# A CLASSIFICATION OF LONG-TERM MAINTAINED NEURAL ACTIVITY IN PORTIONS OF THE SHEEP MEDULLA

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
John R. Haight
1968

THESIS

Q-108

ABSTRACT

A CLASSIFICATION OF LONG-TERM MAINTAINED
NEURAL ACTIVITY IN PORTIONS OF
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By

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A sampling of 57 single neural units exhibiting maintained firing properties was obtained from 8 sheep. The sampling site was the caudal portion of the medulla which was chosen because of the rich variety of neuronal types present within a limited area. Some of the units obtained were drivable when appropriate mechanical stimuli were applied to the body surface. Most had no obvious driving stimulus.

Consideration of the firing rates, averaged over 10 second intervals, gave rise to six distinct types of unit. These types are best characterized by the following description of their firing patterns: 1) regular and smooth; 2) regular but wobbly; 3) irregular, undulating; 4) irregular, stepping; 5) irregular, erratic; and 6) irregular, decremental. Each type of unit described was distributed throughout all anatomical areas of the medulla that were considered.

Interspike interval histograms computed from the same data also yielded six distinct types of interval distribution. Four of these were unimodal and were characterized by: 1) symmetric peak with "pre-modal" or fast intervals; 2) symmetric peak with little or no "pre-modal" activity; 3) asymmetric peak with "exponentially" decreasing tail; 4) asymmetric peak with "ramp-like" tail. Two distributions were multimodal having dominant features: 5) one symmetric major peak with one

or more minor peaks in the histogram tail and 6) two peaks, one symmetric and one asymmetric or both symmetric, all with short tails. These types were all found distributed throughout each discrete anatomical area that could be defined either histologically or electrophysiologically.

Certain correlations of interval distribution type and anatomical location were noted. Units found in the afferent dorsal fiber tracts tend to produce histograms of the symmetric type with emphasis upon multiperiodicity in the tail. Pre-modal activity was common. Ordinary symmetric distributions - those without pre-modal activity or without multimodal tails - were missing altogether. Units in the somatic-sensory nuclei produce the ordinary symmetric interval distributions. The multiperiodic tailed variety were not found in this area. Unit firing pattern types were evenly distributed in all other medullary areas.

It was noted that the type of interspike interval distribution produced remained constant regardless of the length of time sampled. Each maintained neuron appears committed to fire in an established pattern. Units with higher overall mean firing rates tend to produce more multiperiodic interval distributions. Units of slow to moderate firing rate generate symmetric histograms while units firing at extremely slow rates (less than 2 or 3 spikes per second) produce more of the asymmetric variety of interval distribution.

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John R. Haight

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Biophysics

1968

". . . . our theories should be based on the actual biological evidence at hand, so as to prepare the ground for the next step in experimentation, and not primarily on physical analogies."

Ragnar Granit
*Receptors and Sensory Perception*
Chapter 8.

## ACKNOWLEDGEMENTS

I wish to thank Dr. J.I. Johnson, Jr., the laboratory director and chairman of this thesis committee, both for his guidance and forbearance during the sometimes erratic course of this research.

This research was supported by NIH grants GM 10890 and NB 05982 together with NASA contract NSG 475.

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# Introduction

A recording electrode inserted into virtually any portion of the central nervous system will reveal spontaneous or what Kuffler (1956) more aptly calls maintained activity. A recent review by Moore, Perkel and Segundo (1966) discusses maintained activity research throughout the vertebrate nervous system. Bishop's (1967) review covers maintained activities in the afferent portions of the nervous system. Table 1 compares some of the maintained firing properties of neurons found in various nervous system areas as reported by various authors.

Ignoring maintained neural activity in favor of the experimentally more tractible dynamic properties of neural units may be a rather backward approach. Analysis of either steady-state or transient responses to stimuli cannot be considered adequately without first giving some attention to the neural baseline. It is therefore desirable to devise a means of dealing with the maintained or "spontaneous" nerve behavior in such a fashion as to facilitate comparisons with a given unit's driven responses, and with maintained activities from other neural regions (which may or may not be functionally related).

Many investigators have gone further than merely noting the presence of maintained activity. As is well-known from the work of Kuffler (1956) and others (see Bishop, 1967), the receptive field properties of the mammalian (cat) retinal ganglion cell are predicated upon a continuous firing rate modulated by alterations in visual contrast. Similar organizations have been noted in other sensory systems, including the auditory (Katsuki, et al., 1959; Greenwood and Maruyama, 1965) and the somatic sensory (Mountcastle and others, 1961a, 1961b; Gordon and Jukes, 1964).

Table 1

Maintained Unit Activity in the Central Nervous System: A Comparison of Various Regions and Animals

| Investigator | Animal | Area | Average Firing Rate of Population (Spikes/Second) | Range of Rates Reported (Spikes/Second) | Anesthetic Used |
|---|---|---|---|---|---|
| Pfeiffer & Kiang (1965) | Cat | Cochlear Nucleus | less than 100[1] | 0 - 150 | Dial |
| Kiang (1965) | Cat | Auditory Nerve | 40 - 50[2] | 0.03 - 118 | Dial-Urethane |
| Rodieck (1967) | Cat | Retinal Ganglion Cells | 27[3]-30[4] | 1 - 100 | Decerebrate |
| Herz, Creutzfeldt & Fuster (1964) | Cat | Visual Cortex:<br>Lateral Geniculate:<br>Optic Tract: | 6[3]<br>14[3]<br>36[3] | Not Available<br>"<br>" | Encephale-Isole<br>"<br>" |
| Braitenberg, *et al*. (1965) | Frog | Purkinje Cells (Cerebellum) | 11 | 5 - 24 | Syncurina |
| Amassian, *et al*. (1964) | Cat | Cuneate Nucleus | 26[2] | 5 - 97 | Flaxedil |
| Martin & Branch (1958) | Cat | Betz Cells | 6 | 1 - 27 | Midbrain Lesion |
| Cross & Silver (1966) | Rat | Hypothalamus:<br>Thalamus | less than 1[1]<br>between 1 and 2[1] | 0 - 6 plus<br>" | Urethane<br>" |
| Poggio & Viernstein (1964) | Monkey | Ventral Basal Thalamus | 14 - 15[2] | 6 - 35 | Unanesthetized |

Table 1 (cont'd)

| Investigator | Animal | Area | Average Firing Rate of Population (Spikes/Second) | Range of Rates Reported (Spikes/Second) | Anesthetic Used |
|---|---|---|---|---|---|
| Evarts (1964) | Monkey | Pyramidal Tract: | 7 | 4 - 12 | Sleeping[5] |
| | | Non-pyramidal Area | 1 | Not Available | Sleeping[5] |
| Haight (1968) | Sheep | Medulla | 12 | 0.4 - 44.7 | Dial-Urethane |

[1] 90% of population had rates of less than 100 spikes per second.

[2] Calculation from author's data.

[3] Animal in darkness.

[4] Animal in light.

[5] Data available for awake animals

Some neurologic models have been constructed which attempt to relate stimulated or driven and the maintained behavior of neurons. These models generally view the response to the stimulus as either a modulation or a steady-state change in the maintained baseline. Mathematical treatments have been presented; notable among these efforts is the work by Werner and Mountcastle (1963); Finkelstein and Grüsser (1965); Goldberg, et al. (1964); and Mountcastle, et al. (1963). All of these authors have reported a power law relation between stimulus and response with maintained activity providing an irreducible minimum of nerve activity present at a zero stimulus level or at some state of physiological equilibrium.

Other efforts have been concerned with the statistical behavior of spike trains, both in the stimulated and unstimulated (maintained) modes. Kiang (1965); Gerstein and Mandelbrot (1964); and Rodieck (1967) have approached the problem of spike train decoding in this manner. Such techniques do not stand alone, however. These models are useful for determining what a given neuron cannot be doing during some time interval. Their predictive value has generally been nil. Such results can establish guidelines for interpreting experimental results when more data are present. Further discussion concerning the value of these techniques can be found in Bishop's review (1967) and in the paper by Gerstein and Mandelbrot (1964).

Many investigators have not been able to hold maintained units for appreciable periods of time. Movements of tissue due to arterial pulsations and respiratory activity often limit the long-term recording possibilities from a given unit. The movement of the tissue around the electrode causes, at best, spike amplitude changes which can make amplitude discrimination from the baseline noise level very difficult. Progressively more disastrous in nature are the occasions where the unit is removed from the region of the electrode tip altogether. Finally, the moving tissue and the stationary electrode often conspire to stimulate the unit being studied, thus making, for example, "respiratory" units out of these whose primary concern in the organism's life is not respiration or the control thereof. (see Amassian, 1964)

As a consequence, recording sessions with single units seldom extend beyond a few minutes. It should be noted that differing brain regions present varying sets of difficulties in this regard. Rodieck and Smith (1966) and Rodieck (1967) have shown that it is possible to record from the retinal ganglion cell of the cat for periods of an hour or more. This is in a thoroughly paralyzed preparation. Amassian (1964), on the other hand, experienced considerable difficulty in holding single units for more that a minute or two while recording from the cat's medulla.

