inhibition. Orthodromic activation by electrical stimulation applied to the olfactory mucosa produced corroborative results.

A modification of ideas concerning mitral cell inhibition was to come from an analysis of the bulbar field potential in response to lateral olfactory tract shocks. The field potential (which is simply a summed electrical response from the several cell types and recorded at various depths in the bulb with a microelectrode) had been characterized by various workers (von Baumgarten, Green, and Mancia, 1962a; Ochi, 1963; Phillips, Powell, and Shepherd, 1963). Analysis of this potential was reported by Rall, Shepherd, Reese, and Brightman (1966) and by Rall and Shepherd (1968). They attributed the first phase of the potential to flow of extracellular current from mitral cell dendrites to mitral

cell bodies during depolarization by action potentials in these cell bodies, and the second phase to flow of extracellular current from repolarizing mitral cell bodies to mitral cell dendrites which have been depolarized (either by passive electrotonic spread or by active impulse invasion from the cell bodies). The third phase of the potential distribution was assigned to the granule cells since it was found this potential distribution could be reconstructed by assuming that there is a strong membrane depolarization of the granule dendrites in the plexiform layer, coupled with an essentially passive membrane in their deeper processes and cell bodies. Since this depolarization of granule cell dendrites would occur in the region of contact with many mitral cell secondary dendrites and at a time just after the mitral dendrites were depolarized, it was postulated by these workers that the granule cell dendrites are depolarized by excitatory synaptic input from mitral cell secondary dendrites. Furthermore, since this phase of the potential distribution corresponds in time with the onset of mitral cell inhibition, depolarization of granule cell dendrites appeared to be well suited as the source of inhibitory synaptic input to mitral cell dendrites.

This designation of granule cell dendrites as the source of mitral inhibition was not based exclusively upon the field potential analysis. Reese and Brightman (1965) had discussed morphological evidence for dendrodendritic synapses between secondary dendrites of mitral cells and granule cell dendrites obtained from electron microscopic studies of the

rat olfactory bulb. These workers found that the granule endings on mitral dendrites differed from typical synaptic endings in that two separate synaptic contacts with opposite polarities were often found juxtaposed in the same ending, thus implying that there was both mitral-to-granule and granule-to-mitral synaptic transmission. Structural differences between the mitral-to-granule and granule-to-mitral contacts had suggested that these two kinds of synaptic contact could be functionally different.

This morphological evidence, along with the results of their field potential analysis, lead Rall and co-workers to postulate the following scheme for suppression of excitability following impulse discharge in the mitral cells of the mammalian olfactory bulb. In lieu of the concept of recurrent axon collaterals of mitral cells activating granule cells as inhibitory interneurons, they proposed that depolarization spreads from the mitral cell bodies into their secondary dendrites, where it activates excitatory synapses which depolarize granule cell dendrites. This depolarization then activates granule-to-mitral inhibitory synapses. They acknowledge that the granule cells could serve as inhibitory interneurons in a more general sense in that their deeper lying processes receive input from several sources.

With intracellular recording in the rabbit, Nicoll (1969) also observed a hyperpolarization following antidromic invasion of mitral cells due to shocks to the lateral olfactory tract, and he too suggested it was due to an IPSP

across the mitral cell membrane. The latency of this IPSP was sufficient time for at least two synaptic delays. He further demonstrated that a conditioning stimulus to the lateral olfactory tract would lead to a reduction in the IPSP recorded in response to a subsequent test shock to the lateral olfactory tract. Increasing the strength of the conditioning stimulus resulted in an increase in the size of the conditioning IPSP and a decrease in the size of the IPSP evoked by the test shock. As Nicoll pointed out this is consistent with the concept of a dendrodendritic rather than a recurrent pathway for mitral cell inhibition. If a dendrodendritic pathway were responsible for generation of the IPSP, the inhibitory pathway would be blocked by a conditioning lateral olfactory tract stimulus, since the pathway through mitral cell dendrites would be inhibited. In a recurrent collateral pathway, however, the inhibition should not be blocked by a conditioning lateral olfactory tract stimulus as the recurrent collateral pathway would by-pass the site of the inhibition on the mitral cell.

Nicoll also showed that the effect of conditioning lateral olfactory tract shocks on extracellular field potentials was consistent with the dendrodendritic model. It was found that the waves associated with depolarization of the granule dendritic membrane and with inhibitory synaptic currents generated in the dendrites of mitral cells were inhibited by a conditioning lateral olfactory tract stimulus. The wave due to depolarization of the granule dendritic membrane

would be thus inhibited if this depolarization were due to mitral cell dendrites, again because the conditioning IPSP in the mitral cells could inhibit the excitatory pathway from mitral to granule cells. Since the generation of the wave due to inhibitory synaptic currents depended upon depolarization of the granule cell dendrites, both these waves would be suppressed, as observed, if the mechanism were dendrodendritic.

Recording unitary activity in the granular layer, Nicoll found that conditioning orthodromic or antidromic shocks had an inhibitory effect on the response to a test orthodromic shock. Similarly such conditioning shocks inhibited the subsequent response to a test antidromic shock. The inhibitory effect of either conditioning shocks on the test orthodromic response would be expected since impulses in the olfactory nerve can excite neurons in the granular layer only by way of secondary neurons, which are inhibited following the conditioning shocks. However the inhibition of the response to the test antidromic shock following the conditioning shocks would not be predicted if the granular layer cells are assumed to be activated by a recurrent pathway, since inhibition of the mitral cell soma should not block conduction through recurrent collaterals. Thus, Nicoll assigned the responses in the granular layer to granule cells activated by dendrodendritic excitatory synapses between mitral cells and granule cell dendrites. He allowed, in agreement with Rall et al. (1966), that only a small fraction of the total population of granule cell bodies must be activated because the field

potential in response to a lateral olfactory tract shock indicated that the granule cell bodies were predominantly passive.

2.1.3 Microelectrode Recordings in the Frog Olfactory Bulb

Using microelectrodes, Shibuya, Ai, and Takagi (1962) studied discharge patterns of neurons in the olfactory bulb of the frog due to natural stimulation. They found two classes of spike discharge, one in which activity in the neurons was induced by odourous stimulation and the other in which the odourous stimulation modified a previously occurring spontaneous activity. As one might expect, within these two classes they observed a diversity of response patterns. No attempt was made to identify the neurons; but mention was made of the fact that the induced spike discharges were recorded in a relatively shallow layer of the bulb while the spontaneous discharges were generally found deeper. Thus, they assumed a different cell-type origin for the two discharges.

Döving (1964) obtained extracellular records of unit activity in the olfactory bulb of the frog with chemical stimulation of the olfactory mucosa. While no attempts were made to identify the neurons histologically, Döving argued that, since his records were obtained from the anterior portion of the bulb, and since the secondary neurons are more numerous and larger than the periglomerular cells, it was most likely he was recording from the cell soma of the secondary neurons. He reported that all secondary neurons in the bulb

maintain a constant activity in the absence of overt stimulation, some units maintaining a regular rhythmic activity, others firing in bursts at random intervals, and others discharging at a low irregular rate. No further studies were made of this background activity except to report that the patterns of activity remained constant for several hours and that the observed frequency range was from 0.01/sec. to 4/sec.

Natural olfactory stimulation was shown to result either in an augmentation or in an inhibition of the activity of these bulbar neurons, with a small number apparently unaffected. The most common effect, however, was an inhibition of the "spontaneous" activity, frequently followed by an increased activity. With prolonged stimuli, neurons were seen which responded with a maintained discharge or with a discharge of declining frequency. Typically the response to a prolonged stimulus was apparently an initial inhibitory effect preceding the discharge, this discharge then being maintained for considerable time after cessation of stimulation.

As possible sources for the inhibition which was obtained in most of the units, Döving offered the following possibilities:

a) if the spontaneous activity in the bulb were a result of peripherel influx from the receptors, the inhibition could be due to a block of the receptor activity by stimulation. However, Gesteland, Lettvin, and Pitts (1963) had shown some receptors are excited, some unaffected and

some inhibited by odourous stimuli, and it seemed unlikely that the small number of inhibited receptors could be responsible for the large number of inhibited bulbar units,

b) the result of an inhibitory interaction between the neurons in the bulb, or,

c) due to centrifugal fibres from higher centres.

2.1.4 Summary

Shepherd (1970) has revisited the experimental evidence gathered by himself and others on the mammalian bulb. He suggests that the granule cells serve as general interneurons, mediating inhibition to tufted and glomerular cells as well as to mitral cells. Whatever synaptic mechanisms are involved in mediating inhibition to these former cells, the inhibition of mitral cells in the mammalian bulb via dendrodendritic synapses with granule cells seems a well established concept. Indeed such a mechanism is supported by unitary recordings (both intracellular and extracellular), by analysis of the bulbar field potential, and by electron microscopic evidence. Furthermore, such a mechanism can account for the potential oscillations observed by early workers in gross recordings from the bulb, and for the fact that spikes in granule cells have not been generally reported.

The actions of glomerular and tufted cells remain uncertain, although Nicoll (1971) has implicated the recurrent collaterals of secondary neurons, both mitral and tufted cells, in a different process, namely, that of mediating recurrent

excitation directly between other secondary neurons.

Research into neuronal mechanisms in the olfactory bulb of the frog has been extremely limited. Suggestions as to origins of the components of the bulbar surface potential, and postulates put forward to explain the responses of bulbar neurons to odourous stimuli, constitute the complete story to date.

2.2 Review of Extra-Bulbar Control of Olfactory Bulbar Neurons

Central control of sensory systems has come to be studied only recently in electrophysiology. Thus the early concept which assumed impulses from sensory systems were relayed to the cerebral cortex for integration has been supplanted by a recognition that higher centres can modify the transmission of sensory information at many levels in the afferent pathway.

2.2.1 Gross Recordings

Electrophysiologically speaking, a recognition of the influence of centrifugal fibres on the olfactory bulb was apparent from the work of Arduini and Moruzzi (1953), who demonstrated that electrical stimulation of the intralaminar thalamic nuclei could affect the electrical activity recorded from the olfactory bulb of the cat.

Subsequently Kerr and Hagbarth (1955), again in recordings from the cat, showed that stimulation of the basal rhinencephalic area produced a depression of afferent-induced activity in the contralateral bulb. High frequency stimulation

of the anterior commissure was seen to cause a similar depression - Kerr and Hagbarth ascribed these effects to an activation of centrifugal fibres terminating on granule cells. They claimed that the influence of these centrifugal fibres could be a tonic one, in that they observed animals with poorly synchronized activity during olfactory stimulation in which light central anaesthesia or sectioning of the anterior commissure (both of which block the centrifugal influence) resulted in increased synchronization and augmentation of the amplitude of olfactory responses.

Walsh (1959) reported that electrical stimulation of the contralateral bulb evoked a non-propagating potential. This evoked potential was assigned as arising from post-synaptic potentials of granular cells activated by tufted cell processes from the contralateral bulb.

Kerr (1960) investigated the gross electrical activity evoked in the olfactory bulb of the cat by stimulation of the anterior commissure and the lateral olfactory tract. The potentials thus evoked were different in configuration and behaviour; those due to commissural stimulation were seen to summate with repetitive stimulation while those arising from lateral olfactory tract activation did not. Furthermore, bulbar responses evoked by lateral olfactory tract stimulation were depressed by continuous high frequency stimulation of the anterior commissure, while the reciprocal situation did not obtain. The responses due to lateral olfactory tract stimulation were assigned to antidromic mitral cell potentials

or to synaptic effects due to the putative mitral cell recurrent collateral system. The potentials evoked by stimulation of the commissure were said to occur as a result of activation of efferent fibres. The origin of the potential was considered to be either the granule cells themselves or, in view of the fact that they summated, the mitral cell dendrites under the influence of the granule cells, in analogy with the observations of Clare and Bishop (1955) on cortical apical dendrites.

Takagi (1962) studied the centrifugal system to the bulb in the frog and the toad. He found that electrical stimulation of the anterior surface of the diencephalon induced a wave in the olfactory bulb which could normally depress for several seconds the appearance of the wave due to electrical stimulation of the olfactory mucosa. In general agreement with previous workers, Takagi assigned the phenomenon to an activation of centrifugal fibres which synapse on granule cells, these granule cells then affecting the activity of mitral and tufted cells.

On the basis of electrophysiological studies, Takagi traced the centrifugal system from an origin in the anterior diencephalon through the cerebrum. Fibres from the diencephalon of one side were reported to run bilaterally to the bulb. Takagi further claimed that the centripetal fibres take a similar course in the cerebrum, with some of them running as far as the anterior diencephalon. Thus, stimulation of the anterior diencephalon should result not only in activa-

tion of centrifugal fibres but also in some antidromic activation. Takagi claimed, however, that the antidromic component plays a minor role.

2.2.2 Unitary Recordings in the Mammalian Bulb

Mancia, Green, and von Baumgarten (1962) examined the role played by the extra-bulbar system at the level of a single neuron. They observed that mitral, tufted, and granule cells could be modified by high frequency stimulation of the midbrain reticular formation, this modification usually taking the form of an inhibition of spontaneous unitary activity. Perhaps the most interesting observation made by these workers was that reticular stimulation could influence the response of bulbar neurons to natural olfactory stimulation, even to the extent of converting inhibition to excitation, and vice versa.

Subsequently, von Baumgarten, Green, and Mancia (1962b) investigated the effects of antidromic stimulation of commissural fibres on mitral, tufted, and granule cells. Their rationale was the similarity of mitral and tufted cells, both of which give off numerous axon collaterals. Von Baumgarten et al. felt that, just as it was thought the collaterals of mitral cells generated the inhibition observed in the bulb with lateral olfactory tract stimulation, so too might tufted cells, activated antidromically by shocks to the anterior commissure, generate a recurrent effect. Recording extracellularly in the olfactory bulb of the rabbit they found that antidromic impulses evoked by single shock stimulation of the anterior commissure failed to invade the soma of tufted cells, and also

failed to give rise to orthodromic firing of granule cells. Such shocks did, however, produce a pause in the spontaneous firing of a majority of mitral, tufted, and granular cells, while repetitive stimulation of the commissural fibres produced a sustained inhibition. These observations were made both in intact preparations and in chronic preparations in which degeneration of any fibres running to the bulb in the anterior commissure had occurred. Since invasion of tufted cells was not seen, von Baumgarten et al. proposed that inhibition of neurons of the olfactory bulb due to stimulation of axons in the anterior limb of the anterior commissure arose via a direct pathway through recurrent collaterals, just as it was then thought that the inhibition of these neurons by purely antidromic stimulation of the lateral olfactory tract occurred via direct-inhibition through axon collaterals. It should perhaps be included as a post-script here that tufted cell axons may not enter the anterior commissure (Lohman, 1963).

Yamamoto, Yamamoto, and Iwama (1963) observed, in intracellular records obtained from the rabbit, that stimulation of the anterior commissure could produce an IPSP in mitral cells, with an increasing number of shocks apparently summing to yield an IPSP of increasing magnitude and duration. The antidromic spike and subsequent hyperpolarization of mitral cells due to lateral olfactory tract stimulation could be blocked by preceding commissural stimulation. Most of the deep layer cells were observed to be depressed by stimulation of the anterior commissure, while, with extracellular

electrodes, a few interneurons activated by commissural stimulation were observed. As a scheme to account for their observations, Yamamoto et al. proposed that a group of interneurons (which they did not specify) activated by stimulation of the anterior commissure mediated inhibition both to the mitral cells themselves as well as to any recurrent pathway which might be involved in mitral cell inhibition.

2.2.3 Centrifugal System to the Olfactory Bulb of the Burbot

Clarification of the role of the olfactory efferent system seemed promising in studies carried out on the burbot, in which the olfactory tracts, contrary to those of other vertebrates, are not in close spatial relationship to other parts of the forebrain but are separate and accessible (Döving and Gemme, 1963, 1965). They still contain both efferent and afferent fibres, however.

In a study of the efferent system in the burbot, Döving and Gemme (1966) reported that spontaneous centrifugally propagated impulses could be recorded from single fibres split from the central stump of a transected tract. In addition, evoked efferent activity could be recorded in one tract by stimulation of the contralateral tract, or in the central stump of a partially transected tract with ipsilateral stimulation, demonstrating the existence of both crossed and uncrossed bulbar efferents.

Döving (1966a) carried out a study of the secondary

olfactory neurons of the burbot with efferent effects removed by transection of the olfactory tract. He found a low frequency of background discharge which could be augmented, inhibited, or unaffected by natural stimulation of the olfactory epithelium. Döving subsequently extended the investigation to include an examination of the effect of evoked efferent activity on the secondary neurons (Döving, 1966b). Evoked efferent activity, either crossed or uncrossed, could cause either excitation or inhibition of the spontaneous activity of a bulbar unit. Döving supposed a direct termination of the efferent fibres on the observed bulbar neurons.

Döving and Hyvärinen (1969) studied, by statistical time series analysis, the effect of the efferent system on spontaneous activity, as well as on that evoked by natural stimulation, in the secondary neurons of the burbot. In concurrence with earlier work they reported that the efferent system must exert both excitatory and inhibitory effects on the spontaneous activity of these neurons, and that it could influence the post-stimulus response patterns.

2.2.4 Summary

It is difficult to conclude a discussion of centrifugal control of olfactory bulbar neurons with definitive statements. The lack of progress is, of course, partially due to the difficulty of knowing whether one is observing the effects of the centrifugal fibre system alone, or the effects of this plus some other fibre system. Furthermore, comparisons between studies in the burbot and those in higher animals

should perhaps be made with some hesitation in view of the variance in neuronal interconnections. Thus, Allison (1953) has noted that in fishes, granular cells relate the glomeruli to the secondary paths, while in the amphibians this structural organization has given way to the scheme discussed earlier in this review.

CHAPTER III

METHODS

3.1 Introduction

Unitary recordings were obtained from neurons in the anterior regions of the olfactory bulb of the frog by means of extracellular microelectrodes. Such extracellular techniques were chosen since they allowed minimal disturbance of the observed neuron. The methods used to prepare such electrodes are discussed in this chapter. In addition, the histological techniques and electrophysiological criteria used to determine the location of the recording tip of the microelectrode are presented.

Recordings of spontaneous single-neuron activity were obtained from intact, decentralized, and deafferented preparations, and were analyzed by the statistical techniques reviewed earlier. The general electrophysiological system used is presented, and the methods of analysis (including the computer programs used) are discussed. In addition, further studies were carried out on a portion of the neurons by driving them post-synaptically via electrical stimulation of the olfactory nerve. To verify further that the observed

neurons were secondary neurons, antidromic activation of the neurons was accomplished in a few animals by electrically stimulating the forebrain. The techniques used for this stimulation, and the means by which the data was analyzed, are discussed.

3.2 Preparation of Animals

Grass frogs (Rana pipiens) were immobilized by injections of 0.2 ml. to 0.5 ml. Tubarine (3 mg. tubocurarine chloride/ml.; Burroughs Wellcome and Co.)[1] into the dorsal lymphatic sac. Local anaesthesia of the nerve endings in the skin of the dorsal extent of the head was accomplished by regional infiltration of xylocaine hydrochloride (Astra, Pharmaceutical Division)[2]. In several animals no tubocurarine chloride was administered; with these animals it could be determined that xylocaine infiltration was sufficient to locally anaesthetize the regions in which surgery was performed. A small patch of skin was removed from the dorsal surface of the skull in the region of the underlying olfactory bulbs and olfactory nerves. The bone of the skull was opened and removed over as minimal an area as possible. Where necessary the dura mater was opened or removed to allow placement of the microelectrode and the stimulus electrodes. Occasionally an additional injection

[1] Burroughs Wellcome and Co., Montreal, Quebec, Canada.

[2] Astra, Pharmaceutical Division, Mississauga, Ontario, Canada.

of 0.2 ml. Tubarine was administered to prevent muscular twitchings in the limbs of the animal during the course of the microelectrode recordings. In some animals the possible influence of centrifugal fibres was removed by sectioning the forebrain caudal to the olfactory bulbs. In one preparation the possible influence of peripheral influx from the olfactory receptors was removed by sectioning the olfactory nerves.

3.3 The Electrophysiological Recording System

3.3.1 The Microelectrode System

Accounts of the usefulness and the properties of glass and metal microelectrodes have been given by various authors (Gesteland, Howland, Lettvin, and Pitts, 1959; Robinson, 1968; Schanne, Lavallée, Laprade, and Gagné, 1968). Of the possible electrode systems, the tungsten microelectrode of Hubel (1957) was tried initially. However, both tungsten microelectrodes prepared in our own laboratory after Hubel's method and similar such electrodes obtained commercially (Frederick Haer and Co.)[3], proved to be less than successful in our hands, in spite of their reported use by Döving (1964) to record from the olfactory bulb. As a consequence we chose to employ the electrolyte-filled glass microelectrode, first utilized by Ling and Gerard (1949). While originally designed for intracellular measurements, these microelectrodes have seen increasing use for extracellular measurements in brain tissue.

[3] Frederick Haer and Co., Ann Arbor, Michigan, U.S.A.

Glass microelectrodes were pulled from 1 mm. O.D. pyrex glass capillaries (Walter A. Carveth Ltd.)[4] by means of a vertical micropipette puller (David Kopf Instruments)[5]. Criteria for suitable microelectrodes included a slender taper to a tip diameter estimated, by examination through a microscope, to be less than 1 μm. Tip resistances observed were less than 50 MΩ . The microelectrodes were filled with 0.9% aqueous sodium chloride solution which had been filtered through a 0.22 μm Millipore filter assembly (Millipore Filter Corp.)[6]. This filling was accomplished by a typical reduced pressure boiling technique. Thus, the pipettes were immersed completely, with their tips down, in the 0.9% sodium chloride solution. This solution was then boiled gently at 80° for 3 to 5 minutes at reduced pressure, following which the solution and pipettes were returned slowly to atmospheric pressure. The pipettes were allowed to remain submerged until the temperature had returned to ambient values (pipettes were normally allowed to sit undisturbed for a minimum of 12 hours), thereby allowing any minute gas bubbles within the pipette to dissolve in the solution. The resulting microelectrodes were stored submerged in the filling solution.

Mechanical mounting of the microelectrode was

[4] Walter A. Carveth Ltd., Toronto, Ontario, Canada.

[5] David Kopt Instruments, Tujunga, California, U.S.A.

[6] Millipore Filter Corp., Bedford, Massachusetts, U.S.A.

achieved by means of a commercial electrode holder (Bioelectric Instruments Inc.)[7] which was mounted in a Leitz micromanipulator (E. Leitz)[8]. Within this electrode holder a chlorided silver wire made contact with the electrolyte of the microelectrode through a pool of the same electrolyte. A gold plated junction pin soldered to the silver wire facilitated connection of the microelectrode to the preamplifier.

3.3.2 The Stimulus Apparatus

Stimulus electrodes consisted of two 0.020 inch diamter silver wires which were insulated over the majority of their length with teflon sheaths sealed by epoxy resin. Connection to an isolated stimulator (Isolated Stimulator Mk. IV, Devices Instruments Ltd.)[9] was accomplished by ordinary insulated leads. One of these leads, however, incorporated a 10 kΩ resistor in series. The voltage drop across this resistor could be measured on an oscilloscope (Type 564 Storage Oscilloscope, Tektronix, Inc.)[10], thus allowing the stimulus current to be determined. The terminal portions of both silver wires which would contact the preparation were filed to a smooth taper and lightly chlorided. These two

[7] Bioelectric Instruments Ind., Hastings-on-Hudson, New York, U.S.A.

[8] E. Leitz, Wetzlar, Germany.

[9] Devices Instruments Ltd., Hertfordshire, U.K.

[10] Tektronix, Inc., Beaverton, Oregon, U.S.A.

wires were mounted on a second manipulator (Brinkmann Instruments Inc.)[11]. The isolated stimulator could deliver stimuli of various magnitudes and widths. The stimulus magnitude (current) was measured as above; the widths used were 1 msec, and 5 msec. Repetitive stimulation and conditioning-testing shocks were generated by driving the stimulator with a function generator (Multipurpose VCG Model 116, Wavetek)[12].

3.3.3 The Amplification/Recording System

The area in which the electrophysiological experiments were performed was shielded on three sides by steel sheeting, which was grounded by connecting it to the chassis of the recording equipment. Likewise the preparation was shielded from below by a similarly grounded aluminum plate to which the micromanipulators were rigidly affixed.

The signal from the microelectrode was fed into an A.C.-coupled preamplifier with capacity compensation (Model P-15, Grass Instrument Co.)[13]. The input impedance of this preamplifier was 100 MΩ . The half-amplitude frequency range giving rise to the optimum ratio of spike height to background noise was found to be 100-1000 Hz. The output of this preamplifier was fed into a Tektronix Type 2A61 differential amplifier in a Tektronix Type RM 565 dual beam

[11] Brinkmann Instruments, Inc., Great Neck, New York, U.S.A.

[12] Wavetek, San Diego, California, U.S.A.

[13] Grass Instrument Co., Quincy, Massachusetts, U.S.A.

oscilloscope. The 2A61 amplifier was used in the single ended mode with a frequency response of 60 Hz. to 10 kHz. The signal was then taken from the rear of the oscilloscope and put through a variable-gain D.C. amplifier (Accudata 104, Honeywell Test Instruments Division)[14] in order to boost its magnitude to several volts for convenient recording on magnetic tape, and to drive a speaker system for audio monitoring of the action potentials. The frequency response of this amplifier was D.C. to 10 kHz. The magnetic tape system used was a seven channel Hewlett Parkard Sanborn 3900 Series (Hewlett Packard, Sanborn Division)[15] unit, run at 7½ ips, corresponding to a band width of 0 to 2500 Hz. Time calibration was accomplished by recording a continuous signal from a second function generator (Wavetek Model 144 HF Sweep Generator) coincident with the neural activity but on a separate tape channel. Amplitude calibration for the extracellularly recorded action potentials was accomplished by means of the calibrator circuit incorporated in the preamplifier. Monitoring of the output of the Honeywell Accudata amplifier, to assure that the input to the magnetic tape system was satisfactory, could be carried out with the second beam of the dual beam oscilloscope. All connections between various components of the system were made with coaxial cables, except those from the stimulator to the

[14] Honeywell Test Instruments Division, Denver, Colorado, U.S.A.

[15] Hewlett Packard, Sanborn Division, Don Mills, Ontario, Canada.

stimulus electrodes. The overall electrophysiological system is shown schematically in Figure 2.

3.4 Histological Localization of the Microelectrode

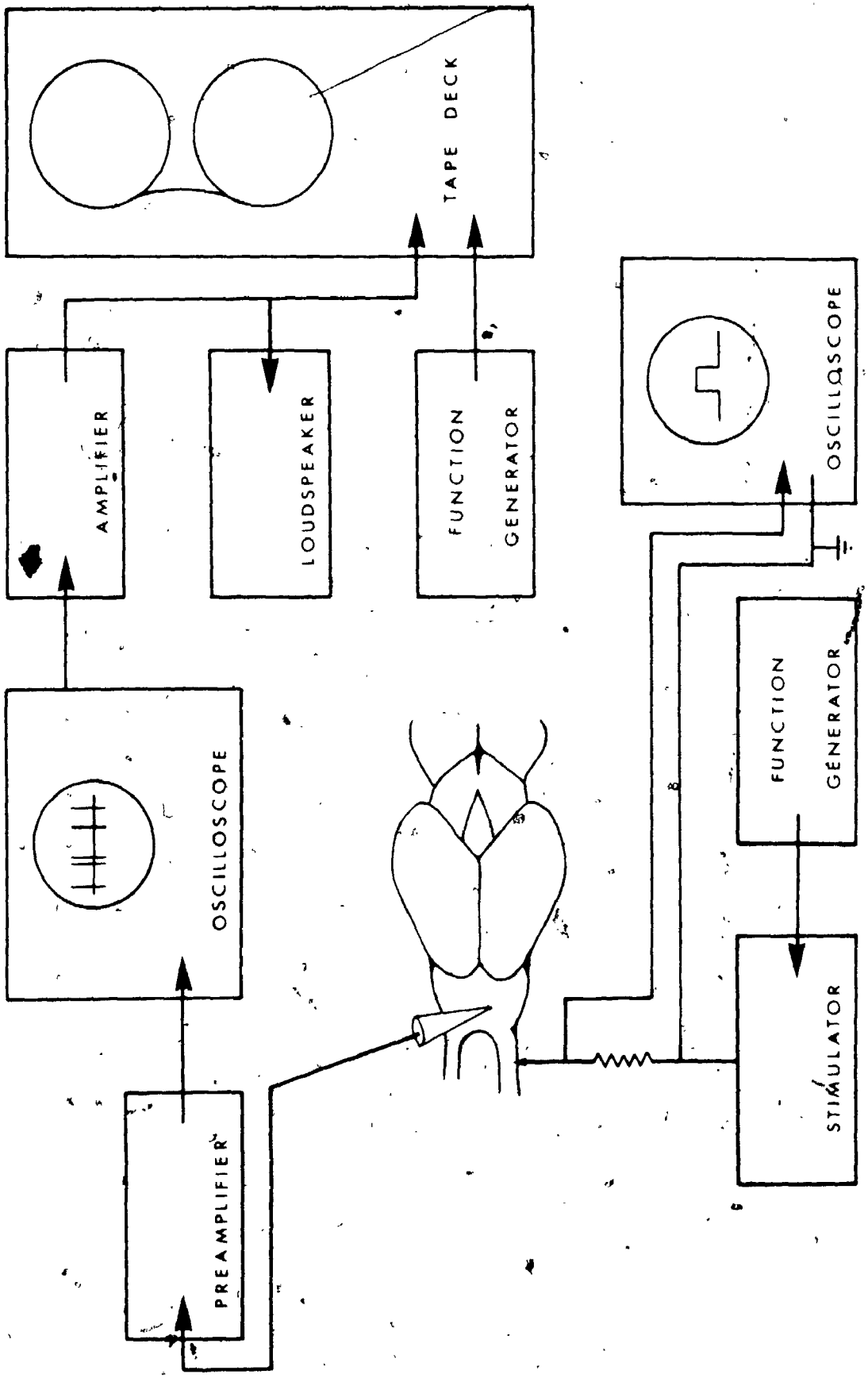
In order to place the identification of the observed spontaneously active neurons in the olfactory bulb on more certain grounds, we carried out localizations of the microelectrode tip in several preparations. Of the many methods reported in the literature for such localization, that of Lee, Mandl, and Stean (1969) was chosen. In brief this method involves the iontophoretic deposition of a quantity of the dye Alcian blue 8GX. The particular advantage of this dye lies in the fact that it does not diffuse away from the site of deposition. Unfortunately the dye will work satisfactorily only with microelectrodes whose tip diameters are larger than 1 μm, since with smaller tips the current densities during extrusion become too high, resulting in heat production and subsequent coagulation of the dye at the tip (Mandl, personal communication). For similar reasons the microelectrodes may not be filled by the reduced-pressure boiling technique. While Lee et al. filled their pipettes by applying pressure from a syringe, the following approach was chosen in this study.

Micropipettes of tip diameters between 1 μm and 5 μm were allowed to stand immersed to the shoulder in a solution of 8% Alcian Blue 8GX (Esbe Laboratory Supplies)[16]

[16] Esbe Laboratory Supplies, Toronto, Ontario, Canada.

FIGURE 2

The Electrophysiological Recording System


TAPE DECK
AMPLIFIER
LOUDSPEAKER
FUNCTION GENERATOR
OSCILLOSCOPE
OSCILLOSCOPE
PREAMPLIFIER
FUNCTION GENERATOR
STIMULATOR

in 0.5 M sodium acetate which had been filtered through a 0.45 m Millipore filter. A period of 24 to 48 hours was sufficient to result in filling of the tip and terminal portions of the pipette with this solution by capillary action. The pipettes were then filled completely by means of a syringe and a 30G hypodermic needle, with a Millipore syringe-fitting filter assembly (0.45 µm) interposed.

Dye extrusion was accomplished after recording activity from a neuron by connecting the microelectrode to a constant current device (Appendix 2). A current of 0.5 µA was passed for 60 seconds, with the microelectrode tip as anode. The entire animal was then fixed in 10% neutral buffered formalin at 4°C, the brain subsequently dissected out, the tissue cleared and dehydrated, and embedded in paraffin. Sections were cut at 7 µm and stained by Vogt's method for nerve cell products (Luna, 1968).

3.5 The Experimental Procedure

Surgically prepared animals were placed on a wooden platform above the aluminum shielding, covered with moist gauze, and connected to the ground terminal of the preamplifier by means of a chlorided silver wire placed in the mouth. Stimulus electrodes were lowered gently into position by means of the micromanipulator system, one electrode being set on the olfactory nerve and the other on the adjacent bone or soft tissue. Under observation through a dissecting microscope the microelectrode was lowered

vertically by means of the second micromanipulator to a position just above the anterior surface of the olfactory bulb. The laboratory room lights were extinguished to avoid the problem of noise pickup from this source. The preamplifier was turned on and the microelectrode lowered further until contact was made with the surface of the brain. The microelectrode subsequently was advanced very slowly into the brain tissue until spontaneously active neurons were encountered. To avoid any effects due to irritation of the neurons by the microelectrodes, only those cells whose activity appeared to be invariant to small movements of the electrode (of the order of a couple of micrometers) were recorded on magnetic tape. The normal routine was to record the spontaneous activity from the neurons for a long enough period to obtain a reasonable sample of this activity (at least several hundred spikes). The effect, on some of these neurons, of electrical stimulation of the olfactory nerve was then examined using single, paired, and repetitive cathodal shocks. In a few cases the forebrain caudal to the olfactory bulbs was stimulated in order to activate the neurons antidromically. Stimulus currents were measured at the end of experiments. Similarly amplitude calibration of the spikes was carried out at this time. To avoid any effect due to odours in the air, the laboratory was kept closed and movement was kept to a minimum. No responses were seen in the spontaneous activity that could be ascribed to odourous stimulation of the mucosa as reported by Döving (1964).

3.6 Analysis of Neural Data

3.6.1 System for Playback and Analysis

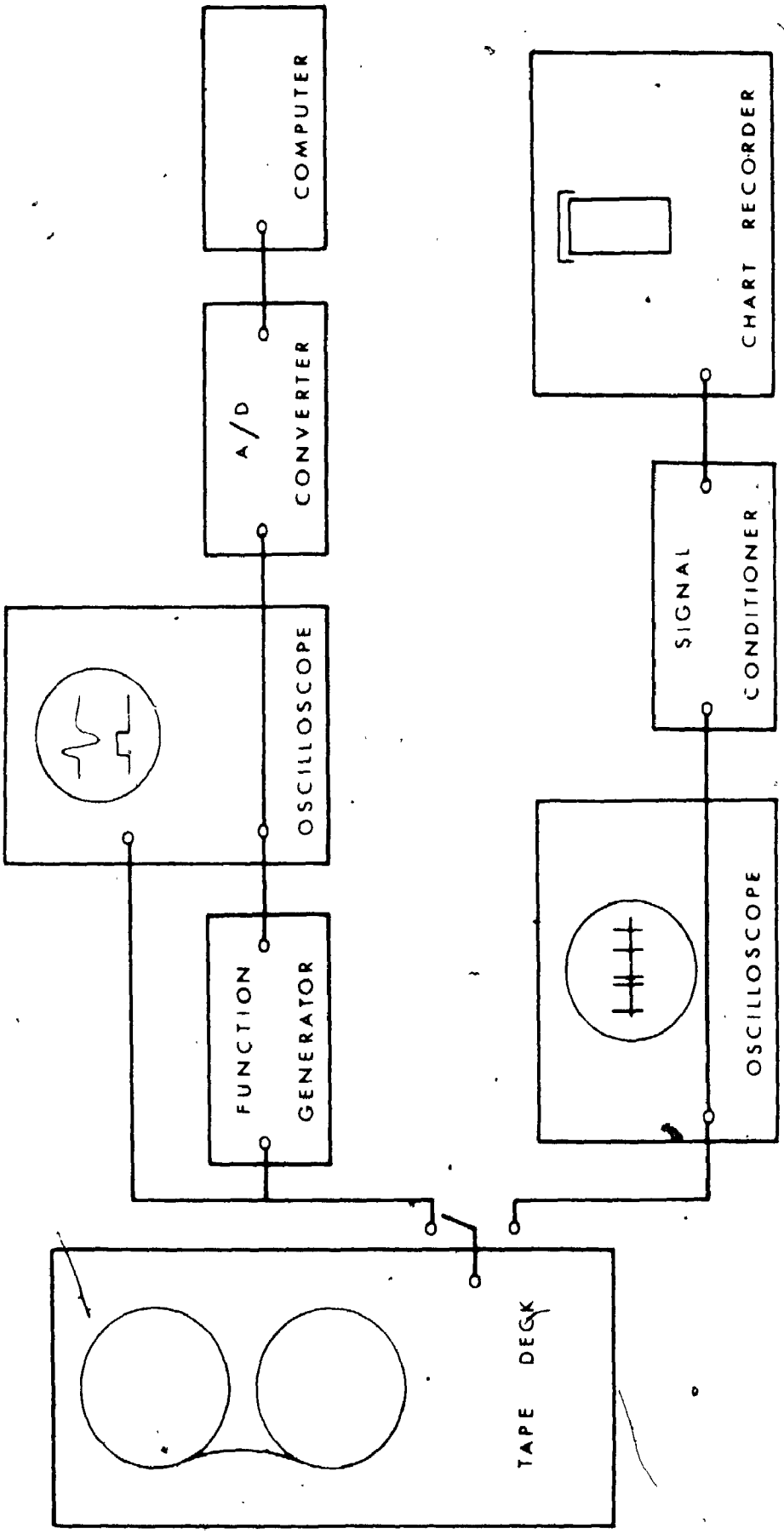
The system for playback and analysis is illustrated in Figure 3. The neural activity recorded on magnetic tape was analyzed in two ways. The spontaneous background activity was analyzed by means of a small computer (Nova 1200, Datagen Canada Ltd.)[17]. Latencies of invasion of the neurons following electrical stimulation were measured directly from the screen of the storage oscilloscope. Paper recordings of both spontaneous and post-stimulus activity were also obtained, however. In the case of spontaneous activity this was done to check for overall stationarity of the spike trains. Stationarity was determined by visual inspection of these paper recordings, the criterion being relative constancy of firing patterns. In addition, there was one record of activity which contained two spontaneously active neurons. Analysis of this record was accomplished from paper recordings. Similar paper recordings of post-stimulus activity were used to assess the overall effect of stimulation upon the neurons. An oscilloscope camera was used to obtain photographs of the extracellular potentials expanded in time and of invasion of the neurons by evoked spikes due to stimulation.

For computer analysis, the action potentials recorded on magnetic tape, now several volts in peak-to-peak

[17] Datagen Canada Ltd., Hull, Quebec, Canada.

FIGURE 3

The System for Playback and Analysis


COMPUTER
A/D CONVERTER
OSCILLOSCOPE
FUNCTION GENERATOR
CHART RECORDER
SIGNAL CONDITIONER
OSCILLOSCOPE
TAPE DECK

amplitude, were played back at the record speed of 7½ ips into one of the function generators (Wavetek Model 144 HF Sweep Generator) set in the triggerable mode. The trigger level of this function generator was set such that one pulse was triggered for each action potential, thus, a simple level detector. Both the signals on the magnetic tape and the resultant triggered pulses were monitored on the storage oscilloscope to ascertain that in fact each action potential did produce one and only one standard pulse. This was important to determine since in some cases the potentials were recorded with an attendant positive after-potential. Were the trigger level not properly established in these cases, the after-potential could result in the appearance of extraneous triggered pulses. The triggered standard pulses were led to an A/D converter (Series AN5800, Analogic Corp.)[18] interfaced with the computer. Computer programs (to be discussed below) were written to determine the lengths of the interspike intervals, and from this data to compute the mean interval, the interval histogram, and the autocorrelogram.

For paper recordings, a fast response chart recorder utilizing ultraviolet light deflected to light sensitive paper by galvanometers was employed (Visicorder Oscillograph Model 2106, Honeywell Test Instruments Division)[19]. Magnetic tapes were played into this chart recorder through

[18] Analogic Corp., Wakefield, Massachusetts, U.S.A.

[19] Honeywell Test Instruments Division, Denver, Colorado, U.S.A.

an amplifier/attenuator system which allowed adjustment of the signal amplitude for convenient paper recording. This playback could be monitored on the dual beam oscilloscope.

3.6.2 Analysis of Background or Spontaneous Activity

Basic to an analysis of the background activity was the measurement of interspike intervals. The computer program which accomplished this was essentially a timing program. (Flow charts for the computer programs are given in Appendix 3.) Each time a pulse corresponding to the occurrence of an action potential was received by the A/D converter, the value in a counter was stored and the timer reset to zero. The program thus generated a series of times corresponding to the interspike intervals. This timing program was calibrated using pulses from a function generator (Wavetek Model 144 HF Sweep Generator); it was capable of resolving pulses with a separation of 2.5 msec., a value far less than the shortest intervals generated by any of the observed neurons. Furthermore, using this program to compare the timing signal on the magnetic tapes with one fed directly into the A/D converter from the function generator, it was found that the record and playback speeds of the tape recorder corresponded within at least 2.5 msec. Thus the intervals measured by the computer could be converted to time in milliseconds, with a resolution of 2.5 msec. These interval values were collected in bins of desired width (between 10 msec. and 500 msec.) to give an interval histogram

for the data, and were further used to obtain the total time of the microelectrode recording and the mean interval. Permanent copies of the interval data were obtained on punched paper tapes generated by the computer. These paper tapes were used subsequently as input for the autocorrelation program.

The manner in which the autocorrelation analysis was performed is indicated in Figure 4. This is easily understood if one recalls that the autocorrelation gives the number of spikes occurring between the times t and $(t + \Delta t)$ after any preceding spike. Once a value has been chosen for the bin width Δt, the first spike of the record is used to define zero time and each bin is incremented by the number of spikes falling within it. The second spike is then used to establish zero time, and then each bin is again incremented by the number of spikes falling within it. This process is continued to the last spike of the record, the resulting time bin values then representing the autocorrelogram. Practically, this analysis was accomplished within the computer by choosing values for the bin width (between 2.5 msec. and 25 msec.) and the length of the autocorrelogram (up to 3 minutes), then using the values of the interspike intervals to calculate into which bins the various spikes fell, under the procedure outlined above.

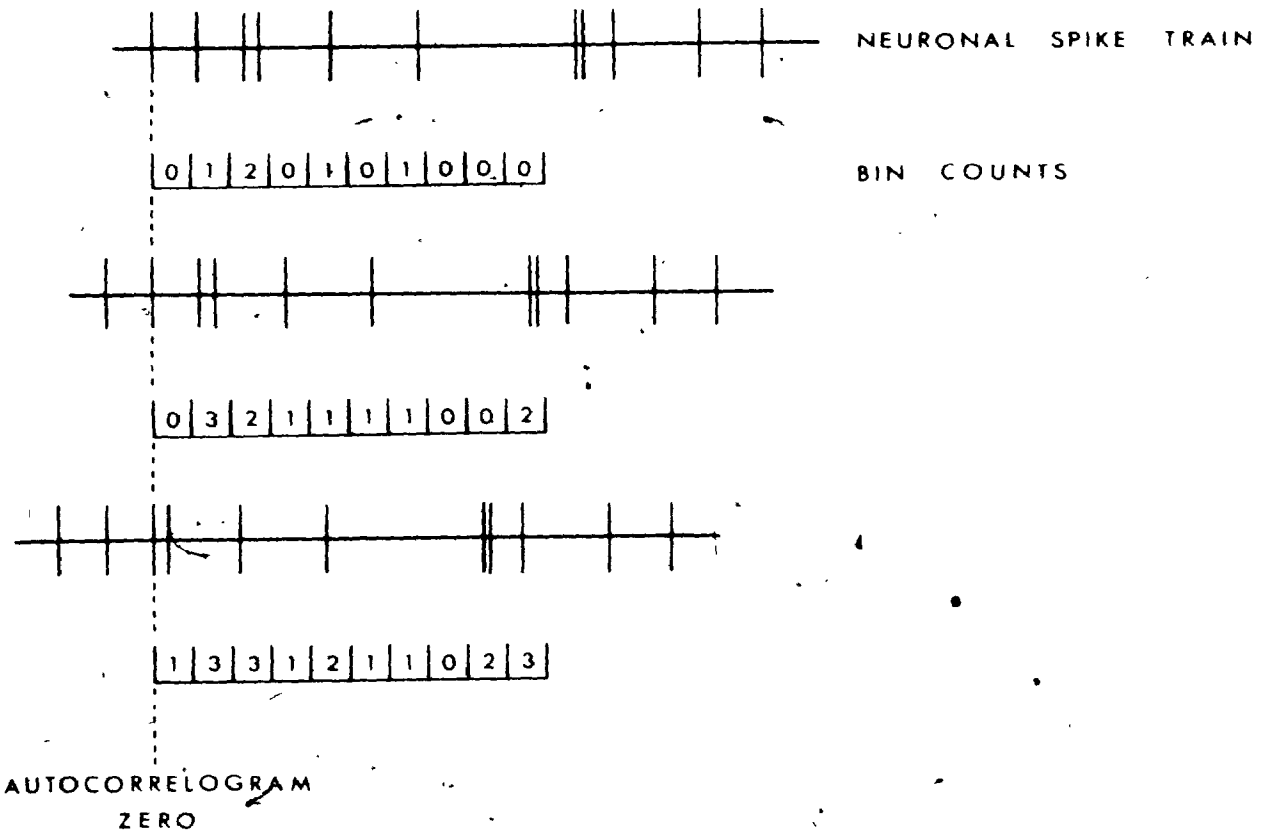
3.6.3 Analysis of Evoked Activity

Measurements of latencies for invasion of the neurons by spikes evoked by stimulation, as mentioned above,

FIGURE 4

Diagrammatic Representation of Calculation

of the Autocorrelogram


NEURONAL SPIKE TRAIN
0 1 2 0 1 0 1 0 0 0
BIN COUNTS
0 3 2 1 1 1 1 0 0 2
1 3 3 1 2 1 1 0 2 3
AUTOCORRELOGRAM
ZERO

were made directly from the storage oscilloscope. The time scale of this oscilloscope had been calibrated by means of a signal from the same function generator used to calibrate the timing cycle of the computer program. Latencies were measured from the end of the stimulus artifact to the rising phase of the unitary potentials. Post-stimulus time histograms were constructed from calibrated paper recordings.

CHAPTER IV

RESULTS

4.1 Description of Unitary Activity Encountered in the Olfactory Bulb

In total, electrical activity was recorded from 118 neurons in 45 frogs. These cells all exhibited spontaneous or background activity in the absence of overt stimulation, and in no instance did stimulation elicit activity which could be attributed to previously quiescent cells. In all cases this electrical activity took the form of a biphasic positive-negative potential of several milliseconds duration. In some cases this biphasic potential was followed by an after-potential. The height and shape of the potential recorded from a given cell would remain constant throughout recording times of many minutes to, in some cases, an hour or more. Plate 1 shows the extracellular potentials, with and without the after-potential. It also illustrates the constancy of the potential configurations. The magnitudes of the potentials observed ranged from several hundred microvolts to several millivolts. It has been on the basis

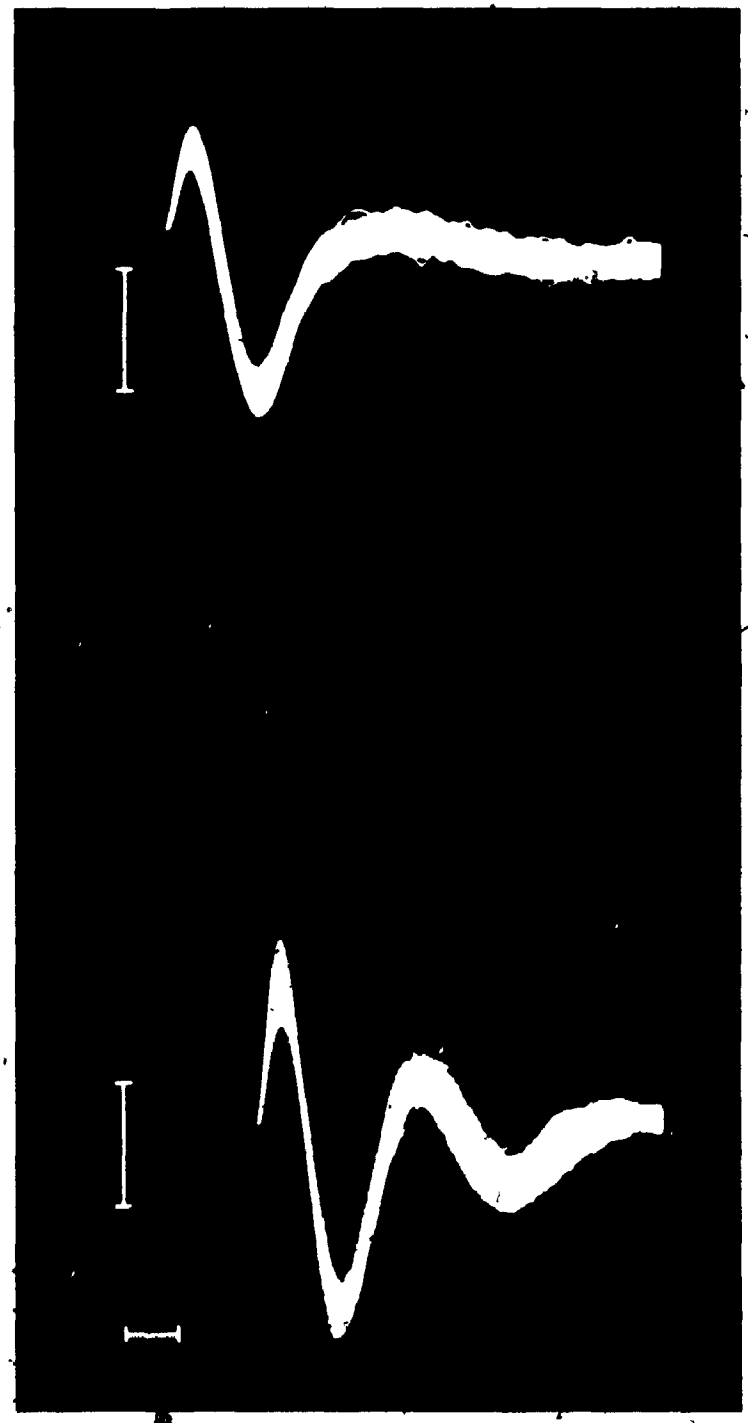
PLATE 1

Repetitive Traces of Spike Potentials

These traces have been obtained by triggering the oscilloscope on the rising phase of the potentials. The upper trace illustrates the positive-negative configuration, while the lower trace shows, in addition, the afterpotential sometimes attendant. Both traces are the superposition of 50 spike potentials. Positivity of the recording tip is the upward direction in this and all subsequent plates.

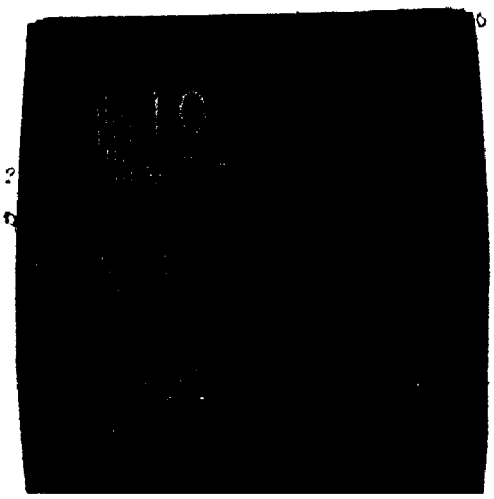
The voltage calibration for the upper trace is 200 μV, that for the lower trace is 250 μV.

Time calibration for both traces is 1 msec.



2 4

OF/DE



of the constancy of size and shape of these potentials, their time duration and amplitude, and their configuration that they have been designated as arising from single neurons in the olfactory bulb, recorded extracellularly. In one instance, two distinct potential configurations could be recognized in the records, as shown in Plate 2. These two configurations were attributed to the recording electrode picking up the activity from two nearby neurons.

4.2 Identification of Source of Unitary Activity

4.2.1 On the Basis of Dye Injection

Using the dye injection technique described earlier, we were able to locate, histologically, the tip of the microelectrode. In Plate 3 a photomicrograph of one of the located cells is presented. The dye extruded from the electrode can be seen as a spot in the layer of secondary neurons, adjacent to the soma of one cell. The potentials recorded from the cell are also shown to illustrate that they were comparable with those seen from other neurons which were not so identified.

Such histological identification of the spontaneously active neurons was accomplished successfully for four cells in two animals. Routine histological identification was not pursued due to the instability of the recordings obtained with the dye-filled micropipettes. In all recordings made with these electrodes it was found to be difficult to maintain the activity for any length of time.

PLATE 2

Extracellular Unit Potentials Recorded

from Two Neurons Simultaneously

The smaller unit potentials occur far more frequently than the large.

Voltage calibration 8 mV.

Time calibration 2 msec.

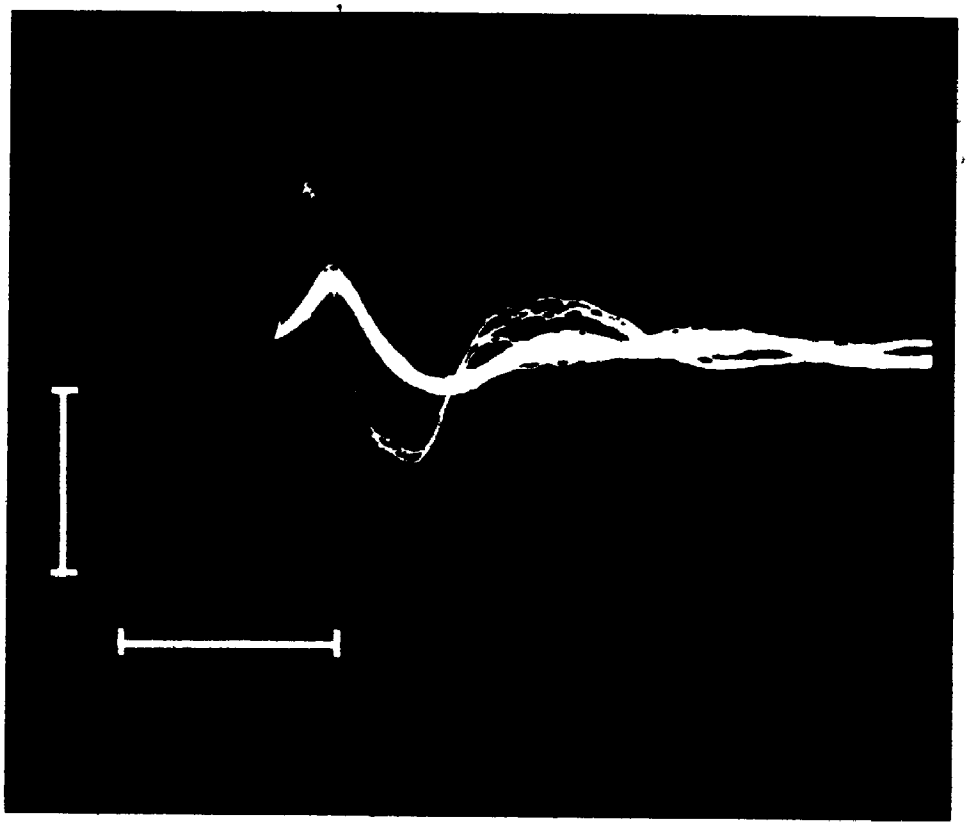


PLATE 3

Photomicrograph of Histologically Identified Neuron, and Unit Potentials Recorded from This Same Neuron

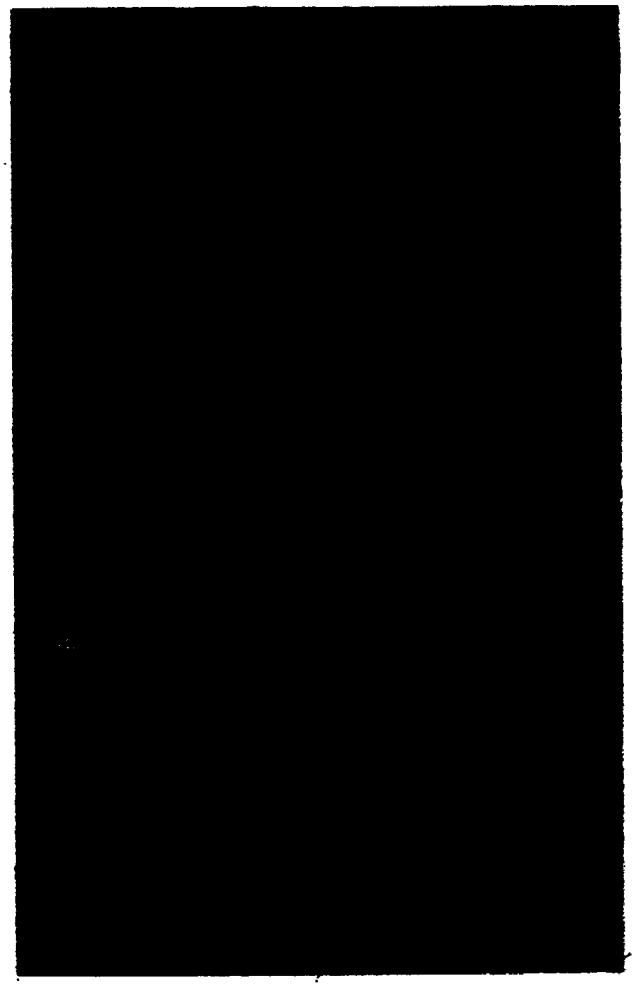
The photomicrograph has been taken from a horizontal section. The dye spot (marked by the arrow) can be seen to be in the vicinity of several secondary neurons, but particularly close to one. The glomerular synapses can be seen in the centre to the upper-right of the photomicrograph; they are the fibrous structures. Granule cells are far to the left and lower-left, out of the photograph. The olfactory nerve enters the bulb from the right. The small, darkly-stained cells to the top of the photomicrograph may be periglomerular cells.

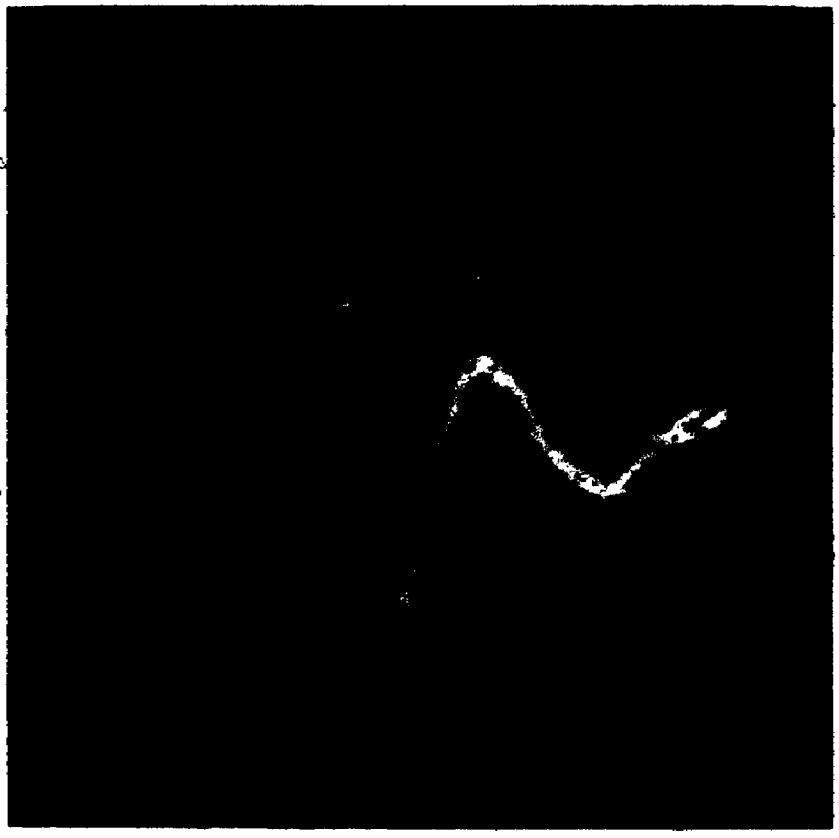
Photomicrograph magnification x160.

Several unit potentials are superimposed in the trace of the extracellular activity.

Voltage calibration 250 μV.

Time calibration 2 msec.





Presumably this problem was due to the relatively larger size of the tip of the dye-filled micropipettes as compared to the routinely used saline-filled ones, or in some way to the difference in electrolyte composition of these micropipettes; the former would seem to be the more likely explanation. None the less, the few neurons which were located provide sound evidence for the argument that the spontaneously active neurons encountered in the bulb were secondary neurons rather than periglomerular or granule cells.

4.2.2 On the Basis of Antidromic Invasion and Spike Fragmentation

While the majority of the biphasic potentials had a smooth rising positive phase, as shown in Plate 4A (upper trace), occasionally spontaneous potentials were encountered with an inflection on the rising phase (Plate 4A, lower trace). In concurrence with Phillips et al. (1963) this inflection has been assigned as arising from two regions of excitable membrane, the A and B membranes, whose invasion is not continuous.

Furthermore, it has been observed that stimulation of the forebrain, through which the axons of the secondary neurons pass to higher centres, could result in invasion of the observed cells by a spike at relatively constant latency, as shown in Plate 4B. This has been assigned as an antidromic impulse arising from invasion of the cell body region by the impulse initiated in the axon of the cell on forebrain stimulation. In view of the fact that this spike always

PLATE 4A

Demonstration of A-B Fragmentation

in Unit Potentials

The upper trace shows two unit potentials which do not exhibit fragmentation. The two spikes in the lower trace both exhibit A-B fragmentation, as indicated by the arrow. All four of these spike potentials were from spontaneously generated activity.

Voltage calibration, upper trace 250 μV., lower trace 300 μV.

Time calibration for both traces, 1 msec.

PLATE 4B

Antidromic Invasion Subsequent to

Forebrain Stimulation

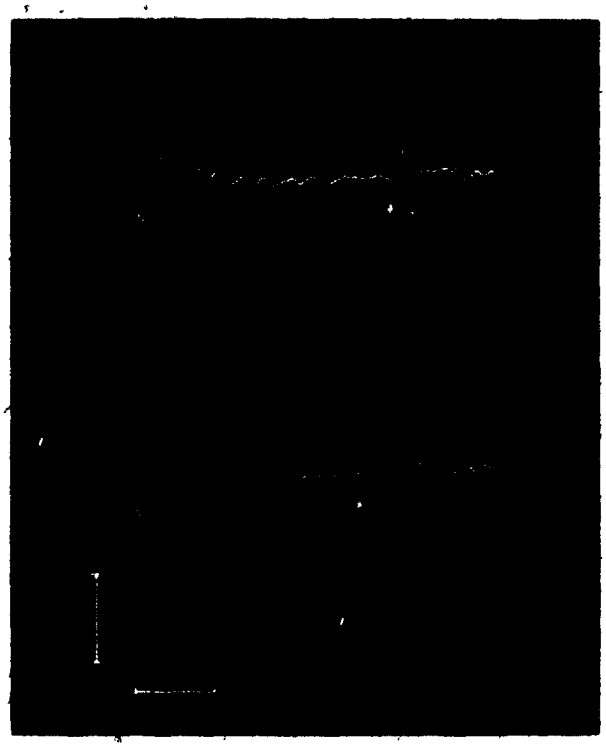
Both traces begin with the shock artifact. Invasion (indicated by the arrows) occurs some 30 msec. subsequent to this artifact. The spike in the upper trace has an inflection on the rising phase, suggestive of fragmentation. In the lower trace there is full-blown resolution into A and B spikes.

Voltage calibration 500 μV.

Times calibration 10 msec.

A

B



exhibited some degree of fragmentation, its assignment as an antidromic spike is reinforced. (Phillips et al. (1963) reported that such fragmentation was seen frequently in antidromic activation of mitral cells in the rabbit olfactory bulb, but rarely in orthodromic activation.)

Thus, on the basis of histological and electrophysiological evidence the biphasic potentials recorded in the anterior region of the olfactory bulb of the frog have been designated as having origin in single secondary neurons.

4.3 Interval Analysis in Intact Preparations

The logical way of viewing a neuron electrophysiologically is to consider its background activity in the absence of stimulation, and subsequently to observe how this background activity is modified by stimulation. It is in this logical sequence that the results of this study are presented. Thus, the results of the statistical analysis of spontaneous activity in secondary neurons in intact, decentralized, and deafferented preparations will be presented first. Subsequently, observations and quantitative measures of the response of the secondary neurons to stimulation will be presented, since these observations and measures disclose further aspects of the interactive neuronal network proposed in this thesis. We begin with the results of statistical analysis of secondary neurons in intact preparations.

Unitary activity was recorded from 103 neurons in 40 intact preparations. Of these neurons, seventy-seven

provided records which were stationary and of sufficient length to allow preliminary analysis. In view of the relatively low mean rate of the spontaneous activity, it is not surprising that obtaining a record of adequate length was a more stringent criterion than was that of stationarity; normally if a cell could be maintained, stationarity obtained. Qualitatively the spontaneous activity, in all but one instance, took the form of an irregular discharge pattern. In the one exceptional neuron the spontaneous activity was seen to occur as a regular discharge. These two discharge patterns are illustrated in Plate 5.

Quantitative measures of the neural activity were begun by obtaining the mean interspike interval for each of the seventy-seven neurons. These mean intervals are listed in Table 1. To demonstrate the cell-to-cell variation in the spontaneous activity the mean intervals are presented in the form of a histogram in Figure 5. The low mean rate of the spontaneous activity alluded to previously is evident from this distribution.

More detailed analysis was carried out in twenty-nine of these neurons for which the time series records consisted of at least several hundred interspike intervals. It is the analysis of these neurons that will be discussed in the remainder of this section. These neurons are listed in Table 2 with their mean interval and with the standard deviation of the mean. It can be seen that the mean intervals of these twenty-nine neurons are distributed throughout those

PLATE 5A

Regular Spontaneous Discharge Seen in One Exceptional Neuron

Consecutive traces form a continuous record.

Voltage calibration 200 μV.

Time calibration 1 sec.

PLATE 5B

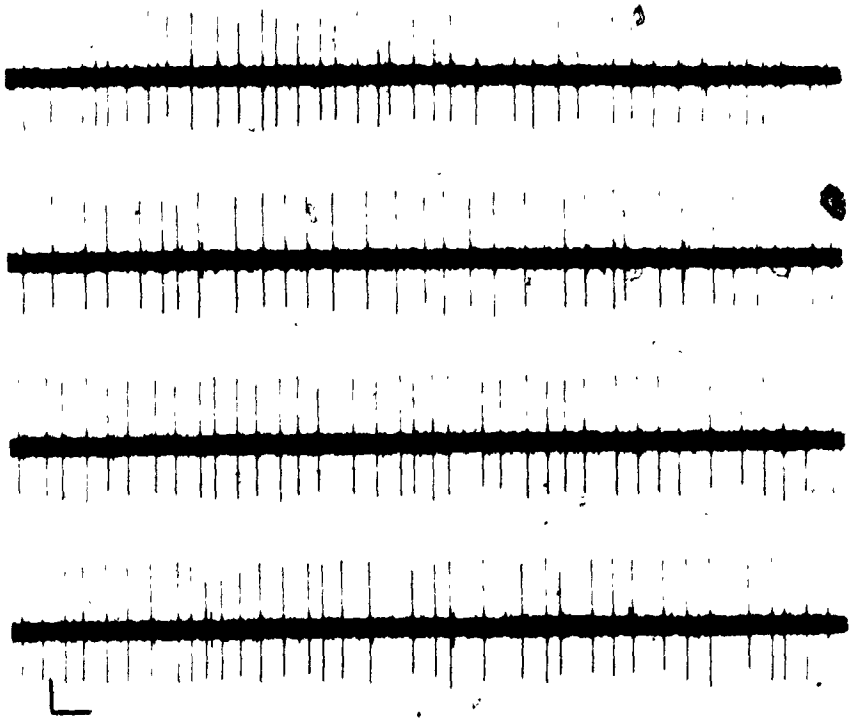
Irregular Spontaneous Discharge Typical of That Recorded from the Secondary Olfactory Neurons

Consecutive traces again form a continuous record.

Voltage calibration 200 μV.

Time calibration 1 sec.

A



B

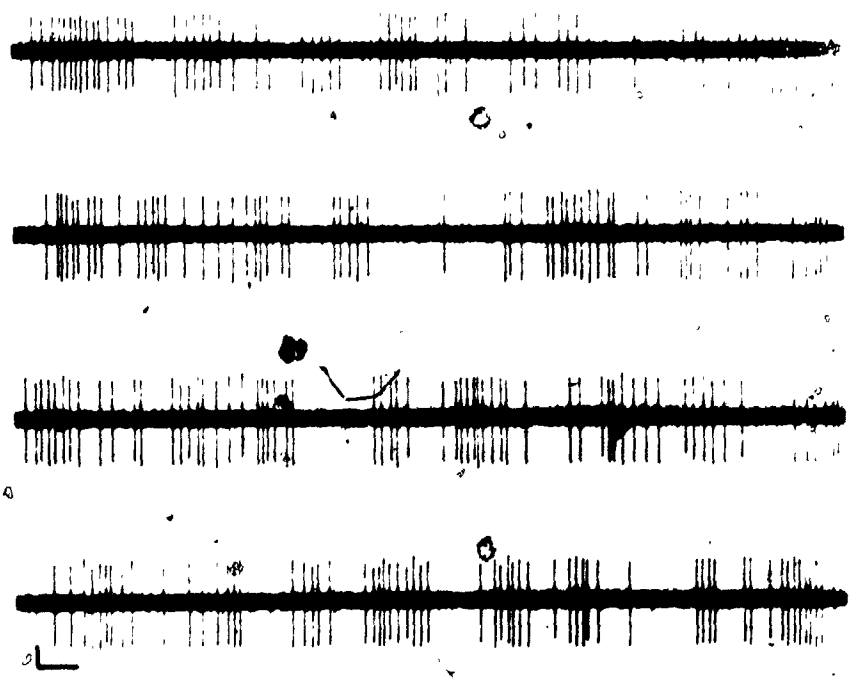


TABLE 1

MEAN INTERSPIKE INTERVALS OF SPONTANEOUS ACTIVITY

FROM NEURONS IN INTACT PREPARATIONS

Neuron	Tape Segment	Mean Interval (msec.)
I-A	50-500	3528
I-B	550-750	2765
I-C	850-1280	2170
I-D	1300-1450	1515
II-B	2000-2600	4852
II-D	0-700	2153
II-E	800-1050	2419
III-A	1300-2950	2879
V-B	250-350	979
V-C	700-900	2376
VI-A	2300-2500	3431
VI-B	2600-2800	1912
VI-C	0-1250	2270
VI-D	1300-2000	1996
VII-A	0-410	1159
VII-B	900-1000	1291
VIII-A	1600-1980	589
IX-A	2000-2450	389
IX-B	120-570	361
IX-C	1200-2400	1174
XI-A	300-850	1732
XI-B	900-1150	1999
XII-A	1500-1900	1299
XIII-A	1900-2300	2064
XIV-B	1500-1700	777
XV-A	1910-2180	3237

TABLE 1 (CONTINUED)

Neuron	Tape Segment	Mean Interval (msec.)
XV-B	2200-2400	1999
XV-D	0-220	1209
XV-E	400-700	1045
XV-F	800-1150	1237
XVI-A	1710-1950	1160
XVI-B	2000-2250	790
XVI-C	2300-2550	2650
XVI-D	2615-3000	1404
XVII-A	2000-2700	2241
XVIII-A	300-700	304
XIX-A	900-1450	1303
XXI-A	2600-2750	1728
XXII-A	0-350	1039
XXII-B	400-550	916
XXII-C	550-850	430
XXII-D	850-1250	1348
XXIV-A	2500-2600	338
XXIV-B	2850-2950	332
XXIV-C	3100-3380	1748
XXIV-D	100-275	1917
XXIV-E	1000-1350	1217
XXIV-F	2125-2350	1814
XXV-A	0-650	1140
XXV-B	900-1500	1441
XXVII-A	2400-2800	585
XXVII-B	0-150	370
XXVIII-A	750-950	610
XXVIII-B	1100-1300	1935
XXVIII-C	1400-1600	1156
XXIX-A	2400-2900	1757
XXIX-C	3200-3350	679

TABLE 1 (CONTINUED)

Neuron	Tape Segment	Mean Interval (msec.)
XXXI-A	800-1050	1504
XXXI-B	1050-1540	1145
XXXI-C	1900-2100	1764
XXXII-A	2200-2330	525
XXXIII-A	2350-2450	459
XXXIII-B	2450-2550	340
XXXIV-A	0-200	1242
XXXIV-B	200-1000	1806
XXXIV-C	1000-1400	1921
XXXV-B	1700-1900	535
XXXV-C	2150-2450	1764
XXXV-D	2450-2850	1079
XXXVI-A	2850-3300	2114
XXXVII-A	0-600	1109
XXXVIII-A	800-1020	644
XXXIX-A	850-1100	1515
XL-A	1900-2400	1391
XL-B	2400-2560	400
XL-C	2600-2900	455
XL-D	3100-3200	1714

Average mean interval $\pm$ standard deviation: 1464 msec. $\pm$ 862 msec.

FIGURE 5

Distribution of Mean

Interspike Intervals Observed in Neurons

from Intact Preparations

This histogram has been constructed from the mean interspike intervals of the seventy-seven neurons from intact preparations.

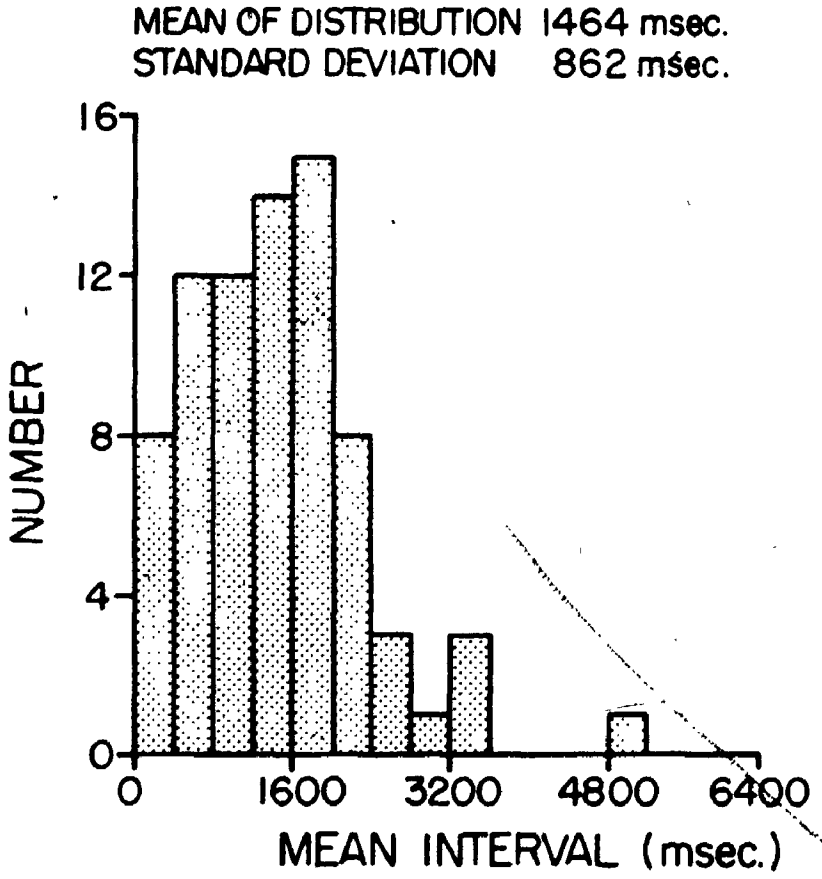

MEAN OF DISTRIBUTION 1464 msec.
STANDARD DEVIATION 862 msec.
16
12
8
4
0
NUMBER
0
1600
3200
4800
6400
MEAN INTERVAL (msec.)

TABLE 2

SPONTANEOUS ACTIVITY IN INTACT PREPARATIONS -

MEAN INTERVALS AND STANDARD DEVIATIONS

Neuron	Tape Segment	Time of Recording (minutes)	Number of Intervals in Record	Mean Interval (msec.)	Standard Deviation (msec.)
III-A	1300-2950	45.2	943	2879	3818
VI-C	0-1250	34.9	922	2270	1981
VII-A	0-410	11.5	593	1159	2680
VIII-A	1600-1980	10.6	1078	589	164
IX-A	2000-2450	13.2	2029	388	486
IX-B	120-570	12.5	2080	361	487
IX-C	1200-2400	33.2	1694	1174	1774
XII-A	1500-1900	11.4	526	1299	1166
XVI-B	2000-2250	7.0	534	790	1018
XVIII-A	300-700	11.2	2216	304	352
XIX-A	900-1450	15.3	706	1307	648
XXII-A	0-350	9.3	537	1039	1650
XXII-C	550-850	8.4	1169	430	237
XXIV-A	2500-2600	2.5	450	340	324
XXIV-B	2850-2950	2.8	498	333	360
XXIV-E	1000-1350	9.6	473	1217	1512
XXV-B	900-1500	16.5	687	1441	2597

TABLE 2 (CONTINUED)

Neuron	Tape Segment	Time of Recording (minutes)	Number of Intervals in Record	Mean Interval (msec.)	Standard Deviation (msec.)
XXVII-A	2400-2800	11.1	1140	585	256
XXVII-B	0-150	4.1	673	370	535
XXVIII-A	750-950	5.5	545	610	502
XXXIII-B	2450-2550	2.8	486	340	602
XXXIV-B	200-1000	22.1	735	1806	1902
XXXV-D	2450-2850	10.8	599	1079	964
XXXVII-A	0-600	16.5	895	1109	1354
XXXVIII-A	800-1020	6.7	620	644	1142
XXXIX-A	850-1100	6.9	272	1515	2161
XL-A	1900-2400	13.6	585	1391	1822
XL-B	2400-2560	4.6	691	400	615
XL-C	2600-2900	8.4	1106	456	621

of the larger sample. Included in this sample of twenty-nine is the cell that was seen to fire regularly, neuron VIII-A.

For this smaller sample analysis was first extended to examine the probability density underlying the distribution of intervals. Thus interspike interval density histograms were obtained. Such histograms for all of these neurons had certain characteristics in common. For the sake of discussion and comparison, several of these histograms have been reproduced in Figure 6. All of the histograms were unimodal and more or less positively skewed, sometimes extremely so. In addition, there was an apparent absence of shorter intervals, intervals which, however, were for longer than one would expect on the basis of refractory periods alone.

In view of the fluctuations evident in the times between spike discharge, the possibility that the intervals are distributed in time as a Poisson process was examined. The first criterion to be considered in this respect is whether the distribution of intervals fits an exponential of the form,

$$P(t' \geq t) \propto \exp(-t/E),$$

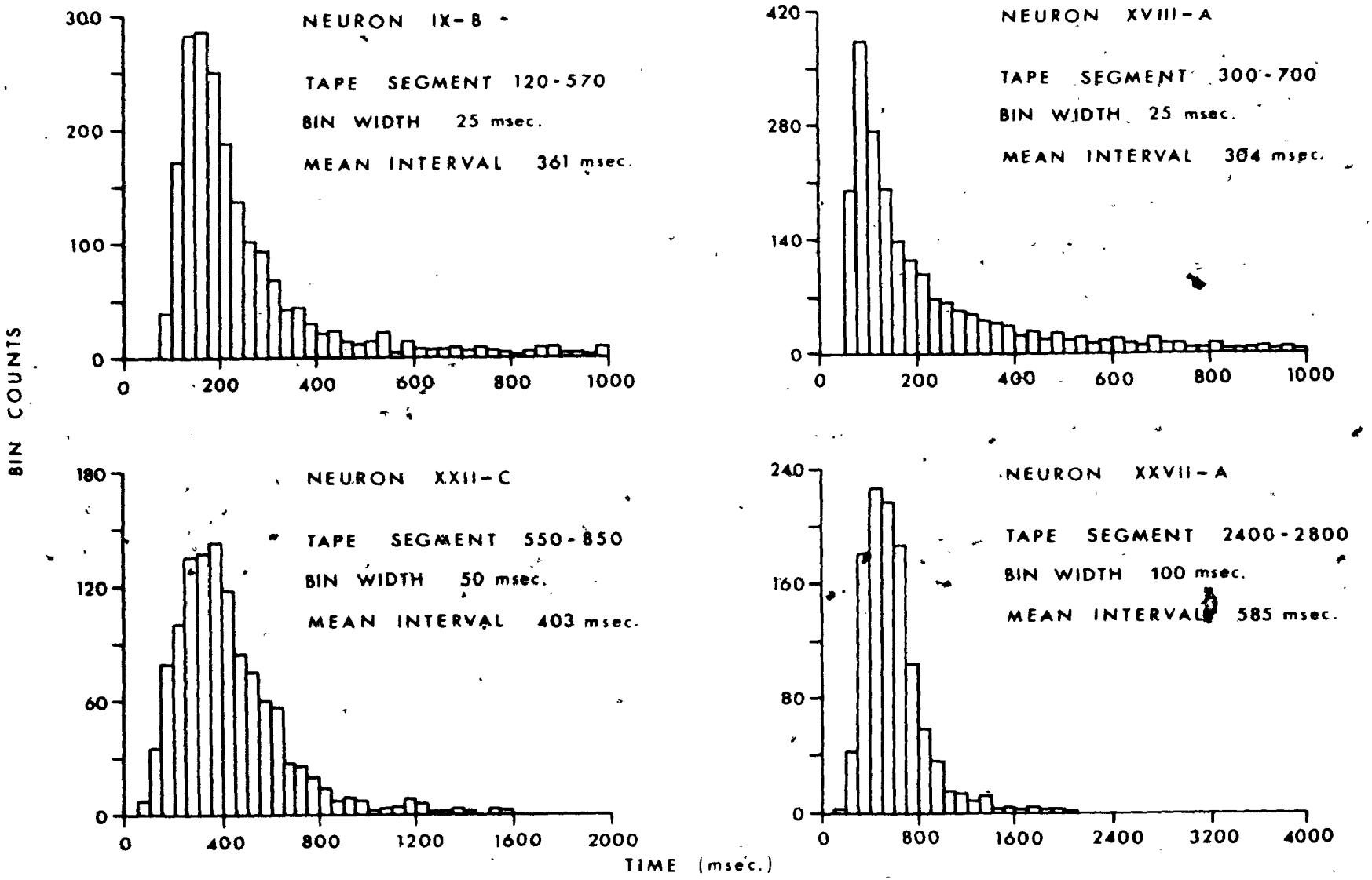
where $P(t' \geq t)$ is the probability of finding an interval greater than or equal to t, and E is the mean interval. Thus

FIGURE 6

Interspike Interval Density Histograms

for Exemplary Neurons from Intact Preparations

The abscissa axes have been labelled, for simplicity, as "time" in this and successive interspike interval density histograms. These axes are in fact "the time duration of interspike intervals".


NEURON IX-B
TAPE SEGMENT 120-570
BIN WIDTH 25 msec.
MEAN INTERVAL 361 msec.
NEURON XVIII-A
TAPE SEGMENT 300-700
BIN WIDTH 25 msec.
MEAN INTERVAL 304 msec.
NEURON XXII-C
TAPE SEGMENT 550-850
BIN WIDTH 50 msec.
MEAN INTERVAL 403 msec.
NEURON XXVII-A
TAPE SEGMENT 2400-2800
BIN WIDTH 100 msec.
MEAN INTERVAL 585 msec.
BIN COUNTS
TIME (msec.)

the interspike interval density histograms[1] were replotted as the number of intervals greater than a given time interval versus that time interval. To examine whether such histograms fit the above exponential distribution, they were plotted on a semi-logarithmic scale. This is shown for the neurons of Figure 6 in Figure 7. These four neurons show a characteristic that was seen in all of the neurons except the regularly firing cell. That characteristic is that the interspike interval distributions, as plotted in Figure 7, possessed a definite point of gradient discontinuity between two approximately linear portions. The histogram for the regularly firing neuron, however, showed a single linear portion when plotted in this way. This is shown in Figure 8.

There are many possible curves which could be fit to the logarithmic distributions of Figure 7. Since, however, the possibility that the intervals are distributed in time in accordance with a Poisson process or processes is being investigated, one wishes to assess whether the distributions of Figure 7 may be considered to consist of several linear portions. This would reflect several underlying exponential distributions. Thus to analyze these distributions further

[1] Interspike interval histograms in which the number of intervals between t and $t + \Delta t$ (where t is a time interval) is plotted versus t will be called interspike interval density distributions; histograms in which the number of intervals greater than or equal to t is plotted versus t are called interspike interval distributions.

FIGURE 7

Interspike Interval Distributions
for Exemplary Neurons from
Intact Preparations

This figure shows the interspike interval data for the four exemplary neurons from intact preparations plotted as the logarithm of the number of neurons greater than or equal to a given time duration versus that time duration. Again, in this and successive interspike interval distributions, "time" has been used as a convenient contraction of "time duration of interspike intervals". The regression lines to the long process have been included.

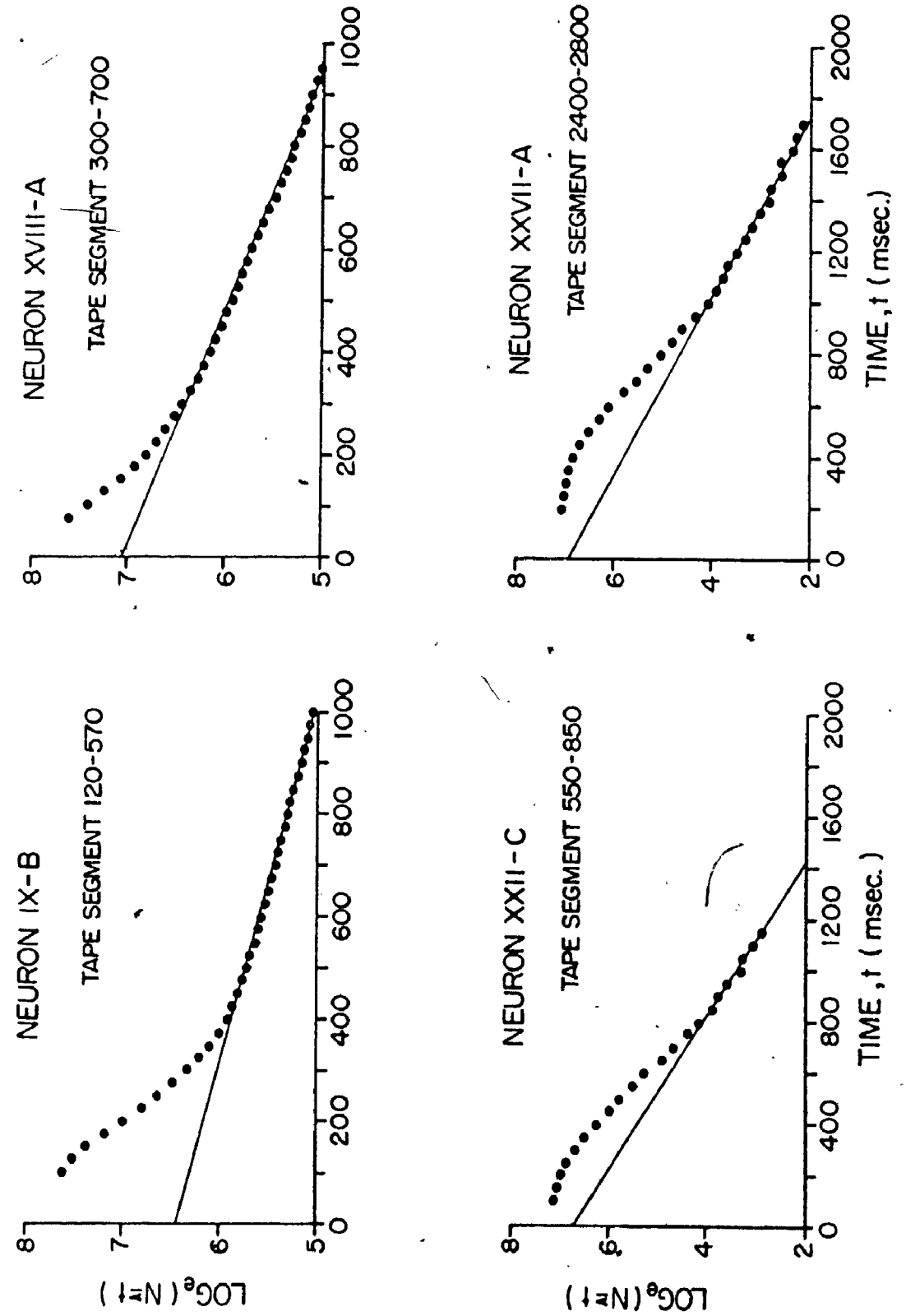
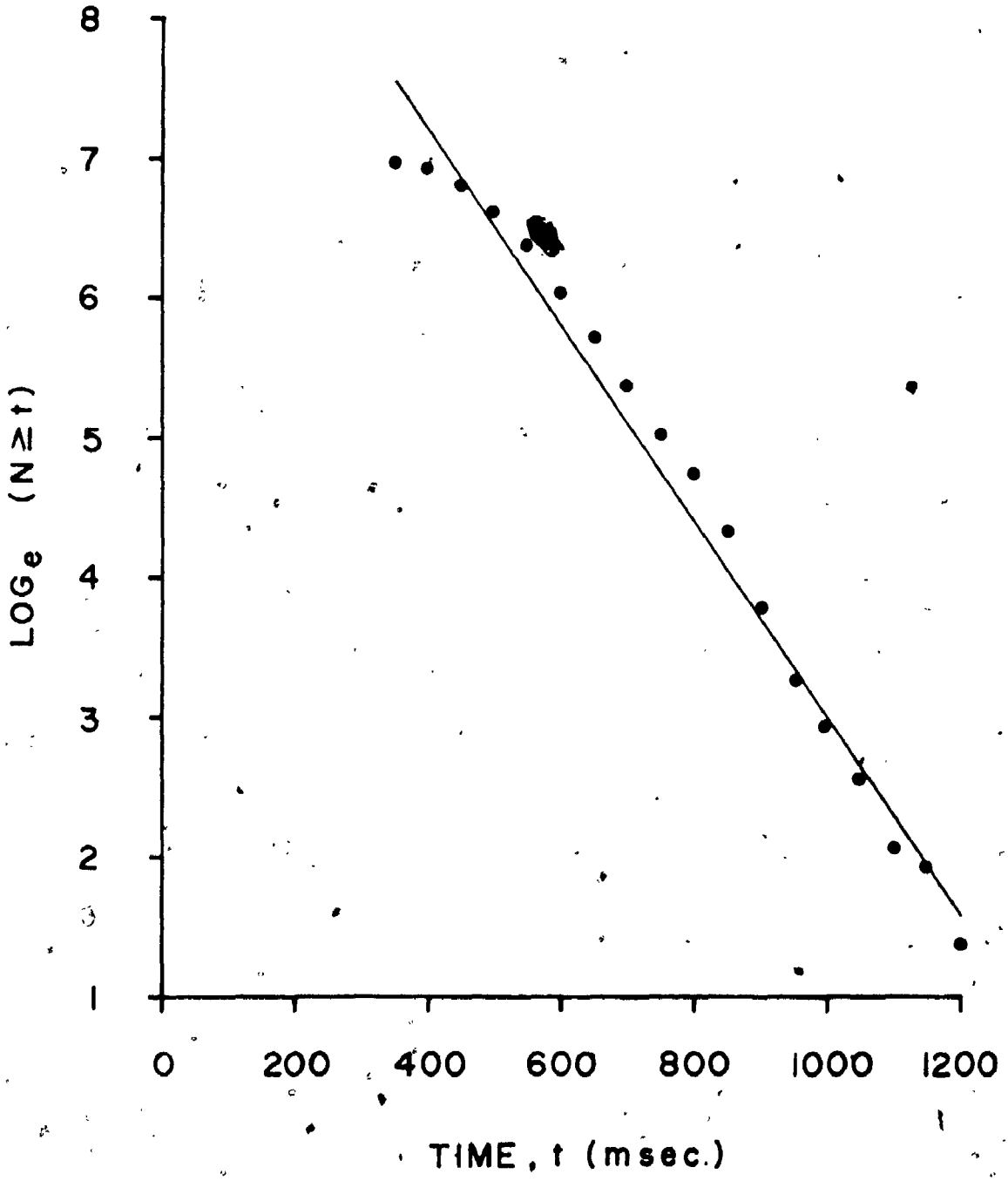

NEURON XVIII-A
TAPE SEGMENT 300-700
NEURON XXVII-A
TAPE SEGMENT 2400-2800
NEURON IX-B
TAPE SEGMENT 120-570
NEURON XXII-C
TAPE SEGMENT 550-850
LOGe (N=1)
TIME, t (msec.)