Comparisons of maintained neural activity from different areas of the same brain are also lacking. To date most investigators have been interested in a particular functional area to the exclusion of surrounding areas. Maintained activity analysis throughout the nervous system as a whole or even in a smaller but multifunctional portion has not been attempted. Some researchers have compared nervous activity in the system of their immediate interest with contiguous areas (see Cross and Silver, 1966; Evarts, 1964). In general, however, this is not attempted.

In the present investigation I obtained a distributed sample of maintained neural activity over relatively long time intervals and from a functionally rich and varied portion of the nervous system.

An elucidation or categorization of baseline activity is necessary before dealing with the dynamic responses of the nervous system to both external and internally generated stimuli. Therefore, the answer to the following question is needed. Can the sequential sampling of a unit's maintained firing rate and the distribution of interspike intervals provide enough information to distinguish consistently and reproducibly between possible classes of units found in functionally distinct systems or structurally differentiated brain regions?

The vehicle for this study was the posterior portion of the medulla of the domestic sheep, Ovis aries. This area of the medulla is desirable from the standpoint of this research because of the myriad of functional systems and distinct morphological entities present in a rather limited space. The sheep medulla was desirable because, unlike the medullas of smaller animals, it is relatively free from movements caused by either the animal's breathing or arterial pulsations.

## Methods

The subjects. Eight female domestic sheep, *Ovis aries*, were used in this study. The animals were anesthetized with dial-urethane or nitrous oxide plus halothane (see Appendix F for details), tracheotomized and positioned for favorable exposure of the medulla. The caudal portion of the cranium and the dorsal aspects of the first two cervical vertebrae were removed and the exposed medulla was positioned using techniques similar to those employed by Johnson, *et al*. (1968). Normal supportive measures including injections of normal saline to prevent dehydration, atropine to prevent excess secretion, and anesthetic as needed. The animals' temperatures were maintained at 34-37°C. by means of a battery of infra-red lamps.

Electrodes. The recording electrodes were modifications of either the Hubel (1957) tungsten microelectrode or the Baldwin, *et al*. (1965) glass insulated tungsten electrode. Electrodes were commonly uninsulated for 30-50 microns back from the tip. These were found to perform adequately with shaft diameters of from 200 to 300 microns. This configuration gave the needed physical strength to penetrate the overlying pia mater but had a small enough tip size to allow reliable access to single units.

Recording and processing equipment. Conventional recording equipment was employed throughout these experiments. A low level AC amplifier received the electrode signals and drove both an oscilloscope and an audio monitor. Signals deemed usable were recorded on magnetic tape using a good quality stereophonic recording machine The second tape channel was used for voice commentary and time marking.

On playback the recorded signals were smoothed and rectified. The spikes were converted to standard pulses by amplitude gates and fed into a multi-channel scaler (CAT 400B) which was programmed to determine either the average firing rate per second (over 10 second intervals) or to construct the appropriate interspike interval histogram.

Histology and track localization. At the termination of the experiments the animals were intercardially perfused with 0.9% saline followed by 10% formalin in 0.9% saline. The medullas were removed, embedded in celloidin, sectioned and stained. Alternate sections were thionin stained (Nissl method) for cell bodies. The remaining sections were alternately stained with hematoxylin (Weil or Sanides-Heidenhain method) for myelinated fibers.

The small number of electrode punctures in any one medulla and the fact that punctures providing useful data showed heavy gliosis because of the time spent in the puncture by the electrode made electrode track tracing relatively simple and certain. A few units were definitely localized as to position in the puncture by means of electrolytic lesions generated by passing small amounts of current through the recording electrode.

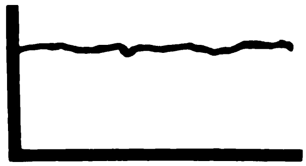
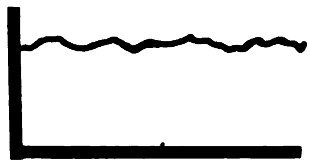
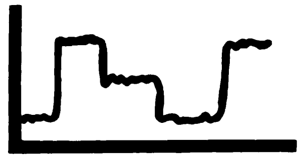
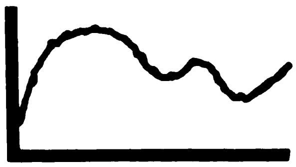
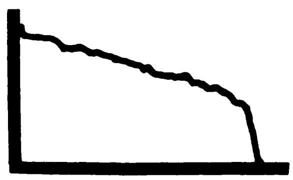
Complete details as to all the methods employed in this study will be found in Appendix F.

## Results

The unit population. Eight sheep provided 57 separate units ranging in overall firing frequency from 0.4 to 44.7 spikes per second. The mean frequency for the entire population was 12.3 spikes per second. Fifty percent of the units fell between 6 and 18 spikes per second. The units comprising this study were held for time intervals of from 80 to over 5000 seconds. Only six of the units were held for periods of less than 300 seconds while over one-half were held for 900 seconds or longer.

With 22 units mechanical stimulation of the body surfaces gave rise either to alteration of the firing rates of the units themselves or to audible differences in the background firing activity as discerned from the audio monitor. Four units were observed to be related to the respiratory cycle of one animal. The remaining units were unaffected by any obvious stimulus, internal or external.

The average firing rate profiles. The plotted average firing rate profiles (see Appendix D) fell into two major classes, regular and irregular. (see Figure 1) Regular (R) units were observed to maintain a more or less constant firing rate during the time the unit's activity was sampled. The regular units were further subdivided, on the basis of topographical features of the profiles, into two subgroups, $R_1$ (even) and $R_2$ (wobbly). $R_1$ (even) units fired with extreme regularity over each ten second sampling. The standard error of their ten second averages was 10% or less in most cases. $R_2$ (wobbly) units tended to exhibit minor fluctuations about a central tendency. The standard errors of these units would often run to 20% or more. (In this text and in the figures the standard error is expressed as a percentage of the mean rate of firing.)

| Type of Average Firing Rate Profile | Mean Firing Rate of Profile Subgroup in Spikes per Second | Mean Standard Error of Profile Subgroup Expressed as % of Mean Rate | Schematic Diagram of Profile | N |
|---|---|---|---|---|
| $R_1$ (even) | 20.4 | 8.7 |  | 15 |
| $R_2$ (wobbly) | 4.6 | 19.4 |  | 7 |
| $I_1$ (stepping) | 10.5 | 55.8 |  | 14 |
| $I_2$ (undulating) | 9.7 | 29.3 |  | 11 |
| $I_3$ (erratic) | 5.0 | 36.9 |  | 4 |
| $I_4$ (decremental) | 14.9 | 35.8 |  | 7 |

Spikes per Second

Time (sec.)

Figure I. The properties of the average firing rate profiles: schematized topographical properties plus the mean firing rate of each category together with the standard error associated with the mean rate.

Irregular (I) units were divided into four subcategories: $I_1$ (stepping), $I_2$ (undulating), $I_3$ (erratic) and $I_4$ (decremental). $I_1$ (stepping) units characteristically jumped from steady-state to steady-state taking a very few seconds to accomplish the change. $I_2$ (undulating) units, on the other hand, did not achieve steady-state firing rates but continuously and slowly changed rate in a sinuous fashion, though most often with no regular periodicity. The $I_3$ (erratic) units tended to change firing rate rather dramatically and did so continuously, often effecting a rate change of 100% or more within the space of a very few seconds. The final group, the $I_4$ (decremental) exhibited a monotonic decrease in firing rate throughout their sampled lifetimes. Eventually these units always "died".

Though the sample size employed in this study is not large enough to allow significant assessments as to the randomness or non-randomness of the distribution of firing rate profile types in various anatomical areas, it should be noted that no profile type was found to be excluded from any of the anatomically defined areas considered in this study. This point will be considered further in the next chapter.

The interspike interval histograms. Three major types of interspike interval histogram (see Figure 2) emerged considered purely on the basis of their plotted shapes. These were distributions with: 1. symmetric peaks, 2. asymmetric peaks and 3. more than one peak. Each of these in turn could be further divided into subgroups.

Taking the symmetric group first, the essential difference was to be found in the presence or non-presence of short intervals, in sufficient quantity to establish a pre-modal plateau in the finished histogram. Distributions lacking this feature were more nearly "normal" in shape.

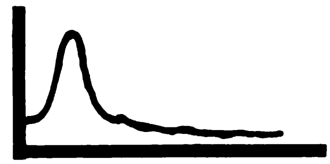
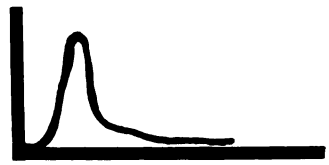
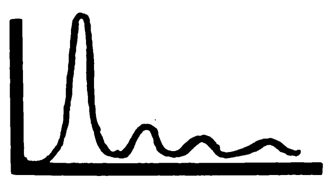
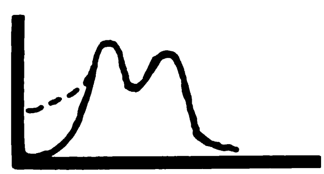
| Abbreviated Symbol for Interval Histogram | Description of Histogram Character | Schematic Diagram of Histogram Type | N |
|---|---|---|---|
| S(M-PT) | Symmetric, unimodal with pre-modal plateau. Moderate tail length. |  | 11 |
| S(ST) | Symmetric, unimodal with no pre-modal plateau. Moderate tail. |  | 10 |
| A(ET) | Unimodal, asymmetric. Fast rise to peak with long, heavy tail which falls off exponentially. |  | 10 |
| A(RT) | Unimodal, asymmetric. Fast rise to peak with a "ramp-like" tail. |  | 4 |
| M(PT) | Multimodal, symmetric major peak with one or more minor peaks in tail. |  | 10 |
| M(BI) | Bimodal, either symmetric-symmetric or asymmetric-symmetric with short tail. |  | 9 |
| N | No interval histogram plotted. (See note at end of Appendix I.) | | 3 |

Figure 2. A description of the various types of interspike interval histograms reported in the present work.

Both types exhibited fairly long tails. These will be referred to as S(P-MT) and S(ST) respectively in the remainder of this report.

Asymmetric interval distributions were characterized by fast linear rises in the short interval side of the peak followed by either a long, heavy "exponential" tail or by a "ramp-like" tail. These types will be henceforth abbreviated as A(ET) and A(RT) respectively.

Finally, the two varieties of multimodal interval distribution will be referred to as M(PT) and M(BI). The first type typically displayed a highly symmetric major peak followed by a lengthly tail with one or more minor peaks superimposed upon it. M(BI) interval histograms had only two peaks. These were usually near to each other in size. With some M(BI)'s both peaks were symmetric and with others one peak was symmetric while the other was not. When this was the case, the longer interval peak was generally the symmetrically shaped one.

It is of interest to consider the nature of the spike trains that go into the makeup of each type of interspike interval histogram. It is found that the generating spike trains are similar for both the symmetric (S) distributions. Both contributing trains are characteristically smooth and even-firing. By triggering an oscilloscope with one spike and measuring the time to its successor, it is possible to obtain a fair estimate of the mean rate of the unit. The S(P-MT) units would appear to slip occasionally and fire two spikes in rapid succession. These short intervals are not uniform, however, and the resulting histogram is consequently characterized by a pre-modal plateau. The highly periodic S(ST) distributions do not show this feature. Their interval histograms rise slowly from the zero level with no irregularities.

The spike trains comprising the asymmetric histograms present a different case. They fired erratically, almost in a "Poisson" sequence. Triggering on one spike and measuring the time interval to the next spike does not reveal the mean firing rate as the interval variation is often several hundred percent. The long, heavy tails typical of these distributions are generated by the relatively frequently occurring long intervals. Both the A(ET) and A(RT) types have a very similar spike train makeup.

Coming to the multimodal (M) types, it is found that these are usually composed of spike trains that exhibit bursting behavior. That is, a cluster of evenly spaced spikes is followed by a pause and then another cluster. When the pauses and the intervals between spikes in a burst are highly periodic, a M(PT) type interval histogram results. When the spike bursts are made of only two or three spikes and the pause between bursts is on the order of the intra-burst interval, a M(BI) interval histogram with two symmetric peaks is usually produced.

Occasionally the clustering consists of unequally spaced spikes within the cluster but with equally spaced intervals. This produces an asymmetric-symmetric type M(BI) distribution that bears some similarity to the symmetric S(P-MT) histogram discussed earlier. The $I_4$ (decremental) average firing rate profile producing units were often observed to display an M(BI) type of interval distribution as well. These will be discussed in the next chapter.

A consideration of anatomical location and interspike interval histogram type will also be left to the next chapter. Appendices D and E contain all of the average firing rate profiles and interspike interval histograms used in this study. Notes are included in these appendices which point out some of the more interesting features of the plotted figures which have come under discussion in this chapter and will come under more discussion in the following chapter.

Histology. An attempt was made to establish an anatomic locus for each unit used in this study. Of the eight sheep used, two were not available for this purpose. (see footnote 3 at end of Appendix B) The remaining six medullas were sectioned, stained and examined for electrode tracks.

Traced units were assigned to four basic medullary locations. These were: a) the ascending afferent tracts overlying the dorsal column nuclei, b) the nuclei themselves including the spinal trigeminal, c) the medullary reticular areas and d) the "other" portions of the medulla. These included the cranial nerve nuclei, the ventral white matter and the ventral horns. The anatomical localizations were based on the descriptions of Palmer (1958) and Woudenberg (1968). Specific locations (in as much as these are possible to claim, see Akert and Welker, 1961) will be found in Appendix C where all the track tracings are presented. Figure 3 shows a typical electrode track as it appeared in a thionin (Nissl) stained section.

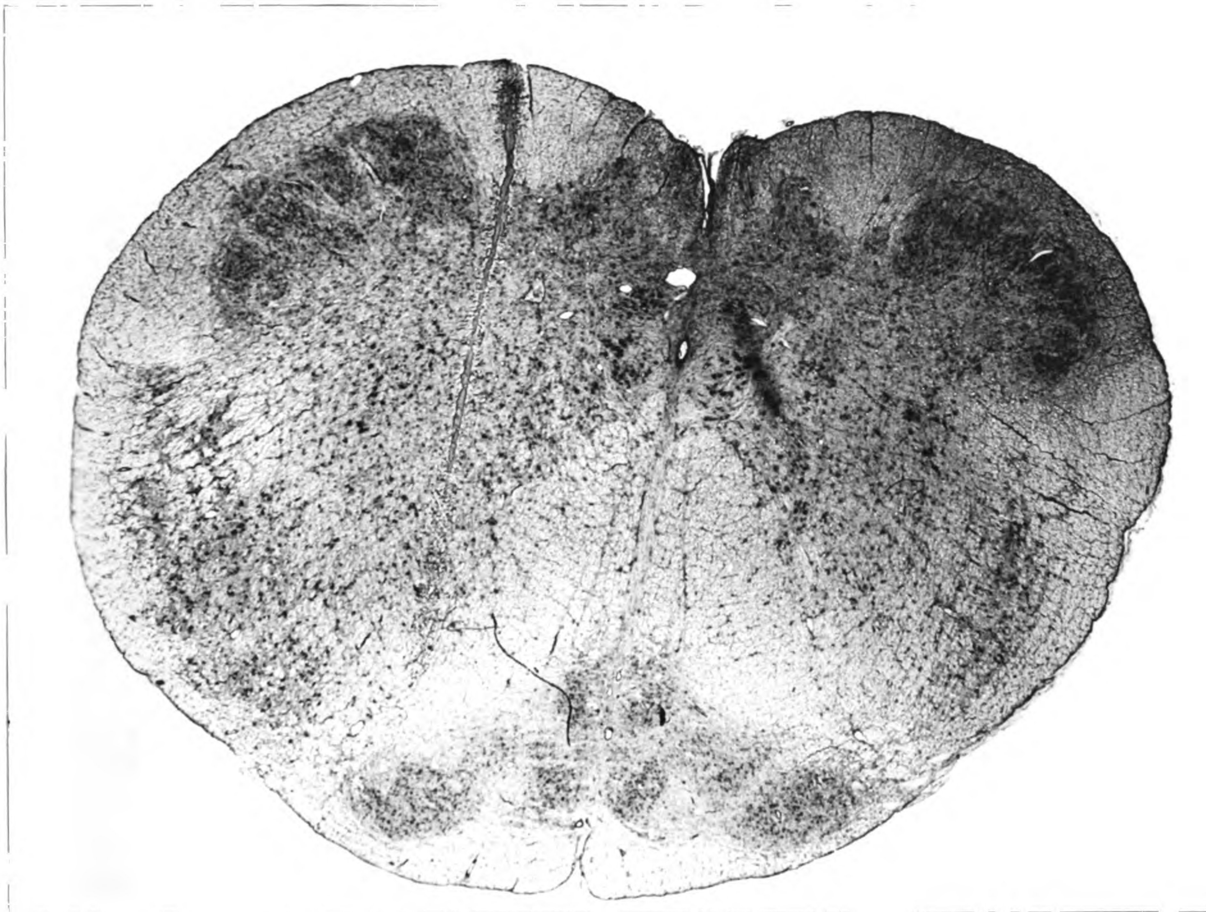


Figure 3. A Thionin (Nissl) stained section of the sheep medulla at the level of the gracile-cuneate complex. Note electrode track passing through cuneate nucleus on the left side.

## Discussion

As was observed in the introductory comments of the preceding chapter, some of the units used in this study were held for periods of one and more hours. A question might arise: does such a unit maintain stationarity with respect to its statistical properties over long time intervals? During data playback the shape of the computed interspike interval histogram was monitored continuously. Once a recognizable form had been achieved, the overall shape of the plotted distribution did not change except for a few irregularly bimodal M(BI) producing units. As a further check upon stationarity, segmental records were made and compared in a manner similar to that employed by Amassian, et al. (1964). Successive 10 minute samplings were compared as to the shape of the interval distribution produced. Minor variations were noted, but no unit could be said to change the type of interval histogram that it characteristically produced.