FIGURE 8

Interspike Interval Distribution for

the Regularly Firing Neuron

This is a similar graph to those of Figure 7 for the regularly firing neuron. The regression line to the data has been included.


8
7
6
5
4
3
2
1
LOG$_e$ (N ≥ t)
0
200
400
600
800
1000
1200
TIME, t (msec.)

the following procedure was used. The "tail" portion of the logarithmic plot of the distribution was fit with a straight line by means of linear correlation-regression techniques. The absolute value of this line was then subtracted from the complete interspike interval distribution, and the remainders replotted semilogarithmically. This semilogarithmic replot of the remainders could then be fit with a second straight line. Thus the total interspike interval distribution could be partitioned into two exponential subprocesses. The process which extends out to the tail of the histogram will be called the "long" process, while the second process whose influence is seen about the mode of the interspike interval density histogram will be called the "short" process. In Table 3 the correlation coefficients for the linear fit to these processes, standard errors of estimate, levels of significance (as determined by the t-test), and the slopes of the regression lines are listed. It can be seen that in all cases the level of significance was 5% or better, with 0.1% being the usual level. The slopes of the regression lines can be taken as a measure of the rates of the two processes. In Figure 9 the distributions of these slopes, or rates, are shown. The rates of the long process are obviously much more narrowly distributed than those of the short process. A significant difference exists at the 0.1% level between the means of these two distributions. In Figure 10 the rate of the long process is plotted versus the rate of the short process. There appears to be no

TABLE 3

STATISTICS FOR THE TWO PROCESSES UNDERLYING SPONTANEOUS ACTIVITY IN NEURONS FROM INTACT PREPARATIONS

		Long Process				Short Process			
Neuron	Tape Segment	R.*	S.E.E.†	Slope^{-1} (sec.$^{-1}$)	Level of Significance	R.	S.E.E.	Slope^{-1} (sec.$^{-1}$)	Level of Significance
III-A	1300-2950	0.99	0.034	0.146	0.1%	0.99	0.242	0.636	0.1%
VI-C	0-1250	1.00	0.052	0.311	0.1%	0.99	0.187	1.118	0.1%
VII-A	0-410	0.99	0.018	0.985	0.1%	0.99	0.063	40.314	0.1%
IX-A	2000-2450	1.00	0.027	1.992	0.1%	0.96	0.544	45.247	2.0%
IX-B	120-570	1.00	0.023	1.401	0.1%	0.99	0.164	13.301	0.1%
IX-C	1200-2400	1.00	0.028	0.634	0.1%	0.99	0.140	3.254	0.1%
XII-A	1500-1900	0.99	0.037	0.922	0.1%	0.93	0.401	1.433	1.0%
XVI-B	2000-2250	0.99	0.032	0.656	0.1%	0.98	0.323	2.479	0.1%
XVIII-A	300-700	1.00	0.042	2.183	0.1%	1.00	0.023	14.755	0.1%
XIX-A	900-1450	0.99	0.048	0.793	0.1%	0.98	0.255	2.628	0.1%
XXII-A	0-350	1.00	0.031	0.791	0.1%	0.98	0.251	3.365	0.1%
XXII-C	550-850	0.99	0.063	3.255	0.1%	0.95	0.508	6.388	0.1%
XXIV-A	2500-2600	1.00	0.049	1.925	0.1%	0.99	0.227	11.910	0.1%
XXIV-B	2850-2950	1.00	0.070	1.877	0.1%	0.94	0.815	21.693	1.0%

TABLE 3 (CONTINUED)

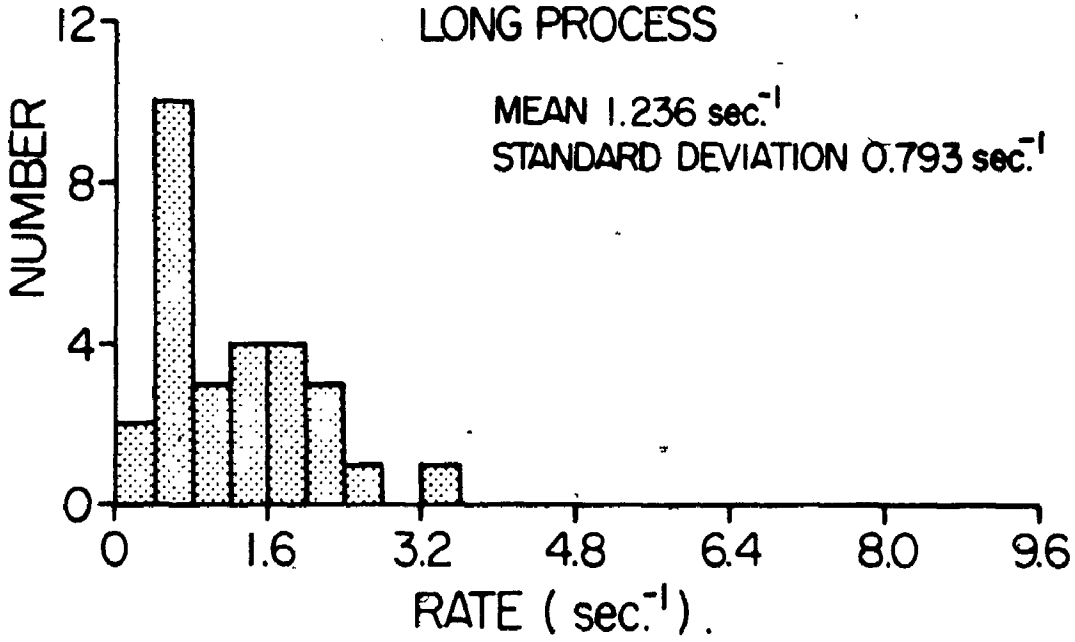
Neuron	Tape Segment	Long Process R.	Long Process S.E.E.	Long Process Slope (sec.$^{-1}$)	Long Process Level of Significance	Short Process R.	Short Process S.E.E.	Short Process Slope (sec.$^{-1}$)	Short Process Level of Significance
XXIV-E	1000-1350	1.00	0.029	0.585	0.1%	0.97	0.447	10.645	1.0%
XXV-B	900-1500	0.98	0.012	1.017	0.1%	0.99	0.148	28.662	0.1%
XXVII-A	2400-2800	0.99	0.072	2.152	0.1%	0.96	0.334	5.537	0.1%
XXVII-B	0-150	0.99	0.031	1.869	0.1%	0.98	0.241	19.425	0.1%
XXVIII-A	750-950	1.00	0.005	1.416	0.1%	0.93	1.422	23.690	5.0%
XXXIII-B	2450-2550	0.99	0.031	2.293	0.1%	0.99	0.174	26.423	0.1%
XXXIV-B	200-1000	0.99	0.034	0.445	0.1%	0.99	0.155	0.988	0.1%
XXXV-D	2450-2850	0.99	0.027	0.528	0.1%	0.99	0.215	1.753	0.1%
XXXVII-A	0-600	1.00	0.010	0.528	0.1%	1.00	0.065	19.962	0.1%
XXXVIII-A	800-1020	0.98	0.015	1.286	0.1%	0.99	0.151	80.269	1.0%
XXXIX-A	850-1100	1.00	0.027	0.430	0.1%	0.98	0.288	9.688	1.0%
XL-A	1900-2400	1.00	0.033	0.435	0.1%	1.00	0.132	11.768	1.0%
XL-B	2400-2560	0.99	0.025	2.431	0.1%	0.97	0.302	23.916	0.1%
XL-C	2600-2900	1.00	0.013	1.317	0.1%	0.99	0.143	19.592	0.1%

* In this and succeeding tables, R. is the linear correlation coefficient.
† In this and succeeding tables, S.E.E. is the standard error of estimate.

FIGURE 9

Distributions of the Rates
of the Two Processes for Twenty-eight Neurons
from Intact Preparations

Notice that the abscissa scales for the two histograms differ by a factor of ten. The process for the regularly firing neuron has not been included.


LONG PROCESS
MEAN 1.236 sec.$^{-1}$
STANDARD DEVIATION 0.793 sec.$^{-1}$
NUMBER
12
8
4
0
0
1.6
3.2
4.8
6.4
8.0
9.6
RATE (sec.$^{-1}$).

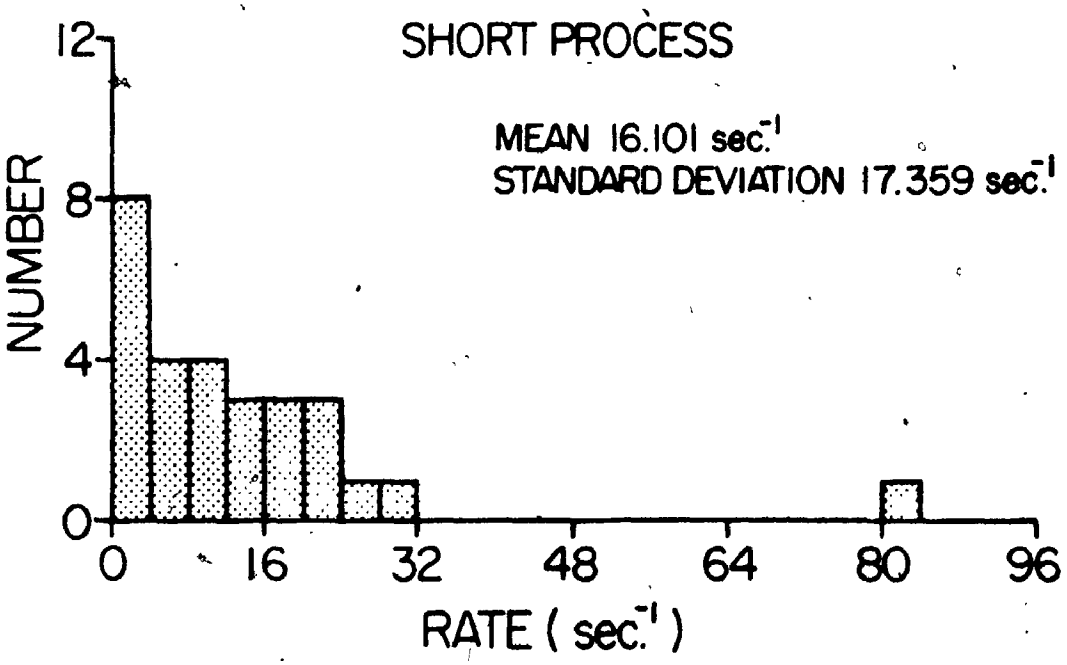
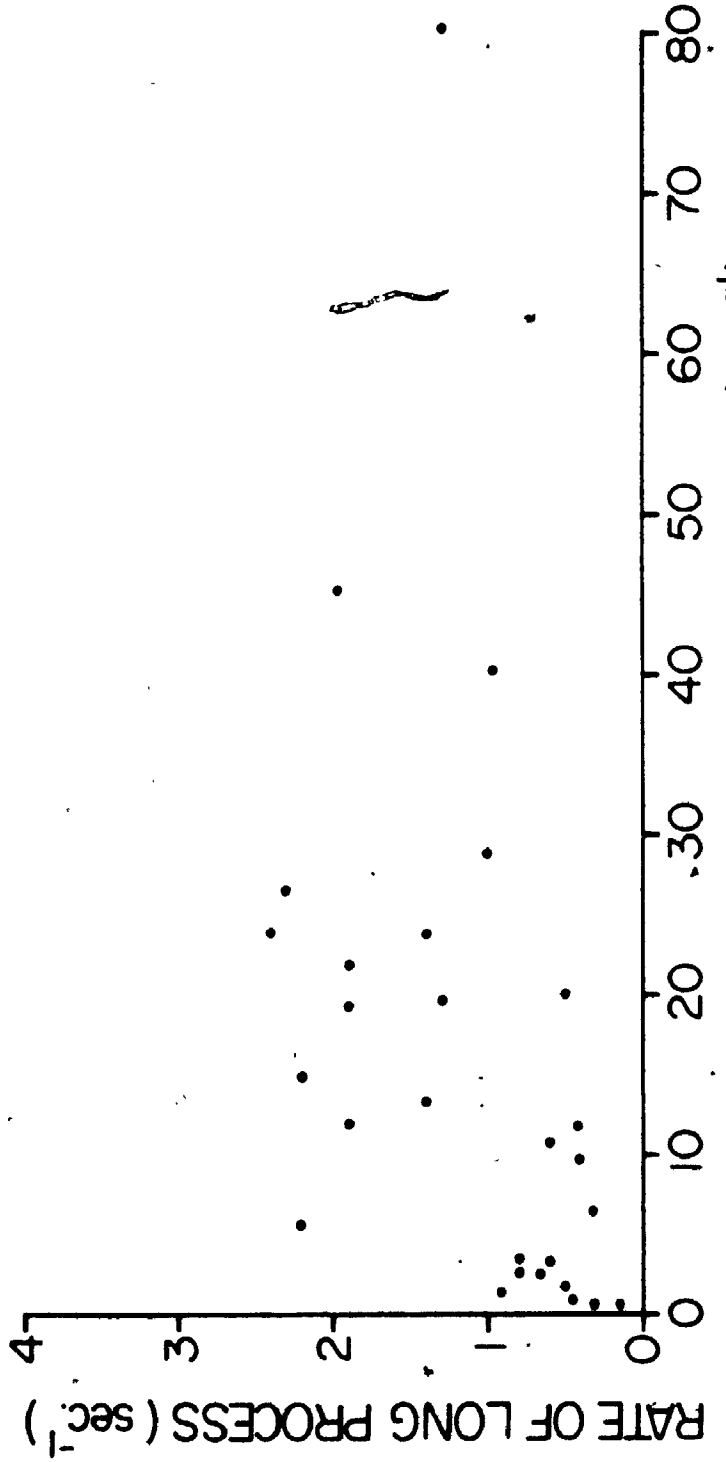

SHORT PROCESS
MEAN 16.101 sec.$^{-1}$
STANDARD DEVIATION 17.359 sec.$^{-1}$
NUMBER
12
8
4
0
0
16
32
48
64
80
96
RATE (sec.$^{-1}$)

FIGURE 10

Rate of the Long Process Plotted Versus the Rate of the Short Process


RATE OF LONG PROCESS (sec^{-1})
0
1
2
3
4
RATE OF SHORT PROCESS (sec.$^{-1}$)
0
10
20
30
40
50
60
70
80

obvious relationship between the two; certainly there is no linear relationship - linear correlation analysis yields a correlation coefficient of only 0.28.

For the regularly firing neuron the interspike interval distribution could be fit by a single straight line. The correlation coefficient for the graph of Figure 8 was 0.99. The slope or rate was found to be 6.999 $sec.^{-1}$ with a standard error of estimate of 0.261; the level of significance was 0.1%.

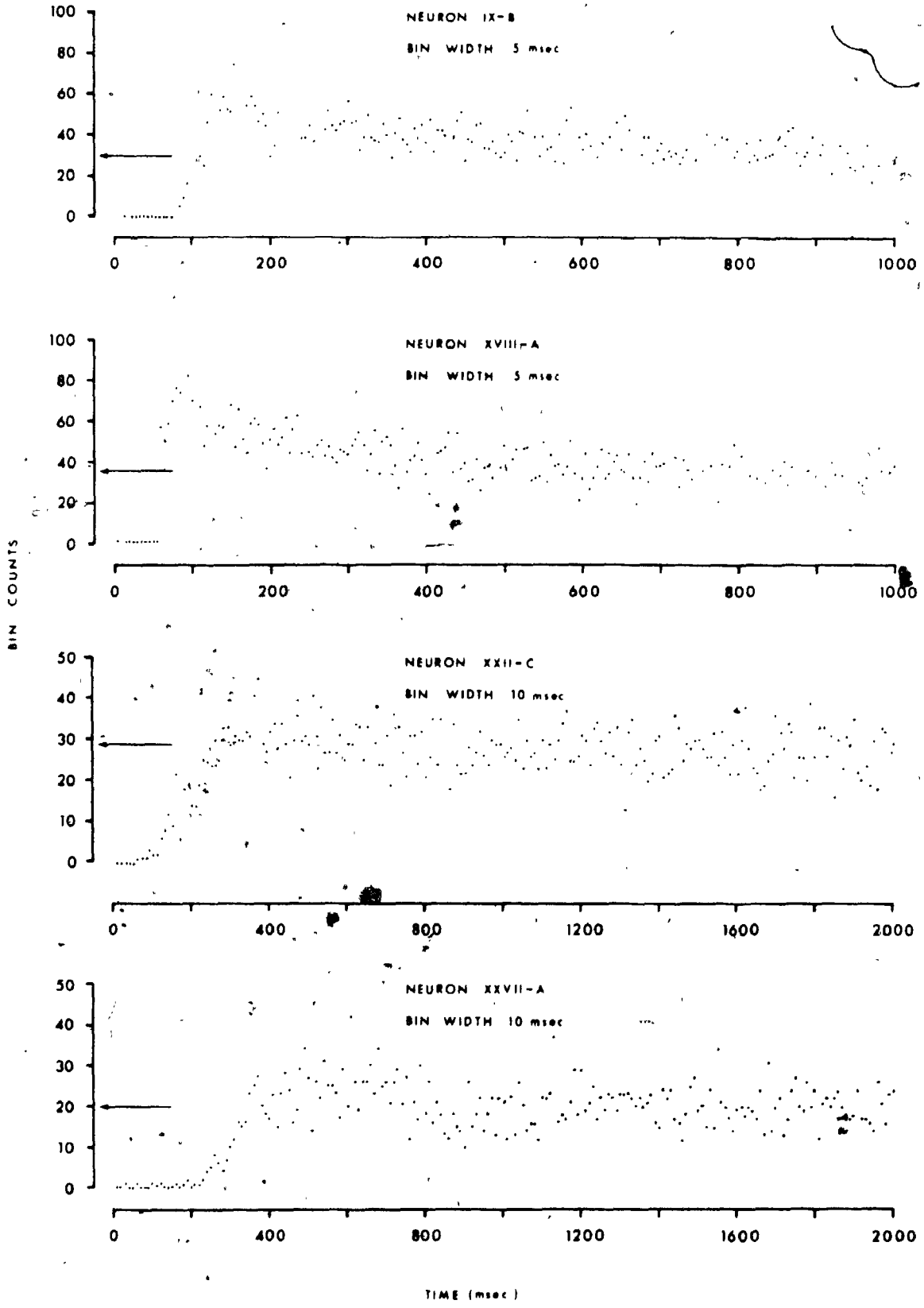
The second criterion to consider with respect to whether the spontaneous activity is generated by a Poisson process is the independence of an event on past events. To assess this, the autocorrelation technique was used. Autocorrelograms for the neurons of Figure 6 are given in Figure 11. The autocorrelograms, which present a measure of the probability of occurrence of a spike at any time after a preceding spike, indicated that for all but the one regularly firing neuron this probability was constant in time. Furthermore, this probability could be calculated from the mean intervals (as will be discussed in greater detail in the next chapter). The autocorrelograms for a few neurons (for example, those for neurons IX-B and XVIII-A in Figure 11) showed an initial period during which the probability of spike occurrence appeared to be somewhat higher than in the remainder of the correlogram. This was obviously not due to any maintained trend in the firing pattern of these neurons, since there was no evidence of a cyclic probability of spike

FIGURE 11

Autocorrelograms for Exemplary Neurons

from Intact Preparations

Arrows indicate the bin counts expected on the basis of the mean intervals.


NEURON IX-B
BIN WIDTH 5 msec
NEURON XVIII-A
BIN WIDTH 5 msec
NEURON XXII-C
BIN WIDTH 10 msec
NEURON XXVII-A
BIN WIDTH 10 msec
BIN COUNTS
TIME (msec)

occurrence. Such initial peaking, when it occurred, was always seen at times around those corresponding to the position of the modal value of the histogram. Beyond this initial period, the probability of spike occurrence was again constant at the value expected from the mean interval. Characteristics of the autocorrelograms are summarized in Table 4.

For the one neuron whose spontaneous activity was a regular discharge, the autocorrelogram indicated a sustained cyclic course for the probability of post-spike occurrences. The autocorrelation, as well as the interspike interval density histogram, are given in Figure 12 for this neuron. It is apparent that the times of maximum probability in the autocorrelogram are determined by the mean interspike interval; these times are integral multiples of the mean interval. The interval density histogram for this neuron appears to be Goussian in shape, at least compared to those for the other twenty-eight neurons. However, as Figure 8 indicates, the interspike interval distribution is exponential.

To obtain further information about the probability density function underlying the interspike intervals, the following analysis was performed. If the intervals between consecutive impulses are statistically independent, as in the Poisson model, the mean, m, and the standard deviation, s, of the sample of intervals stand in the relation

$$m=s^2.$$

Consequently a plot of log(s) versus log(m) would have a

TABLE 4

SPONTANEOUS ACTIVITY IN INTACT PREPARATIONS -

CHARACTERISTICS OF AUTOCORRELOGRAMS

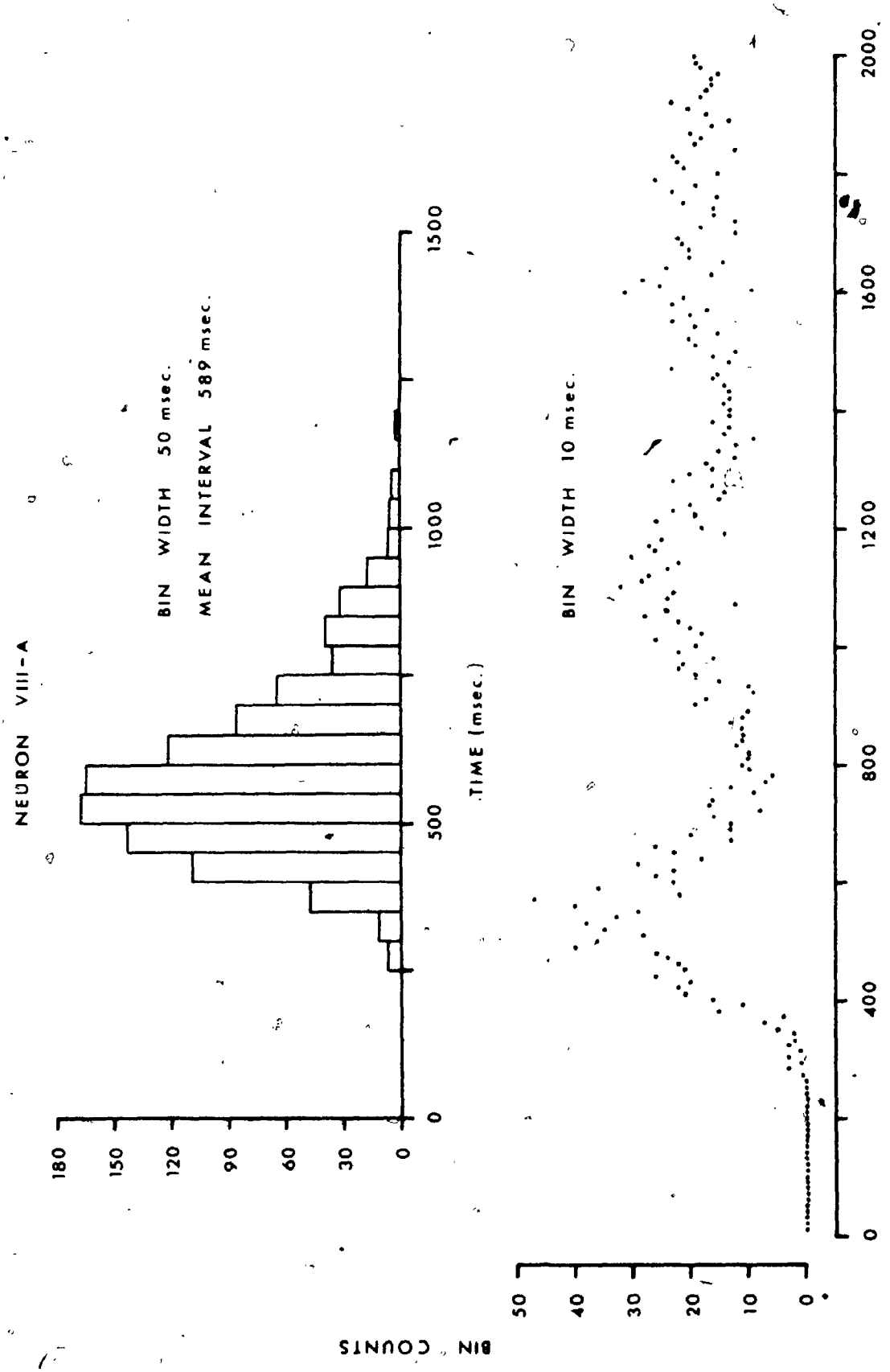
Neuron	Tape Segment	Description of Autocorrelogram
III-A	1300-2950	flat, determined by mean interval
VI-C	0-1250	flat, determined by mean interval
VII-A	0-410	flat, determined by mean interval
VIII-A	1600-1980	oscillates at mean interval
IX-A	2000-2450	initial peaking
IX-B	120-570	slight initial peaking
IX-C	1200-2400	flat, determined by mean interval
XII-A	1500-1900	initial peaking
XVI-B	2000-2250	flat, determined by mean interval
XVIII-A	300-700	slight initial peaking
XIX-A	900-1450	flat, determined by mean interval
XXII-A	0-350	flat, determined by mean interval
XXII-C	550-850	flat, determined by mean interval
XXIV-A	2500-2600	flat, determined by mean interval
XXIV-B	2850-2950	flat, determined by mean interval

TABLE 4 (CONTINUED)

Neuron	Tape Segment	Description of Autocorrelogram
XXIV-E	1000-1350	flat, determined by mean interval
XXV-B	900-1500	flat, determined by mean interval
XXVII-A	2400-2800	flat, determined by mean interval
XXVII-B	0-150	flat, determined by mean interval
XXVIII-A	750-950	flat, determined by mean interval
XXXIII-B	2450-2550	flat, determined by mean interval
XXXIV-B	200-1000	flat, determined by mean interval
XXXV-D	2450-2850	flat, determined by mean interval
XXXVII-A	0-600	initial peaking
XXXVIII-A	800-1020	initial peaking
XXXIX-A	850-1100	flat, determined by mean interval
XL-A	1900-2400	flat, determined by mean interval
XL-B	2400-2560	flat, determined by mean interval
XL-C	2600-2900	flat, determined by mean interval

FIGURE 12

Interspike Interval Density Histogram and Autocorrelogram for Regularly Firing Neuron


NEURON VIII-A
BIN WIDTH 50 msec.
MEAN INTERVAL 589 msec.
BIN WIDTH 10 msec.
TIME (msec.)
TIME (msec.)
BIN COUNTS
0
30
60
90
120
150
180
0
500
1000
1500
0
10
20
30
40
50
0
400
800
1200
1600
2000

slope of 0.5. Such a plot for the twenty-nine neurons is given in Figure 13. The correlation coefficient is high and significant at the 0.1% level (tested on the t-distribution), but the value for the slope of the regression line, 1.05, is far removed from the value of 0.5 expected on the basis of a simple Poisson model. It can also be seen from this figure that the interspike interval density histogram for the regularly firing neuron had the smallest standard deviation.

In three cases in which the samples of spontaneous activity were particularly large, the records were divided into two portions which were analyzed separately. In these cases, the mean intervals and their standard deviations were constant between the two portions, as shown in Table 5A. As shown in Figure 14 and Table 5B, the interspike interval density histograms and autocorrelograms demonstrated themselves to be stable measures of the spontaneous activity. And, as one might expect, histograms replotted as interspike interval distributions were seen to be constant from one portion to the second (Figure 15 and Table 5C).

4.4 Interval Analysis in Decentralized Preparations

Unitary activity was recorded from thirteen neurons in four preparations in which the olfactory bulb was isolated from the rest of the forebrain by complete bilateral transection. The region of this transection is indicated in Figure 16. Qualitatively the spontaneous activity recorded from these neurons was indistinguishable from that recorded

FIGURE 13

A Plot of the Logarithm of the Standard Deviation Versus the Logarithm of the Mean Interval for Neurons from Intact Preparations

This graph is for the twenty-nine neurons from intact preparations whose activity was analyzed in detail. The point corresponding to the regularly firing neuron is marked with an arrow. Parameters for linear correlation-regression analysis are as follows:

slope of regression line: 1.05

intercept of regression line: -0.22

correlation coefficient: 0.83 (significant at the 0.1% level)

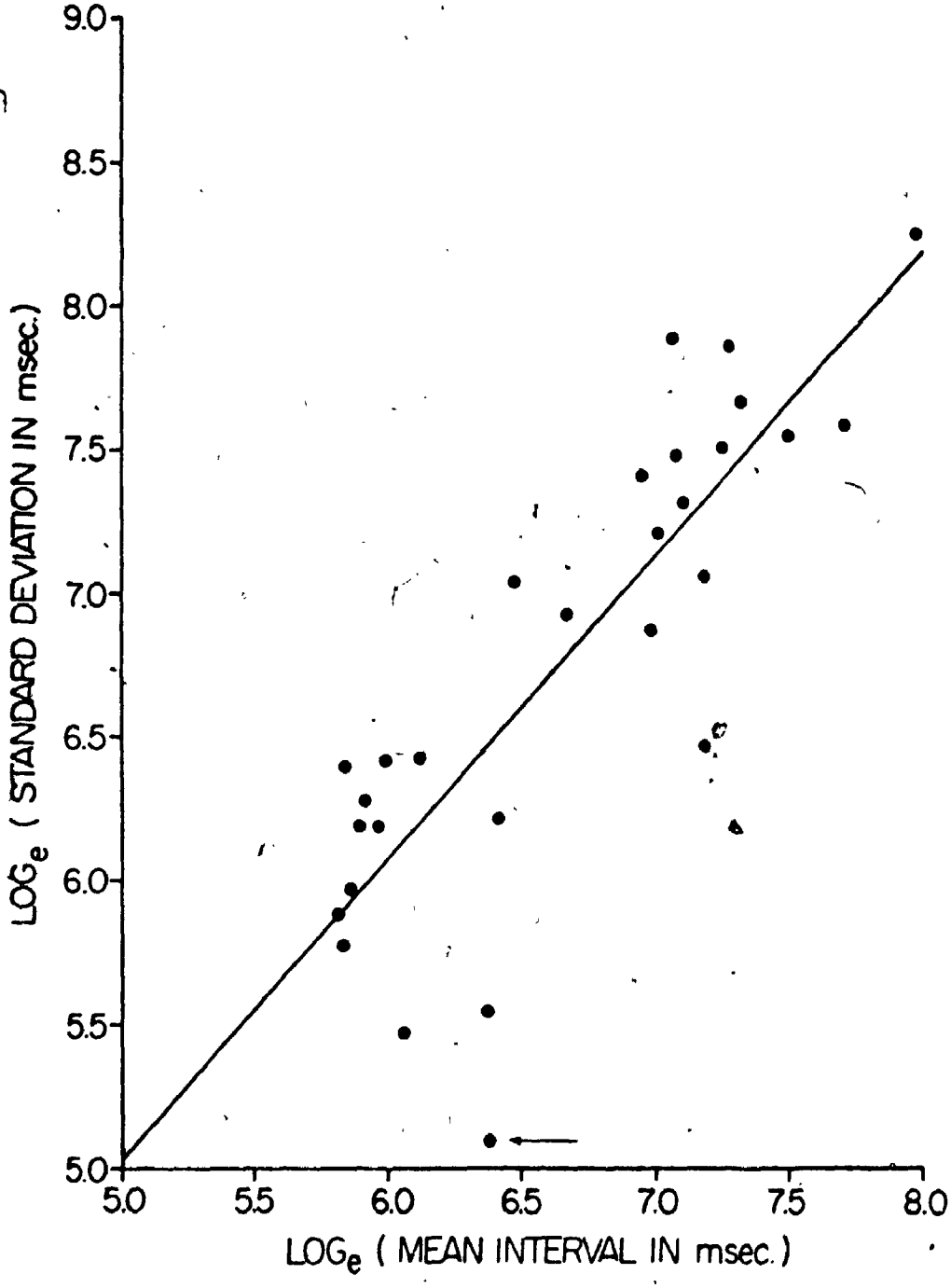

9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
LOG$_e$ (STANDARD DEVIATION IN msec.)
5.0
5.5
6.0
6.5
7.0
7.5
8.0
LOG$_e$ (MEAN INTERVAL IN msec.)

TABLE 5A

COMPARISON OF TWO SAMPLES FROM THE SAME NEURON -

MEAN INTERVALS AND STANDARD DEVIATIONS

Neuron	Tape Segment	Time of Recording (minutes)	Number of Intervals in Record	Mean Interval (msec.)	Standard Deviation (msec.)
IX-B	120-570	12.5	2080	361	487
IX-B	615-965	9.6	1528	378	483
XVIII-A	20-300	7.8	1335	352	391
XVIII-A	300-700	11.2	2216	302	352
XXVII-B	0-150	4.1	673	370	535
XXVII-B	455-600	4.0	666	365	551

FIGURE 14

Comparison of Interspike Interval Density Histograms and Autocorrelograms for Two Samples of Activity

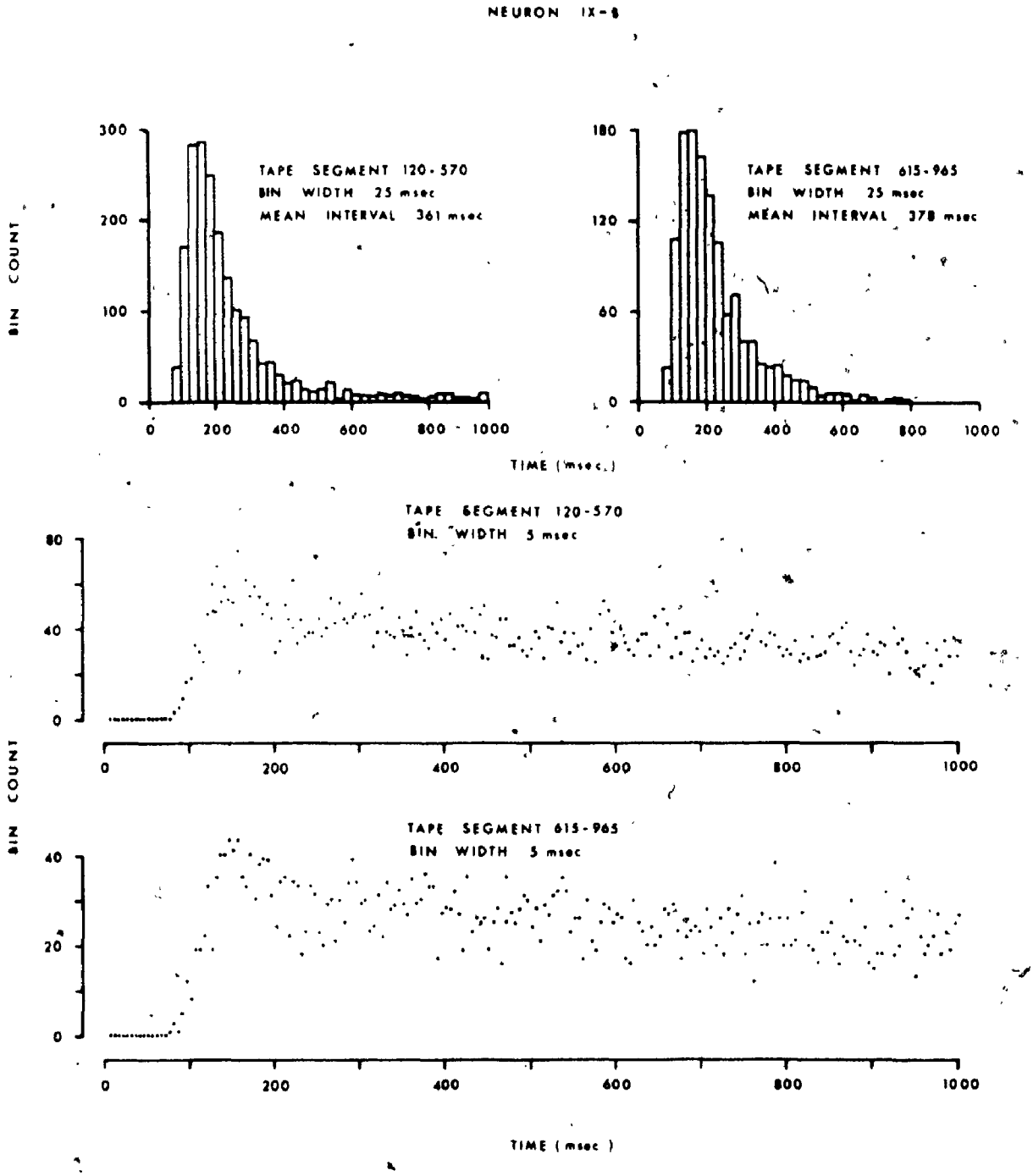

NEURON IX-8
TAPE SEGMENT 120-570
BIN WIDTH 25 msec
MEAN INTERVAL 361 msec
TAPE SEGMENT 615-965
BIN WIDTH 25 msec
MEAN INTERVAL 378 msec
BIN COUNT
TIME (msec.)
TAPE SEGMENT 120-570
BIN WIDTH 5 msec
TAPE SEGMENT 615-965
BIN WIDTH 5 msec
BIN COUNT
TIME (msec)

TABLE 5B

COMPARISON OF TWO SAMPLES FROM THE SAME NEURON -

CHARACTERISTICS OF THE AUTOCORRELOGRAMS

Neuron	Tape Segment	Description of Autocorrelogram
IX-B	120-570	slight initial peaking
IX-B	615-965	flat, determined by mean interval
XVIII-A	20-300	flat, determined by mean interval
XVIII-A	300-700	slight initial peaking
XXVII-B	0-150	flat, determined by mean interval
XXVII-B	455-600	flat, determined by mean interval

FIGURE 15

Comparison of Interspike Interval Distributions

for Two Samples of Activity

The interspike interval distributions presented here are for the same two samples of activity as Figure 14. The regression lines to the long process have been included.

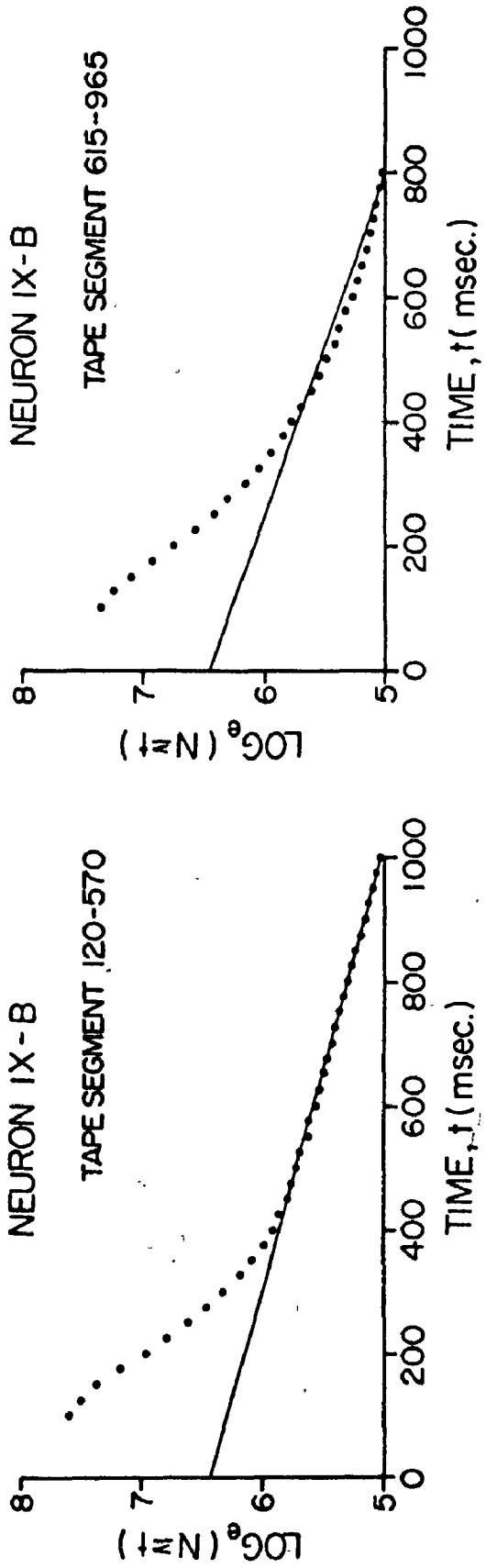

NEURON IX-B
TAPE SEGMENT 615-965
LOG$_e$ (N≠)
TIME, t (msec.)
8
7
6
5
0
200
400
600
800
1000
NEURON IX-B
TAPE SEGMENT 120-570
LOG$_e$ (N≠)
TIME, t (msec.)