The M(BI) type units, it will be remembered, were produced by units having two distinct types of spike train. One of these was an irregularly bursting train while the other, superficially at least, was rather even and regular in discharge rate. Observations made on these latter units indicated that they were strongly correlated with the $I_4$ (decremental) firing rate profile producing units. These, while gradually diminishing in firing rate, appeared to fire with extreme regularity over shorter time intervals. However, the "slowing down" of these units was typified by a preference on the part of the unit for a certain modal interval. Ever longer intervals were interspersed among the "preferred" intervals such that a facade or more-or-less constant interval could be maintained. Eventually, this was no longer possible and the unit would shift to some new modal value with a longer interval. As a rule not more than two of these shifts were noted because the units in question would stop firing altogether, usually rather suddenly.

Similar behavior, i.e. the production of M(BI) interval histograms, was not observed with the remaining types of irregularly profiled units, at least not in appreciable quantity. For instance, two type $I_1$ (stepping) units displayed M(BI) type interval histograms. However so did two $R_1$ (smooth) profile producing units.

A better view of the trends is contained in the cross comparison tables presented below. All the units used in this study are compared as to:

1. Anatomical location and the type of interspike interval histogram produced. (Table 2)
2. Anatomical location and the type of average firing rate profile produced. (Table 3)
3. Interspike interval histogram and average firing rate profile produced. (Table 4)

The data are presented as the number of observed units sharing any two properties as described above. The number in parentheses following each observed value is the "expected" value which would be obtained if the relation between the variables were completely independent. The significance of the "expected" value is predicted upon the randomness of the method in which the units were sampled in the medulla - a matter about which this study makes no claims. The basis for the calculation of the "expected" values arises from contingency table constructions, the details of which can be found, for example in Freund (1962). The obvious next step of determining the $chi^2$ test for independence of the variables was not feasible because of the limited sample size. Proper usage demands that the expectation values in each cell of the contingency table be greater than five.

In spite of the testing limitations, trends can be noted. Table 3 shows that M(PT) interval histograms are preferentially produced by fibers of the dorsal fiber tracts. This indicates that such fibers tend, as a class, to fire in regular bursts. A few S(P-MT) or symmetric, pre-modal histograms are produced by the fibers in this area. These can be construed as units which fire short bursts of two spikes with regular intervals between bursts and irregular intervals within the burst. The other symmetric S(ST) type of interval histogram is apparently not produced by units comprising the primary afferents of the dorsal column tracts.

Neurons in the somatic sensory nuclei would seem to favor S(P-MT) irregular bursting but symmetric firing patterns together with the S(ST) symmetrics. Both of these are highly "periodic" in nature and can be distinguished from each other only by the absence of the "pre-modal" or fast activity in the case of the S(ST) units. The regular bursting multimodal M(PT) units are missing from these nuclear areas though they were common enough in the pre-synaptic areas.

Reticular and "other" areas of the medulla appear to be distributed very close to expectation across the various interval histogram types.

The average firing rate profiles receive the same treatment in Table 3. Again, significance tests cannot be applied, but it is reasonably apparent that firing rate profile patterns are distributed very close to expectation throughout all areas of the medulla that were considered as separate entities.

Identical techniques were used to compare the occurrence of a given average firing rate profile category within a particular interspike interval histogram class. These results are presented in Table 4. The

Table 2

The Distribution of Interspike Interval Histogram Types Found in Various Medullary Regions as Determined Histologically.

| Interspike Interval Histogram Type | The Number of Units of a Given Interspike Interval Histogram Type Identified Histologically in Various Medullary Locations | | | |
|---|---|---|---|---|
| | Dorsal Fiber Tracts | Somatic Sensory Nuclei | Medullary Reticular Areas | "Other"[1] |
| S(P-MT) | 3 (3.8)[2] | 5 (3.2) | 1 (1.8) | 1 (1.2) |
| S(ST) | 0 (2.7) | 4 (2.2) | 0 (1.3) | 2 (0.8) |
| A(ET) | 4 (3.8) | 2 (3.2) | 3 (1.8) | 1 (1.2) |
| A(RT) | 0 (1.2) | 1 (1.0) | 1 (0.6) | 1 (0.4) |
| M(PT) | 8 (3.4) | 0 (2.9) | 1 (1.6) | 1 (1.1) |
| M(BI) | 3 (3.0) | 3 (1.6) | 2 (1.4) | 0 (1.0) |
| N | 1 (1.2) | 1 (1.0) | 1 (0.6) | 0 (0.4) |

[1] This category includes units located in:
a. The cranial nerve nuclei.
b. The ventral white matter.
c. The ventral horn.

[2] Expectation value as discussed in text.

Table 3

The Distribution of Average Firing Rate Profile Categories Found in Various Medullary Regions as Determined Histologically

| Average Firing Rate Profile Type | The Number of Units of a Given Average Firing Rate Profile Category Identified Histologically in Various Medullary Locations | | | |
|---|---|---|---|---|
| | Dorsal Fiber Tracts | Somatic Sensory Nuclei | Medullary Reticular Areas | "Other"[1] |
| $R_1$ even | 6 (5.5)[2] | 3 (4.2) | 3 (2.4) | 1 (1.6) |
| $R_2$ wobbly | 2 (2.7) | 4 (2.2) | 0 (1.3) | 1 (0.8) |
| $I_1$ stepping | 5 (5.5) | 3 (4.2) | 2 (2.4) | 3 (1.6) |
| $I_2$ undulating | 2 (3.4) | 5 (2.9) | 2 (1.6) | 0 (1.1) |
| $I_3$ erratic | 1 (1.1) | 1 (1.0) | 1 (0.6) | 0 (0.4) |
| $I_4$ decremental | 3 (2.0) | 0 (1.6) | 1 (0.9) | 1 (0.6) |

[1]This category includes units located in:
a. The cranial nerve nuclei.
b. The ventral white matter.
c. The ventral horn.

[2]Expectation value as discussed in text.

Table 4

A Cross-Comparison of the Occurrence of the Various Interspike Interval Histogram Types and the Average Firing Rate Profile Categories

| Interspike Interval Histogram Type | Number of Times a Unit of Specified Average Firing Rate Profile Category is Found within a Given Interspike Interval Histogram Class | | | | | |
|---|---|---|---|---|---|---|
| | $R_1$ even | $R_2$ wobbly | $I_1$ stepping | $I_2$ undulating | $I_3$ erratic | $I_4$ decremental |
| S(P-MT) | 2 (2.9)[1] | 4 (1.4) | 3 (2.7) | 2 (2.1) | 0 (0.7) | 0 (1.2) |
| S(ST) | 4 (2.6) | 0 (1.2) | 2 (2.5) | 3 (1.9) | 0 (0.7) | 1 (1.1) |
| A(ET) | 1 (2.6) | 2 (1.2) | 4 (2.5) | 2 (1.9) | 1 (0.7) | 0 (1.1) |
| A(RT) | 0 (1.1) | 1 (0.5) | 0 (1.0) | 1 (0.8) | 2 (0.3) | 0 (0.4) |
| M(PT) | 5 (2.6) | 0 (1.2) | 4 (2.5) | 0 (1.9) | 0 (0.7) | 1 (1.1) |
| M(BI) | 2 (2.4) | 0 (1.1) | 0 (2.2) | 2 (1.7) | 1 (0.6) | 4 (1.0) |
| N | 1 (0.8) | 0 (0.4) | 1 (0.7) | 1 (0.6) | 0 (0.2) | 0 (0.3) |

[1] Expectation value as discussed in text.

Figure 4. A topographical comparison of the interspike interval histograms reported by various workers.

Data are presented from three studies in addition to the present effort. The six different types of interspike interval distribution discussed in the present study are represented by the six figures under Haight, 1968. These are presented in the order:

| | |
|---|---|
| S(M-PT) | Symmetric peak, pre-modal fast activity. |
| M(PT) | Multimodal with regular symmetric peaks. |
| S(ST) | Symmetric peak, moderate tail, no pre-modal activity. |
| A(ET) | Asymmetric peak, long tail. |
| A(RT) | Asymmetric peak, "ramp-like" tail. |
| M(BI) | Bimodal, irregular. |

(see Figure 2 for more details) Data from the work of other investigators have been expanded or compressed to best fit the format used in this study. The unit identifications used are the author's own with the exception of the data from Amassian. Here the three digit number refers to the page upon which the figure is to be found and the single digit following the dash is the figure number used in the article. The uits from Kiang's study will be found on page 97 of his monograph.

The short table below directly compares each author's data with the categorization scheme proposed in this study. Under each author's name are listed the units reported by him arranged in accordance with the classification used to distinguish units in the sheep medulla.

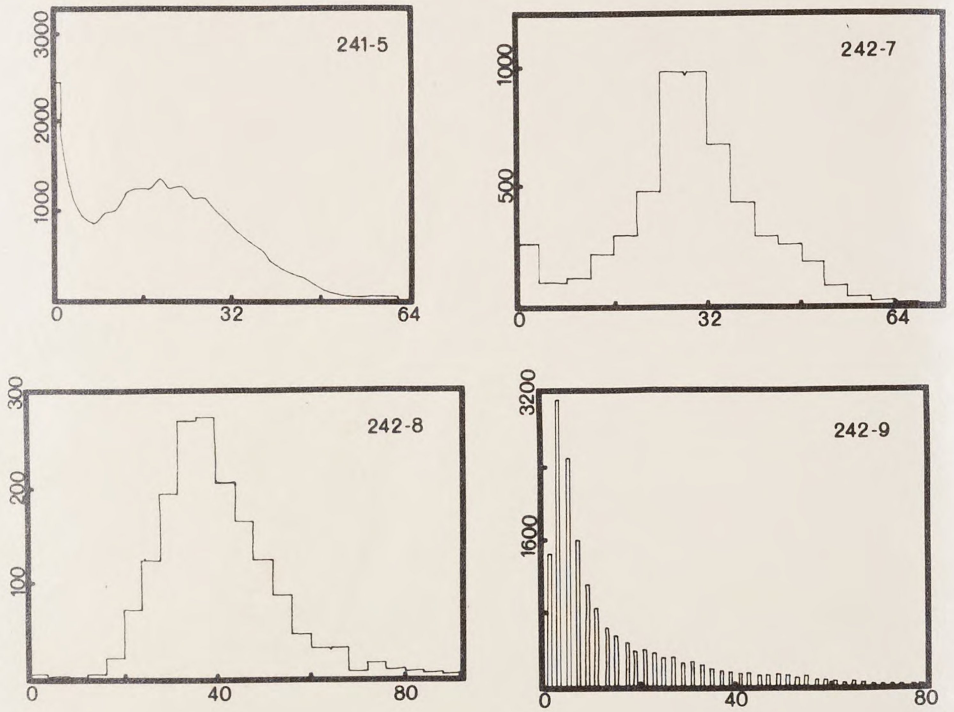
| Type of Unit (Haight Classification) | Amassian (1964) | Kaing 1965) | Rodieck (1967) | Haight (1968) |
|---|---|---|---|---|
| S(M-PT) | 241-5; 242-7 | x | x | 68145-11A |
| M(PT) | x | x | x | 68145-1A |
| S(ST) | 242-8 | x | E; F | 68145-13D |
| A(ET) | 242-9 | 299-25 | A | 68145-16A-1 |
| A(RT) | x | 299-19 | B; D | 67136-12B |
| M(BI) | x | x | C | 68144-8B |