TABLE 5C

COMPARISON OF TWO SAMPLES FROM THE SAME NEURON - PARTITION OF INTERSPIKE INTERVAL DISTRIBUTIONS INTO TWO UNDERLYING PROCESSES

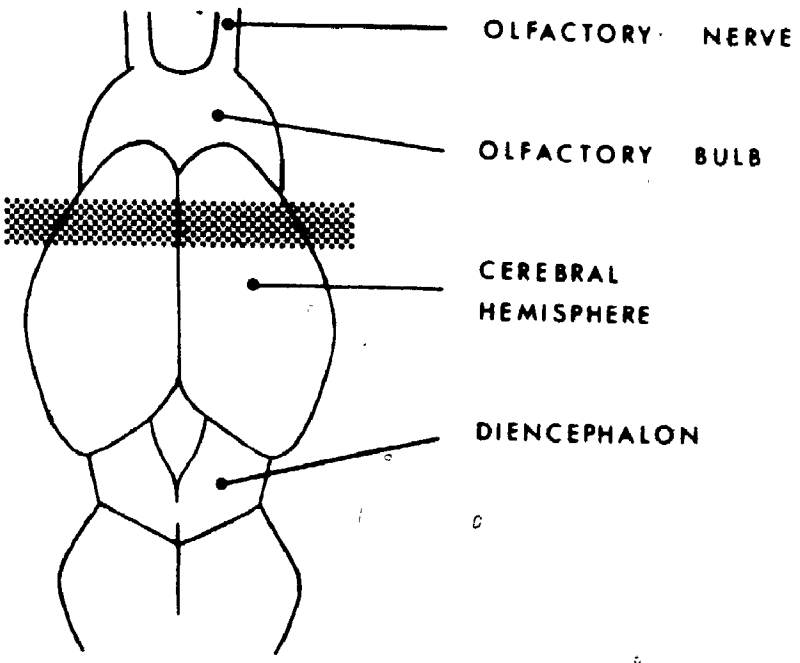
		Long Process				Short Process			
Neuron	Tape Segment	R.	S.E.E.	Slope$_{-1}$ (sec.$^{-1}$)	Level of Significance	R.	S.E.E.	Slope$_{-1}$ (sec.$^{-1}$)	Level of Significance
IX-B	120-570	1.00	0.023	1.401	0.1%	0.99	0.164	13.30	0.1%
IX-B	615-965	0.99	0.035	1.537	0.1%	1.00	0.094	11.00	0.1%
XVIII-A	20-300	1.00	0.039	1.951	0.1%	1.00	0.071	11.92	0.1%
XVIII-A	300-700	1.00	0.042	2.183	0.1%	1.00	0.023	14.75	0.1%
XXVII-B	0-150	0.99	0.031	1.869	0.1%	0.98	0.241	19.42	0.1%
XXVII-B	455-600	0.98	0.040	1.920	0.1%	0.98	0.257	16.67	0.1%

FIGURE 16

Illustration Showing the Region of

Forebrain Transection

DORSAL VIEW


OLFACTORY NERVE
OLFACTORY BULB
REGION OF FOREBRAIN TRANSECTION
CEREBRAL HEMISPHERE
DIENCEPHALON

from neurons in intact preparations. The activity was always seen to occur as an irregular discharge which was stationary over long-term recordings of many minutes. Plate 6 shows the activity recorded from a secondary neuron at two different times after forebrain transection.

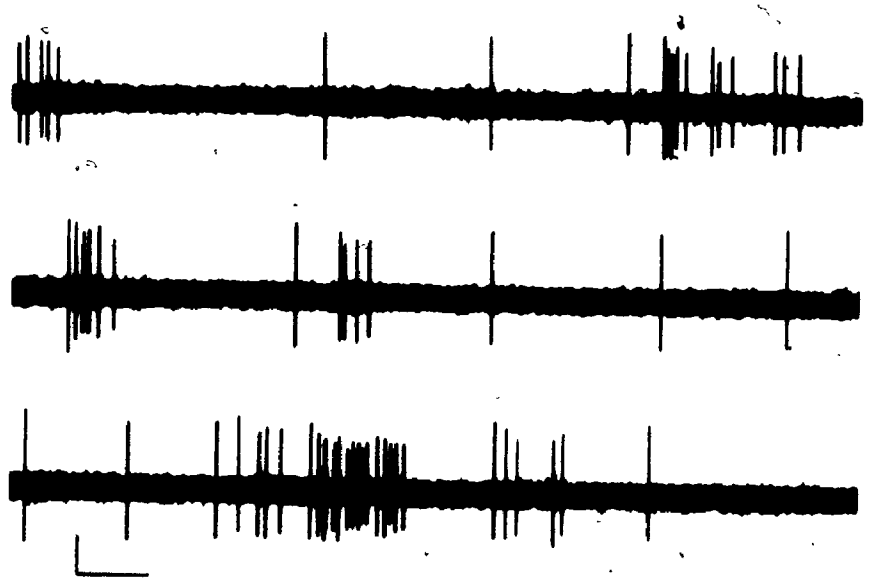
Of these thirteen neurons, seven provided suitable samples of spontaneous activity for mean interspike interval calculations. These mean intervals are listed in Table 6. In Figure 17 the mean intervals have been included in the distribution first given in Figure 5 for mean intervals from intact preparations. It can be seen that they fall throughout the distribution obtained for intact preparations. The average mean interval for the intact preparations was compared with that for the decentralized preparations with a t-test, and no significant difference was seen at the 5% level. The mean of the overall combined distribution of eighty-four neurons (seventy-seven from intact preparations, seven from decentralized preparations) is 1431 msec., the standard deviation is 859 msec.

More detailed analysis was pursued in five of these neurons whose records consisted of at least several hundred interspike intervals. These neurons are listed in Table 7 with their mean intervals and standard deviations. As in intact preparations, interspike interval density histograms and autocorrelograms were obtained. These measures for decentralized secondary neurons were in accord with those for neurons from intact preparations. As an example, Figure

PLATE 6

Spontaneous Activity Recorded from a Neuron in a Decentralized Preparation

Consecutive traces form a single record in each case. Upper record begins 150 minutes after decentralization, lower record begins 160 minutes after decentralization. For both records, the voltage calibration is 250 μV., the times calibration is 1 sec.



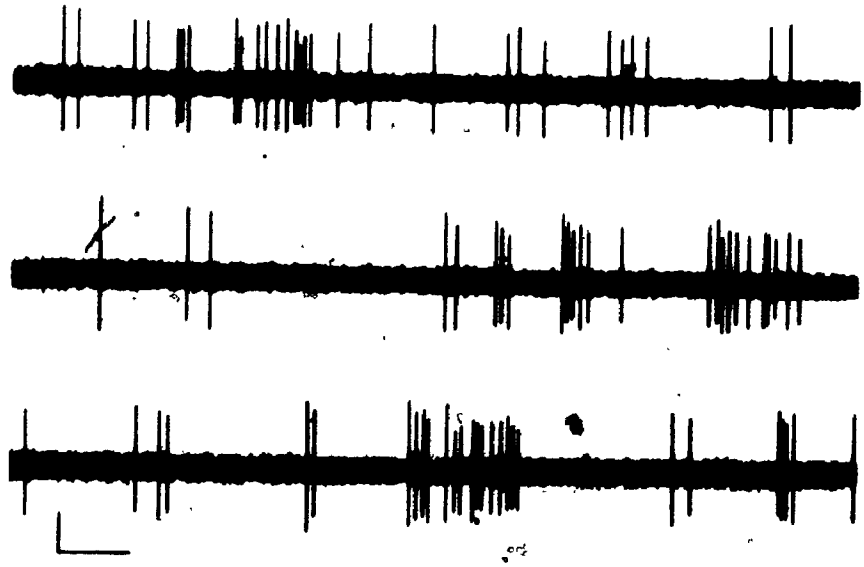


TABLE 6

MEAN INTERSPIKE INTERVALS OF SPONTANEOUS ACTIVITY FROM NEURONS IN DECENTRALIZED PREPARATIONS

Neuron	Tape Segment	Mean Interval (msec.)
CF-I-B	500-650	584
CF-I-C	650-800	711
CF-I-D	800-850	191
CF-IV-A	2210-2590	1606
CF-V-A	2480-3240	1322
CF-V-D	750-1540	2496
CF-V-E	1600-2000	522

Average mean interval $\pm$ standard deviation: 1062 msec. $\pm$ 798 msec.

FIGURE 17

Distribution of Mean Interspike Intervals from Neurons in Intact and Decentralized Preparations

This histogram has been constructed from the mean interspike intervals of the seventy-seven neurons from intact preparations and the seven neurons from decentralized preparations.

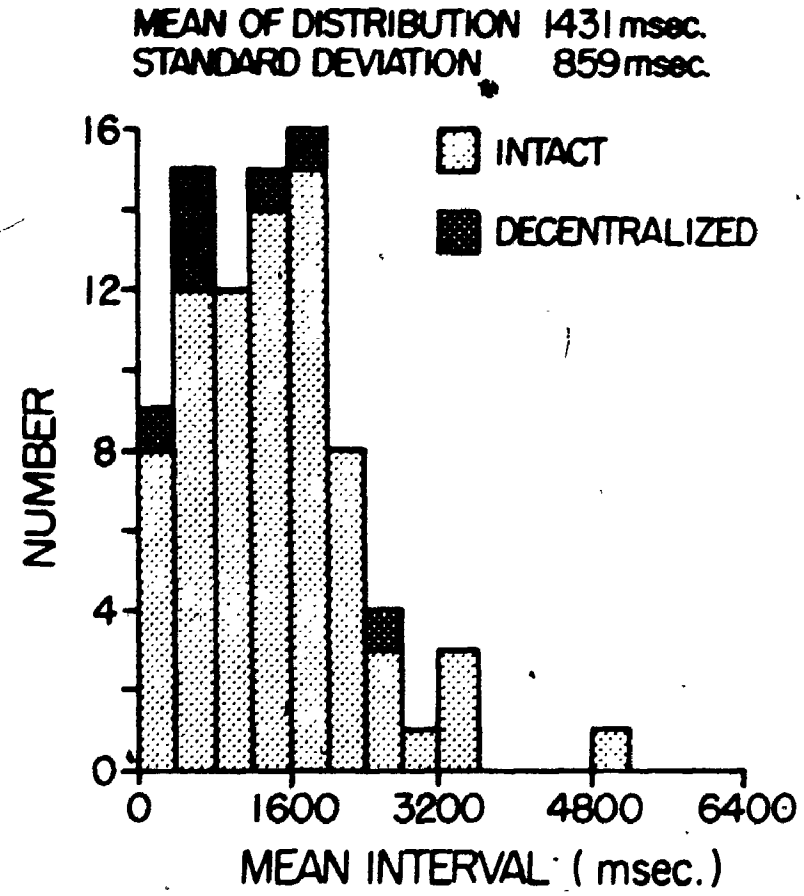

MEAN OF DISTRIBUTION 1431 msec.
STANDARD DEVIATION 859 msec.
INTACT
DECENTRALIZED
NUMBER
16
12
8
4
0
0
1600
3200
4800
6400
MEAN INTERVAL (msec.)

TABLE 7

SPONTANEOUS ACTIVITY IN DECENTRALIZED PREPARATIONS -

MEAN INTERVALS AND STANDARD DEVIATIONS

Neuron	Tape Segment	Time of Recording (minutes)	Number of Intervals in Record	Mean Interval (msec.)	Standard Deviation (msec.)
CF-I-B	500-650	4.1	421	584	677
CF-I-C	650-800	3.9	329	711	1039
CF-V-A	2840-3240	11.0	501	1322	1601
CF-V-D	750-1540	21.8	524	2496	3760
CF-V-E	1600-2000	11.2	1283	522	1058

18 shows the interspike interval density histogram and autocorrelogram for the neuron of Plate 6. Such histograms were again seen to be unimodal, positively skewed, and to demonstrate an absence of short intervals. Autocorrelograms indicated that the probability of post-impulse occurrence was constant at that value determined by the mean intervals. Characteristics of the autocorrelograms are given in Table 8.

Plotting the interspike interval density histograms as the number of intervals greater than or equal to a given time versus that time again revealed the presence of two approximately linear portions. This is shown in Figure 19 for the neuron of Plate 6. Using the same techniques as discussed in the previous section, straight lines were fitted to the "tail" portion and the "modal-minus-tail" portion of these distributions. Slopes of the regression lines fitted to these two portions, along with statistical measures, are listed in Table 9. Significance was again at least at the 5% level. These slopes or rates have been added to the distributions of the rates for intact preparations, and these joint distributions are shown in Figure 20. It can be seen that the rates for the long process for decentralized neurons maintain the narrowness of the distribution seen for intact preparations. Similarly the rates for the short process are widely scattered, as they were for neurons from intact preparations. Again there is a significant difference at the 0.1% level between the mean rates of the long and short processes.

FIGURE 18

Interspike Interval Histogram
and Autocorrelogram for an Exemplary Neuron
from a Decentralized Preparation

The arrow in the autocorrelogram indicates the level expected on the basis of the mean interval. This level is lower, in this particular instance, than the observed level due to the presence of very long intervals in the sample (note the large value for the mean interval). The autocorrelogram still indicates a random firing pattern however.

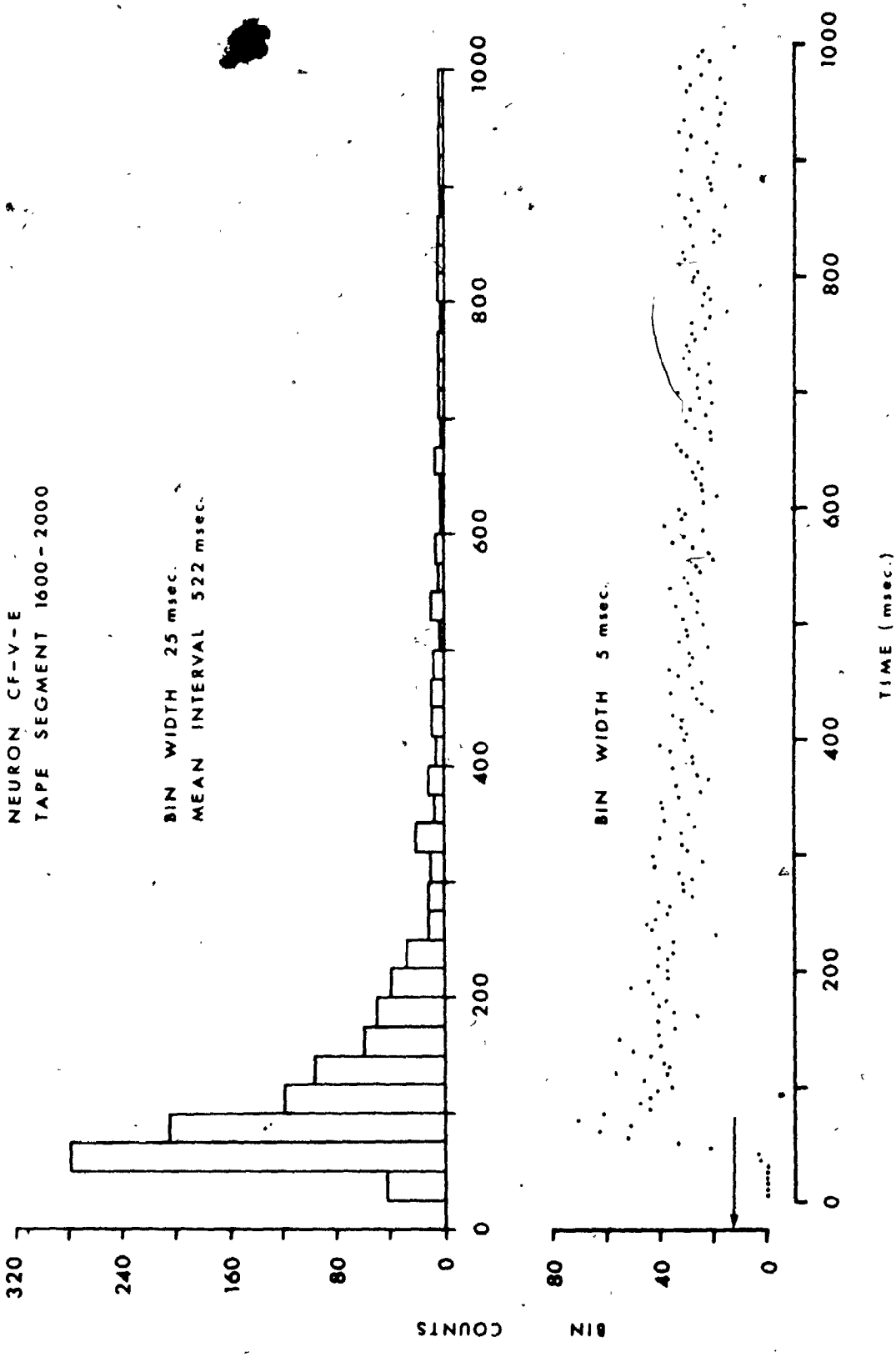

NEURON CF-V-E
TAPE SEGMENT 1600-2000
BIN WIDTH 25 msec.
MEAN INTERVAL 522 msec.
BIN WIDTH 5 msec.
COUNTS
BIN
TIME (msec.)
0
80
160
240
320
200
400
600
800
1000
40

TABLE 8

SPONTANEOUS ACTIVITY IN DECENTRALIZED PREPARATIONS -

CHARACTERISTICS OF AUTOCORRELOGRAMS

Neuron	Tape Segment	Description of Autocorrelogram
CF-I-B	500-650	flat, determined by mean interval
CF-I-C	650-800	flat, determined by mean interval
CF-V-A	2840-3240	flat, determined by mean interval
CF-V-D	750-1540	flat, determined by mean interval
CF-V-E	1600-2000	flat, determined by mean interval

FIGURE 19

Interspike Interval Distribution for the Exemplary Neuron from a Decentralized Preparation

The graph is a plot of the logarithm of the number of intervals whose length is greater than or equal to a given time versus that time. The regression line to the long process has been included.

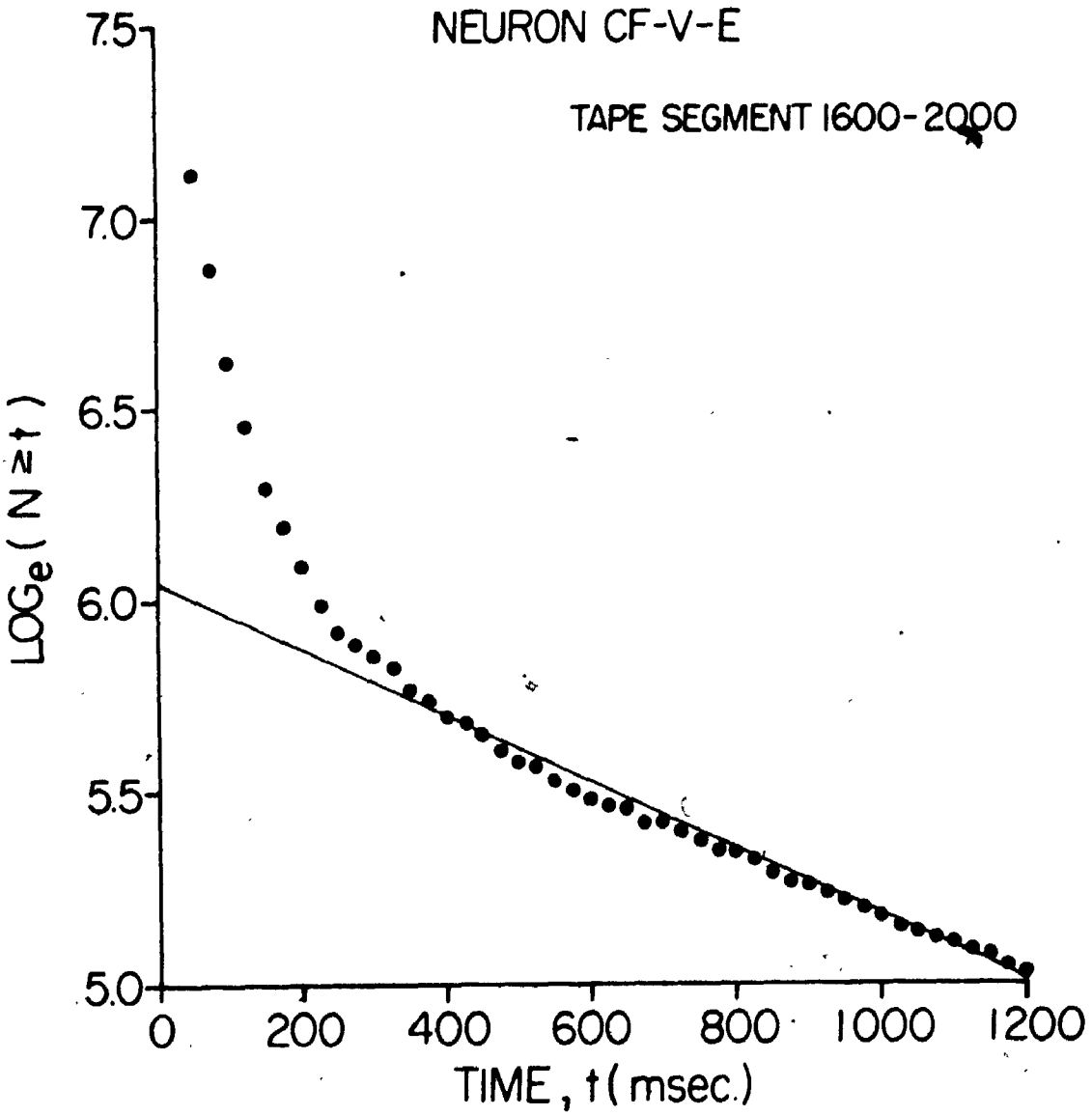

NEURON CF-V-E
TAPE SEGMENT 1600-2000
7.5
7.0
6.5
6.0
5.5
5.0
LOGe (N ≥ t)
0
200
400
600
800
1000
1200
TIME, t (msec.)

TABLE 9

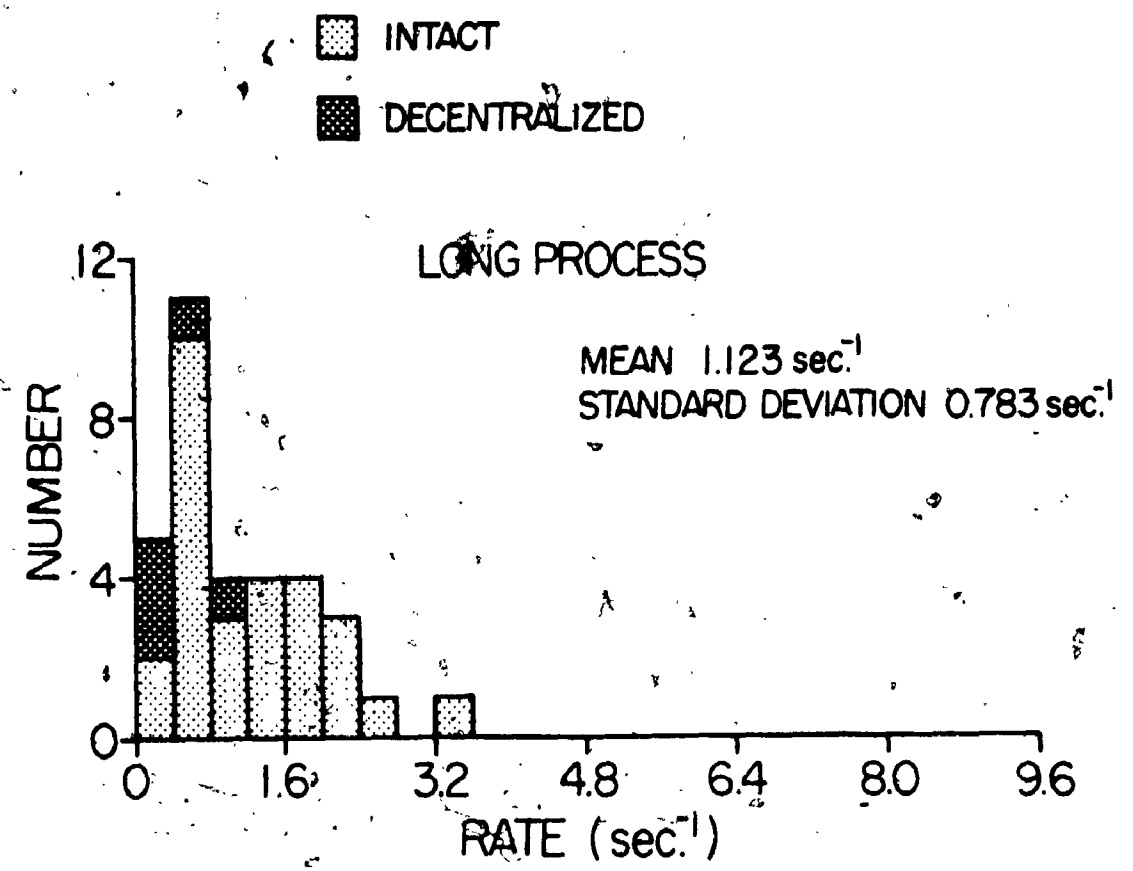
STATISTICS FOR THE TWO PROCESSES UNDERLYING SPONTANEOUS ACTIVITY IN NEURONS FROM DECENTRALIZED PREPARATIONS

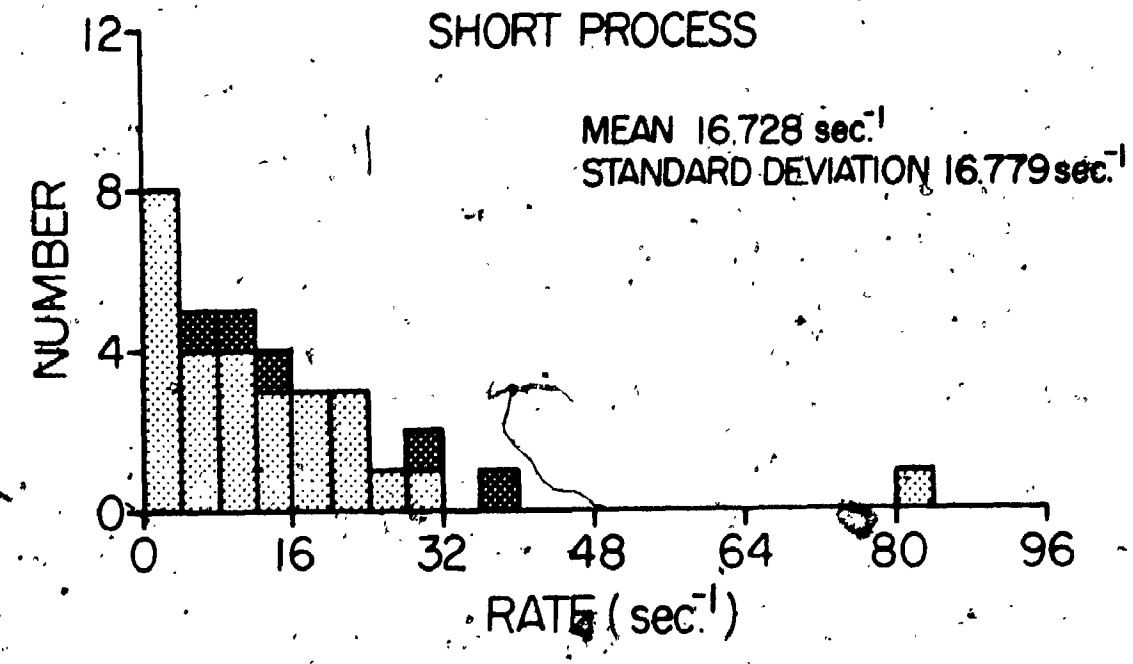
		Long Process				Short Process			
Neuron	Tape Segment	R.	S.E.E.	Slope$_{-1}$ (sec.$^{-1}$)	Level of Significance	R.	S.E.E.	Slope$_{-1}$ (sec.$^{-1}$)	Level of Significance
CF-I-B	500-650	0.98	0.011	0.328	0.1%	0.99	0.166	31.34	0.1%
CF-I-C	650-800	0.98	0.009	0.298	0.1%	1.00	0.137	38.70	0.1%
CF-V-A	2840-3240	1.00	0.044	0.682	0.1%	0.88	0.611	8.155	5.0%
CF-V-D	750-1540	1.00	0.019	0.267	0.1%	0.96	0.384	7.208	1.0%
CF-V-E	1600-2000	0.99	0.030	0.870	0.1%	1.00	0.041	15.77	0.1%

FIGURE 20

Distributions of the Rates of
the Two Processes in Neurons from Intact
and Decentralized Preparations

Comprising these histograms are the rates obtained for the twenty-eight neurons from intact preparations and the five neurons from decentralized preparations. The process for the regularly firing neuron has not been included. Notice that the abscissa scales for the two histograms differ by a factor of ten.


INTACT
DECENTRALIZED
LONG PROCESS
MEAN 1.123 sec.⁻¹
STANDARD DEVIATION 0.783 sec.⁻¹
NUMBER
12
8
4
0
0
1.6
3.2
4.8
6.4
8.0
9.6
RATE (sec.⁻¹)


SHORT PROCESS
MEAN 16.728 sec.⁻¹
STANDARD DEVIATION 16.779 sec.⁻¹
NUMBER
12
8
4
0
0
16
32
48
64
80
96
RATE (sec.⁻¹)

In Figure 21 the five neurons from decentralized preparations have been added to the graph of the logarithm of the standard deviation versus the logarithm of the mean interval. The regression line calculated with these neurons included is shown along with that obtained on the basis of neurons from intact preparations only. The correlation coefficient for the line including the decentralized neurons, tested on the t-distribution, is significant at the 0.1% level.

4.5 Deafferented Preparations

In one animal the olfactory nerves were sectioned in order to isolate the secondary neurons from any peripheral input which might arise as a result of spontaneous activity in the olfactory receptors. Two spontaneously active neurons were recorded from this preparation. The interspike interval density histograms for these neurons are shown in Figure 22. The histogram for one of these neurons (ON-I-A) has a moderate increase in bin counts between 500 and 1000 msec. The increased firing in this range is also apparent in the autocorrelogram for this neuron (Figure 23). Since this single neuron was the only one in the entire study demonstrating such a phenomenon, it is presumed that this phenomenon was due to some injury discharge in the central olfactory nerve stumps. The second neuron, recorded at a much later time after nerve sectioning, did not show such a phenomenon. However, since the firing patterns of these neurons could both be affected by injury discharge in the olfactory nerves,

FIGURE 21

A Plot of the Logarithm of the Standard Deviation Versus the Logarithm of the Mean Interval for Neurons from Intact and Decentralized Preparations

This graph is for the twenty-nine neurons from intact preparations and the five neurons from decentralized preparations whose activity was analyzed in detail. The point corresponding to the regularly firing neuron is denoted by an arrow. The parameters for linear correlation-regression analysis for data from intact preparations only (as reported in Figure 13) are as follows:

slope of regression line: 1.05

intercept of regression line: -0.22

correlation coefficient: 0.83 (significant at 0.1% level)

Similar parameters for analysis of the combined data from intact and decentralized preparations are as follows:

slope of regression line: 1.05

intercept of regression line: -0.18

correlation coefficient: 0.83 (significant at 0.1% level)

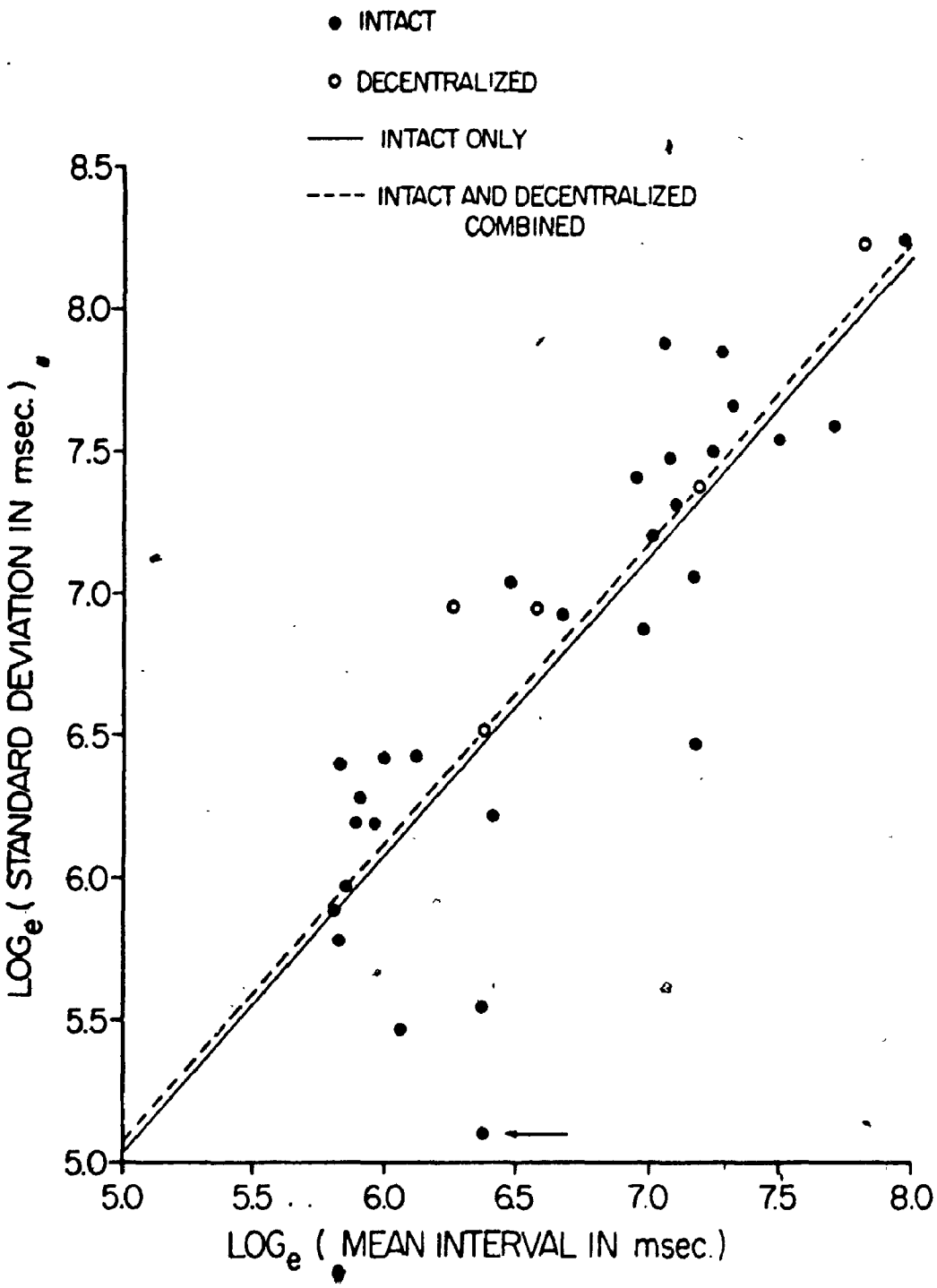
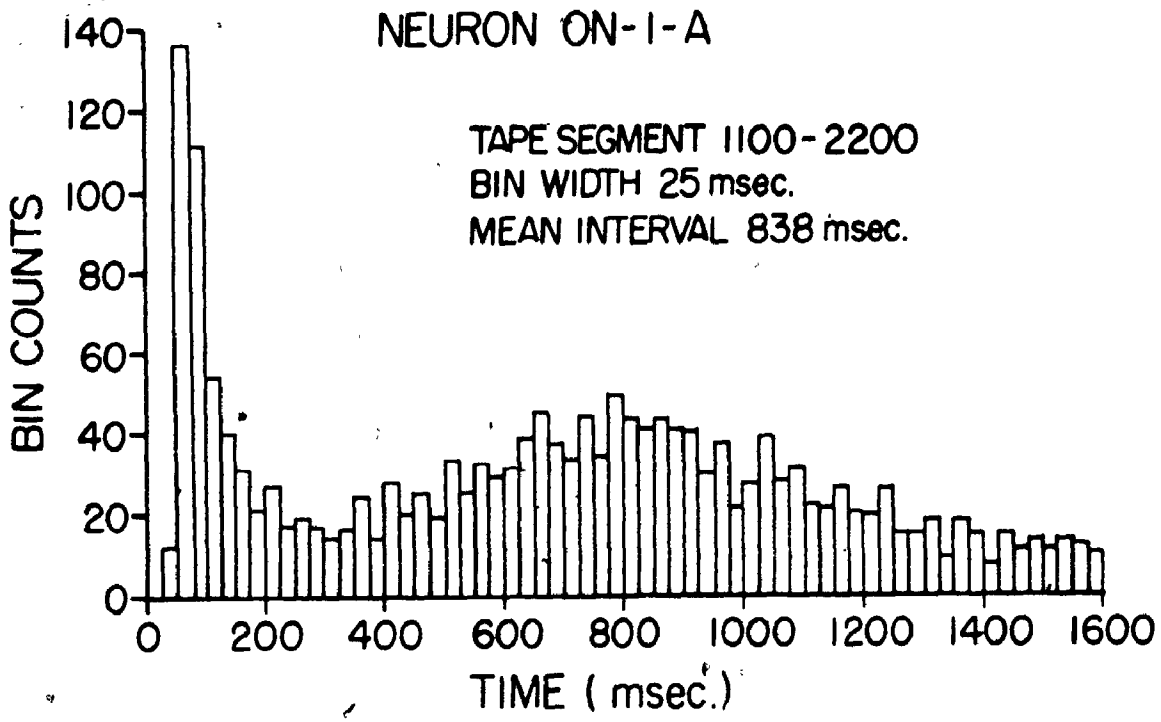

INTACT
DECENTRALIZED
INTACT ONLY
INTACT AND DECENTRALIZED COMBINED
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
LOGe (STANDARD DEVIATION IN msec.)
5.0
5.5
6.0
6.5
7.0
7.5
8.0
LOGe (MEAN INTERVAL IN msec.)

FIGURE 22

Interspike Interval Density Histograms for Two Neurons From a Deafferented Preparation

For neuron ON-I-A (upper figure), the record begins 18 minutes after the olfactory nerves had been sectioned and extends over a period of 28.5 minutes. For neuron ON-I-B, the record begins 85 minutes after the nerves had been sectioned and extends over a period of 10.7 minutes.


NEURON ON-1-A
TAPE SEGMENT 1100-2200
BIN WIDTH 25 msec.
MEAN INTERVAL 838 msec.
BIN COUNTS
140
120
100
80
60
40
20
0
0
200
400
600
800
1000
1200
1400
1600
TIME (msec.)

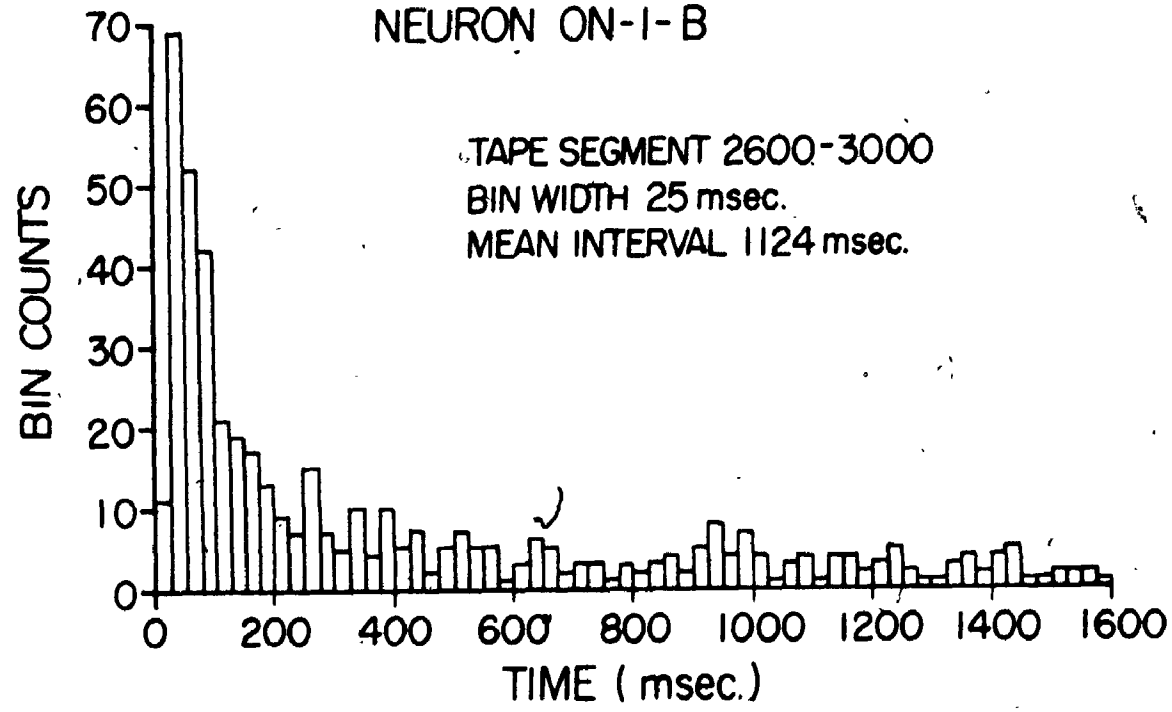
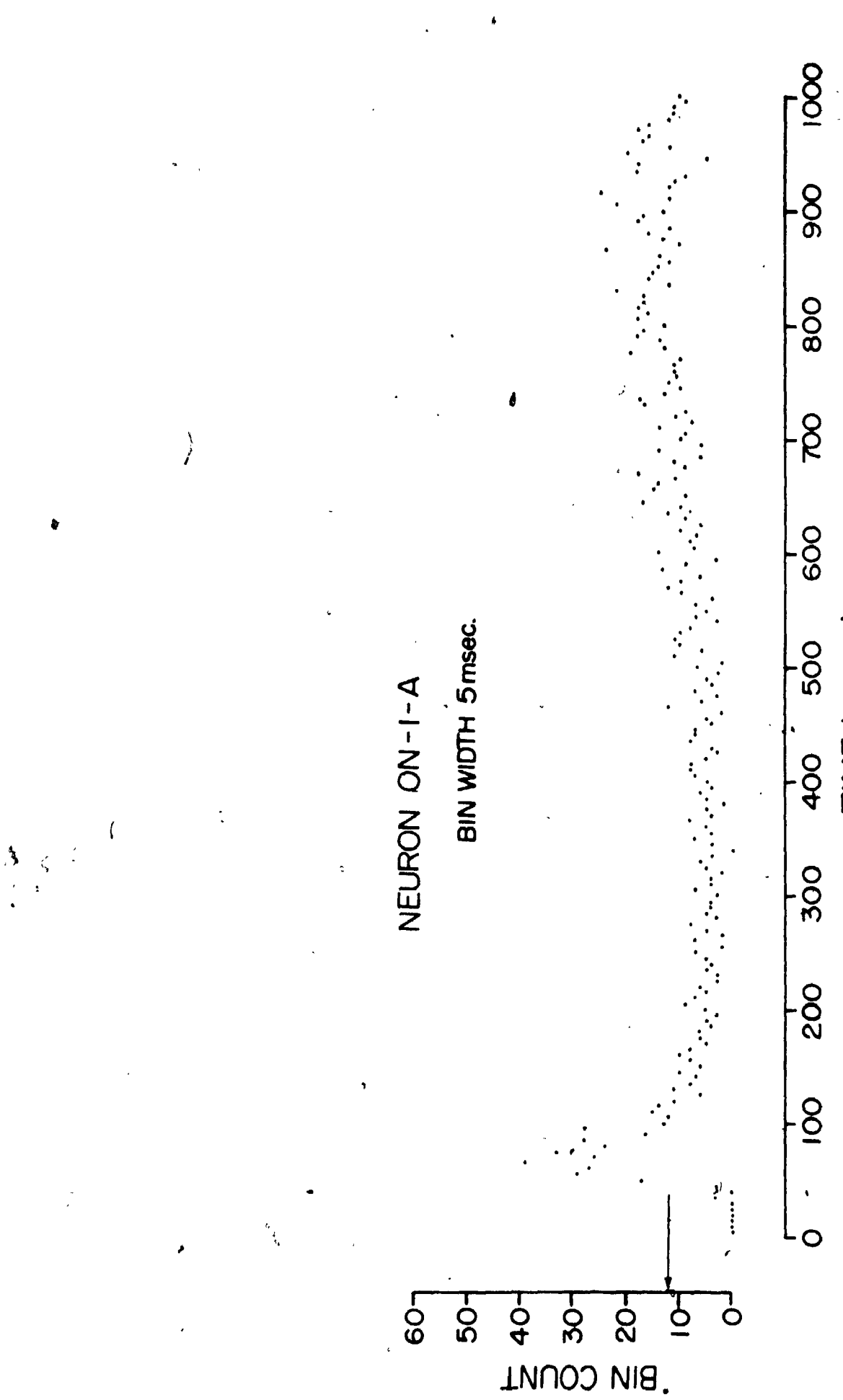

NEURON ON-1-B
TAPE SEGMENT 2600-3000
BIN WIDTH 25 msec.
MEAN INTERVAL 1124 msec.
BIN COUNTS
70
60
50
40
30
20
10
0
0
200
400
600
800
1000
1200
1400
1600
TIME (msec.)

FIGURE 23

Autocorrelogram for a Neuron from the Deafferented Preparation

This figure shows the autocorrelogram for neuron ON-I-A. The record is the same as that used to construct Figure 22 (upper figure). After a period of initial peaking the probability of spike occurrence is flat to approximately 500 msec. From 500 msec. to 1000 msec. there is a gradual increase in spike occurrence. The arrow indicates the constant bin count expected on the basis of randomness and calculated from the mean interspike interval.


NEURON ON-1-A
BIN WIDTH 5msec.
BIN COUNT
60
50
40
30
20
10
0
TIME (msec.)
0
100
200
300
400
500
600
700
800
900
1000

these two neurons have not been included in the overall statistics. Nonetheless, mean interspike intervals (Table 10A) for these neurons were distributed among those for all other neurons seen in this study (Figure 24). Furthermore, interspike interval distributions demonstrated that the two underlying processes were still present (Table 10B), and that the rates of these processes were distributed in accord with those from other neurons (Figure 25).

4.6 Analysis of a Record Showing Two Spontaneous Neurons

In the course of the experiments, one record was obtained which showed two neurons discharging spontaneously. A sample of the activity from these neurons is shown in Plate 7. To determine if there was any dependency of the firing of the neuron generating the small spikes upon the neuron generating the large spikes, cross-correlation analysis was performed on this record. The resulting cross-correlogram is shown in Figure 26. Departure of this correlogram from that expected on the basis of independence was tested by means of the χ^2-distribution. It was found that the probability that the null hypothesis is true was between 0.80 and 0.95. Thus there is no significant dependency of the firing of the neuron generating the small spikes upon the firing of the neuron generating the large spikes.

That the reciprocal situation (the neuron generating the small spikes driving the neuron generating the

TABLE 10A

MEAN INTERSPIKE INTERVALS OF SPONTANEOUS ACTIVITY FROM NEURONS IN DEAFFERENTED PREPARATIONS

Neuron	Tape Segment	Time of Recording (minutes)	Number of Intervals in Record	Mean Interval (msec.)	Standard Deviation (msec.)
ON-I-A	1100-2200	28.5	2048	838	842
ON-I-B	2600-3000	10.7	576	1124	1596

FIGURE 24

Distribution of Mean Interspike Intervals from Neurons in Intact, Decentralized, and Deafferented Preparations.

This histogram has been constructed from the mean interspike intervals of the seventy-seven neurons from intact preparations, the seven neurons from decentralized preparations, and the two neurons from the deafferented preparation. The mean and standard deviation of the distribution are those calculated on the basis of neurons from intact and decentralized preparations only. Deafferented neurons were excluded since there may be an influence due to nerve injury potentials in these neurons.

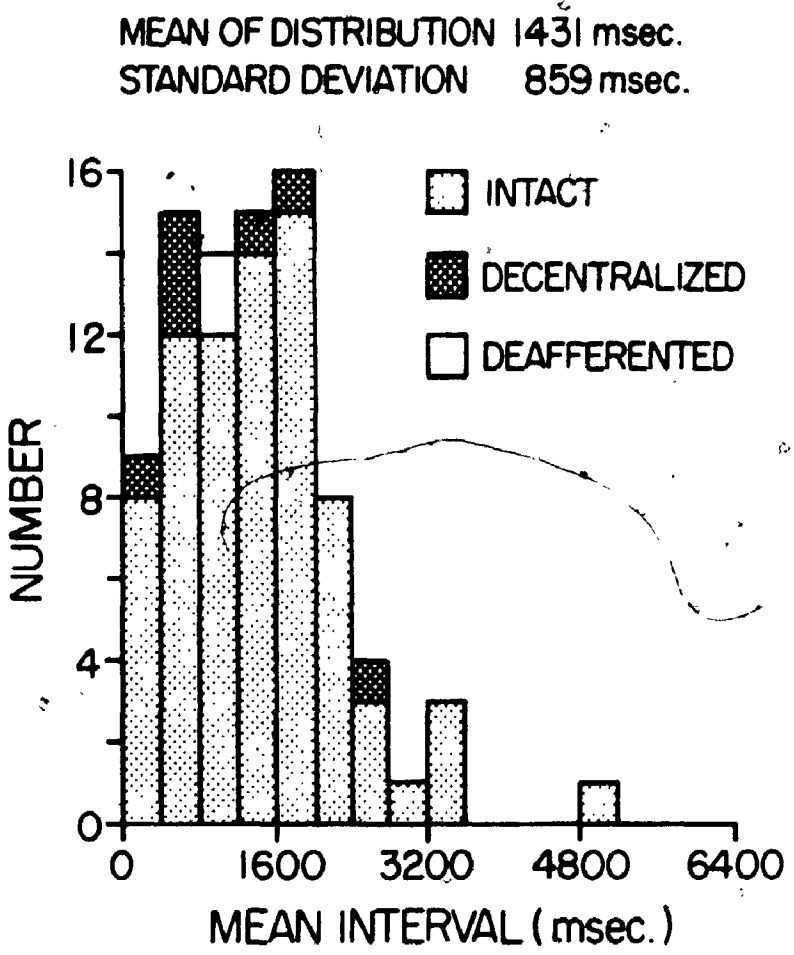

MEAN OF DISTRIBUTION 1431 msec.
STANDARD DEVIATION 859 msec.
INTACT
DECENTRALIZED
DEAFFERENTED
NUMBER
16
12
8
4
0
0
1600
3200
4800
6400
MEAN INTERVAL (msec.)

TABLE 10B

STATISTICS FOR THE TWO PROCESSES UNDERLYING SPONTANEOUS ACTIVITY IN NEURONS FROM THE DEAFFERENTED PREPARATION

		Long Process				Short Process			
Neuron	Tape Segment	R.	S.E.E.	Slope $(sec.^{-1})$	Level of Significance	R.	S.E.E.	Slope $(sec.^{-1})$	Level of Significance
ON-I-A	1100-2200	1.00	0.003	0.488	0.1%	1.00	0.110	30.779	0.1%
ON-I-B	2600-3000	1.00	0.025	0.917	0.1%	1.00	0.048	15.620	0.1%

FIGURE 25

Distributions of the Rates of the Two Processes in Neurons from Intact, Decentralized, and Deafferented Preparations

Comprising these histograms are the rates obtained for the twenty-eight neurons from intact preparations, the five neurons from decentralized preparations, and the two neurons from the deafferented preparation. The means and standard deviations are those calculated on the basis of intact and decentralized preparations only. The process for the regularly firing neuron has not been included. The abscissa scales for the two histograms differ by a factor of ten.

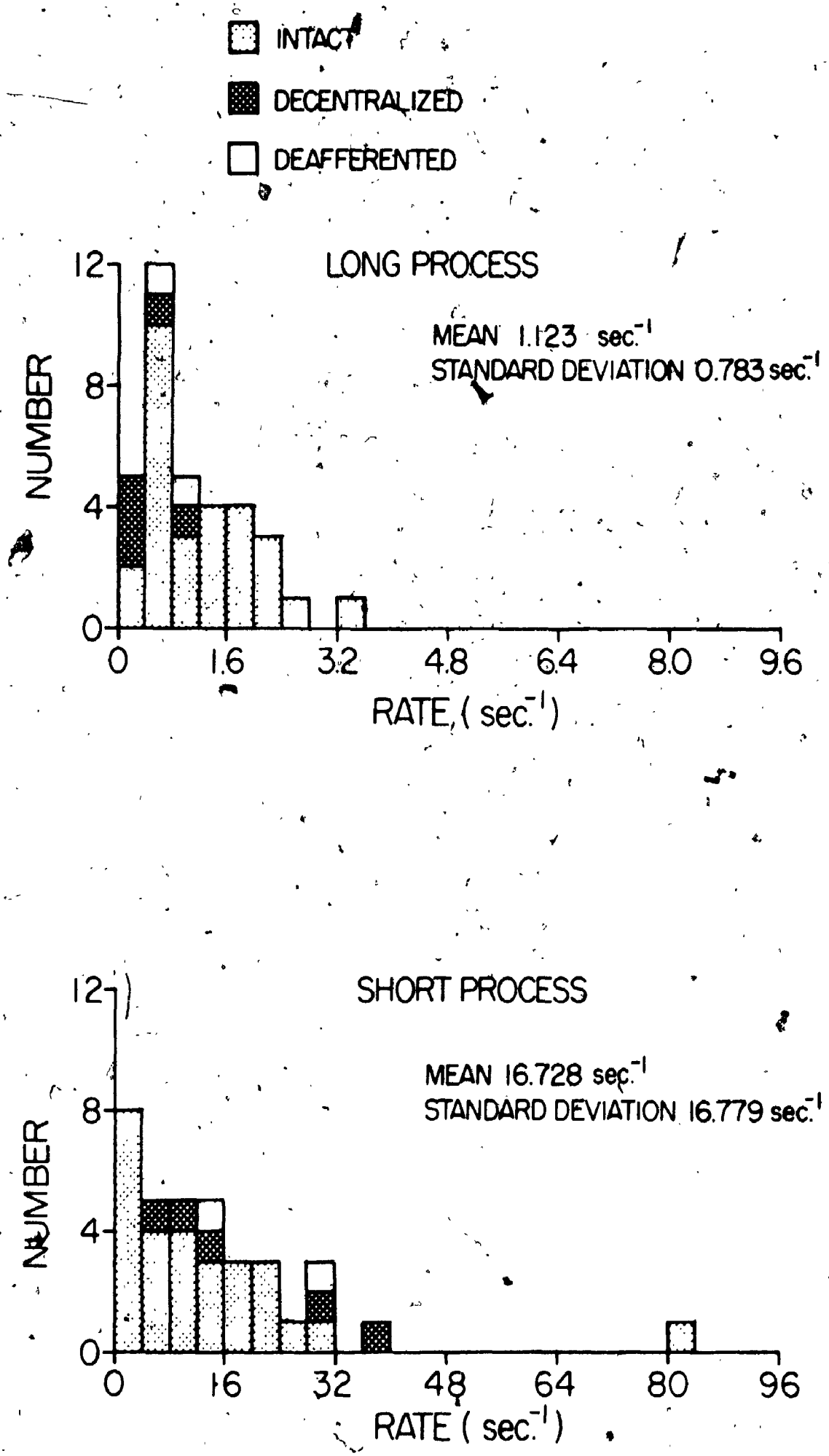

INTACT
DECENTRALIZED
DEAFFERENTED
LONG PROCESS
MEAN 1.123 sec.⁻¹
STANDARD DEVIATION 0.783 sec.⁻¹
NUMBER
12
8
4
0
0
1.6
3.2
4.8
6.4
8.0
9.6
RATE, (sec.⁻¹)
SHORT PROCESS
MEAN 16.728 sec.⁻¹
STANDARD DEVIATION 16.779 sec.⁻¹
NUMBER
12
8
4
0
0
16
32
48
64
80
96
RATE (sec.⁻¹)

PLATE 7

Record Showing Two Spontaneously Active Secondary Neurons

Consecutive traces form a single record.

Voltage calibration 7 mV.

Time calibration 1 sec.

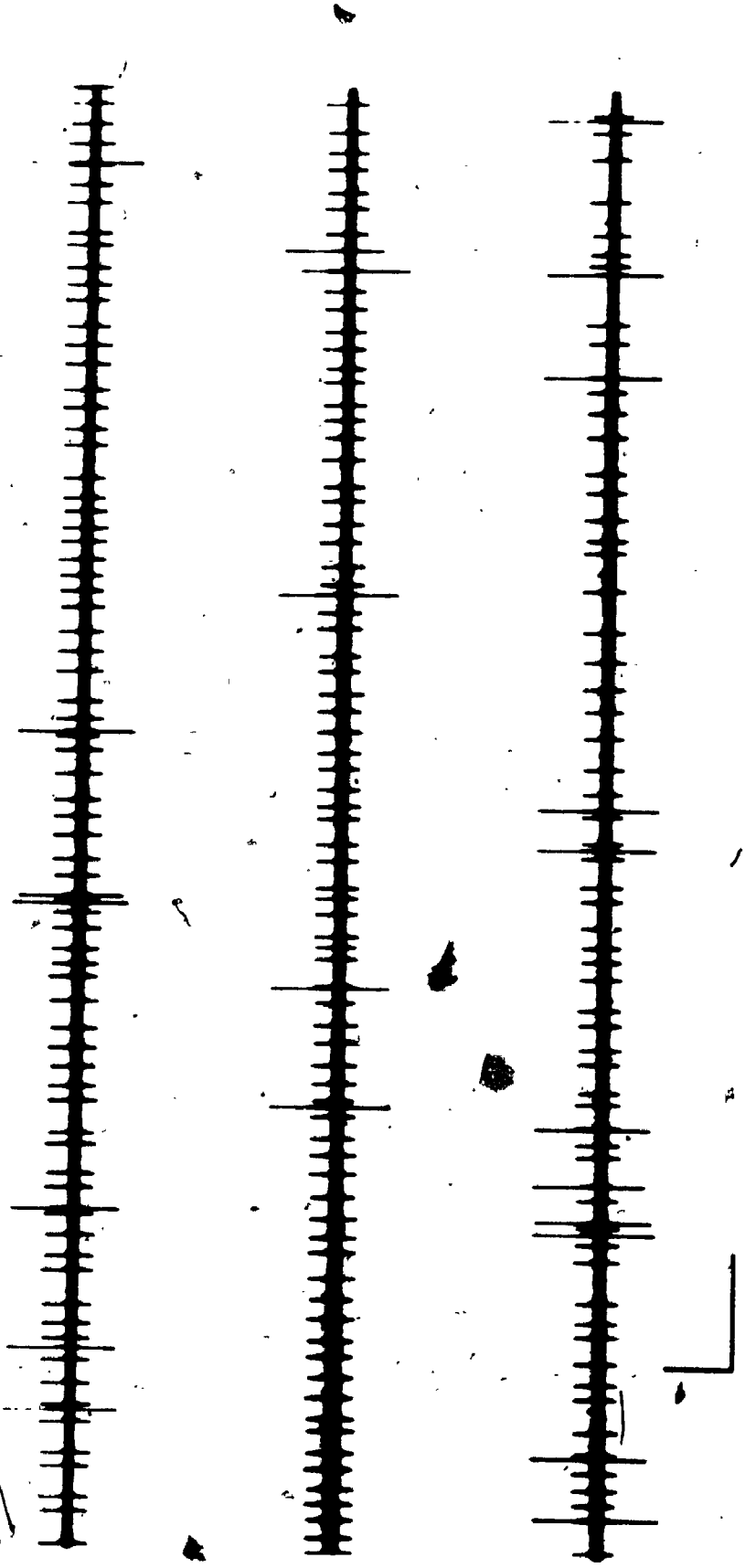
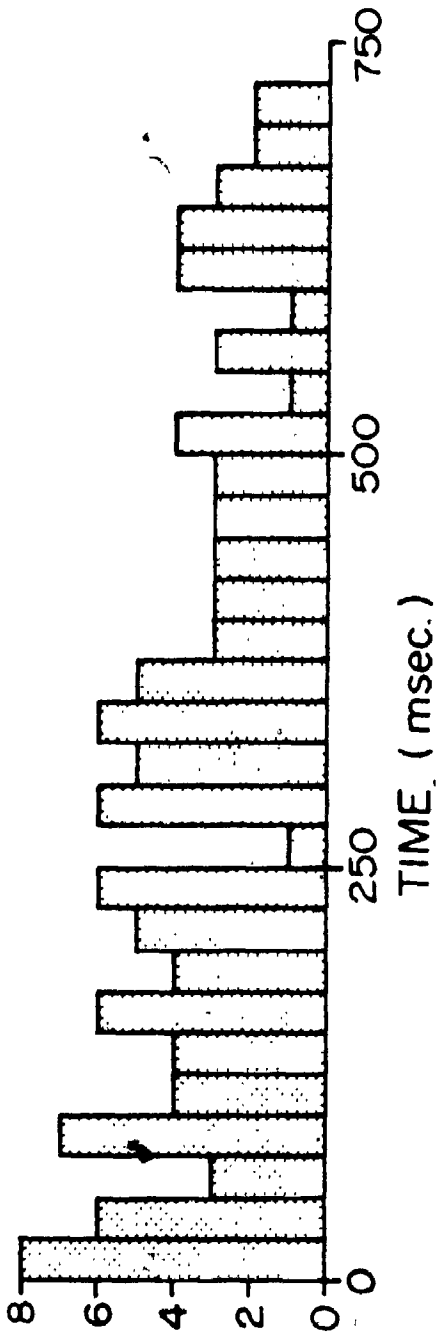


FIGURE 26

Cross-Correlogram for the

Record Containing Two Spontaneously Active

Secondary Neurons


NO. OF SMALL SPIKES PER 25 msec.
FOLLOWING 55 LARGE SPIKES
8
6
4
2
0
0
250
500
750
TIME (msec.)

large spikes) does not obtain can be seen from a consideration of the spike trains and from the number of small spikes preceding each large spike. Were the small spikes somehow driving the neuron generating the large spikes, the number of small spikes preceding each large spike should be essentially constant. In fact the number of small spikes preceding each large spike varied from 29 to 0 about a mean of 5.2 small spikes/large spike. Thus there is no apparent causal dependence of the neuron generating the large spikes upon the other neuron.

4.7 Evoked Activity in Intact Preparations - Inhibition

Electrical stimulation of the olfactory nerve was seen to cause a depression of the spontaneous activity of the secondary neurons for times as long as the order of one second. This depression was followed either by a direct return to the pattern of activity seen before stimulation (Plate 8A), or by a transient increase in discharge rate followed by a return to previous spontaneous levels (Plate 8B). Such effects could occur whether or not the sampled neuron was invaded by a post-synaptic spike due to nerve stimulation. That is, depression of the activity of the neuron subsequent to nerve stimulation did not require its prior firing by the stimulus. Furthermore, these post-stimulus effects were extremely constant, as shown in Plate 9 for a neuron which exhibited depression followed by a transient increase in discharge rate. These post-stimulus

PLATE 8A

Depression of Spontaneous
Activity Following Electrical Stimulation of the
Olfactory Nerve

The shock artifact near the beginning of the second trace (and marked by the superposed dot) is followed by a single spike (not visible as separate from the shock artifact on this time scale), and then depression of activity. The three traces form a continuous record (the large spikes are due to the presence of another neuron in the record).

Voltage calibration 3 mV.

Time calibration 1 sec.

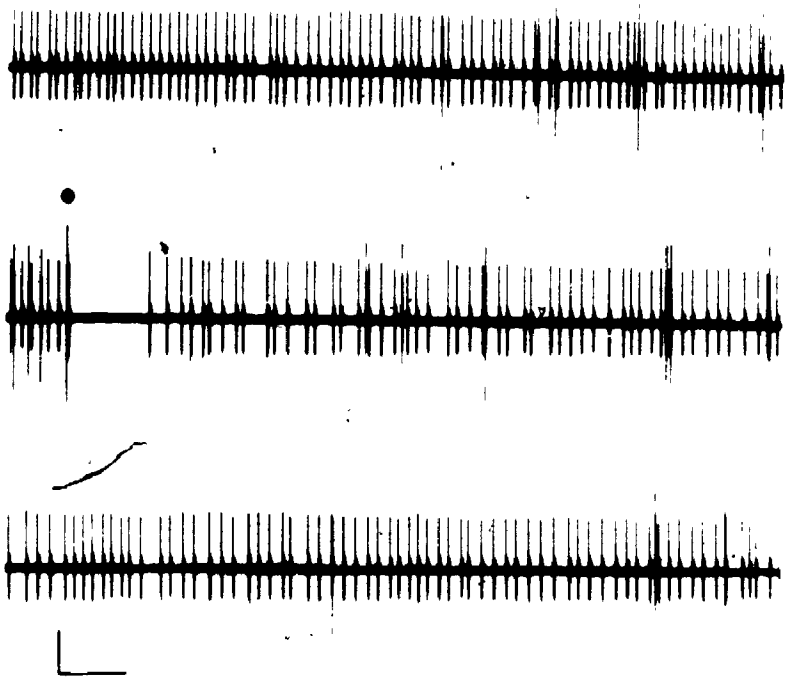
PLATE 8B

Depression of Spontaneous
Activity Following Electrical Stimulation of the
Olfactory Nerve

In this case the response to stimulation is a period of depression followed by a transient increase in discharge. The stimulus artifact is marked as in Plate 8A. The neuron is not invaded by a spike subsequent to stimulation in this case.

Voltage calibration 500 μV.

A



B

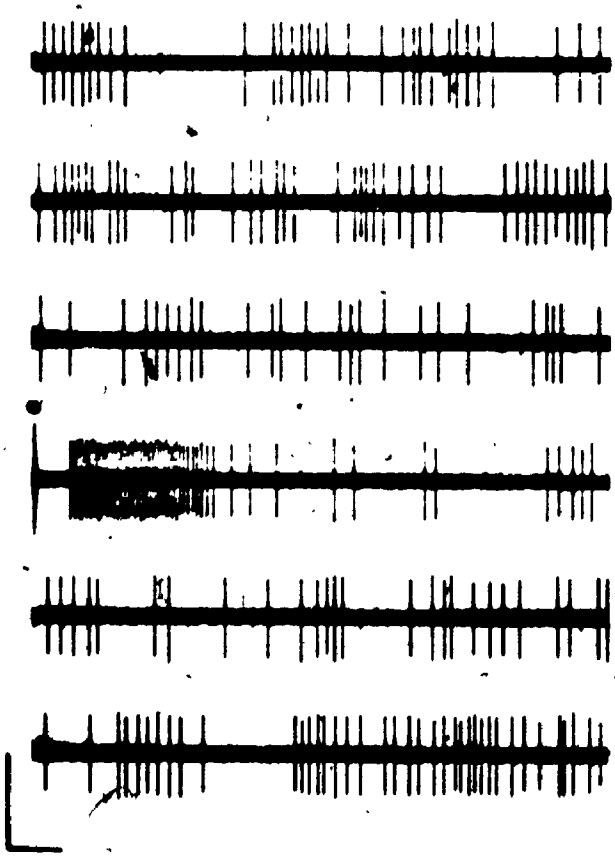


PLATE 9

Constancy of Post-Stimulus

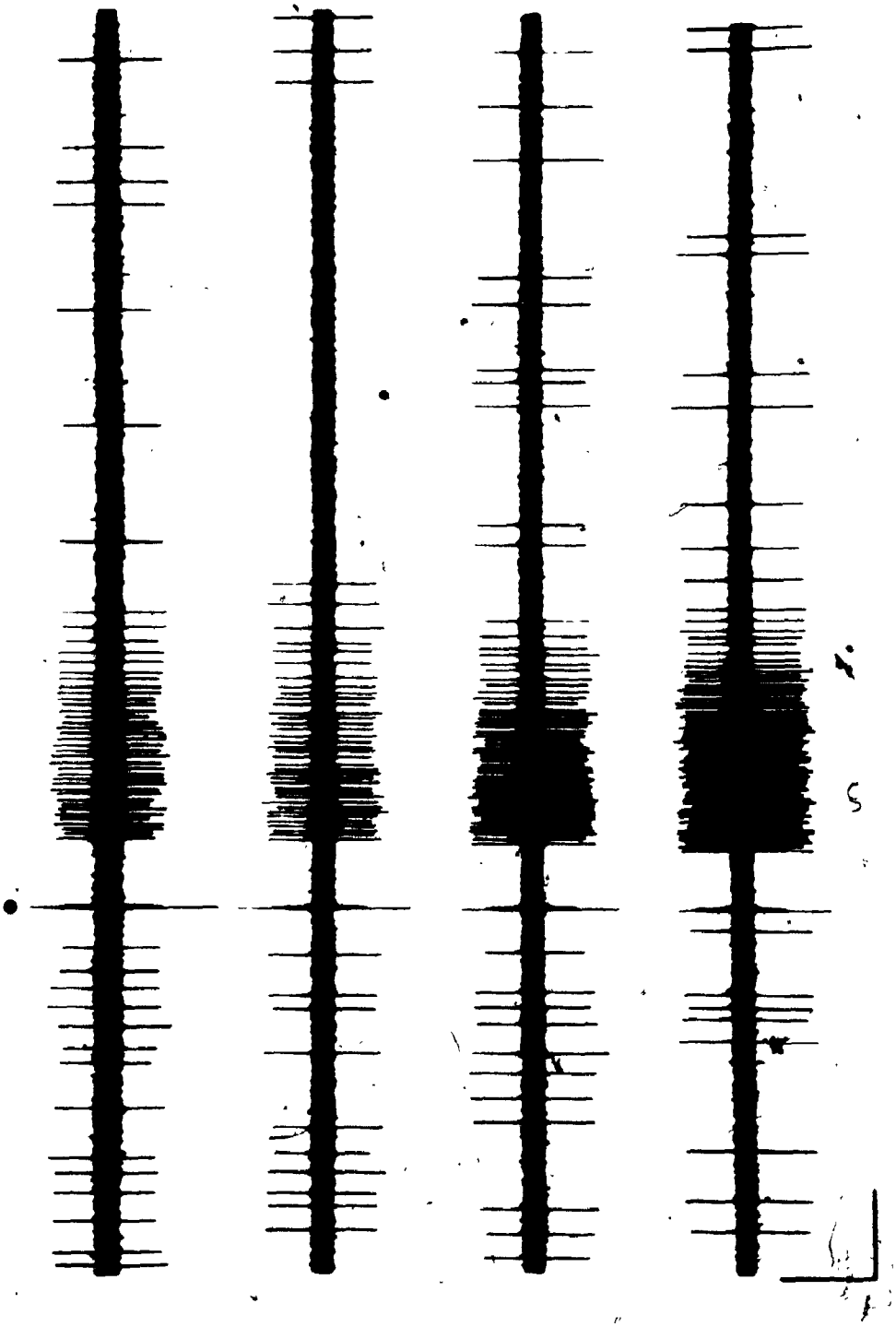
Response to the Secondary Neurons to Olfactory

Nerve Stimulation

The four traces show four stimulus presentations to the neuron at approximately 3-minute intervals. The superposed dot marks the shock artifacts.

Voltage calibration 300 μV.

Time calibration 1 sec.



effects are consistent with the results of Döving (1964) who studied the response of secondary neurons of the frog to natural odourous stimulation, although this author also reported seeing secondary neurons which responded to odourous stimulation with a simple increase in discharge rate (see figures 1 and 7 in Döving, 1964). To verify that the response patterns of neurons seen in this study with electrical stimulation of the olfactory nerve were consistent with those seen using natural stimulation of the nasal mucosa, several experiments were carried out using such natural stimuli. As an approximate measure of the time of onset of the stimuli, the electro-olfactogram was used. This is simply a summed potential recordable from the surface of the nasal mucosa, and due to the electrical activity of the receptors. In these experiments, the response patterns of the secondary neurons were similar to those seen using electrical stimulation of the nerves. As examples of the response patterns with natural stimulation, those for two different neurons are shown in Plate 10. The neuron in Plate 10A was seen to be inhibited by the odour, while the one in Plate 10B was seen to demonstrate inhibition followed by a transient increase in discharge rate. Neurons directly excited by natural stimulation were not seen.

To obtain a somewhat more quantitative picture of the response of the secondary neurons to electrical stimulation of the olfactory nerve, post-stimulus time histograms were plotted. These are shown in Figure 27 for the

PLATE 10A

Response of a Secondary Neuron to Natural Stimulation of the Olfactory Mucosa

This plate shows the response of a secondary neuron to stimulation of the olfactory mucosa with n-butanol vapour.[1] Consecutive traces form a single record. Onset of mucosal stimulation is indicated by the electro-olfactogram (EOG)[2] below the third trace of neuronal spike activity (the EOG pertains to the third trace).

Time calibration for both traces is 1 sec.

Voltage calibration for EOG is 1 mV.

Voltage calibration for spike activity is 100 μV.

[1] Vapour directed onto exposed nasal mucosa by means of a syringe.

[2] The EOG was recorded by means of glass pipettes whose tip diameters were of the order of 0.1 mm. These pipettes had been filled with a solution of gelatin in 0.9% sodium chloride. The pipettes were connected to a D.C. amplifier through an Ag/AgCl junction.

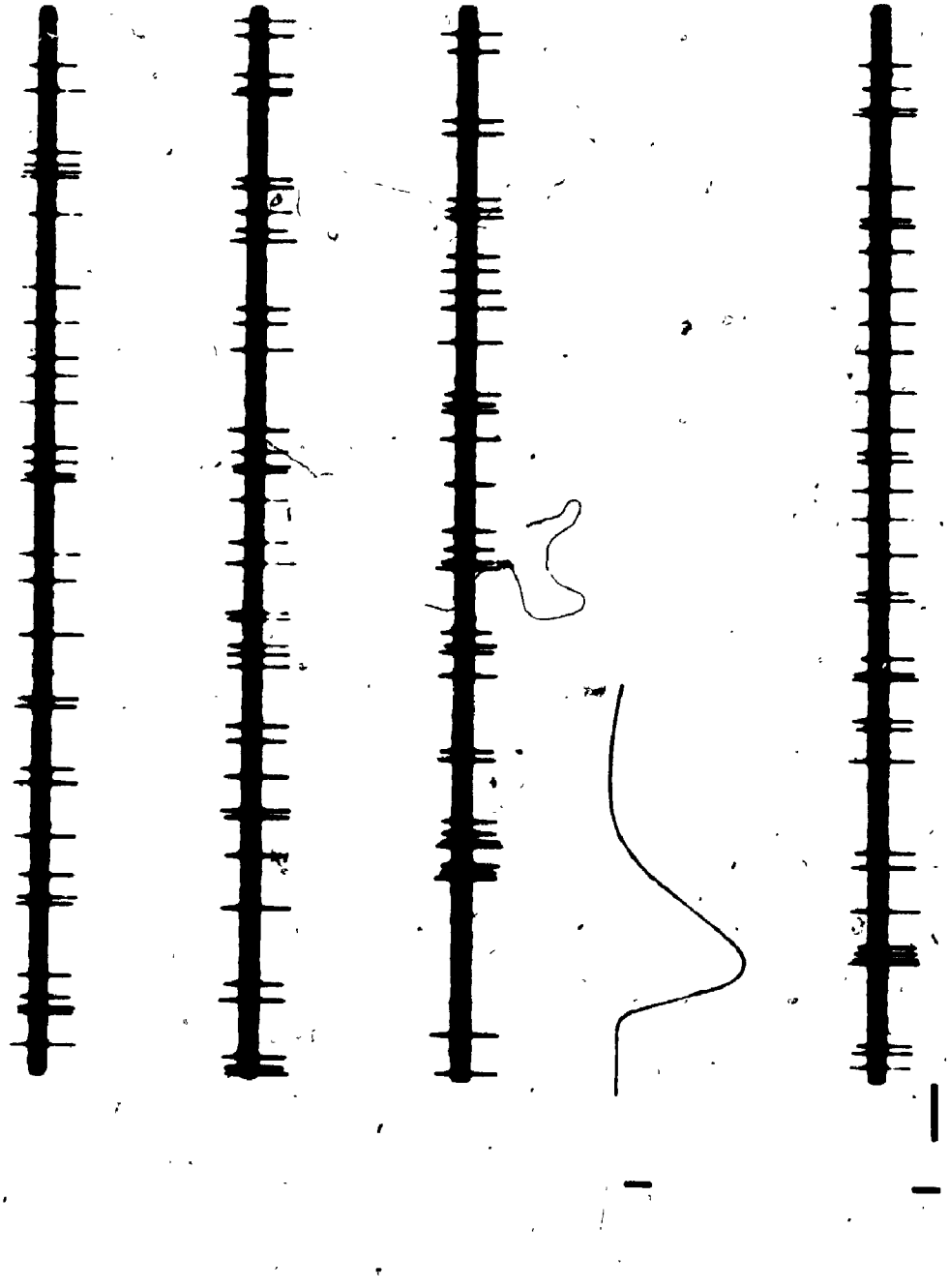


PLATE 10B

Response of a Secondary Neuron to Natural Stimulation of the Olfactory Mucosa

This plate shows the response of another secondary neuron to stimulation of the mucosa with pyridine vapour. Consecutive traces form a single record. The EOG again refers to the third trace.

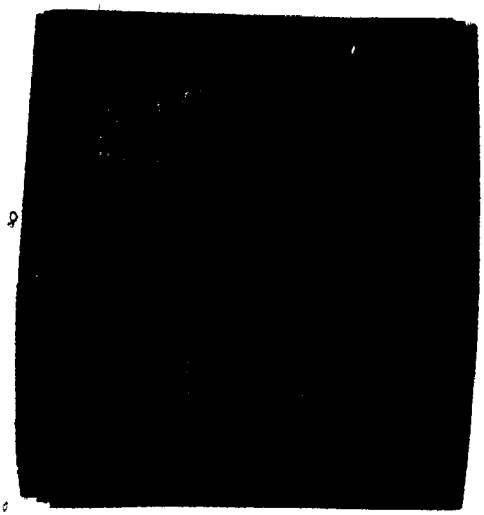
Time calibration for both traces is 1 sec.

Voltage calibration for EOG is 1 mV.

Voltage calibration for spike activity is 100 μV.

3 4

OF/DE



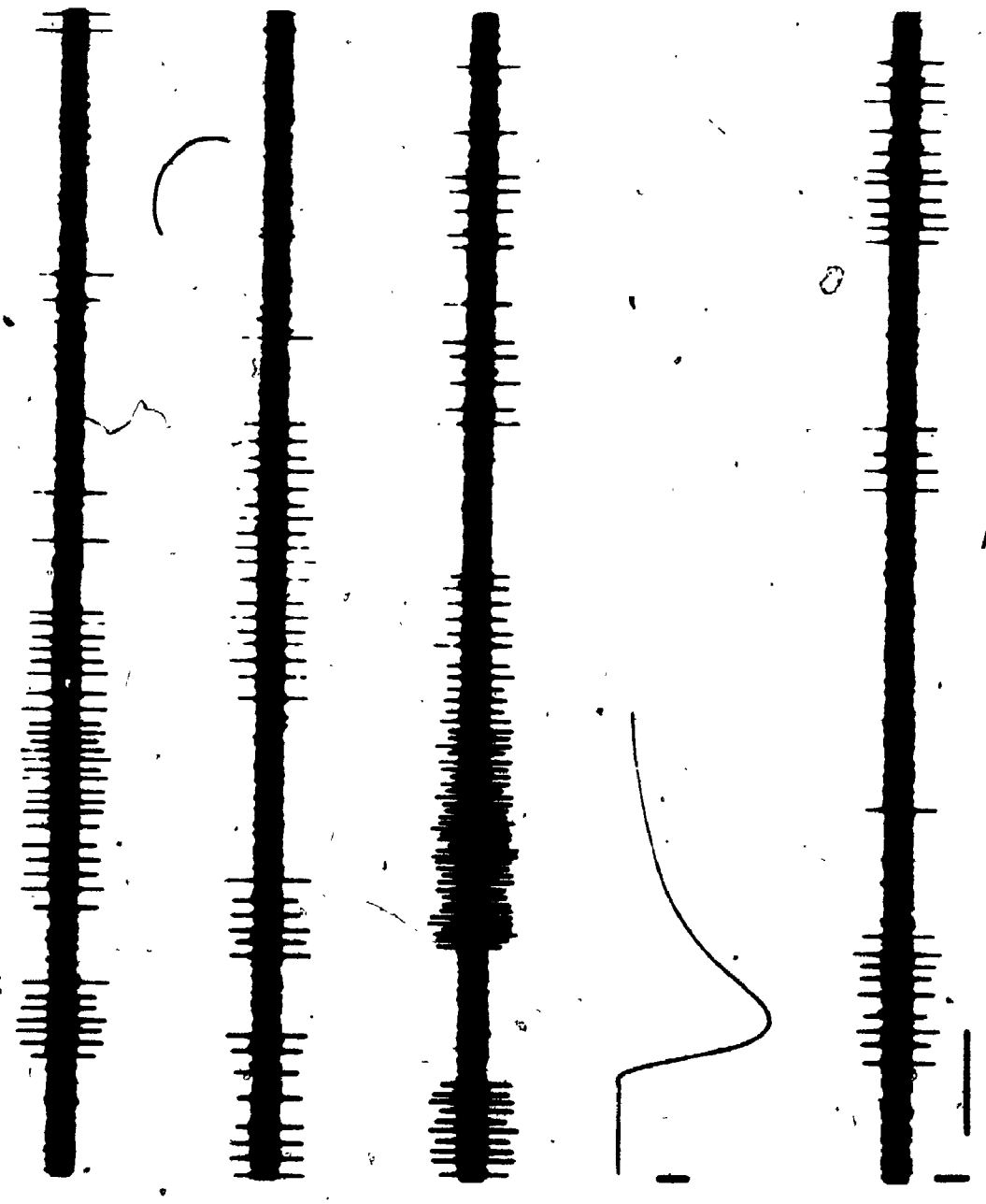
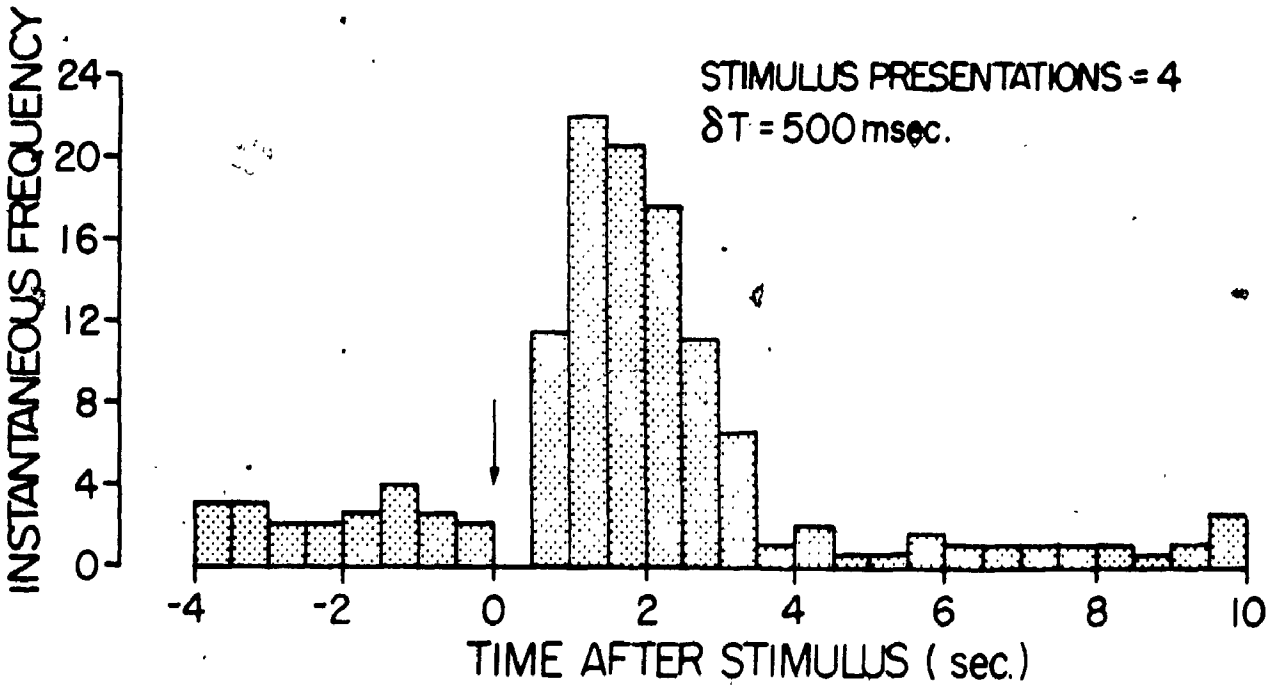


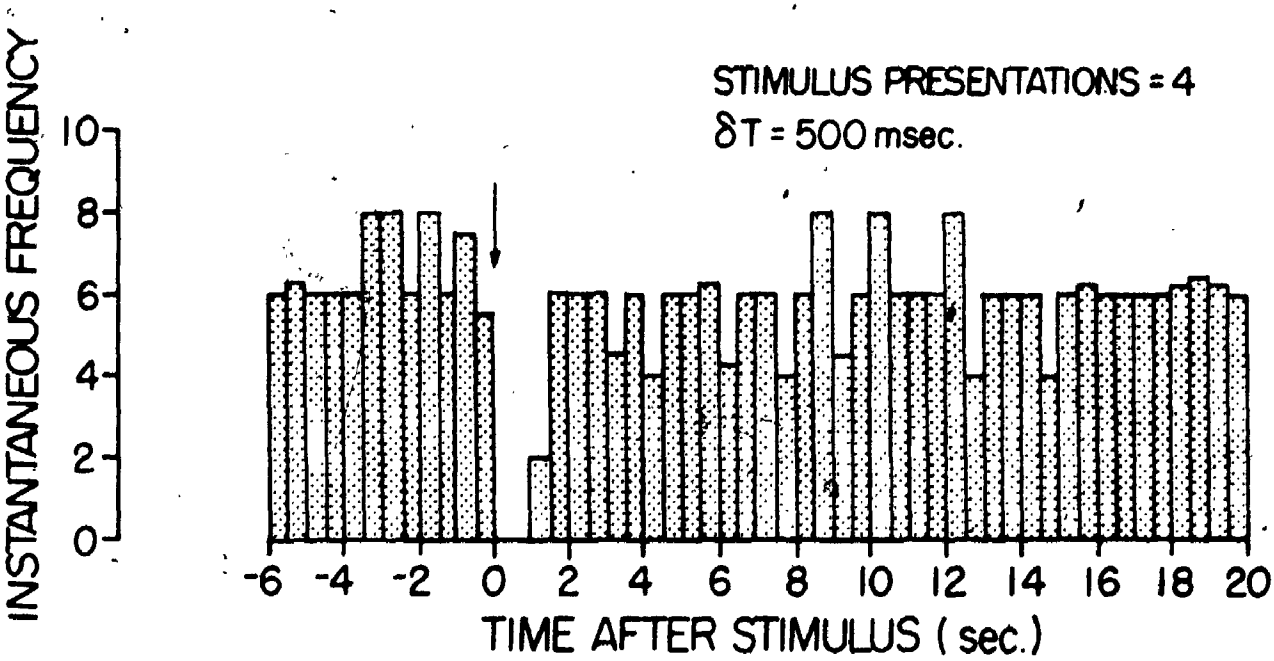
FIGURE 27

Post-stimulus Time Histograms for Two Neurons Giving Characteristic Response Patterns to Electrical Stimulation of the Olfactory Nerve

The upper post-stimulus time histogram is for a neuron demonstrating inhibition followed by rapid rebound firing. The lower histogram is for a neuron demonstrating inhibition followed by a return to normal firing with no overshoot. Arrows indicate the time of stimulus presentation. Both post-stimulus time histograms have been calculated from four stimulus presentations. The units of the ordinate of instantaneous frequency arise as follows:

$$\text{frequency} = \frac{\text{count in each time bin of } \delta T \text{ duration}}{(\text{number of stimuli}) \times (\delta T \text{ in seconds})}$$


INSTANTANEOUS FREQUENCY
24
20
16
12
8
4
0
STIMULUS PRESENTATIONS = 4
δT = 500 msec.
-4
-2
0
2
4
6
8
10
TIME AFTER STIMULUS (sec.)


INSTANTANEOUS FREQUENCY
10
8
6
4
2
0
STIMULUS PRESENTATIONS = 4
δT = 500 msec.
-6
-4
-2
0
2
4
6
8
10
12
14
16
18
20
TIME AFTER STIMULUS (sec.)

neuron generating the small spikes in Plate 8A and for the neuron of Plate 8B. These post-stimulus time histograms are characteristic for the secondary neurons observed in this study. It can be seen from such post-stimulus time histograms that the instantaneous frequency of firing of the neuron in both cases is suppressed following stimulation. When the neuron demonstrates a rebound firing after the period of inhibition, the instantaneous frequency rises considerably above the spontaneous level for a short time. When there is no rebound firing, the instantaneous frequency simply rises to its previous spontaneous level with no overshoot.

When post-synaptic firing did occur, it was seen to take the form of a single spike at long but constant latency from the stimulus artifact, as shown in Plate 11. Such invasion was observed in seven of the neurons studied. The mean latencies observed in these neurons are listed in Table 11. In Figure 28 this latency is plotted versus stimulus current for neuron XXXIX-A. From this graph it appears that the latency is independent of stimulus strength. For a comparison with these latencies of orthodromic invasion, latencies of antidromic invasion were examined in two neurons. The mean latencies for these neurons are given in Table 12: In this section, studies of the period of depression are presented. In the next section, a study of the period of rebound firing is presented.

The depression of activity observed in the

PLATE 11

Invasion of a Neuron Subsequent to Olfactory Nerve Stimulation

The invasion (indicated by the arrows) occurs at long latency from the shock artifact which begins either trace. Both traces are from the same cell, and each trace is the superposition of three shock presentations.

Voltage calibration 250 μV.

Time calibration 10 msec.