# INTERSPIKE INTERVAL HISTOGRAMS

## AMASSIAN, 1964

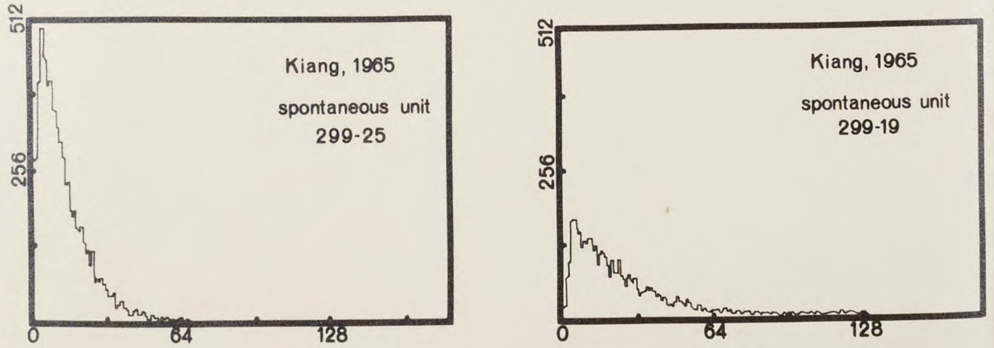
### CAT, CUNEATE NUCLEUS

NUMBER OF SPIKES PER INTERVAL




## KIANG, 1965

### CAT, COCHLEAR NERVE



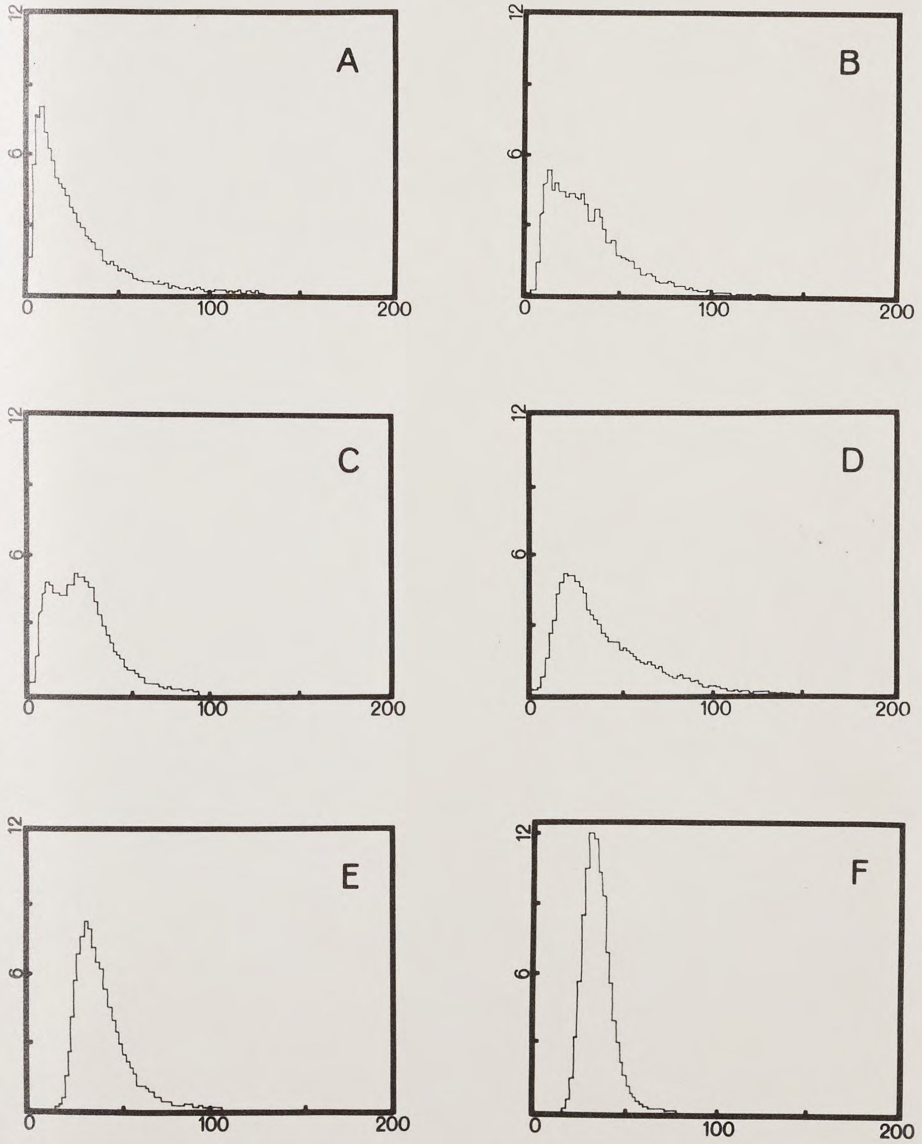

INTERVAL IN MILLISECONDS



# INTERSPIKE INTERVAL HISTOGRAMS

## RODIECK, 1967

### CAT, RETINAL GANGLION CELLS



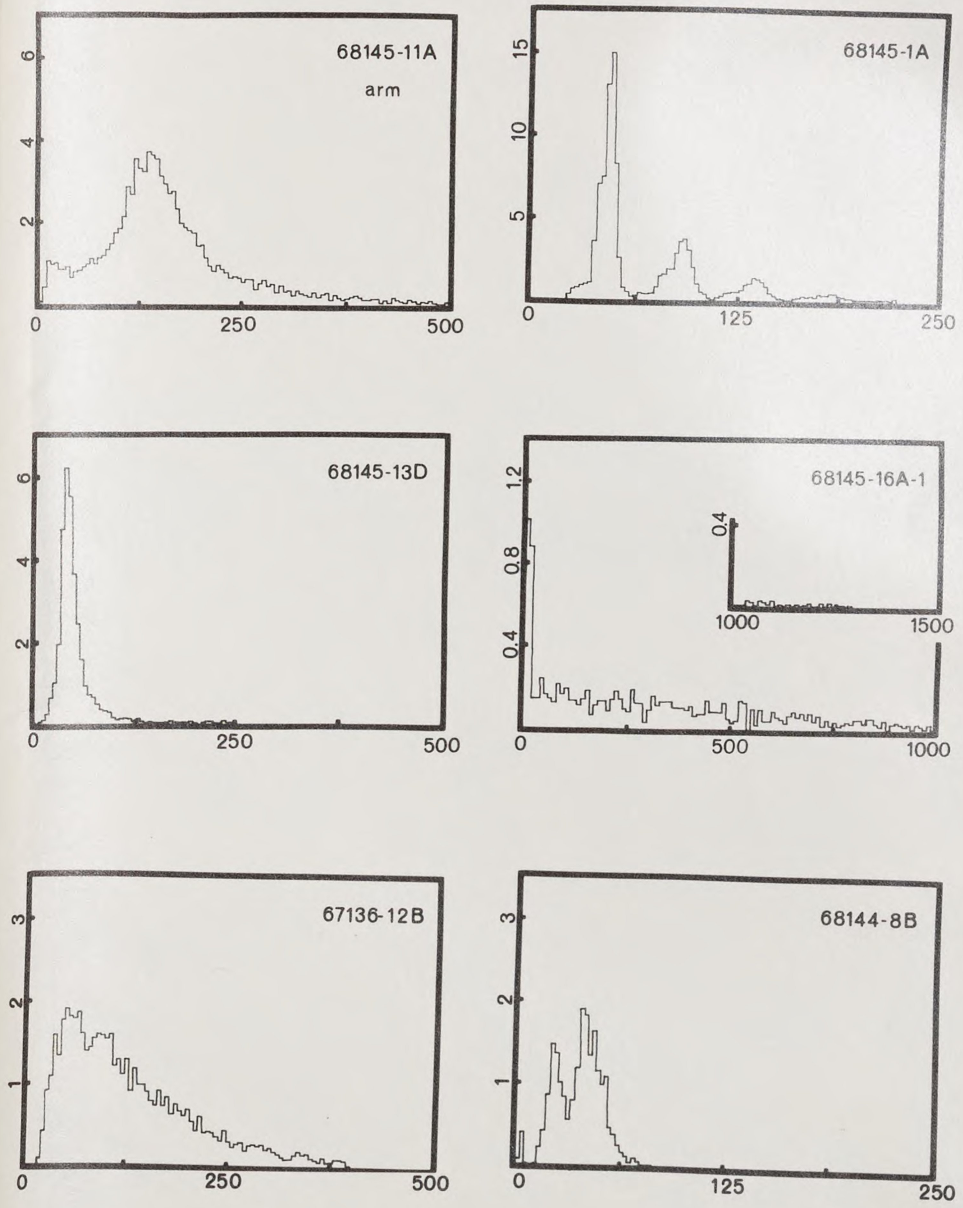

INTERVAL IN MILLISECONDS

# INTERSPIKE INTERVAL HISTOGRAMS

## HAIGHT, 1968

### SHEEP, MEDULLA

X 100




INTERVAL IN MILLISECONDS

statistical dependence or independence of the properties which produce a given type of interspike interval distribution and those which are responsible for a specific pattern of average firing rates are, in themselves, independent of sampling prejudices. Therefore, the observed versus the expected differences seen in Table 4 can be viewed with less suspicion than was possible in Tables 2 and 3.

Examination of Table 4 will show that S(P-MT) or the symmetric, pre-modal type of interval histogram is heavily "over represented" in the $R_2$ (wobbly) profile class. The bursting M(PT) or multiperiodically tailed interval histograms are represented heavily in the $R_1$ (even) average firing rate profiles. They are also present in excess within the $I_1$ (stepping) category but are totally absent in the $R_2$ (wobbly) and $I_2$ (undulating) profile groups. This is contrary to random or independent expectation. Further perusal of table 4 shows that over or under-representation is the case in about one-half of the cells with the remainder being rather close to the calculated expectation.

Finer anatomical distinctions may bring out other properties, allowing for more concrete assignments of spike train character with both function and anatomy. In the main, however, Tables 2-4 do not serve as much to answer questions as to pose new ones.

On an overall basis the interspike interval histograms described in the present study are not appreciably different from those described by other workers. Figure 4 compares the "typical" interval histograms discussed in this study with those reported by three other investigators. The findings of this study and the reports of others, see Braitenberg, et al., 1964; Amassian, et al., 1964; Herz, et al., 1964; and Rodieck, 1967) indicate the following:

1. The variety of interspike interval histograms found by manipulating neuronal spike data is limited to a few basic types which can be characterized by: a) symmetry or asymmetry of the major peak, b) the number of peaks present, c) the length and weight of the histogram tail and d) the presence or absence of premodal or fast activity.

2. These basic qualities are not mutually exclusive. Most investigators have arrived at descriptive schema which incorporate these features to a greater or lesser degree.

3. The limited number of histogram types found in the central nervous system are found in all sites thus far considered.

Unfortunately, data are lacking with respect to the average firing rate profiles. Most workers do not discuss long-term sequential properties of the units with which they are working. When they do, it generally is not in terms of easily analyzed sequential plots such as are used in the present study. (see Rodieck, 1967 for an exception.)

One must not lose track of the fact that it is the spike train which is received and acted upon by the recipient neuron and not the interspike interval histogram. Keeping the foregoing in mind plus the projudicial sampling techniques employed in this study, the comments which follow summarize the essential findings. In the portions of the sheep medulla considered a limited number of interspike interval patterns were found. This limited repertoire was found distributed throughout the anatomical areas that were delineated either histologically or electrophysiologically. Certain correlations of interval histogram type and anatomical location were noted:

1. Units producing multiperiodic interval histograms appear to be most prevalent in the afferent dorsal fiber tracts of the medulla.

2. Symmetric peaked histograms with or without pre-modal or semi-burstlike activity are found predominantly in the dorsal column nuclei. Multimodal types such as are found in the overlying afferent tracts are not found in these nuclei.

3. There are no obvious trends to be noted concerning the nature of the unit producing a given interval histogram type in other areas of the medulla, though all observed histograms were noted in these regions.

With respect to the average firing rate profiles it can be said that if these measures are related to function, it is not solely on the basis of anatomical location. Each profile type is to be found in each medullary area as nearly as can be determined from the present effort.

It would be desirable to be able to make a quantitative statement about the probabilities of finding maintained activity of a specific kind at a particular medullary locus - or, indeed, anywhere else in the nervous system. The results of the present research indicate that, using the techniques described herein, such statements cannot be made with high degrees of confidence. It should be emphasized that while relative representation of both profile and histogram categories defined in this study do vary from site to site, maintained activity appears to be present everywhere. It is perhaps significant that such activity is not associated exclusively with the proprioceptive steady-state units of the somatic afferent system or with heart and respiratory units. Maintained activity was commonly found associated with units whose response to external stimuli were decidedly transient in nature. This tends to indicate that functional subdivisions within a given anatomical area are going to have to be considered before the role of maintained neural processes is completely understood.

Future work might be concerned with a more equal emphasis upon all portions of the medulla or other brain areas that happen to be under investigation. The more ventral portions of the medulla, for example, were rather neglected in this study. With larger unit samples statistical methods might be utilized to better advantage. This study has shown that certain tendencies do exist; more information is needed to determine whether these tendencies are important either neurophysiologically or behaviorally.

More research also needs to be done on the effects of anesthetic agents. The difficulties experienced in comparing the efforts of various authors is in great part due to differential anesthetic effects. In this regard, Evart's (1964, 1968) work with awake and naturally sleeping animals is encouraging in that it presages the eventual establishment of a technique which utilizes a reproducible, yet biologically natural state of the animal for examining nervous behavior. Sometimes artificial agents must be used. Paralytic agents for retinal research provide an example. It would be desirable to be able to quantify some of the effects of these agents in such a manner that they could be used to insure experimental reproducibility.

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# APPENDICES

## Appendix A

I. Abbreviations to be used in the following appendices

Al. N., alar nucleus (dorsal motor nucleus of the Xth cranial nerve).

Ar. Post., area postrema.

Cu.-Gr. N., cuneate-gracile nuclear complex.

Cu. N., cuneate portion of cuneate-gracile nuclear complex.

Ex. Cu. N., external cuneate nucleus.

Gel. Su., gelatinous substance.

Gr. N., gracile portion of cuneate-gracile nuclear complex.

H.N., hypoglossal nucleus.

I.O.N., inferior olivary nuclear complex.

L.R.N., lateral reticular nucleus.

M.L.F., dorsal portion of median longitudinal fascicle.

Pyr. Dec., pyramidal decussation.

R.F., reticular formation

Sp. Trg. N., spinal trigeminal nucleus.

Sp. Trg. Tr., spinal trigeminal tract.

S. Tr., solitary tract.

Vn. Hn., ventral horn of caudal medulla.

Vn. Wh., ventral white matter of caudal medulla.

II. An explanation of the unit identification system used in this research.

The first two identifying digits represent the year in which the recording session was run. The next digit denotes the category of animal, marsupials being zero, ruminants being one, and so on. The next two digits stand for the "nth" animal in a given series.

Following the hyphen is the puncture number. The letters denote the number and positions of units obtained within a given puncture. Occasionally two or more units were recorded simultaneously from the same electrode position. When this happened a second hyphen followed by a number was used to separate the units involved.

Hence:

Unit 67129-2L refers to an experiment run in 1967 upon a sheep which happened to be the 29th in its series. The unit obtained came from the second electrode puncture and was the Lth response.

Unit 68145-15C-3 would likewise refer to an experiment performed in 1968 upon a sheep, 45th in its series. The third (C) response derived from the 15th puncture yielded four distinct units. The unit in question here was the third of these.