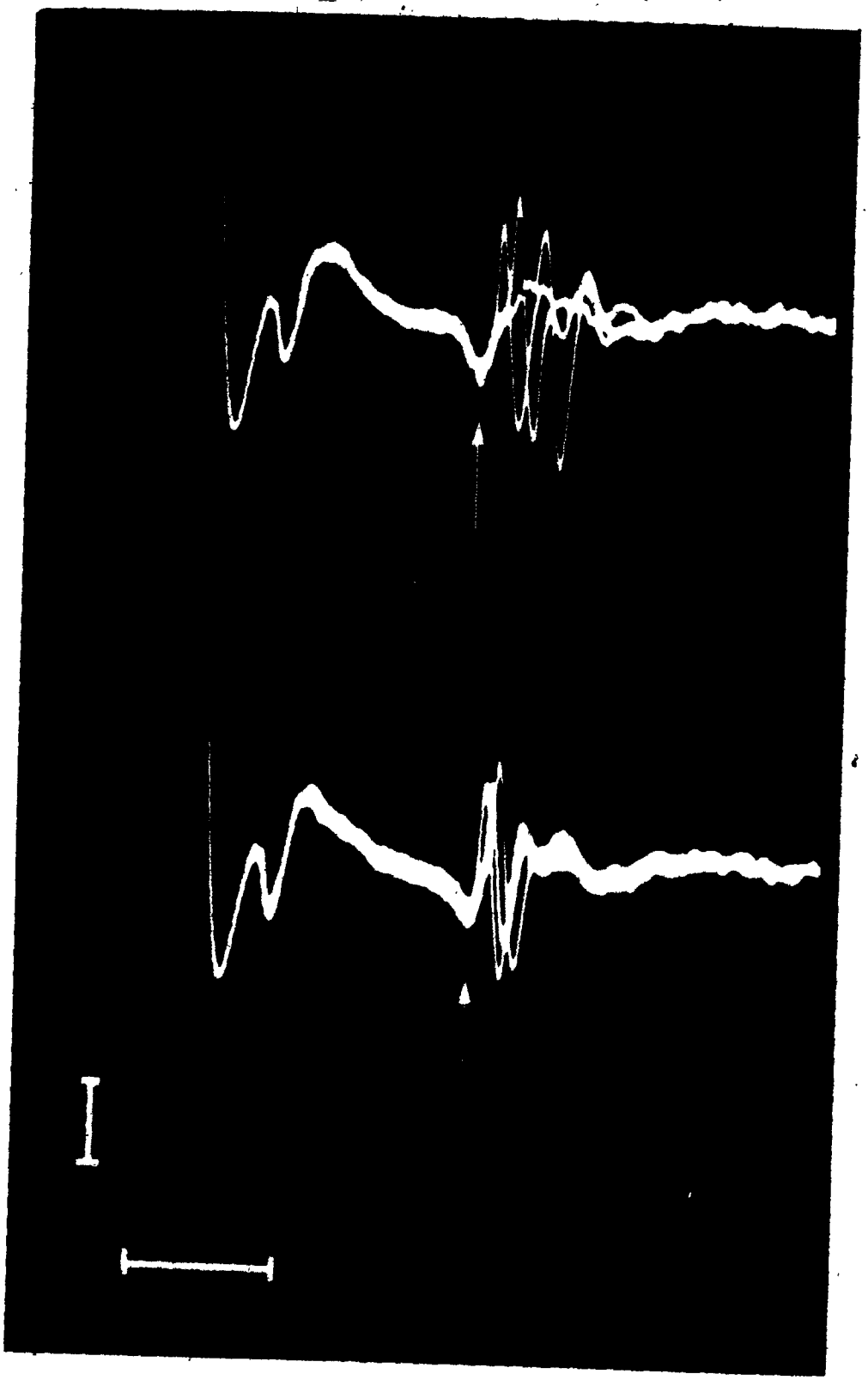

I

TABLE 11

MEAN LATENCIES FOR ORTHODROMIC INVASION

Neuron	Stimulus Current (μA.)	Mean Latency ± S.D.* (msec.)
XIX-A	70	23.9 ± 3.8
XX-A	100	17.8 ± 0.4
XXIV-C	80	22.4 ± 1.6
XXIV-D	80	24.5 ± 1.3
XXIV-D	120	24.2 ± 0.7
XXIV-E	80	25.3 ± 1.1
XXIV-F	160	19.8 ± 0.4
XXXIX-A	300	no invasion
XXXIX-A	500	no invasion
XXXIX-A	600	36.3 ± 7.8
XXXIX-A	800	36.4 ± 2.6
XXXIX-A	1000	38.6 ± 4.0
XXXIX-A	1200	39.9 ± 3.0
XXXIX-A	1400	41.6 ± 4.2
XXXIX-A	1600	40.1 ± 2.5
XXXIX-A	2000	43.3 ± 1.7
XXXIX-A	4000	32.3 ± 3.7

* On this and succeeding tables, S.D. is the standard deviation.

FIGURE 28

Constancy of Latency with

Stimulus Strength

The graph is for neuron XXXIX-A. Error bars indicate plus or minus one standard deviation.

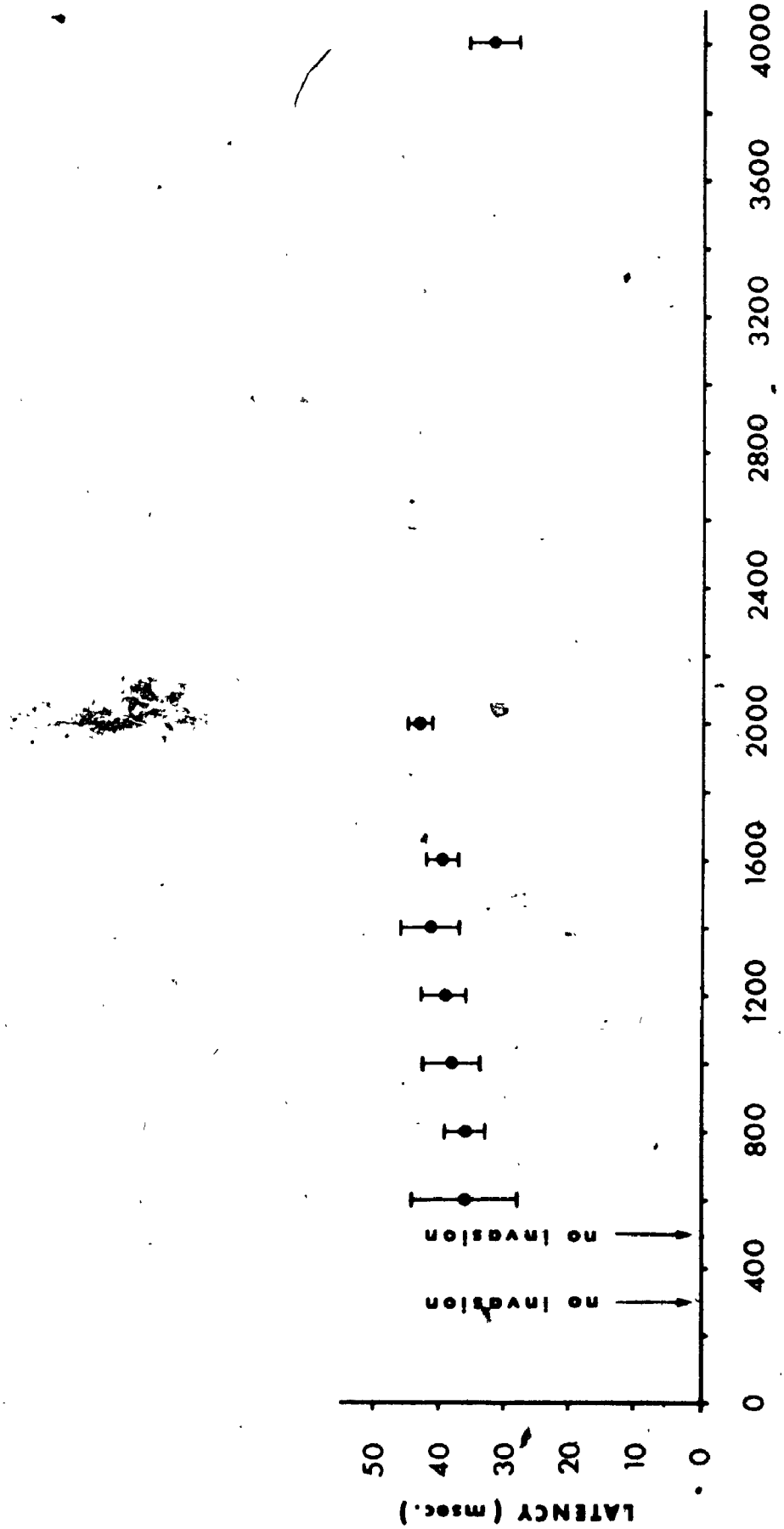

LATENCY (msec.)
0
10
20
30
40
50
no invasion
no invasion
0
400
800
1200
1600
2000
2400
2800
3200
3600
4000
STIMULUS CURRENT (μA)

TABLE 12

MEAN LATENCIES FOR ANTIDROMIC INVASION

Neuron	Stimulus Current (μA.)	Mean Latency ± S.D. (msec.)
XXXIII-C	120	20.5 ± 2.0
XXXVII-A	50	39.0 ± 0.9

secondary neurons following olfactory nerve stimulation could not be studied in depth in those neurons which were not fired by such stimulation. Further study of this depression was restricted to those neurons which could be driven by nerve stimulation. The state of these neurons following impulse generation was tested using paired conditioning and testing shocks applied to the nerve. Of the seven neurons which were driven by olfactory nerve shocks, six were examined using a conditioning-testing interval of two seconds. It was found that in four of the cells the testing shock would fail to result in production of a spike in the cell. Such failure is shown for one of the cells in Plate 12. The two cells that would generate a testing impulse at two second intervals (neurons XXIV-E and XXIV-F) both did so at increased latency over that at which they generated the conditioning spike. At conditioning-testing intervals of one second neuron XXIV-E also consistently failed to generate a testing spike. This is shown in Plate 13A. The second cell unfortunately was lost before conditioning-testing intervals shorter than two seconds could be attempted. Neuron XXXIX-A was not examined by means of paired stimuli.

The influence on the cells of repetitive stimulation of the nerve at 0.5 Hz. was also examined. In the four neurons which failed to generate a testing impulse at two second intervals, such repetitive stimulation resulted either in a maintained failure of impulse generation for all shocks past the first or in alteration between periods of

PLATE 12

Failure of Testing Shock to Elicit a Spike

This plate is the superposition of the traces for the conditioning and testing shocks to the olfactory nerve. The conditioning shock can be seen to cause invasion of the cell by a post-synaptic spike at a latency of approximately 20 msec. (indicated by the arrow). The testing shock, occurring 2 seconds later, fails to drive the neuron. Both traces begin with the shock artifact.

Voltage calibration 400 μV.

Time calibration 10 msec.

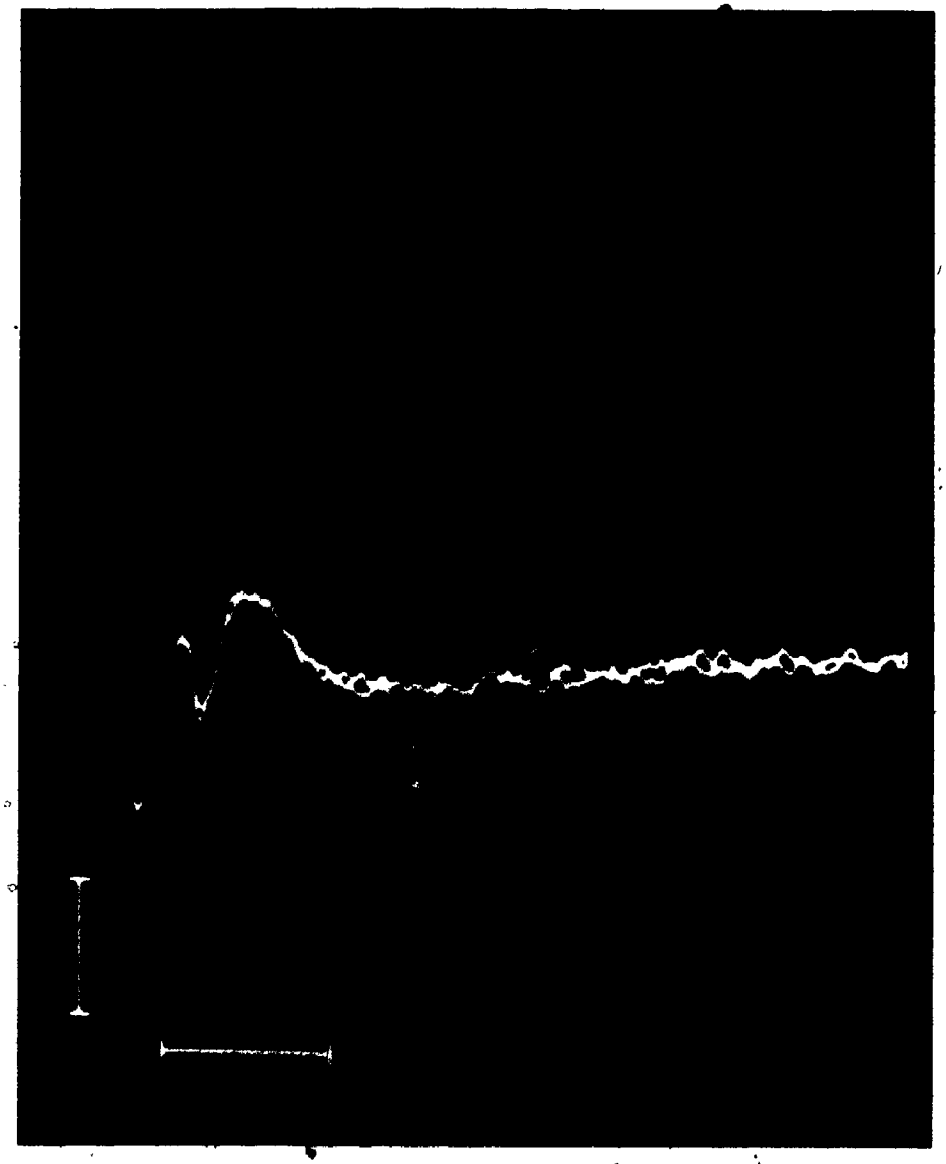


PLATE 13A

Response to Paired Conditioning-Testing Shocks to the Nerve

Neuron XXIV-E is driven by both conditioning and testing stimuli (marked by the arrows) at a shock separation of 2 seconds (upper trace, the testing shock produces the spike at longer latency). At a separation of 1 second between the conditioning and testing shocks (lower trace), the cell is driven by the conditioning shock (marked by the arrow) but fails to produce a spike in response to the testing shock.

Voltage calibration 250 μV.

Time calibration 10 msec.

PLATE 13B

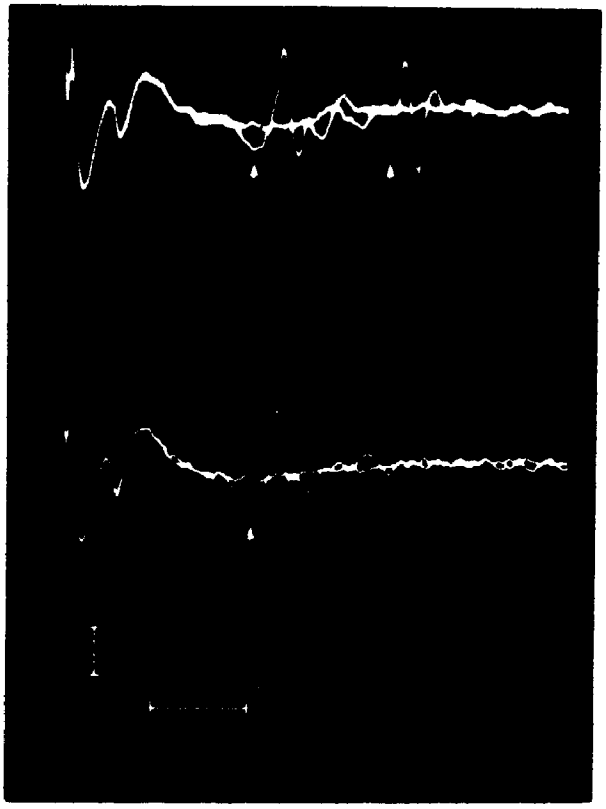
Response to Repetitive Nerve Stimulation

Each trace is the superposition of four consecutive stimuli. Neuron XXIV-E follows a rate of 0.5 Hz., producing a spike subsequent to each nerve shock (upper trace). At 1 Hz. however, the cell cannot follow and produces a spike in response to only two of the four shocks (lower trace).

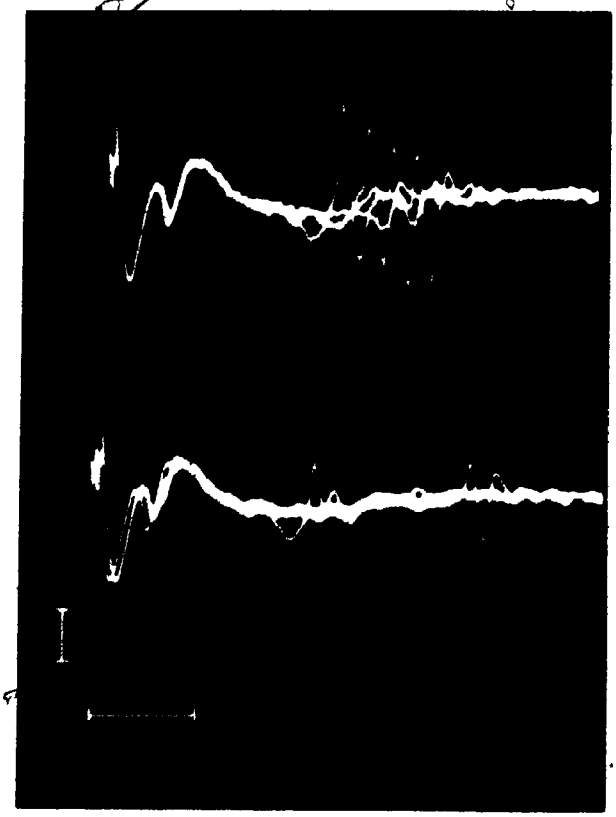
Voltage calibration 250 μV.

Time calibration 10 msec.

A



B



failure of invasion, generation of a spike, and again failure. In this latter case spike generation, when observed, occurred at longer latency than for the conditioning spike. Plate 14 illustrates a cell which consecutively followed and failed to follow shocks to the nerve. The results of repetitive stimulation are summarized in Table 13. The two cells which generated testing impulses at two second intervals both followed repetitive stimulation at 0.5 Hz., but at increased latencies. At 1 Hz. neuron XXIV-E would follow and fail as shown in Plate 13B (XXIV-F had been lost before repetitive stimulation at 1 Hz. could be attempted; XXXIX-A was not examined with repetitive stimulation). Differences between the mean latencies of invasion following conditioning and repetitive stimuli (beyond the first) were tested on the t-distribution and found significant at the 1% level in all cases.

4.8 Evoked Activity in Intact Preparations - Rebound Firing

In one neuron, the effect of stimulus strength upon the period of rebound firing was studied. This was neuron XXXIX-A (Table 11 and Figure 28 of the previous section). At low stimulus strengths (600 μA nerve shock), the response of this neuron to electrical stimulation of the olfactory nerve consisted of a single spike at a mean latency of 36.3 msec. (Plate 15, upper trace). When the stimulus strength was raised to 800 μA, the neuron again responded with a single spike. In addition, however, after a delay of the order of

PLATE 14

Consecutive Following and Failing in Repetitive Stimulation of the Nerve

Response of neuron XXIV-D to six consecutive (A, B, C, D, E, and F) nerve shocks at 0.5 Hz. The cell can be seen to follow (A, C, and E) and fail (B, D, and F) alternately.

Voltage calibration 250 μV.

Time calibration 10 msec.

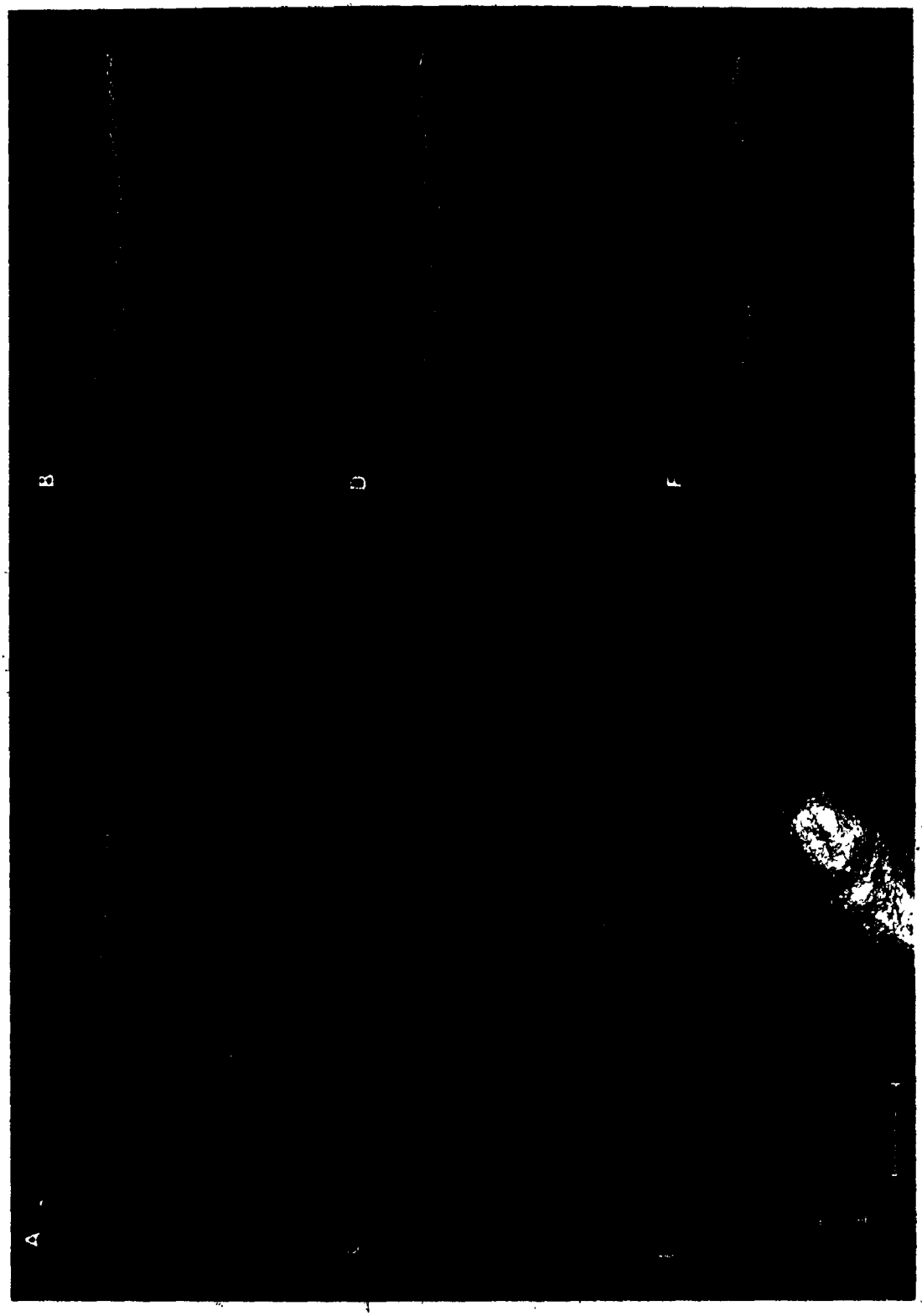

B
D
E
A

TABLE 13

SUMMARY OF REPETITIVE ORTHODROMIC STIMULATION

Neuron	Stimulus Current (μA.)	Frequency of Repetitive Stimulation (Hz.)	Description of Response	Mean Latency of Firing Beyond First ± S.D. (msec.)	Mean Latency* of Conditioning Spike ± S.D. (msec.)
XIX-A	70	0.5	Fails		23.9 ± 3.8
XX-A	100	0.5	Follows and Fails	20.5 ± 0.5	17.8 ± 0.4
XXIV-C	80	0.5	Follows and Fails	35.7 ± 1.2	22.4 ± 1.6
XXIV-D	80	0.5	Follows and Fails	38.8 ± 3.4	24.5 ± 1.3
XXIV-D	120	0.5	Follows and Fails	37.0 ± 3.2	24.2 ± 0.7
XXIV-E	80	0.5	Follows	32.7 ± 3.5	25.3 ± 1.1
XXIV-E	80	1.0	Follows and Fails	36.0 ± 4.3	25.3 ± 1.1
XXIV-F	160	0.5	Follows	21.8 ± 1.0	19.8 ± 0.4

* From Table 10

PLATE 15

Effect of Stimulus Strength upon the

Period of Rebound Firing

This plate shows the response of neuron XXXIX-A to three nerve shocks of different magnitudes. The stimulus artifacts are marked by the superposed dot. The stimulus strengths are as follows:

upper trace: 600 μA

middle trace: 800 μA

lower trace: 4000 μA

Voltage calibration is 2 mV.

Time calibration is 100 msec.

two hundred milliseconds the neuron produced a short burst of spikes (Plate 15, middle trace). As the stimulus strength was increased further, the period between the single initial spike and the first spike of the burst decreased (Plate 15, lower trace). In Figure 29B, the latency of occurrence of the burst (measured from the single initial spike) has been plotted versus stimulus current. In Figure 29C, a reproduction of Figure 28 showing the latency of the initial spike from the stimulus artifact is presented. It can be seen that, while the latency of invasion of the neuron by the initial spike is constant, the latency of the burst response (measured from this spike) steadily declines, eventually to attain a constant value of approximately 100 msec. In Figure 29A, the number of spikes in the burst response is plotted versus stimulus current. With increasing stimulus current the number of spikes in the burst response increases to finally settle at a value of approximately 8 spikes/burst.

4.9 Evoked Activity in Intact Preparations - Effect of Repetitive Stimulation on Response Patterns

The response patterns of secondary neurons were also examined using repetitive stimulation. From records such as those of Plate 16 it can be seen that stimuli falling within the period of inhibition did not lengthen this period. Furthermore, stimuli falling early in the period of rebound firing do not produce a recurrence of the inhibition. Stimuli falling later in the rapid firing can suppress the rapid

FIGURE 29

Effect of Stimulus Current on

Rebound Firing

These graphs are for neuron XXXIX-A. Error bars are in all cases plus or minus one standard deviation. The point at 4000 μA in the uppermost graph has no error bar since at this stimulus current the neuron consistently produced a burst of eight spikes.

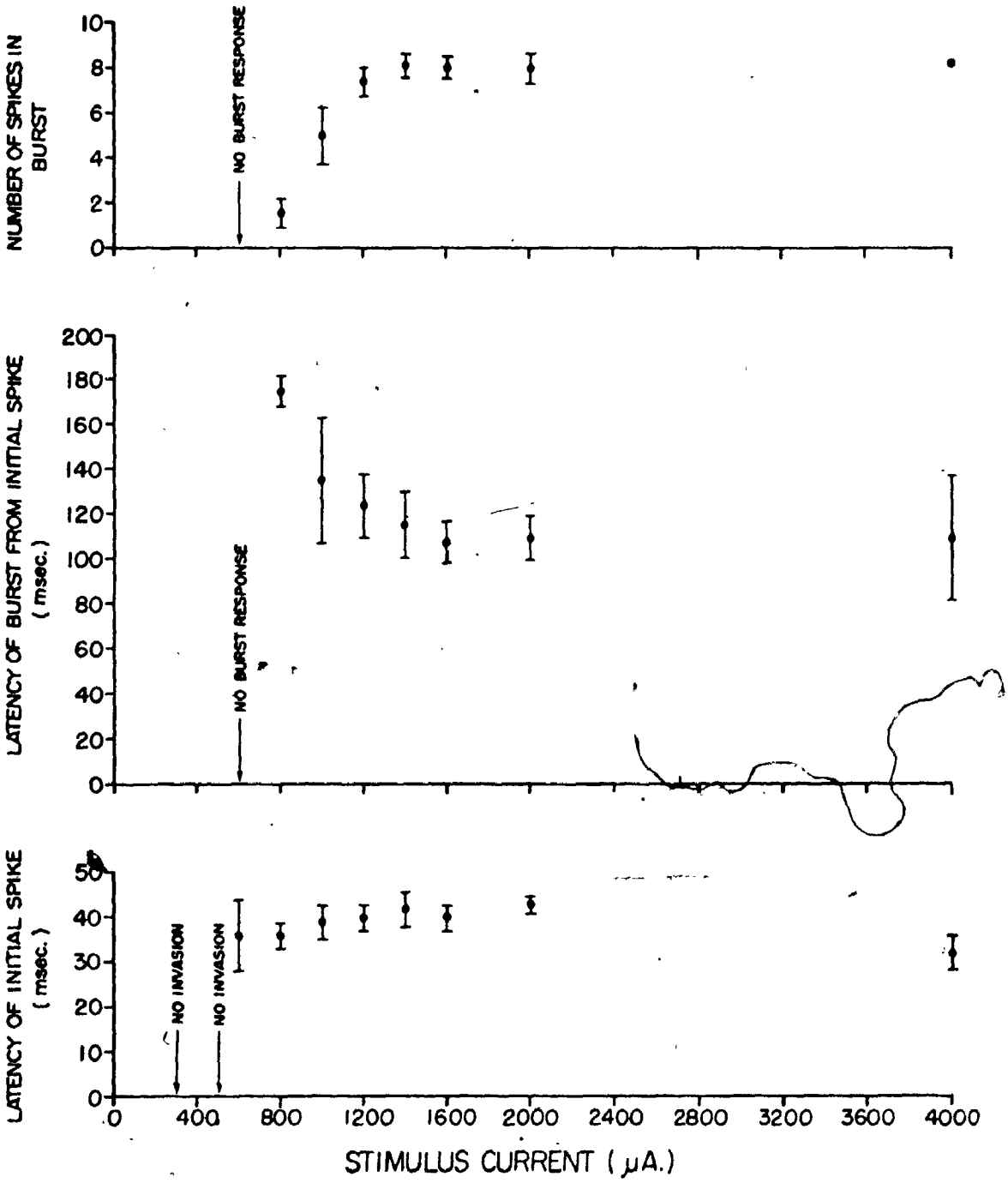

NUMBER OF SPIKES IN BURST
NO BURST RESPONSE
LATENCY OF BURST FROM INITIAL SPIKE (msec.)
NO BURST RESPONSE
LATENCY OF INITIAL SPIKE (msec.)
NO INVASION
NO INVASION
STIMULUS CURRENT (μA.)

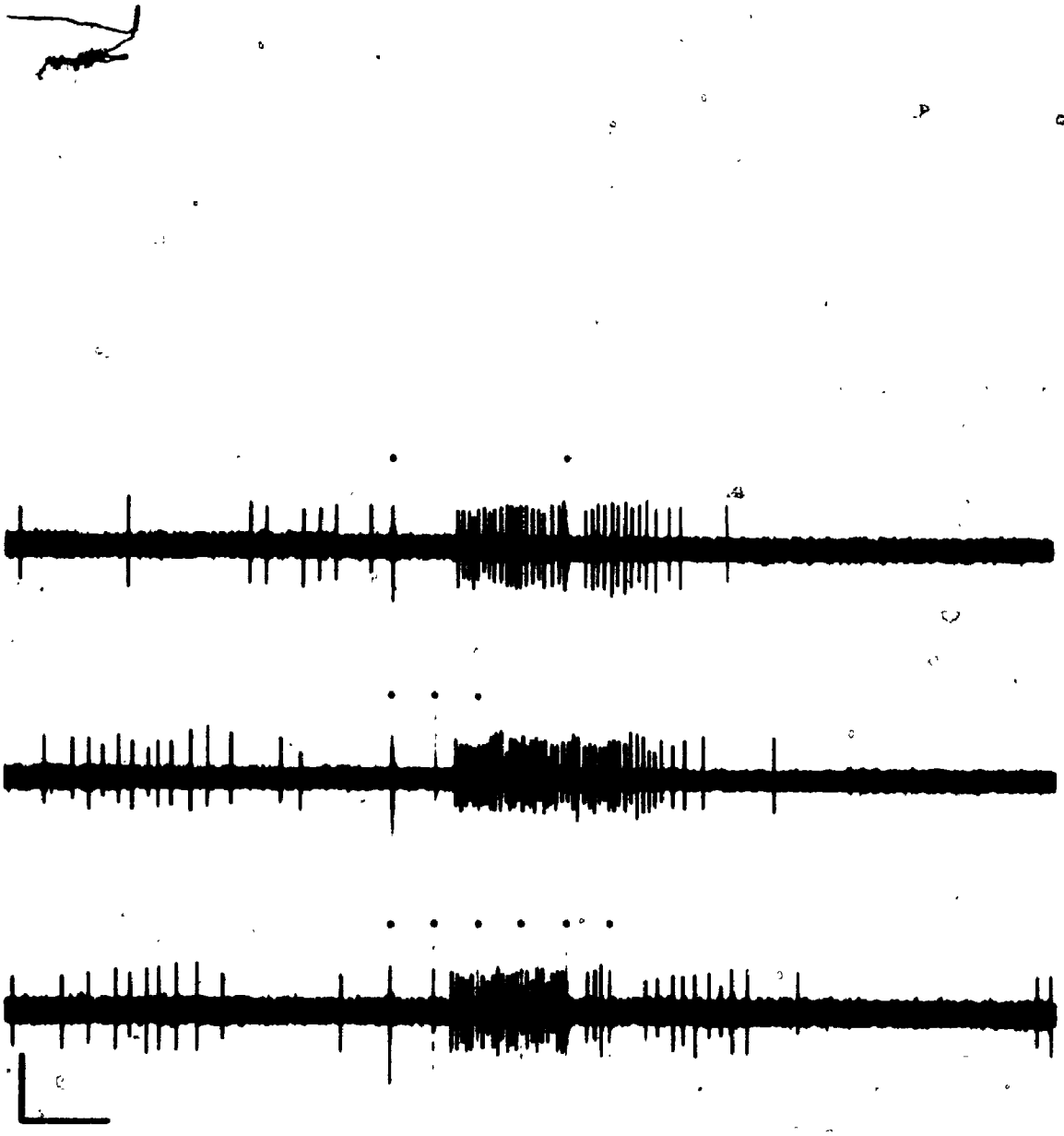
PLATE 16

Effect of Stimuli on the Periods of Inhibition and Transient Rapid Firing

These records illustrate the effect of successive nerve shocks on the periods of inhibition and transient rapid or rebound firing.

Voltage calibration 500 μV.

Time calibration 1 sec.



firing for a short time, but it subsequently returns.

4.10 Evoked Activity in Decentralized Preparations

Long-lasting depression of activity due to olfactory nerve stimulation was also seen in decentralized preparations, as shown in Plate 17. In the few neurons studied, depression was not seen to be followed by rebound firing. In addition, invasion by the post-synaptic spike due to nerve stimulation did not occur in the neurons studied in decentralized preparations. Consequently, the depression could not be studied by paired conditioning-testing stimuli.

PLATE 17

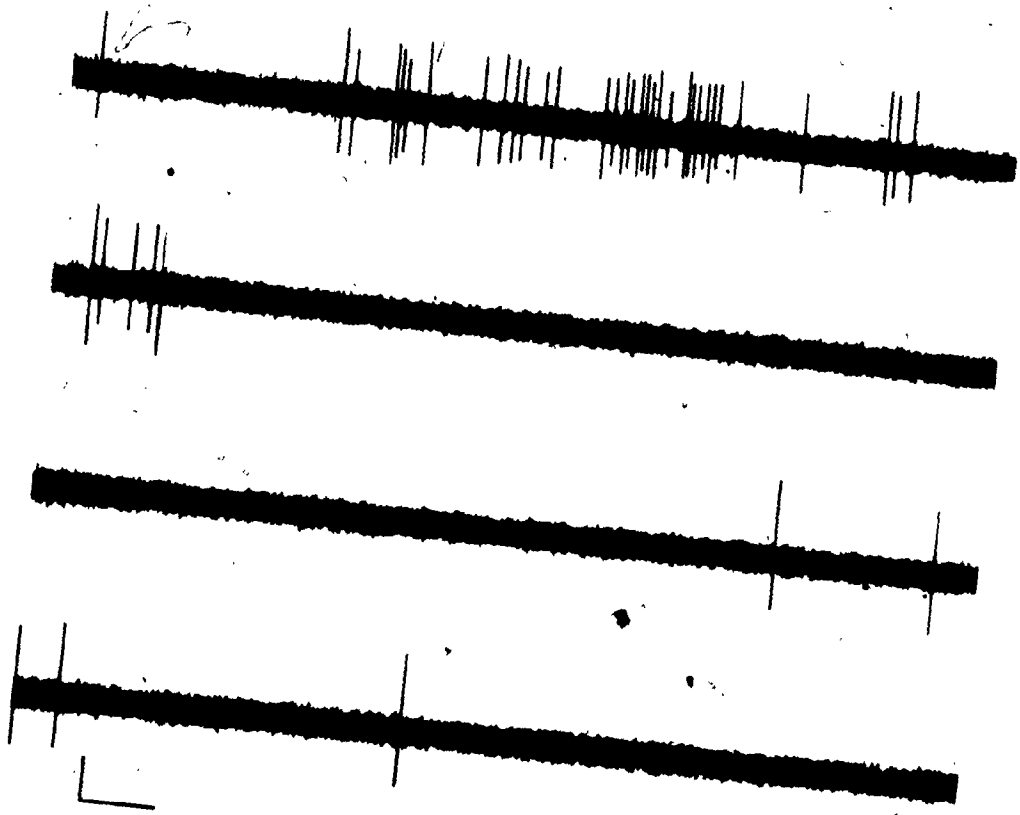
Inhibition Following Nerve Stimulation in a Neuron from a Decentralized Preparation

The stimulus artifact is marked by the superposed dot.

Consecutive traces form a single record.

Voltage calibration 250 μV.

Time calibration 1 sec.



CHAPTER V

DISCUSSION AND CONCLUSIONS

5.1 Identification of Secondary Neurons

All unitary activity recorded in this study was from the anterior region of the olfactory bulb. Dye extrusion was used to locate four neurons, which were unquestionably in the layer of secondary neurons. The larger and less densely packed secondary neurons distinguish this layer from the periventricular layer of granule cells. The other neurons of the bulb, the periglomerular cells, are restricted to the interglomerular regions. Thus, identification of the secondary neurons in histological sections may be made quite simply.

The histologically identified neurons gave extracellular potentials which were identical in configuration to those obtained from neurons which were not so located. These observations confirm the conclusion that the neurons recorded in the general study were secondary neurons. Further support for this conclusion has come from the demonstration that the sampled neurons could be invaded antidromically

subsequent to electrical stimulation of the pathways of the axons of secondary neurons. In addition, the spontaneous extracellular potentials recorded from the neurons would occasionally exhibit spike fragmentation. That Phillips et al. (1963) saw this fragmentation in the mitral cells of the mammalian bulb offers further corroborative evidence that the sampled neurons were secondary neurons. Observation of this fragmentation also suggests that the extracellular potentials were recorded from the cell soma/axon hillock region.

Additional evidence that it was secondary neurons whose activity was being recorded may be seen in a comparison of the present study with that of Döving (1964). This latter study reports a mean frequency of spontaneous activity of 0.7/sec. This corresponds to an average mean interspike interval of approximately 1400 msec., which is in excellent agreement with the value of 1431 msec. for the average mean interspike interval seen in this study (Figure 24).

5.2 Spontaneous Activity in Secondary Neurons

Except for the one regularly-firing neuron, no pattern or temporal organization in the spike trains of spontaneously active secondary olfactory neurons was evident on visual inspection. This fact implied that the spike generation resulted from a random process. To test this hypothesis the spike activity was examined using the Poisson process as a basis for analysis.

There are four criteria for events to be distributed

in time as a Poisson process. These are:

1. the chance of two or more events occurring simultaneously is negligible,
2. the probability of occurrence of events does not change with time, thus, there are no trends in the series,
3. the distribution of intervals between events should fit an exponential of the form

$$P(t' \geq t) \propto \exp(-t/E)$$

where $P(t' \geq t)$ is the probability of observing an interval t' which is larger than or equal to t, and E is the mean interval,

4. the time of occurrence of an event is totally independent of all past events.

We shall begin the discussion of this section by examining whether or not the spike trains obtained from the secondary olfactory neurons of the frog satisfy these criteria. The first criterion, that the probability of simultaneous occurrence is negligible, is obviously satisfied for a nerve cell generating all-or-nothing spikes.

The second point with which one has to contend is, then, the absence of trends in the series, or, stationarity. As has been disclosed above, stationarity was determined by visual inspection of the overall record. This is certainly the most direct and most commonly used method of determination since no satisfactory quantitative test for stationarity is available for finite records. Consequently one simply

examines the overall record to ascertain that there is no sustained trend in the data. Thus, for example, the spike train shown in Figure 30A would be declared nonstationary, since there is a sustained trend of increasing instantaneous mean rate in the data. However, if the overall spike train consisted of repeating segments of increasing mean rate, such as is shown in Figure 30B, it would be declared stationary, since there is no sustained trend in the data. All of the neurons analyzed in this study were seen to be stationary in such terms.

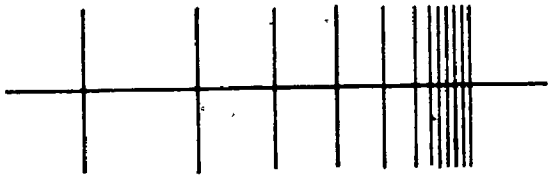
Interspike interval density histograms were seen, for neurons from intact, decentralized, and deafferented preparations, to be unimodal and positively skewed, the former characteristic suggesting a single population of interspike intervals, or at least not suggesting any contradictions to such an hypothesis. However, when the histograms were replotted semilogarithmically as interspike interval distributions, all except that for the regularly firing neuron demonstrated not one linear portion as would be expected on the basis of a single underlying Poisson process, but two distinct linear portions. This must be interpreted as denoting two distinct processes underlying spike generation, both of which are Poisson insofar as they both satisfy this third criterion of a Poisson process. The fact that a similar plot for the regularly firing neuron demonstrated a single linear portion suggests that there is a single process underlying spike generation in this neuron.

FIGURE 30

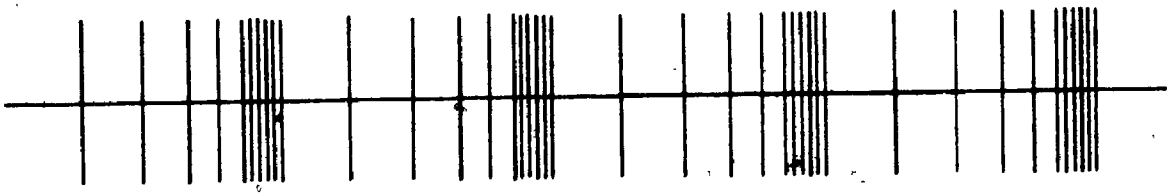
Illustration of Nonstationary and

Stationary Spike Trains

A



B



The final criterion of a Poisson process to be examined is the independence of a given event of all past events. To test for this criterion, the technique of autocorrelation has been used. It has been shown (Huggins, 1957; Poggio and Viernstein, 1964) that events generated by a random process will give an autocorrelogram of constant value. In the present study the autocorrelogram has been expressed in terms of occurrences per bin. Consequently, if the process is random the bin count for every bin is given by

$$n = N \cdot \Delta t \cdot (1/E),$$

where N is the number of spikes in the record, Δt is the autocorrelogram bin width, and E is the mean interspike interval. These expected counts were shown on the autocorrelograms presented in the preceeding chapter. It is apparent that the observed bin counts are constant about the expected counts, except for the following cases:

a) the autocorrelogram for the regularly firing neuron had a definite, sustained oscillation at a frequency determined by the mean interval. In other words, in this neuron the occurrence of a given spike was more or less time-locked to the occurrence of previous spikes.

b) the autocorrelograms for some neurons, while flat in general, demonstrated a period of initially higher bin counts which has been referred to as initial peaking. This will be

discussed below.

To summarize thus far, then, it is proposed that the spontaneous activity of the secondary olfactory neurons can be described as being due to two Poisson generators. This conclusion, which has been reached by the above considerations of criteria for a Poisson process, is in agreement with other evidence derived from this study. Thus, the greater standard deviation of the mean interspike intervals than expected on the basis of a single Poisson model can be considered as a result of the greater scatter in the interval data since such data is generated by two distinct processes.

The flatness of the autocorrelograms (beyond any initial peaking) suggests that the two underlying processes generate spikes independently of one another. Were this not the case, and generation of a spike by one process, statistically at least, resulted in the generation of a spike by the other process, then peaking would be expected in the autocorrelogram. Such peaking was in fact seen in the autocorrelograms of some neurons, and it occurred at times about those of the modal values of the corresponding interspike interval density histograms. This indicates a greater number of spikes following other spikes at an interval approximately determined by the position of the mode of the histogram than expected on the basis of absolute randomness. This then could be an indication of interaction between the processes. However, it could also be a manifestation of the fact that there is maximal overlap of the two generating processes for

producing intervals of such length - consequently more spikes could be produced at approximately such intervals after previous spikes and yet the processes still be independent. Evidence suggesting the independence of the two processes is obtained from the plot of the rate of one process against the rate of the other. Such a graph showed no obvious relation between the rates of the two processes. Smith and Smith (1965) observed a partitioning of the interspike interval distributions of cortical neurons into two processes, as we have reported in this study. On the basis of results obtained with microelectrode polarization techniques they suggested that the one process gates on a second random process at random moments and for variable times. However, it appears that the two processes observed in these secondary olfactory neurons are independent of one another.

The difference in the distributions of the rates of the two processes is interesting. The narrowness of the distribution of the rates of the long process indicates that this process would be a generator of spikes whose interspike intervals would have a much smaller cell-to-cell variation than that observed (Figures 24 and 25). However, the superposition of the short process introduces a good deal of the cell-to-cell variation.

At this stage of the discussion it is necessary to consider what the origin of the generating processes might be, in terms of the components of the nervous system, the neurons, and their interactions. The first point about the

generating processes to consider in this respect is that neither are of central origin, since the processes were seen in decentralized secondary neurons as well as in neurons from intact preparations. Thus decentralization did not remove either of the processes. In fact, decentralization did not even modify either process, at least as far as the measures used in this study are concerned. The following evidence supports this opinion:

a) mean intervals of neuronal spike data recorded from decentralized neurons were scattered throughout the distribution of mean intervals from neurons in intact preparations.

b) the relationship between mean intervals and standard deviations was unaffected by decentralization.

c) the rates for the long and short processes in decentralized neurons were distributed in agreement with those for neurons in intact preparations.

Spontaneously active neurons were also recorded from the preparation in which the olfactory nerves had been sectioned, and analysis demonstrated that the two processes were again present. While in one of these neurons there was some suggestion of interference by injury discharge in the sectioned nerve, it is untenable that such an injury discharge could be responsible for generation of interspike interval data which is in such agreement with that obtained from

neurons in intact preparations. Consequently, neither generating process is the result of peripheral influx from the olfactory receptors. It must be accepted, therefore, that the generators of the spontaneous activity seen in the secondary olfactory neurons are within the olfactory bulb.

In view of the excellent research on the mammalian olfactory bulb by neurophysiologists (discussed in the review chapters of this thesis), the granule cells can be eliminated as a source of excitatory input - they can be described as inhibitory interneurons. The periglomerular cells conceivably could drive the secondary neurons through the glomerular synapses. However, spontaneously active neurons whose electrical activity could not be assigned to secondary neurons were not encountered. Were the periglomerular cells spontaneously active, this activity should have been recorded at least occasionally. It seems doubtful, then, that these cells are the generators of the spontaneous activity. Indeed in microelectrode recordings from single glomeruli in the rabbit, Leveteau and MacLeod (1965, 1966, 1969) do not report any sort of spontaneous activity from these structures. Since periglomerular cells interconnect the glomerular synapses, were the periglomerular cells spontaneously active and driving the secondary neurons, one would expect to see some evidence of this activity in recordings from the synapses. However, there is disagreement as to the existence of spontaneous activity in the mitral cells of the mammalian bulb.

The most suitable accounting of the generating

processes underlying the spontaneous activity in the secondary olfactory neurons of the frog is as follows:

a) an intrinsic mechanism which is autochthonous to each secondary neuron such as a fluctuating membrane potential, or oscillating metabolic process, or whatever, and which can lead to spike generation whenever a threshold is reached and the neuron is not refractory. This is supported by the work of Yamamoto and Iwama (1962) on the mammalian olfactory bulb. These workers reported that mitral cells demonstrated a fluctuating membrane potential with spike generation occurring whenever a critical level was reached.

b) the second generating process is then considered to be extrinsic to the sampled neuron and is assigned to cell-to-cell excitatory interaction between the secondary neurons. There is both anatomical and other electrophysiological evidence that such interaction may occur. Thus, secondary dendrites and recurrent axon collaterals appear to provide the necessary pathways for such interaction, and Nicoll (1971) has proposed that one mitral cell (in the rabbit olfactory bulb) may excite another via recurrent axon collaterals following orthodromic and antidromic stimulation.

Thus, what we are proposing is that the spontaneous activity seen in a given secondary neuron is not independent of its neighbouring secondary neurons, but rather that the secondary neuron and its neighbours form a functional unit insofar as maintenance of the spontaneous activity is concerned. The functional unit can be defined as the sampled neuron plus the number of surrounding neurons which interact with it. Such a unit is illustrated schematically in Figure 31.

Of course it is still possible to think in terms of individual neurons. This thinking, however, must include the interaction. Thus, suppose one assumes that the intrinsic process is describable by a relaxation oscillator model. Then, under the influence of the intrinsic process alone, the cell would produce a spike whenever the oscillator reached threshold, as shown in Figure 32A. Variability in the interspike intervals is, then, due to inherent variability in the rate of the oscillator. If, however, one allows excitatory interactions between the neurons, so that the oscillators are coupled, the rise of any oscillator to threshold will be modified. In the simplest case, the modification can be thought of as instantaneous perturbations, due to excitatory post-synaptic potentials, which move the oscillator closer to threshold. This is shown in Figure 32B. Now the variability in the interspike intervals is due to a combination of inherent variability in the rate of the oscillator and to variability introduced by cell-to-cell interactions.

FIGURE 31

Schematic Illustration of Proposed

Functional Unit

Circles represent the secondary neurons of the olfactory bulb as viewed down the input pathway (olfactory nerve fibres). The interconnections are meant to show that the neurons can affect each other reciprocally, although not necessarily via the same pathway. The figure should not be construed as purporting to suggest the number of neurons in a functional unit or the number of interconnections - it is merely a schematic illustration.

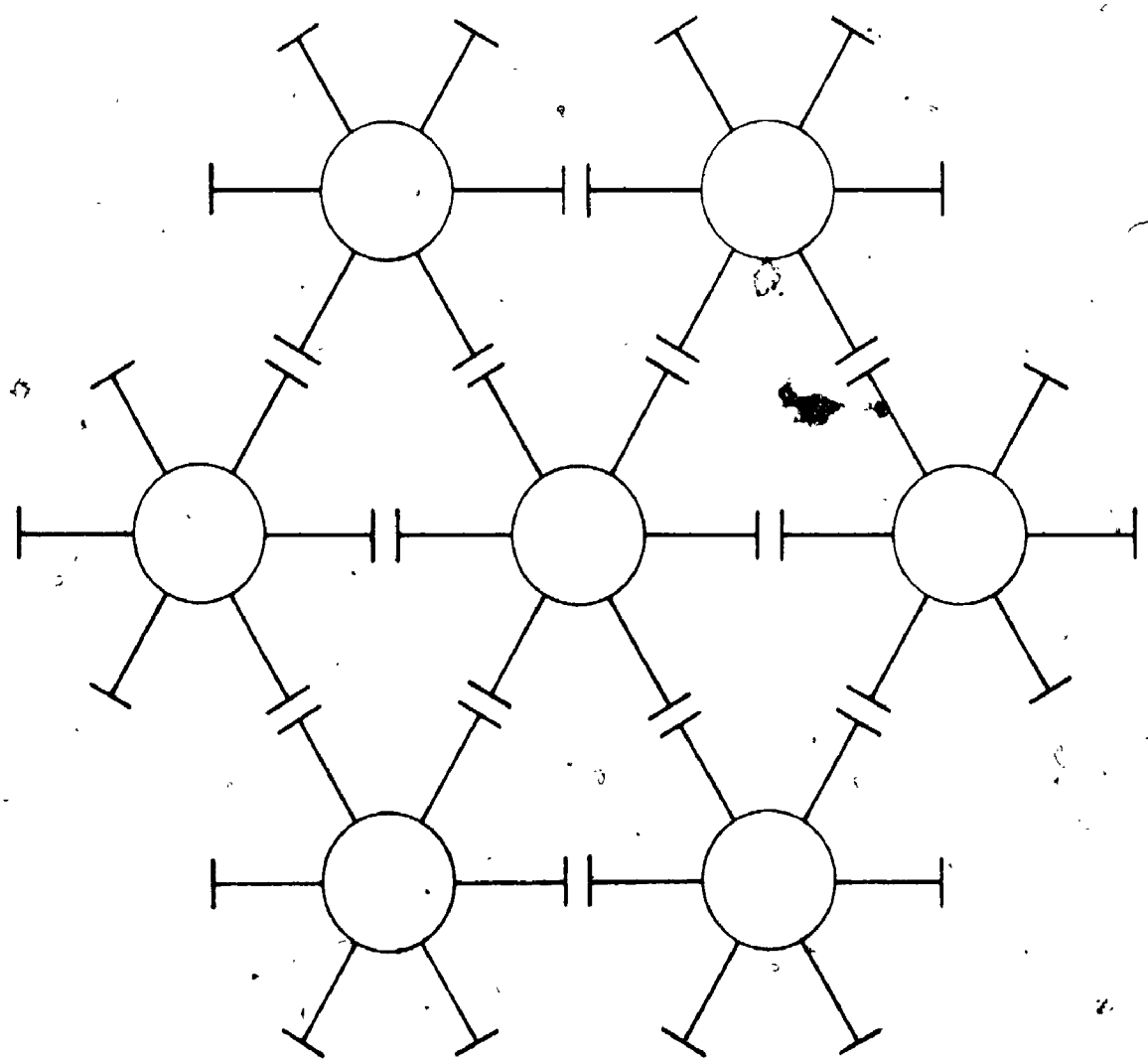


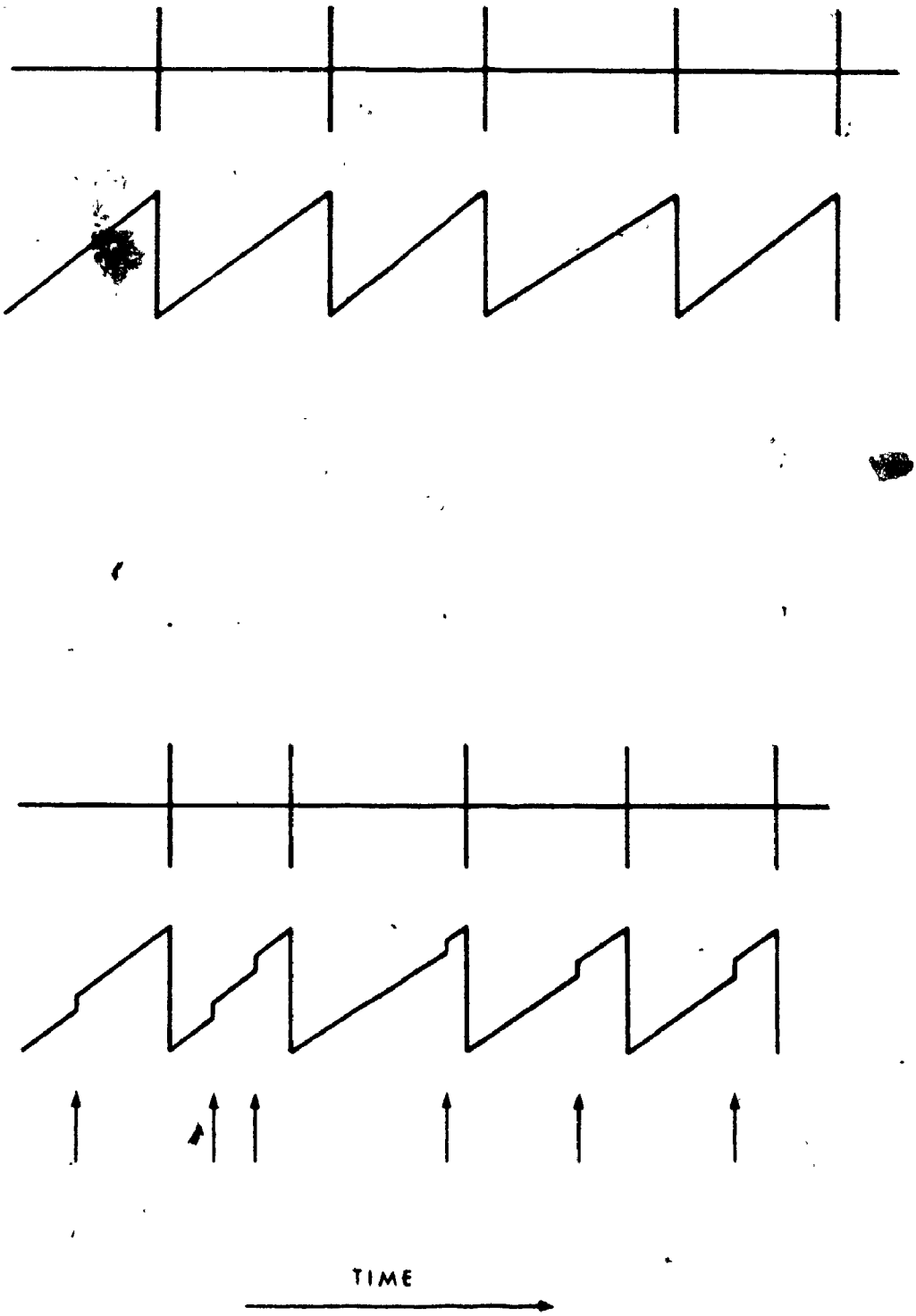
FIGURE 32A

Illustration of Spike Generation by an Isolated Relaxation Oscillator

FIGURE 32B

Illustration of Spike Generation by a Relaxation Oscillator Receiving Excitatory Inputs from Neighbouring Oscillators

The arrows indicate the occurrence of excitatory inputs to the oscillator.


TIME

To cast the interspike interval data in the form of rates of rise of the oscillator, it is necessary only to obtain the reciprocals of these intervals. Doing so, one may observe a measure of the variability of these rates within a given cell in the standard deviation of its distribution of such rates. In Figure 33, distributions of the rates of rise of the oscillator are shown for two cells, the regularly firing neuron and one cell from the general study. Variability within the cell was seen to be the smallest for the regularly firing neuron[1], suggesting minimal perturbations due to interaction. Furthermore, its distribution of rates of rise of the oscillator is consistent with the model of a spike generator with Gaussian fluctuations about the mean. Consequently the regularly firing neuron appears to be a cell which is isolated from interaction with other secondary neurons. The regularity of the spike activity seen in this neuron suggests that without the interaction process spontaneous spike generation might be regular in all secondary neurons.

The question of which generating process (long or short) is the intrinsic process and which is the extrinsic process may now be answered with reference to the regularly firing cell. This cell demonstrated only one underlying generating process, the short process. Furthermore, it has been suggested above that the generating process of this cell

[1] Coefficients of variability are listed in Appendix 4.

FIGURE 33

Distributions of the Rates

of Rise of the Relaxation Oscillator

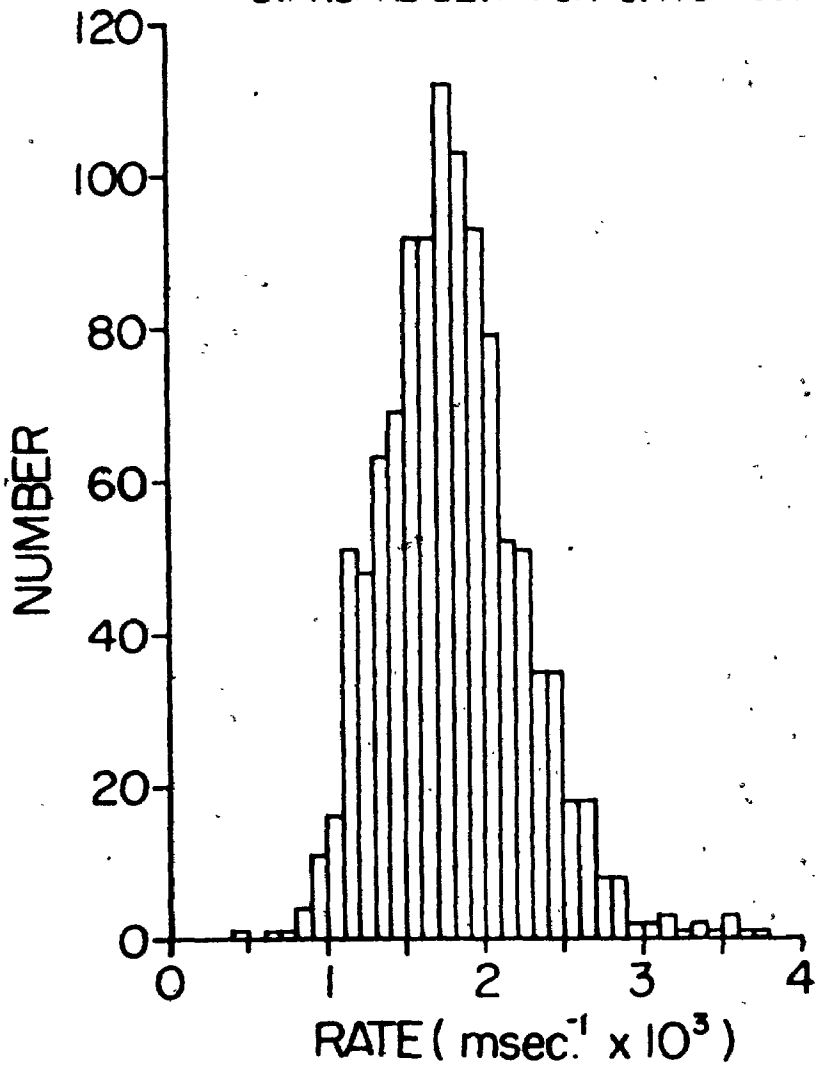
for Two Neurons

The histogram on the left is for the regularly firing neuron, neuron VIII-A.

NEURON VIII - A

MEAN RATE 1.813 msec.$^{-1}$ x 10^3

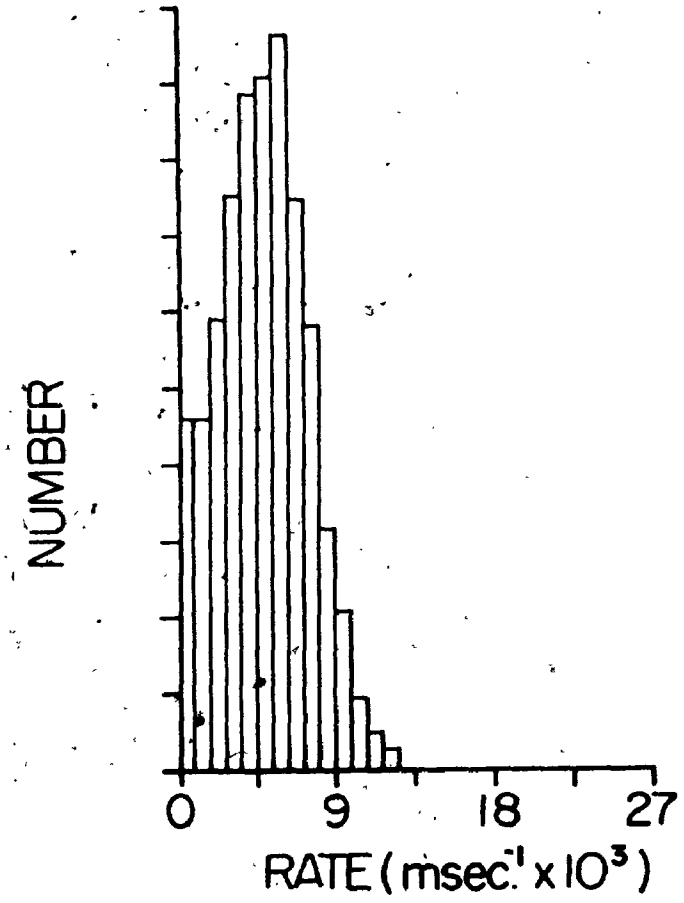
STANDARD DEVIATION 0.475 msec.$^{-1}$ x 10^3



NEURON IX - B

MEAN RATE 4.916 msec.$^{-1}$ x 10^3

STANDARD DEVIATION 2.483 msec.$^{-1}$ x 10^3



may be considered as an intrinsic relaxation oscillator with Gaussian fluctuations in its rate. One may consider, in addition, that cell-to-cell interactions can scarcely be expected to drive a neuron so regularly. Thus the short process must be the process intrinsic to the secondary neurons. Thus, cell-to-cell excitatory interaction remains as the physical basis of the long process. Such assignments are reasonable from another consideration as well, that is, the intrinsic process might be expected to have a more or less fixed range (short process) whereas cell-to-cell excitation could generate intervals of all lengths (long process)

It is interesting and fruitful to consider the implications of the characteristics of the two processes. Thus, the intrinsic or short process, in view of the widely-scattered distribution of rates seen in Figure 25 is where a great portion of the cell-to-cell variation in mean firing rate arises. That is, the intrinsic process has widely different rates in different neurons. The rates for the interaction process, the long process, were, however, distributed extremely narrowly relative to those for the intrinsic process. Thus, due to interaction with other neighbouring secondary neurons, any given secondary neuron must receive roughly the same amount of excitation per unit time as any other secondary neuron. In view of the fact that there is a substantial cell-to-cell variation in the mean firing rates observed in the secondary neurons (Figure 24), the neurons must be spatially distributed and interact

such that no neuron is receiving input from all rapidly firing or all slowly firing cells. Each secondary neuron is presumably connected to a number of neighbouring neurons whose mean rates of activity are sufficiently varied that the overall excitatory input is an average which is roughly representative of that seen by any other secondary neuron. In view of the broad distribution of the mean intervals, it would appear that the probability of a given neuron receiving inputs from cells whose spontaneous activity is quite diverse is high. Thus the narrowness of the distribution of rates for the interaction process suggests that the dimensionality of the interconnectivity is rather constant throughout the bulb. That is, the number of secondary neurons whose spontaneous activity impinges upon, and therefore affects the activity of, a given secondary neuron is approximately the same as for any other secondary neuron.

In section 4.6 analysis was presented for a record which showed two neurons firing spontaneously. One would think that these two neurons might well be interconnected, and therefore interacting, since the neurons must be in close proximity for the activity to be monitored by a single microelectrode. Cross-correlation analysis showed, however, that the null hypothesis could not be rejected, that is, there was no significant dependency of the firing of one neuron upon the other. There are several considerations as to why the two neurons appeared independent of each other. First of all, if a sampled secondary neuron receives excitatory

input from a number of other secondary neurons, the detection of the influence of one of these neurons on the sampled neuron, even statistically, is difficult. Furthermore, the generation of a spike in one of the surrounding neurons cannot necessarily be expected to cause a spike in the sampled neuron. Rather, the neurons affecting the sampled secondary neuron probably generate only excitatory post-synaptic potentials (EPSP's) in the sampled neuron, thereby moving it closer to threshold. This type of interaction would render the probability of detecting the interaction on the basis of simultaneous spike trains from two cells even lower. Consequently the failure to observe a dependency between the two spike trains of Plate 7 does not offer any concrete evidence for or against the model. Indeed if one has already accepted the model, the analysis offers evidence that a sampled neuron is influenced by a number of neighbouring neuron.

Further support for the contention that a given secondary neuron is influenced by a large number of neighbouring neurons is to be found in the independence of the two generating processes. If only a few neurons were coupled, then the intrinsic spontaneous activity of one neuron (the short process in this neuron) could markedly affect the spontaneous activity of a second neuron, and the activity of this second neuron feeding back to the first neuron (the long process in this neuron) would be dependent upon the first neuron. However, in a large network such dependencies would be overriden by the large number of neurons generating the

feedback, and by the coupling between these neurons.

A few words about the number of neurons in the proposed functional unit are in order. If one assumes that the interactions occur via secondary dendrites and/or recurrent axon collaterals, then the dimensionality of the functional unit depends upon the spread of these processes. In the absence of quantitative anatomical studies of this, one is hard pressed to even guess at a rational number of interacting neighbours. As a lower limit one might make an order of magnitude assumption of ten on consideration of the possible number of immediately surrounding neurons. However, a much larger number of interacting neighbours is certainly possible.

We shall now proceed with a discussion of the activity induced in the secondary neurons following electrical stimulation of the olfactory nerve. Subsequently we shall return to further discussion of the spontaneous activity.

5.3 Evoked Activity in Secondary Neurons

5.3.1 Controls Using Natural Stimulation

In order to compare the effects of electrical stimulation with those of natural stimulation of the olfactory receptors, the response of several secondary neurons to such natural stimulation was examined. The electro-olfactogram (EOG) was used as a measure of the time of onset of the stimulus. Response patterns seen with odourous stimulation were the same as those seen with electrical stimulation of the

olfactory nerve; the activity of the secondary neuron could be simply depressed, or it could be depressed and it would subsequently rebound. Furthermore, in terms of the depression they produce, natural and electrical stimulation appear to be similar. Although it is difficult with natural stimulation to determine the time of onset of the stimulus, if the rising phase of the EOG is chosen as this time, the depression of activity produced in the secondary neurons is substantial, both as seen in the few neurons in this study and in Döving's (1964) work. This similarity of depression seen with the two types of stimuli may, at first glance, seem surprising since electrical shocks to the olfactory nerve may be expected to produce a massive effect, activating many olfactory nerve fibres more or less synchronously. However, natural stimulation may do much the same thing; the discrimination of individual receptors in the frog is very low, a given receptor responding to many different substances (Gesteland, Lettvin, and Pitts, 1965). Thus any odour may activate a significant proportion of the receptors. In fact, O'Connell and Mozell (1969) have proposed that the pattern of activity developed across the entire ensemble of receptors could be an olfactory code.

5.3.2 Pathways for Driving Neurons

The single spike evoked in the secondary neurons by electrical stimulation of the olfactory nerve is conspicuous in its long latency. Yamamoto (1961) observed similar long latencies after stimulation of the olfactory mucosa in the

rabbit. These were due to the long conduction distances involved and the slow conduction velocity in the nerve fibres (estimated as 0.2 m./sec. on the basis of Gasser's (1956) work). It is instructive to perform a simple calculation to determine if the latencies seen in the present study can be explained on the basis of known parameters for conduction and trans-synaptic excitation.

Ottoson (1959c) reported an average conduction velocity of 0.14 m./sec. for the action potential in the olfactory nerve of the frog. The position of the stimulus electrode on the nerve in the present experiments allowed a distance of approximately 3 mm. to 5 mm. for conduction from the point of stimulation to the glomerular synapses. (This is obviously a crude estimate, since it is impossible to determine the conduction distance from where the nerve fibre enters the bulb to where it terminates in a glomerulus.) Based on a conduction velocity of 0.14 m./sec., the time for propagation over this distance is in the range of 21 msec. to 36 msec. Addition of 1 msec. as a nominal figure for the synaptic delay in the glomeruli brings this calculated range to 22 msec. to 37 msec. The observed latencies range from 17.8 to 43.0 msec. Comparison of the observed and calculated latencies suggests that there are at best only a few milliseconds available for the depolarization generated post-synaptically at the tip of the dendrite to produce a spike in the cell body (the presumed location of the tip of the microelectrode) of the secondary neuron. Considering the

approximate nature of the calculation, it is not possible to say whether the depolarization reaches the cell body via an active process, or electronically. Chang (1951) proposed that a dendritic potential was conducted slowly along the apical dendrites of pyramidal cells, while Grundfest (1957) has allowed that such potentials are non-propagating post-synaptic potentials. Yamamoto (1961) supported the view that potentials do not propagate in the dendrites of mammalian mitral cells but rather spread electrotonically. Long duration dendritic spikes observed by Tasaki, Polley, and Orrego (1954) were not seen in the present study. However, the microelectrodes were positioned on the basis of the spontaneous activity, presumably generated in the cell soma/axon hillock region (viz., the configuration of the potentials, and the A-B fragmentation). Consequently, if dendritic spikes did occur, they probably would not be observed.

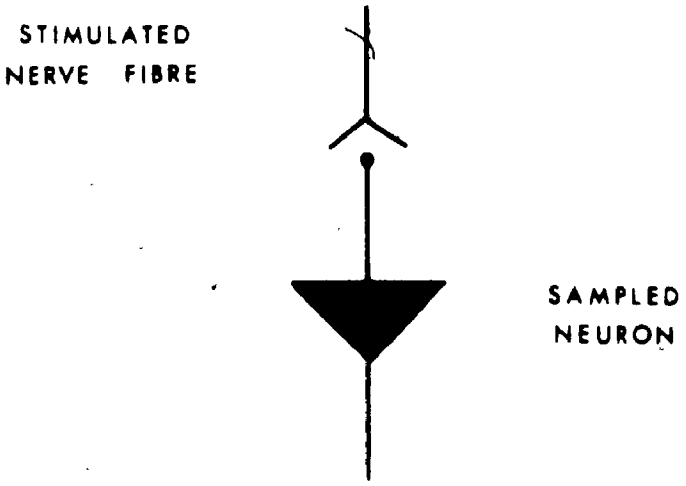
The correspondence between the observed and calculated ranges for the orthodromic latency suggests that activation of the secondary neurons seen in this study with electrical stimulation of the olfactory nerve occurred directly across the glomerular synapse between a stimulated nerve fibre and the sampled neuron, as in Figure 34A, rather than by activation of periglomerular cells or other secondary neurons as excitatory interneurons, as in Figures 34B or C, respectively. Pathways involving such interneurons would result in longer latency of invasion due to the addition of at least one more synaptic delay and of conduction times

FIGURE 34

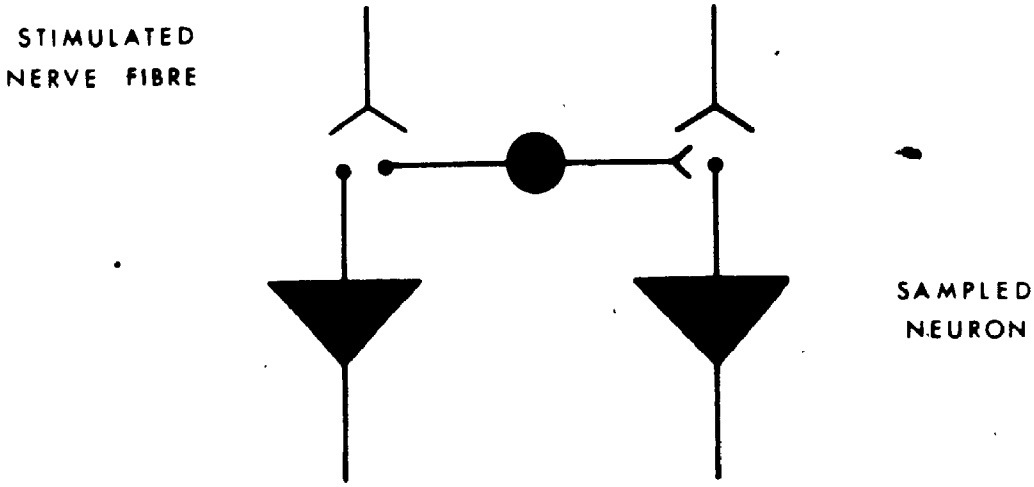
Possible Pathways for Orthodromic Activation of Secondary Neurons

These are based upon known anatomy of the bulb. The triangular figures represent the secondary neurons, while the circle represents a periglomerular cell.

A


STIMULATED
NERVE FIBRE
SAMPLED
NEURON

B


STIMULATED
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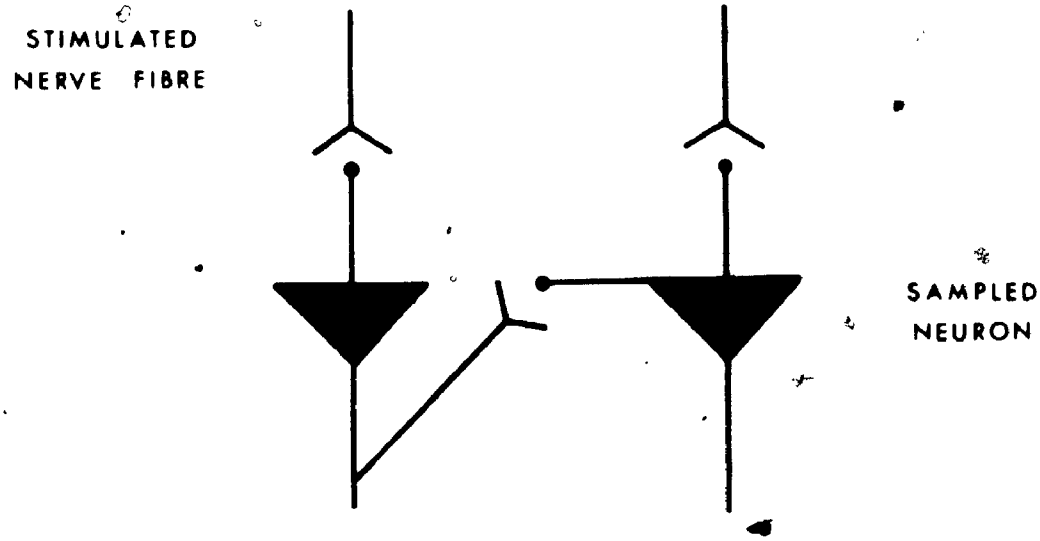
C


STIMULATED
NERVE FIBRE
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NEURON

through these neurons.

The constancy of the latency with stimulus magnitude also favours the direct pathway. Were the activation dependent initially upon networks such as those in Figure 34B or C, it would be expected to change to the network of Figure 34A as the increasing stimulus strength activated more and more nerve fibres.

In antidromic stimulation of the neurons the stimulus electrodes were on the forebrain at least one centimeter caudal to the recording microelectrode. Thus the antidromic conduction distance was at least this long. The antidromic latencies are, then, reasonable for small axons. This suggests that the designated antidromic invasion is, in fact, just that, and not a synaptic excitation via recurrent collaterals or accessory dendrites of another secondary neuron driven antidromically. The A-B fragmentation seen in the spikes elicited by forebrain shocks supports this.

5.3.3 Inhibition of Secondary Neurons

The long-lasting depression of spontaneous activity in the secondary neurons subsequent to olfactory nerve shocks could be ascribed, without further information, either to a long lasting inhibition produced in the secondary neurons or to an inhibition of normal duration followed by a lag in resetting the generators of the spontaneous activity. The use of paired conditioning-testing stimuli to the olfactory nerve allowed a determination of which of the above two alternatives were valid.

The failure of testing shocks to elicit a spike following an effective conditioning shock has been assigned to the occurrence of a long-lasting inhibition in the secondary neurons subsequent to generation of the conditioning spike. As noted above, the latencies of invasion following a single stimulus to the nerve, and the constancy of the latency with stimulus magnitude, indicate that the secondary neurons observed in this study were driven directly across the glomerular synapses by nerve shocks, rather than indirectly by periglomerular cells or other secondary neurons acting as interneurons. Consequently the failure of the testing spike cannot be due to any depression in such pathways. The long time separation of the paired shocks (a minimum of 1 second) removes any question of the observed effects being due to local polarization at the stimulus electrode or to synaptic effects in the glomeruli. Neither can the effects be due to the refractory period of the nerve. Ottoson (1959c) reported that the olfactory nerve of the frog has an absolute refractory period of 30 msec. and a relative refractory period of 200 msec. One is thus left with an inhibition in the secondary neurons as explanation. That invasion of the secondary neurons during repetitive stimulation, when it occurred, was at significantly longer latency than that following single stimuli has been taken as further evidence that these neurons are inhibited following spike generation.

The question remains as to the neuronal network responsible for the observed inhibition. The fact that

depression of spontaneous activity could be seen in neurons in decentralized preparations indicates that the inhibition must be of bulbar origin. In view of the great amount of research implicating the granule cells to an inhibitory role in the mammalian bulb, it does not seem unreasonable to suppose that these same neurons serve an inhibitory function in the olfactory bulb of the frog. The lack of detailed structural information for the frog olfactory bulb at the electron microscopic level makes definitive conclusions impossible, yet some speculation may be made. Dendrodendritic synapses between mitral and granule cells in the mammalian bulb have been seen to occur at the gemmules or spines on the granule dendrites. While these gemmules are not seen on the granule dendrites of all vertebrates, they are found on the dendrites in the frog olfactory bulb. Consequently dendrodendritic synapses may occur, and the dendrodendritic mechanism may be responsible for the inhibition. However, this study is not concerned with the synaptic structures involved. Indeed, this is not important to our argument. What is important concerning the neuronal network involved is that the inhibition of a given secondary neuron did not require its prior firing post-synaptically due to olfactory nerve input. Since granule cells, the putative inhibitory interneurons, do not synapse with incoming olfactory nerve fibres, this must be taken as evidence for the existence of pathways for lateral or neighbouring inhibition in the frog olfactory bulb. Thus the inhibition mediated to a sampled secondary

neuron may occur via a self-inhibitory pathway (Figure 35A) or via a lateral-inhibitory pathway (Figure 35B).

5.3.4 Rebound Firing in Secondary Neurons

The period of rebound firing seen in some secondary neurons were studied in detail in neuron XXXIX-A. It has already been mentioned that the initial spike in the records from this neuron has been taken to register post-synaptic driving of the neuron by the nerve shock. Thus, by means of its primary dendrites which contact incoming nerve fibres at the glomerular synapses, this neuron is activated following nerve stimulation. It has been shown above that the latency of this spike from the shock artifact is consistent with such an argument. Furthermore, the constancy of this latency with stimulus strength supports this position.

Now following this initial spike the cell was quiet for a period which was initially of the order of 200 milliseconds. The cell could then produce a short burst of spikes. Whether the quiet period was a period of inhibition was not tested for this neuron; in accord with other neurons seen in this study one may assume that this is the case. However, this is not important to the following argument. With increasing stimulus strength the latency of the burst response (measured from the initial spike to the first spike of the burst) decreased progressively to finally attain a constant value. The burst does not appear, then, to be associated with activation of the sampled cell by the nerve shock. Thus, the burst response has been assigned to

FIGURE 35

Schematic Representation

of Pathways for Self- and Lateral Inhibition

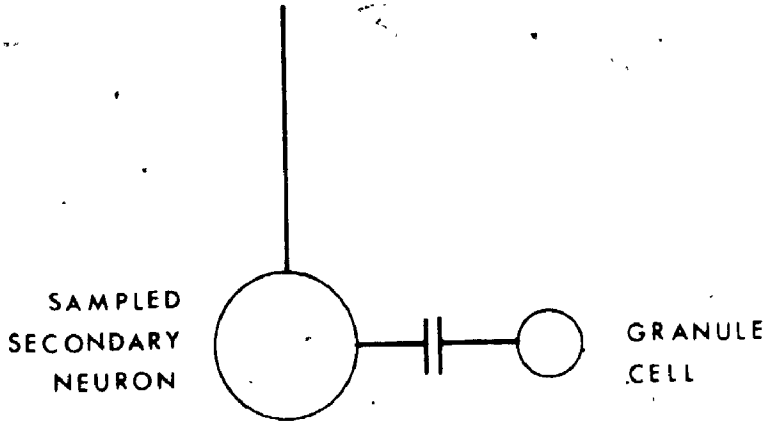
Secondary Neurons

The interconnections between secondary neurons and granule cells are meant to indicate that these two cell types can affect each other reciprocally, possibly by the same pathway as proposed by Rall et al., 1966.

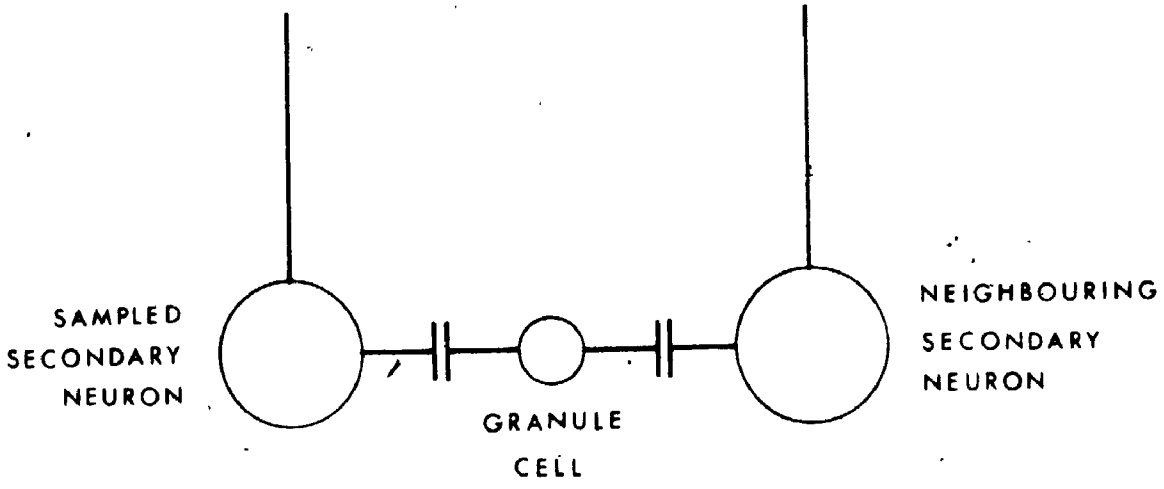
A. In this figure the sampled neuron is driven by the stimulus and this neuron activates granule cells which can then mediate inhibition back to the sampled cell.

B. This figure illustrates that a neighbouring secondary neuron, driven by the stimulus, could activate granule cells which could inhibit the sampled neuron.

A


SAMPLED
SECONDARY
NEURON
GRANULE
CELL

B


SAMPLED
SECONDARY
NEURON
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CELL
NEIGHBOURING
SECONDARY
NEURON

excitation mediated back to the sampled secondary neuron by neighbouring secondary neurons, either via secondary dendrites, recurrent axon collaterals, or both. The excitation could arise from the neighbouring secondary neurons as a result of either:

a) summation of excitation from the neighbouring neurons which are activated by the stimulus but at a later time than the sampled neuron because of asynchrony in the nerve volley set up by the stimulus. Since the rebound firing can occur one second or more after the stimulus, this is not a likely source.

b) summation of postinhibitory rebound[2] in the secondary neurons. If each secondary neuron would generate only a single spike as post-inhibitory rebound when isolated, when interconnected in an interactive network like that of Figure 31 there could be a substantial

[2] Postinhibitory rebound refers to the period of greater excitability that occurs in many neurons following experimental hyperpolarization or hyperpolarizing synaptic inhibition. It has been seen, for example, in spinal motor neurons (Fukami, 1962), in crayfish stretch receptors (Terzuolo and Washizu, 1962), in hippocampal neurons (Kandel and Spencer, 1961), and in Aplysia neurons (Kandel, Frazier, and Wachtel, 1969).

excitatory influence distributed over the network.

In any case, the decrease in latency of onset of the burst response with increasing stimulus strength can then be accounted for by either or both of the following rationales:

a) as the stimulus magnitude is increased, nerve fibres responsible for driving the neighbouring neurons are activated more in phase with nerve fibres driving the sampled neuron, or,

b) as the stimulus magnitude is increased, more nerve fibres are activated and, as a consequence, more neighbouring neurons are recruited. Thus a summation of feedback excitation in the sampled neuron would reach threshold sooner.

Evidence that the burst response is due to excitation mediated back to the sampled secondary neuron by neighbouring secondary neurons is found in the observation that the number of spikes in the burst increases with stimulus strength. The greater stimulus strength will activate a greater number of olfactory nerve fibres; consequently a greater number of secondary neurons will be driven by the stimulus, and greater feedback excitation to the sampled neuron will result. The number of spikes per burst eventually reaches a plateau as the stimulus current increases. This is presumably due to the fact that there is a maximal number of surrounding, interacting neurons, and once these have all been activated the feedback excitation is maximal. The diminuition of spike

amplitude seen to occur from the first to last spike of the burst is consistent with the idea of a wave of excitation in the sampled neuron.

Thus it would appear that excitatory interactions between secondary neurons play a role not only in generating the spontaneous activity of these neurons, but also in their response to stimuli. Thus, excitatory interactions between secondary neurons would appear to be a fundamental part of olfactory processing occurring within the bulb, and it is our proposal here that units such as those proposed in Figure 31, with perhaps the addition proposed below, be considered as the basic information processing and transferring unit of the olfactory bulb.