## Appendix B

A catalogue of the units used in this study

| # | Unit Number | Mean Firing Rate per Second | Median Firing Rate per Second | % Deviation of Median from Mean | Standard Error as % of Mean | Average Firing Rate Profile Category | Inter-spike Interval Histogram Class | Anatomical Location | Comments |
|---|---|---|---|---|---|---|---|---|---|
| 1. | 67129-2L | 11.4[1] | 11.5 | 0.9 | 7.9 | $R_1$ | S(ST) | Sp. Trg. N. | Jaw |
| 2. | 67132-7A | 11.3[1] | 9.5 | -5.9 | 25.7 | $I_2$ | S(ST) | Gr. N.[2] | Leg |
| 3. | 67133-1A | 6.7 | 13.3 | 98.5 | 53.7 | $I_1$ | S(P-MT) | Not Avail.[3] | Cheek ($N_2O$) |
| 4. | 67133-1B | 19.9 | 20.0 | 0.5 | 10.6 | $R_1$ | S(ST) | Not Avail. | ($N_2O$) |
| 5. | 67133-6A | 8.0 | 9.3 | 16.3 | 25.0 | $I_2$ | S(ST) | Not Avail. | ($N_2O$) |
| 6. | 67136-12A | 34.5 | 35.7 | 3.5 | 11.3 | $R_1$ | S(ST) | Not Avail.[3] | |
| 7. | 67136-12B | 6.9 | 9.2 | 33.3 | 18.8 | $I_3$ | A(RT) | Not Avail. | |
| 8. | 67141-3B | 4.4 | 4.8 | 9.1 | 18.1 | $I_2$ | A(RT) | Reticular[4] | |
| 9. | 67141-10B-1 | 3.4 | --- | --- | 52.9 | $I_1$ | N[5] | Reticular | |
| 10. | 67141-10B-2 | 10.2 | 37.0 | 262.7 | 22.5 | $I_2$ | A(ET) | Reticular | |
| 11. | 67141-14A | 8.4 | 9.6 | 14.3 | 20.2 | $I_1$ | S(P-MT) | Sp. Trg. N. | Neck |
| 12. | 67143-3A | 4.7 | 6.8 | 44.7 | 63.8 | $I_4$ | M(PT) | Cu. Tr. | Arm |

| # | Unit Number | Mean Firing Rate per Second | Median Firing Rate per Second | % Deviation of Median from Mean | Standard Error as % of Mean | Average Firing Rate Profile Category | Inter-spike Interval Histogram Class | Anatomical Location | Comments |
|---|---|---|---|---|---|---|---|---|---|
| 13. | 67143-3B | 5.5 | 5.5 | 0.0 | 25.4 | $R_2$ | S(P-MT) | Cu. N. | Arm |
| 14. | 67143-4B | 5.7 | 7.2 | 26.3 | 43.8 | $I_4$ | S(ST) | Vn. Hn. | |
| 15. | 67143-4C | 13.0 | 12.7 | -2.3 | 10.0 | $R_1$ | S(P-MT) | Vn. Hn. | |
| 16. | 67143-4D | 10.0 | 9.7 | -3.0 | 15.0 | $I_1$ | S(ST) | Vn. Hn. | |
| 17. | 68144-1A | 14.4 | 13.3 | -8.3 | 6.9 | $R_1$ | M(PT)[6] | Cu. Tr. | Wrist |
| 18. | 68144-1B | 20.5 | 23.8 | 16.1 | 42.9 | $I_1$ | M(PT) | Vn. Wh. | |
| 19. | 68144-3A | 20.8 | 21.3 | 2.4 | 28.8 | $I_4$ | M(BI) | Cu. Tr. | |
| 20. | 68144-5A | 4.6 | 6.6 | 43.5 | 39.1 | $I_3$ | M(BI) | Cu. Tr. | |
| 21. | 68144-8A | 13.5 | 18.5 | 37.0 | 38.5 | $I_4$ | M(BI) | Cu. Tr. | |
| 22. | 68144-8B | 28.0 | 25.6 | -8.6 | 12.9 | $I_4$ | M(BI) | Reticular | |
| 23. | 68144-11A | 30.7 | 23.3 | -24.1 | 23.5 | $I_2$ | M(BI) | Cu. N. | Thorax |
| 24. | 68145-1A | 18.2 | 20.0 | 9.9 | 13.7 | $R_1$ | M(PT) | Cu. Tr. | |
| 25. | 68145-1B | 44.7 | 41.7 | -6.7 | 6.9 | $R_1$ | M(PT) | Cu. Tr. | |
| 26. | 68145-2A | 7.6 | 13.5 | 77.6 | 15.8 | $R_2$ | S(P-MT) | Gr. Tr. | Calf |

| # | Unit Number | Mean Firing Rate per Second | Median Firing Rate per Second | % Deviation of Median from Mean | Standard Error as % of Mean | Average Firing Rate Profile Category | Inter-spike Interval Histogram Class | Anatomical Location | Comments |
|---|---|---|---|---|---|---|---|---|---|
| 27. | 68145-2C | 23.1 | 23.3 | 0.9 | 30.7 | $I_1$ | S(P-MT) | Gr. Tr. | Calf |
| 28. | 68145-3A-1 | 15.9 | --- | --- | 5.0 | $R_1$ | M(PT)[6] | Cu. Tr. | Arm |
| 29. | 68145-3A-2 | 21.8 | --- | --- | 8.7 | $R_1$ | N[5] | Cu. Tr. | Arm |
| 30. | 68145-8A | 18.3 | 47.6 | 160.1 | 29.0 | $I_1$ | M(PT) | Reticular | |
| 31. | 68145-8B | 16.8 | 17.5 | 4.2 | 25.0 | $I_4$ | M(BI) | Not Avail.[7] | |
| 32. | 68145-9A | 3.0 | 5.9 | 96.7 | 50.0 | $I_1$ | A(ET) | Gr. Tr. | Hoof |
| 33. | 68145-9B | 2.6 | 3.5 | 34.6 | 15.4 | $R_2$ | A(RT) | Al. N. | |
| 34. | 68145-10A-1 | 7.0 | 8.1 | 15.7 | 35.7 | $I_3$ | A(RT) | Gr. N. | Calf |
| 35. | 68145-10A-2 | 1.8 | 2.6 | 44.4 | 55.6 | $I_2$ | M(BI) | Gr. N. | Calf |
| 36. | 68145-10A-3 | 0.4 | --- | --- | 50.0 | $I_2$ | N[4] | Gr. N. | Calf |
| 37. | 68145-11A | 6.2 | 6.9 | 11.3 | 17.7 | $R_2$ | S(P-MT) | Cu. N. | Wrist |
| 38. | 68145-11B | 11.7 | 12.8 | 9.4 | 9.4 | $R_1$ | M(BI) | Cu. N. | |
| 39. | 68145-12A | 13.4 | 13.5 | 0.7 | 9.7 | $R_1$ | S(ST) | Not Avail.[7] | |
| 40. | 68145-13A | 16.6 | 20.8 | 25.3 | 25.3 | $I_1$ | M(PT) | Cu. Tr. | |
| 41. | 68145-13B | 21.1 | 16.4 | -22.3 | 28.9 | $I_1$ | M(PT) | Cu. Tr. | |

| # | Unit Number | Mean Firing Rate per Second | Median Firing Rate per Second | % Deviation of Median from Mean | Standard Error as % of Mean | Average Firing Rate Profile Category | Inter-spike Interval Histogram Class | Anatomical Location | Comments |
|---|---|---|---|---|---|---|---|---|---|
| 42. | 68145-13C | 3.7 | 8.1 | 118.9 | 102.7 | $I_1$ | A(ET) | Cu. N. | |
| 43. | 68145-13D | 7.8 | 20.8 | 166.7 | 130.8 | $I_1$ | S(ST) | Cu. N. | |
| 44. | 68145-14A | 10.9 | 10.8 | -0.9 | 22.0 | $I_2$ | S(P-MT) | Cu. Tr. | |
| 45. | 68145-14B-1 | 4.5 | 4.9 | 8.9 | 15.6 | $R_2$ | S(P-MT) | Cu. N. | Arm |
| 46. | 68145-14B-2 | 10.4 | 9.2 | 11.5 | 39.4 | $I_2$ | S(P-MT) | Cu. N. | Arm |
| 47. | 68145-15A | 2.6 | 6.1 | 134.6 | 30.8 | $R_2$ | A(ET) | Gr. N. | Knee |
| 48. | 68145-15C-1 | 41.6 | 33.3 | -20.0 | 13.2 | $R_1$ | M(BI) | Reticular | Respiratory |
| 49. | 68145-15C-2 | 1.3 | 5.0 | 284.6 | 53.8 | $I_3$ | A(ET) | Reticular | Respiratory |
| 50. | 68145-15C-3 | 8.4 | 19.2 | 128.6 | 7.1 | $R_1$ | S(P-MT) | Reticular | Respiratory |
| 51. | 68145-15C-4 | 11.4 | 43.5 | 281.6 | 5.3 | $R_1$ | A(ET) | Reticular | Respiratory |
| 52. | 68145-16A-1 | 3.1 | 4.0 | 29.0 | 16.1 | $R_2$ | A(ET) | Gr. Tr. | |
| 53. | 68145-16A-2 | 8.4 | 10.4 | 23.8 | 15.4 | $I_2$ | A(ET) | Gr. Tr. | |
| 54. | 68145-17A | 3.6 | 5.4 | 50.0 | 38.9 | $I_1$ | A(ET) | Cu. Tr. | Trunk |
| 55. | 68145-18A | 9.8 | 11.2 | 14.3 | 25.5 | $I_2$ | S(ST) | Cu. N. | Trunk |
| 56. | 68145-18B | 1.0 | 8.2 | 720.0 | 160.0 | $I_1$ | A(ET) | Vn. Hn. | |
| 57. | 68145-18C | 26.6 | 26.3 | -1.1 | 4.9 | $R_1$ | M(PT) | Cu. Tr. | |

Footnotes for Appendix B

[1]All numerical data was calculated from the "unstimulated" position of the limb. See Appendices D and E following.