We have demonstrated that excitatory interactions between the secondary neurons play a role in the generation both of the spontaneous activity of these neurons and of their post-stimulus response. Furthermore, we have shown that the secondary neurons are inhibited following nerve stimulation; that is, inhibitory interactions also play a role in the generation of the post-stimulus response. The question to be raised here is, do inhibitory interactions also play a role in the spontaneous mode of these secondary neurons? Since the pathways exist for self- and lateral inhibition, as seen in responses to stimulation, there is no reason to deny that inhibition could exert an influence upon the spontaneous discharge patterns. Of course it is not being argued that the same extremely long inhibition seen to

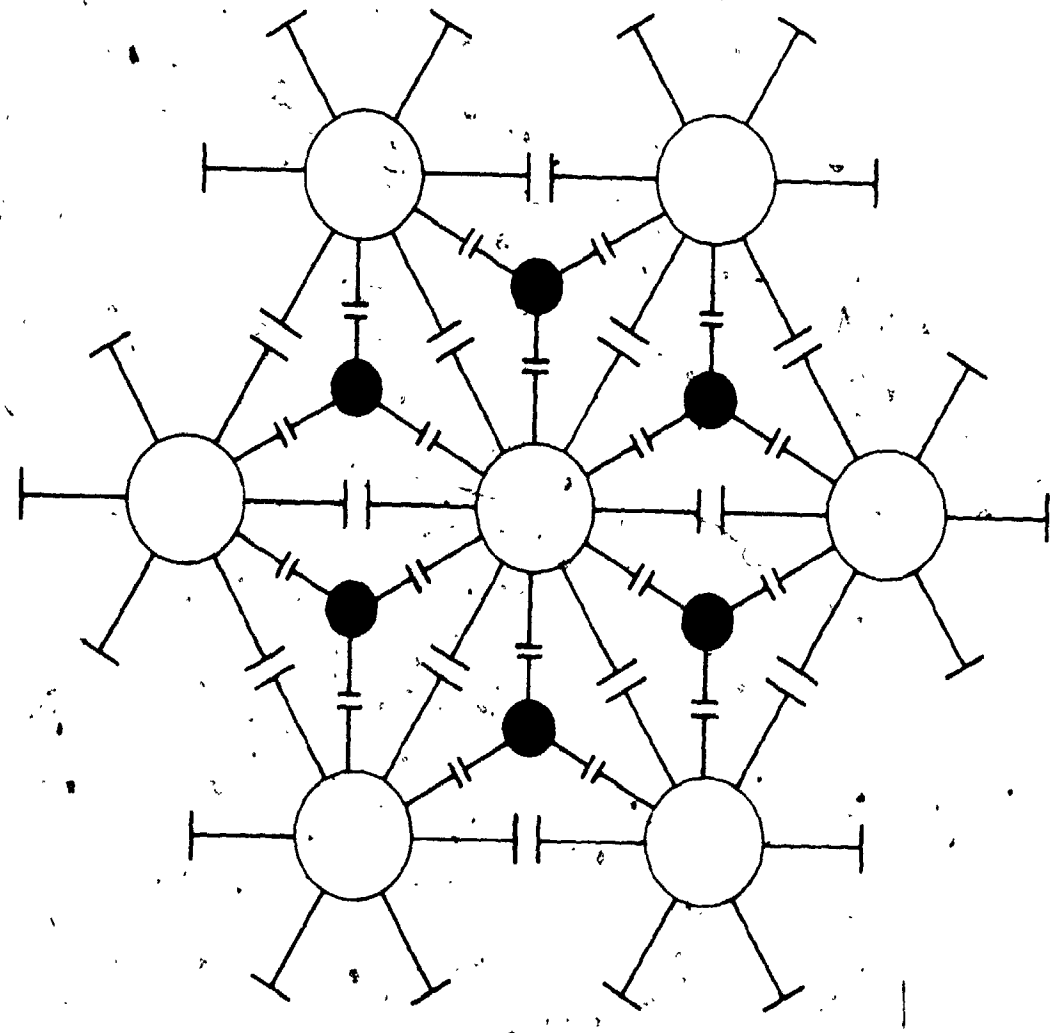
follow olfactory nerve shocks is operational. What is being argued is that the same neuronal networks responsible for the inhibition seen following nerve shocks can operate, but locally, to exert a small inhibitory influence. Thus, spikes in the sampled neuron and/or neighbouring neurons in the unit may activate granule cells (the putative inhibitory neurons) which mediate inhibition in terms of inhibitory post-synaptic potentials (IPSP's) back to the sampled neuron.

It is, of course, difficult to determine that an inhibitory control is present in spontaneous activity. However, as Burns (1968) has pointed out, the rising phase of the interspike interval density histogram gives some measure of the recovery of excitability from the last impulse. For these secondary olfactory neurons there is a noticeable absence of short intervals, that is, a delay of the rising phase of the histogram. Obviously the shortest of these intervals can be discounted on the basis of neuronal refractory periods. Yet these bulbar neurons display an absence of intervals longer than can be attributed to neuronal refractoriness, and it is reasonable to assume that this absence of short intervals is due to such local inhibitory effects as discussed above. Thus our "unit" as defined in Figure 31 must be modified to include this local inhibition. This is shown schematically in Figure 36. The unit is now defined as the sampled secondary neuron, all surrounding secondary neurons whose spontaneous activity can excite the sampled neuron, and all surrounding inhibitory units which can mediate

FIGURE 36

Illustration Showing Inhibitory Neurons Included in the Model of the Functional Unit

Open circles represent secondary neurons, filled circles represent inhibitory neurons. Connections are again meant to show that the neurons can affect each other reciprocally, and are not meant to represent any anatomical pathway. As with Figure 31, no attempt is being made to suggest the numbers of interacting neurons or of interconnections in the functional unit.



inhibition to the sampled neuron.

The period of post-inhibitory rebound seen in the secondary olfactory neurons could be accounted for by explanations other than the model offered in this thesis. For example, this rebound could be a manifestation of Mach bands, as observed in the visual system (Ratliff and Hartline, 1959). Patterns such as Mach bands can be successfully accounted for by strictly inhibitory fields. Such an explanation avoids the necessity of evoking excitatory fields to allow for the rebound firing in the secondary neurons. It does not, however, allow the same unification of explanation as does the model proposed in this thesis, in that the model proposed here can be used to explain both pre- and post-stimulus activity. Nonetheless, strictly inhibitory fields must be considered as a source of the post-inhibitory rebound. Alternately the post-inhibitory overshoot could arise as a result of differential patterns of current flow within the secondary neurons. It is easily imagined that the various membrane areas of these neurons could attain resting depolarization at different rates, thereby resulting in current flow patterns between the regions, and excitation. In this case, excitatory fields are again unnecessary, since the rebound is generated entirely within the secondary neuron. While this is a feasible alternative, it also avoids the conciseness of the proposed model. In the following section we shall show that the model has some interesting consequences.

5.4 Consequences of Interactive Bulbar Units

In this thesis evidence has been presented for a model of interacting groups of neurons in the olfactory bulb. In this section the consequences of this model will be considered insofar as they explain or elucidate the observations made by this author and others on the bulb.

We begin by considering the spontaneous activity of the secondary neurons, specifically, the implications of this activity for higher brain centres. Of course it may be argued that this activity has no role, or that it is simply serving to prime, as it were, the bulbar nervous system. However, closer consideration reveals that the spontaneous activity probably serves an extremely important function - it must represent the non-signal to the higher-order olfactory neurons upon which the axons of the secondary neurons impinge. That is, absence of activity in the secondary neurons could not constitute the non-signal, for this would be interpreted by the higher-order neurons as the inhibitory part of the post-stimulus response. Similarly, regular activity could not constitute the non-signal, for this could be interpreted as the excitatory rebound part of the post-stimulus response. In this latter respect the importance of the interaction to the spontaneous activity is apparent, since the regularly firing cell suggests that without such interactions the intrinsic process can drive the neurons regularly. With the

interaction process, a randomized spontaneous activity is guaranteed as the non-signal.

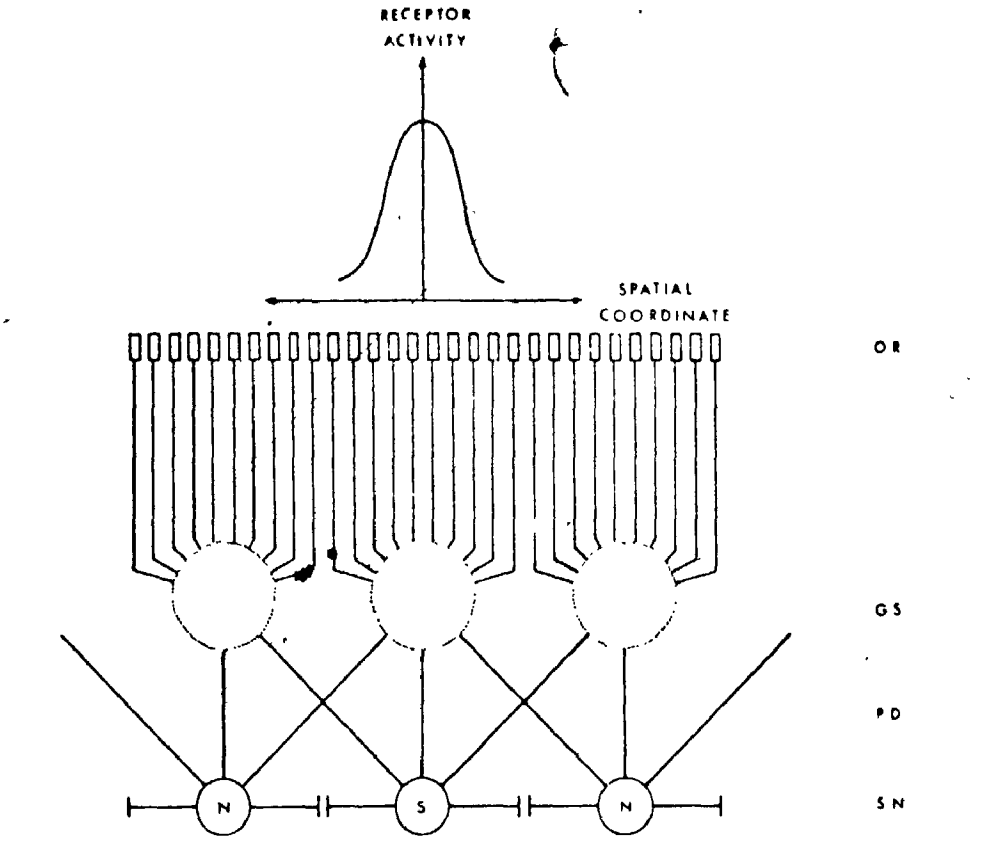
It is appropriate to offer some speculation as to how our proposed interacting groups of neurons could encode the receptor activity which is the input signal to the olfactory bulb. Since workers obtained the first records from single olfactory receptors in the frog, there has been general agreement as to the lack of receptor specificity (Gesteland, Lettvin, Pitts, and Rojas, 1963; Gesteland, Lettvin, and Pitts, 1965; Gesteland, 1971). That is, any particular receptor cell in the frog will respond to many odours. In addition no two receptors will rank a group of stimuli the same way. Such apparent lack of specificity in individual receptors has led O'Connell and Mozell (1969) to propose that odour quality may be encoded by "the relative activity of many individual receptor cells resulting in a unique across-fibre pattern of discharge for each particular odorant". If one considers, then, that the pattern in an ensemble of receptors encodes the message to the olfactory bulb, the role of the interactive neural groups proposed in this thesis becomes clearer. Since a number of nerve fibres input to a single glomerulus, and since a secondary neuron may contact several glomeruli, a single isolated secondary neuron would receive input from a wide spatial array of receptors. However, with no inhibitory interactions from granule cells and no excitatory interactions from neighbouring secondary neurons, this isolated secondary neuron could merely register the presence or absence of input

from this array of receptors by producing or not producing a spike or spikes. Including the inhibitory interaction, the secondary neuron can now register the receptor inflow as an inhibition of some time duration. Including as well the excitatory interaction between secondary neurons, the secondary neuron can now register the receptor activity as an inhibition followed in time by an excitation. That is, the interactive unit can convert the spatial array of activity across the receptors into a temporal pattern of activity within a secondary neuron. Furthermore, the parameters of the temporal response pattern in a given secondary neuron could depend upon the ensemble of receptor activity. Thus, as shown in Figure 37, if all the receptors are strongly activated there might be strong rebound firing in the sampled secondary neuron since neighbouring secondary neurons would be activated. If, however, the odour activated only receptors in a local area, the rebound firing could be less or absent, reflecting the fact that neighbouring secondary neurons were only partially recruited or were not recruited at all. If one considers that, in our model study with electrical stimulation, increasing the stimulus current activates more and more nerve fibres, then the observations made on neuron XXXIX-A with increasing stimulus currents mimic the case in which more and more receptors are activated. These observations are consistent with the above arguments and with Figure 37. Neuron XXXIX-A showed that a secondary neuron may exhibit no burst response at low stimulus strengths

FIGURE 37

Illustration Showing the Implications of the Proposed Model of Interacting Groups of Neurons for Coding Receptor Activity

This figure illustrates how a spatial code in the olfactory receptors (OR) may, by the model of interacting neuron groups proposed in this thesis, become in the secondary neurons (SN) a temporal code whose parameters depend upon the receptor activity. For simplicity the inhibitory neurons of the interacting neural groups have been omitted. Also, the figure has been simplified to one dimension, ie., a linear array of receptors, glomerular synapses, and secondary neurons. Were there a narrow spatial distribution of receptor activity (upper figure), the neighbouring secondary neurons (N) of the group would be excited only weakly since they contact only one active glomerulus via their primary dendrites (PD). Consequently, feedback excitation from these neighbours to the sampled cell (S) might not be able to overcome the self- and lateral inhibition generated by the inhibitory neurons. However, as the receptor activity broadens spatially (lower figure), neighbouring secondary neurons would be more strongly activated, and feedback excitation to the sampled cell would be greater. Such feedback excitation might now overcome the inhibition, and rebound firing could occur. Maximal feedback excitation at the shortest latency would occur when neighbouring secondary neurons were maximally excited.


RECEPTOR ACTIVITY
SPATIAL COORDINATE
OR
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PD
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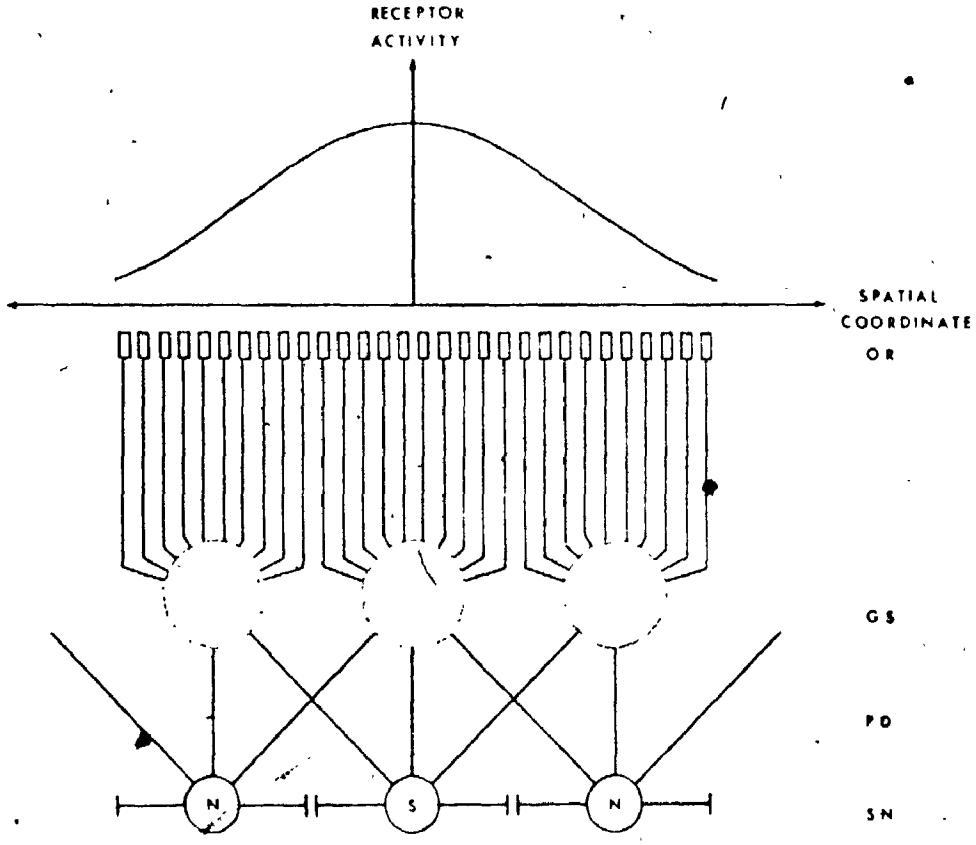
N
S
N


RECEPTOR ACTIVITY
SPATIAL COORDINATE
OR
GS
PD
SN
N
S
N

when, however, the neuron is driven by the stimulus. Presumably the recurrent excitation due to other neurons in the interactive group either does not occur because these other neurons are not driven by the low stimulus or the recurrent excitation is not strong enough to overcome the inhibition generated in the neuron by the inhibitory part of the network. As the stimulus strength is increased, however, a burst response occurs in neuron XXXIX-A, and the latency and magnitude of the burst depends upon the stimulus strength up to a saturation. Now, presumably, the stimulus is great enough that other secondary neurons in the interactive group are progessively recruited, and these neighbouring neurons generate feedback excitation which can eventually overcome the inhibition. Saturation would occur when all of the neighbouring neurons are maximally recruited. Consequently, making use of the balance between inhibition and excitation within the interactive unit, a given secondary neuron could, to reiterate our earlier statement, produce a temporal response whose parameters encode the olfactory signal contained in an ensemble of receptor activities. Thus the model of interacting neurons can explain how a model of receptor coding (due to O'Connell and Mozell, 1969) can be converted into the temporal sequence of inhibition-rebound firing observed for these secondary neurons.

It is worth noting that the interactive groups of neurons proposed here should result in adjacent secondary neurons having similar patterns of activity, while more

widely separated secondary neurons might have quite different patterns. Intriguingly, Adrian (1950, 1951, 1953) observed such localization within the olfactory bulb - he noted that esters tended to excite the rostral part of the bulb while hydrocarbons had a greater effect on the caudal region.

The effects of repetitive stimulation as seen in records such as those of Plate 16 become clearer when viewed in the light of the model of interactive neurons. In such records the ineffectiveness of successive stimuli falling within the period of inhibition in lengthening this inhibition can be assumed to be due to the widespread nature of the inhibition. Thus the secondary neurons cannot transmit the information registering the successive stimuli, and consequently the source of the inhibition is not reactivated. Such general inhibition can be thought of as being generated by the participation of entire groups of inhibitory neurons. Indeed the long time duration of the inhibition seen in this study supports such a concept. In the records of Plate 15 it can also be seen that stimuli falling early in the period of rebound firing are ineffective in producing a recurrence of the inhibition, while stimuli falling later in the rebound firing produce only a partial recurrence of the inhibition. Stimulation during this rebound firing should be able to reactivate the source of the inhibition, since the secondary neurons, the pathway from the olfactory nerve to this source, are no longer inhibited. Indeed one would expect that the rapid firing itself should be able to activate feedback

inhibition. It would appear, therefore, that the secondary neurons are now in a state of excitation that can overcome any inhibition. This state of excitation is most readily rationalized by assuming that the excitatory interactions of the network are swamping the inhibitory system. For example if each secondary neuron could, on its own, generate a small postinhibitory rebound, when these secondary neurons are interconnected to form an interacting group there could be a massive excitatory influence distributed over the network of the group. Such a large excitatory influence could be sufficient to overcome any inhibitory influence.

5.5 Summary

Observations and measurements have been made on spontaneous and post-stimulus activity in the secondary olfactory neuron of the frog. The results of this study may be summarized as follows:

a) spontaneous activity recorded from secondary neurons has been shown, in all but one instance, to be random in nature and to be the result of two oscillators which are independent of one another. These two oscillators have been shown to be within the olfactory bulb. In the one exceptional instance, the neuron generated spikes very regularly and was seen to have only a single underlying oscillator.

b) following electrical stimulation of the

olfactory nerve there is widespread inhibition of the secondary olfactory neurons for times of the order of one second.

c) following the inhibition arising as a result of nerve stimulation the secondary neurons may demonstrate transient high frequency rebound firing. The latency and magnitude of this rebound firing has been shown to depend upon stimulus strength.

These observations have been incorporated into a biophysical model in which groups of secondary neurons and inhibitory neurons are said to interact to form a functional unit in both the spontaneous and post-stimulus states. The two oscillators generating the spontaneous activity are:

a) a within-the-cell oscillator or intrinsic mechanism, and,

b) a mechanism external to the sampled cell and due to the summation, within the sampled cell, of excitatory influences from other cells which contact it.

The regularly firing cell, it is argued, is isolated from the interaction with other secondary neurons. The rebound firing seen in the post-stimulus response of the secondary neurons can then be ascribed to feedback excitation from other secondary neurons within the functional unit. The action of groups of inhibitory neurons within the functional unit can account for the inhibition seen in the secondary neurons

following nerve stimulation. It has been shown that the existence of such functional units, as well as accounting for the above observations, allows further insight into possible mechanisms of olfactory information processing.

CHAPTER VI

SUGGESTIONS FOR FURTHER STUDIES TO EXAMINE THE MODEL

Evidence has been presented in this thesis for integrative cell assemblies in the olfactory bulb which influence both the spontaneous activity and the post-stimulus response of the secondary neurons. That is, such cell assemblies have been proposed as the information processing units of the olfactory bulb. In this section some further studies are proposed which might shed additional light on the validity of this proposal.

As a biophysicist one first thinks of a more mathematical test of the model. Consequently one suggestion for further work in this area is to set up a computer study of an individual interactive unit involving secondary neuron and inhibitory neurons. One could use this to examine what parameters such a system must have to generate the observed spontaneous activity and post-stimulus responses. For example, one might determine the ratio of the number of inhibitory neurons to the number of secondary neurons necessary to generate the interplay of inhibition and

excitation seen in the response patterns. Such parameters could then be compared with established data on the bulbar nervous system. Such comparisons should allow an assessment of the validity of the model.

Experimentally, of course, it would be extremely useful if one could record the activity of a number of adjacent secondary neurons simultaneously. Thus, correlation analysis could be used to determine more directly if interactions are evident between the secondary neurons in their spontaneous mode.

Earlier in this thesis, the ability of the model to account for olfactory input to the bulb was discussed. In addition, the usefulness of the model in generating a random non-signal was mentioned. However, no detailed discussion was made of the implications of the model to higher centres. This aspect was intentionally neglected, the reason being the paucity of studies on higher olfactory centres reported in the literature. Thus there is little information available on the structure and function of these centres. Haberly (1969) has reported that neurons in the prepyriform cortex of the frog display less specificity to natural stimulation than do secondary neurons or receptors. If, indeed, the model advanced in this thesis is valid, then the message due to a given odour molecule may be carried by all the neurons of the group. Depending upon how the axons of the secondary neurons of an interacting group synapse with tertiary olfactory neurons (ie. is it a convergent network, divergent network,

one-to-one projection, etc.?) the olfactory message might be stronger, weaker, or unchanged in a given tertiary neuron. While Stevens (1969) and Biedenbach and Stevens (1969a, b) have investigated the structure and function of the olfactory cortex in the cat, there have been no detailed studies of projections to or function within higher centres in the frog. Were such studies carried out they could shed light on the validity of the model proposed in this thesis.

APPENDIX 1

Mathematical Background for Statistical Analysis of Neural Spike Data

The development of this appendix is essentially that of Moore, Perkel, and Segundo (1966).

Consider a spike train in which the N spikes are treated as indistinguishable, instantaneous events. Then the spike train is completely represented by the series of times of spike occurrence, t_0, t_1, t_2, ..., t_N. The interspike intervals are defined as the times between adjacent spikes, thus, t_1-t_0, t_2-t_1, t_3-t_2, ..., t_N-t_{N-1}. The mean interval E is given by

$$E = (t_N-t_0)/N \ .$$

The mean firing rate ρ is simply the reciprocal of the mean interval,

$$\rho = 1/E \ .$$

The probability density function f(x) underlying the distribution of interspike intervals is estimated by a histogram. There are several functions which are mathematically equivalent to the probability density function. Thus,

a) the cumulative distribution function,

$$F(x) = \int_0^x f(u)du = \text{prob } (X \leq x),$$

measures the probability that the random variable X, corresponding to an interspike interval, is less than or equal to x.

b) the survivor function,

$$\Xi(x) = 1 - F(x),$$

is simply the complement of F(x), and measures the probability that X is greater than x.

c) the age-specific failure rate,

$$\Phi(x) = f(x)/\Xi(x),$$

measures the instantaneous risk of failure of a component of age x.

In the present study, f(x) has been estimated on the basis of the interspike interval density histogram, and $\Xi(x)$ has been called the interspike interval distribution.

The renewal density h(x) specifies the probability of encountering a spike as a function of time after a given spike, irrespective of the number of intervening events, if any. Thus,

$$h(x)dx = \text{prob}\ \{\ \text{an event in } (x,x+dx)\ |\ \text{an event at } 0\ \}\ .$$

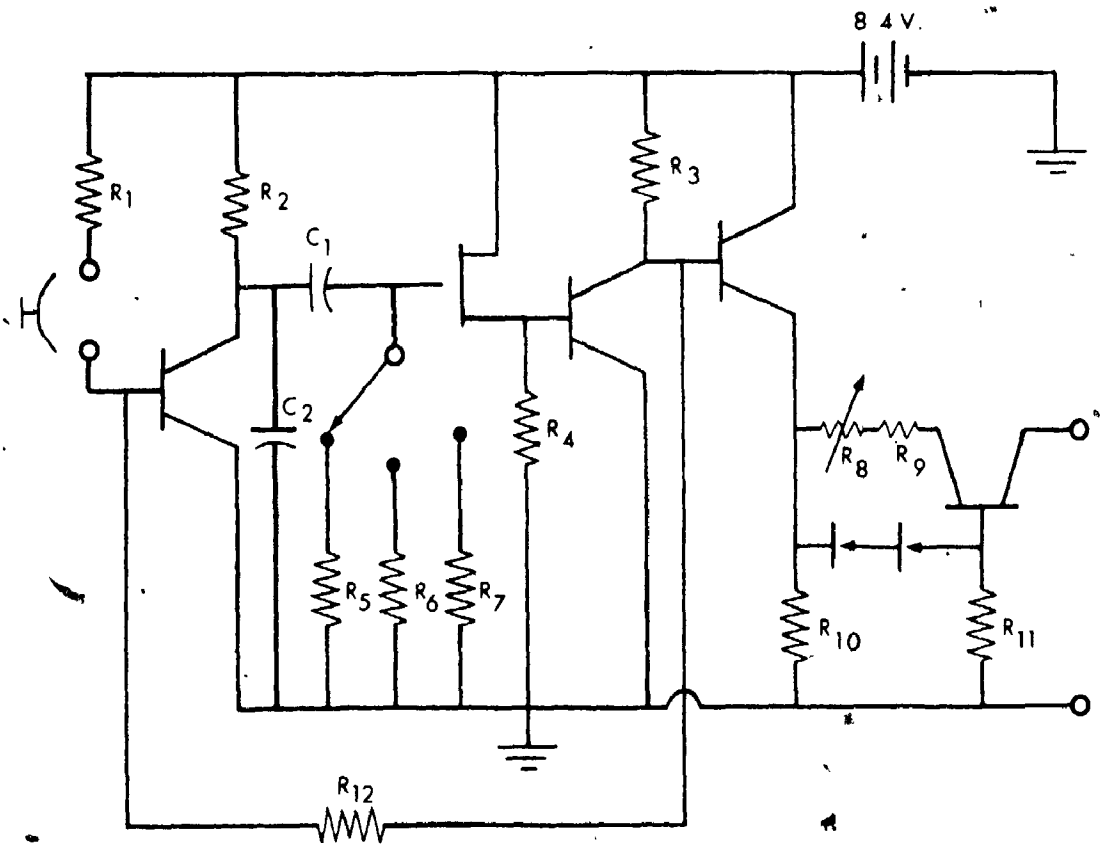
Since any event that is encountered must be the first, second, third, ..., etc., after the event of zero time, the renewal density is the sum of the interval densities of all orders,

$$h(x) = \sum_{k=1}^{\infty} f_k(x)\ ,$$

where $f_k(x)$ is the k-th order interval density, that is, the density defined by all the intervals spanned by a given spike and the k-th following spike. In this study, as is common in spike train analysis, the renewal density has been called the autocorrelogram.

APPENDIX 2

Constant Current Device for Dye Extrusion



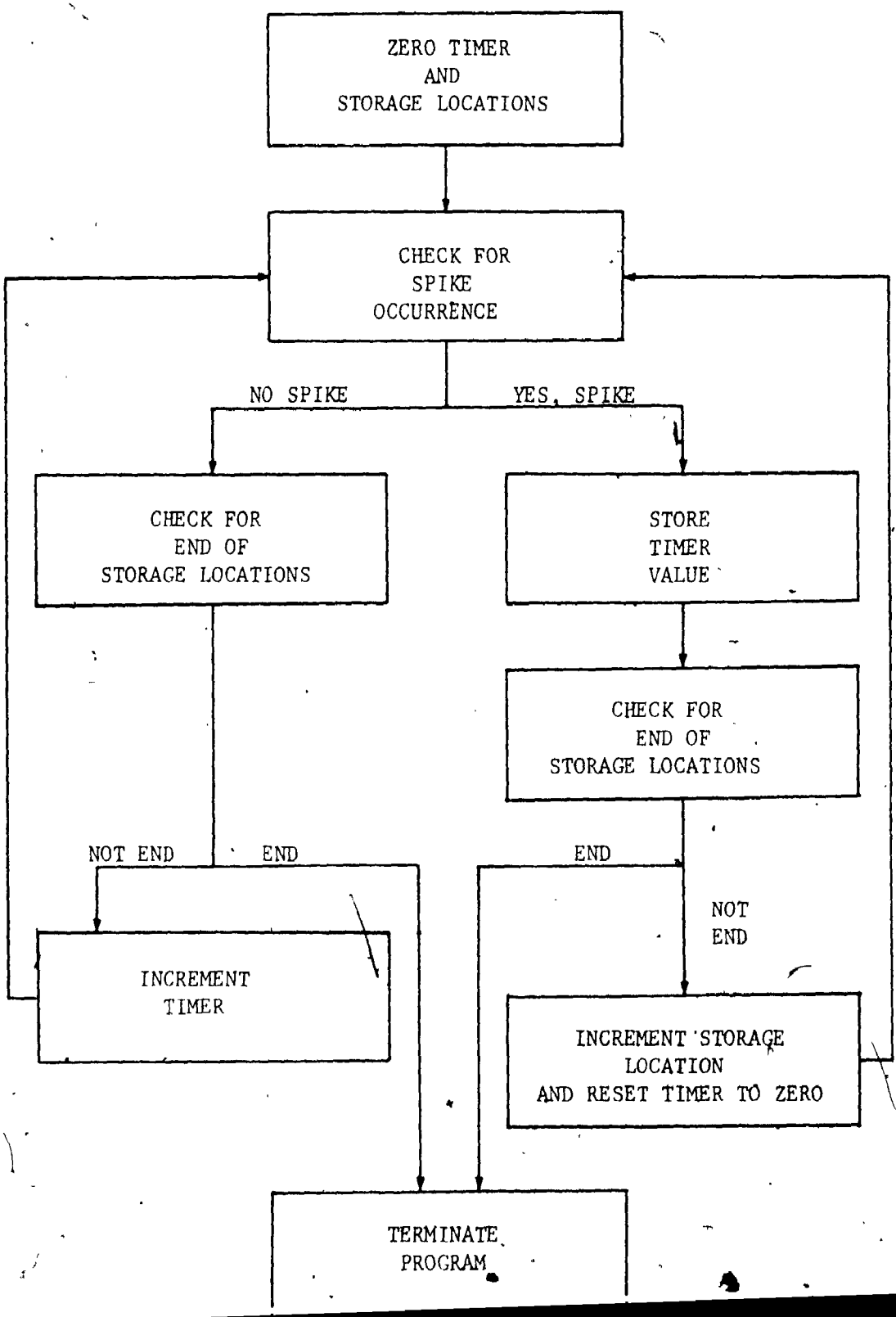
R_1 = 6.8 KΩ.
R_2 = 10 KΩ.
R_3 = 1 KΩ.
R_4 = 1.5 KΩ
R_5 = 330 KΩ
R_6 = 660 KΩ.
R_7 = 1 MΩ.
R_8 = 2 MΩ.
R_9 = 2 MΩ
R_{10} = 0 KΩ.
R_{11} = 2 2 KΩ
R_{12} = 10 KΩ.

C_1 = 30 μF
C_2 = 0.15 μF.

APPENDIX 3

Flow Charts for Computer Programs

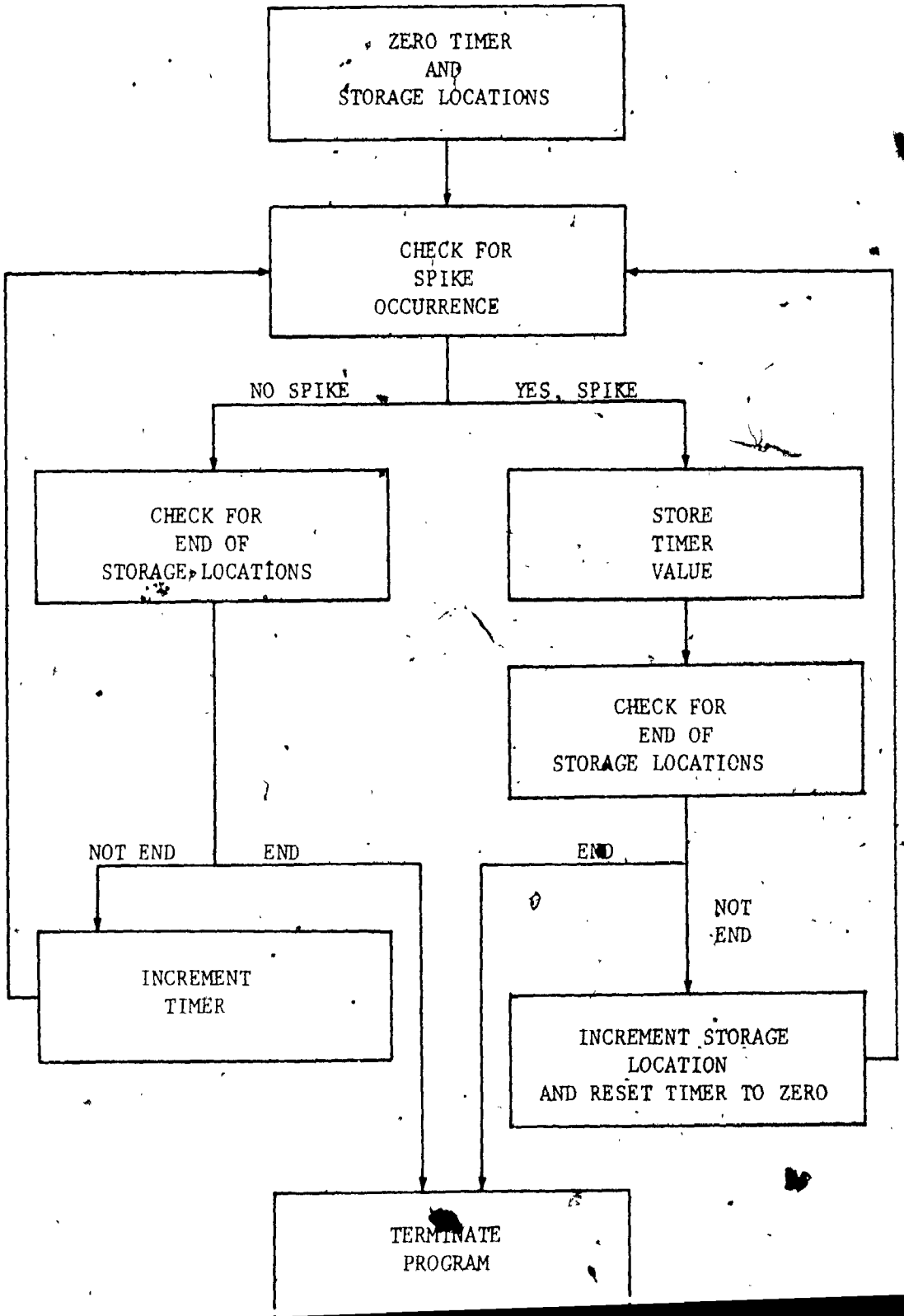
a) Interspike interval measurement, and histogram

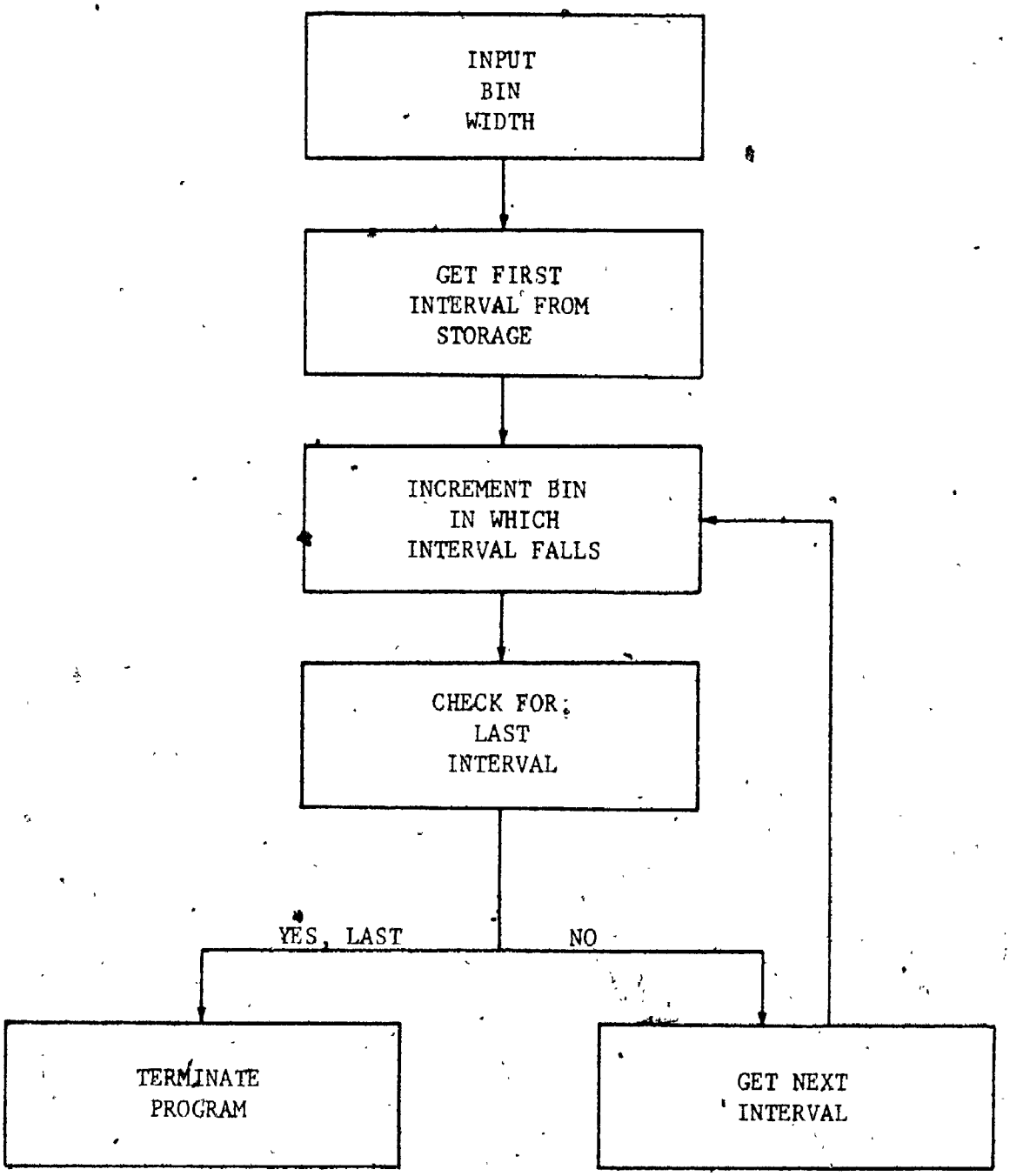


APPENDIX 3

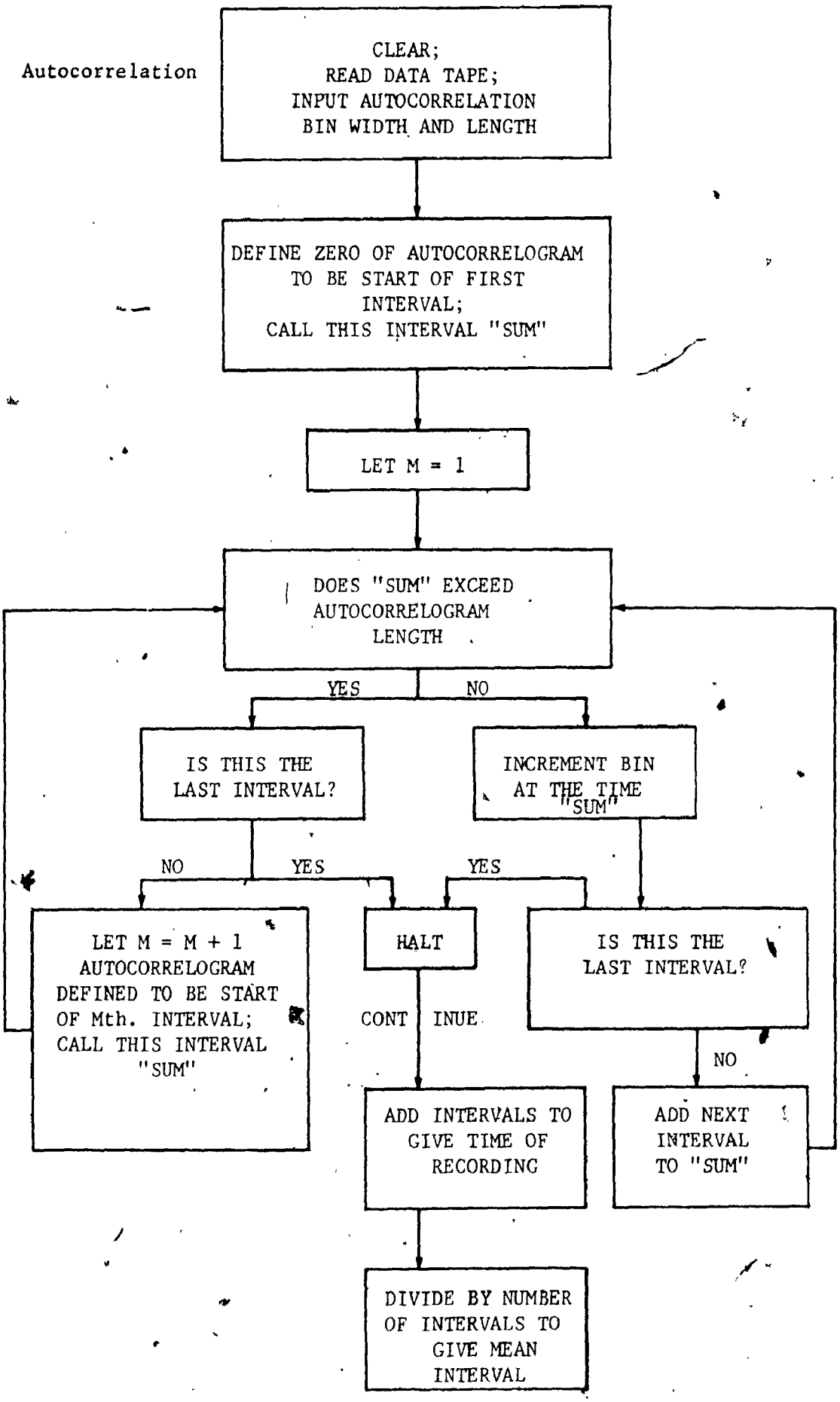
Flow Charts for Computer Programs

a) Interspike interval measurement, and histogram




INPUT
BIN
WIDTH
GET FIRST
INTERVAL FROM
STORAGE
INCREMENT BIN
IN WHICH
INTERVAL FALLS
CHECK FOR
LAST
INTERVAL
YES, LAST
NO
TERMINATE
PROGRAM
GET NEXT
INTERVAL

b) Autocorrelation



APPENDIX 4

Coefficients of Variability* for Neurons Modeled as Relaxation Oscillators

Neuron	Tape Segment	Coefficient of Variability
III-A	1300-2950	144
VI-C	0-1250	243
VII-A	0-410	92
VIII-A	1600-1980	26
IX-A	2000-2450	94
IX-B	120-570	51
IX-C	1200-2400	136
XII-A	1500-1900	98
XVI-B	2000-2250	350
XVIII-A	300-700	65
XIX-A	900-1450	177
XXII-A	0-350	102
XXII-C	550-850	56
XXIV-A	2500-2600	51
XXIV-B	2850-2950	50
XXIV-E	1000-1350	111
XXV-B	900-1500	94
XXVII-A	2400-2800	71
XXVII-B	0-150	52
XXVIII-A	750-950	87
XXXIII-B	2450-2550	77
XXXIV-B	200-1000	149
XXXV-D	2450-2850	134
XXXVII-A	0-600	127
XXXVIII-A	800-1050	84
XXXIX-A	850-1100	121
XL-A	1900-2400	176
XL-B	2400-2560	69
XL-C	2600-2900	76

APPENDIX 4 (CONTINUED)

Neuron	Tape Segment	Coefficient of Variability
CF-I-B	500-650	87
CF-I-C	650-800	88
CF-V-A	2840-3240	118
CF-V-D	750-1540	124
CF-V-E	1600-2000	77

* Coefficient of variability = $\frac{\text{standard deviation}}{\text{mean}} \times 100$

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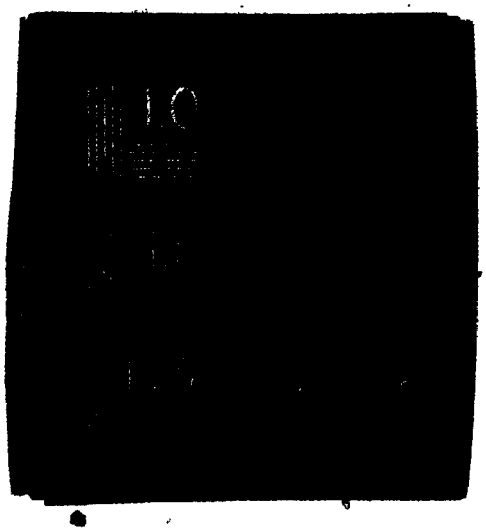

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