[2]This unit was located in the border region between the cuneate-gracile nuclear complex and the external cuneate nucleus. As a consequence, it might be associated with either nucleus. See Appendix C, track 67132-7

[3]This animal was not processed through histology. Animal 67133 was a pilot study for a nitrous oxide anesthesia project. The animal was disposed of before the recordings were found to be of value. Animal '-36 was utilized in a teaching experiment. An excessive number of electrode punctures made track tracing impossible.

[4]The track containing this unit skims the hypoglossal nucleus. Localization of puncture within the track was not precise enough to allow a definite attribution.

[5]Due to inability of the electronic apparatus used to discriminate spikes of differing amplitude imposed upon a wavering baseline, these histograms could not be plotted.

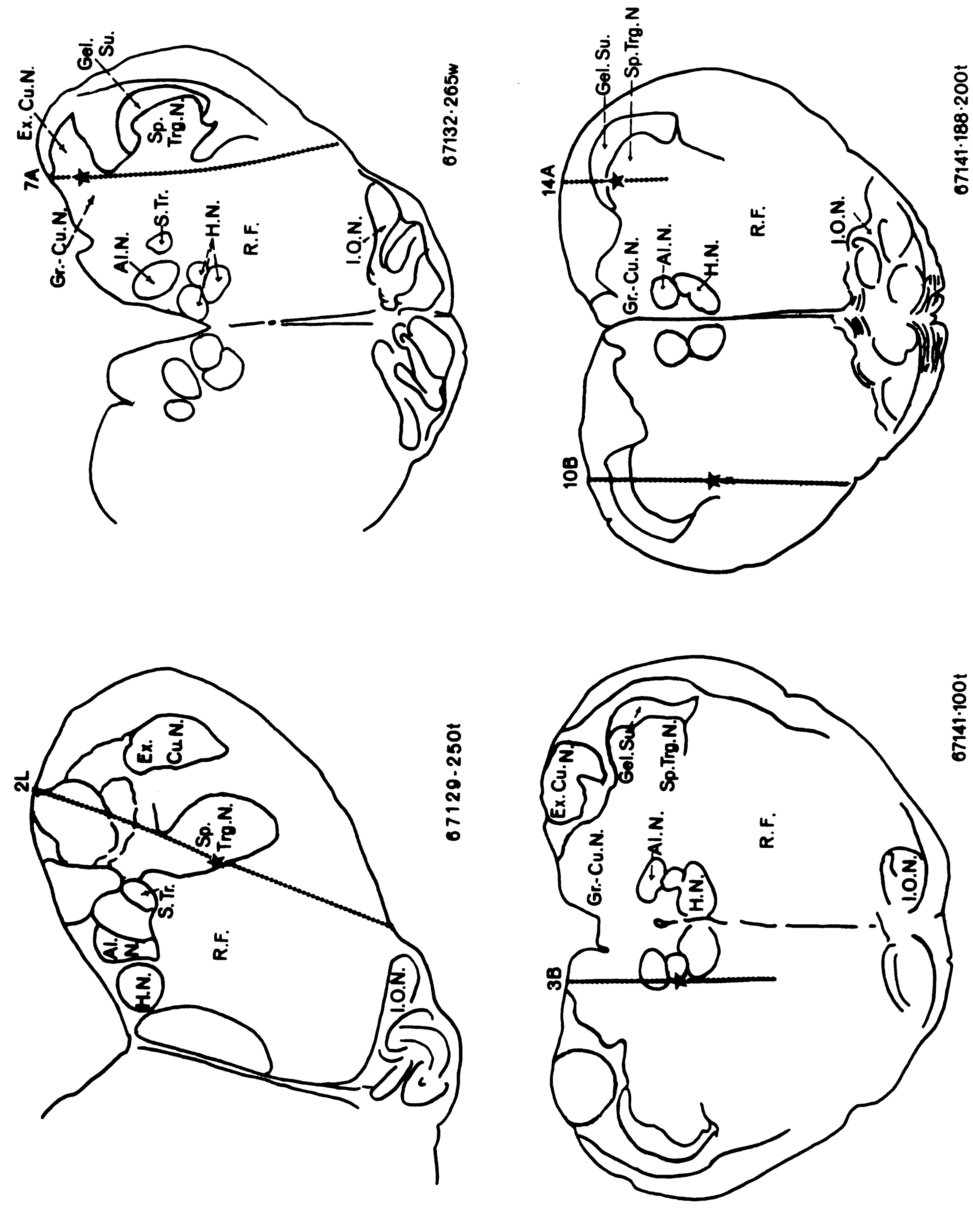
[6]This unit is placed in the M(PT) or periodic tailed histogram class because of the extreme symmetry of the major peak - a characteristic peculiar to this type of interval histogram alone. Both units involved are "somatic". 68144-1A may not be in a physiologically "neutral" state. Unit 68145-3A was being "stimulated" while the interval histogram was plotted because it was difficult to obtain data in the neutral position due to the presence of extraneous activity.

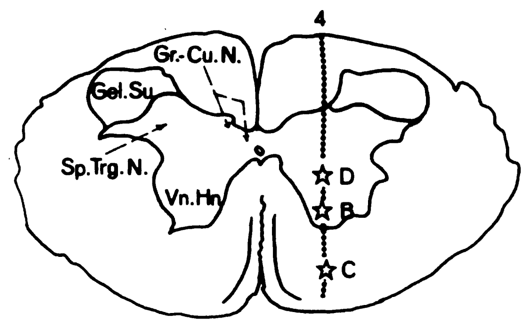
[7]These units could not be found. The microelectrode left no apparent track.

## Appendix C

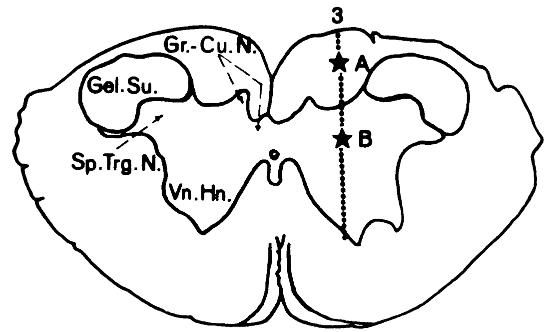
### Tracings of the electrode tracks

The tracings are presented by animal in the order experimented upon. The sections pertaining to a given animal are presented in the order in which they were sectioned. This may be in a rostro-caudal direction in one animal and the opposite in another. Thus: 67143-584t is the 584th section (thionin) from animal 67143. This section shows the 4th electrode puncture which contains three single unit recording sites.

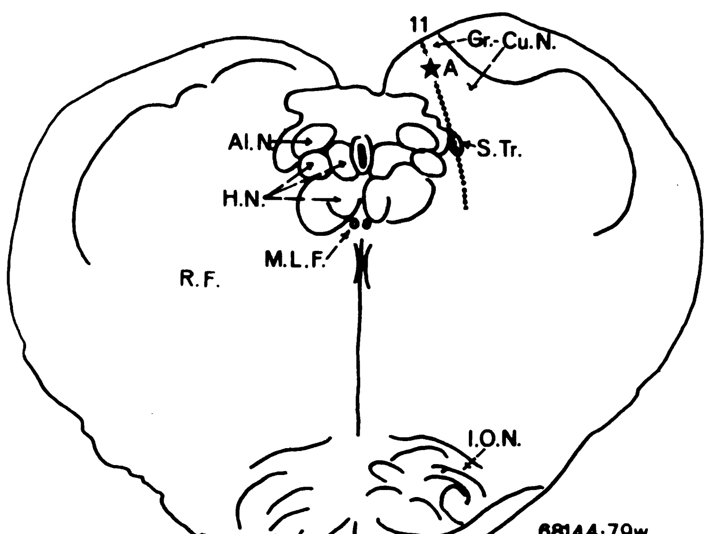

2L
Ex. Cu.N.
Sp. Trg.N.
S.Tr.
Al. N.
H.N.
R.F.
I.O.N.
67129·250t
7A
Ex. Cu.N.
Gel. Su.
Sp. Trg.N.
Gr.-Cu.N.
Al.N.
S.Tr.
H.N.
R.F.
I.O.N.
67132·265w
3B
Ex.Cu.N.
Gel.Su.
Sp.Trg.N.
Gr.-Cu.N.
Al.N.
H.N.
R.F.
I.O.N.
67141·100t
10B
14A
Gel. Su.
Sp.Trg.N
Gr.-Cu.N.
Al.N.
H.N.
R.F.
I.O.N.
67141·188·200t



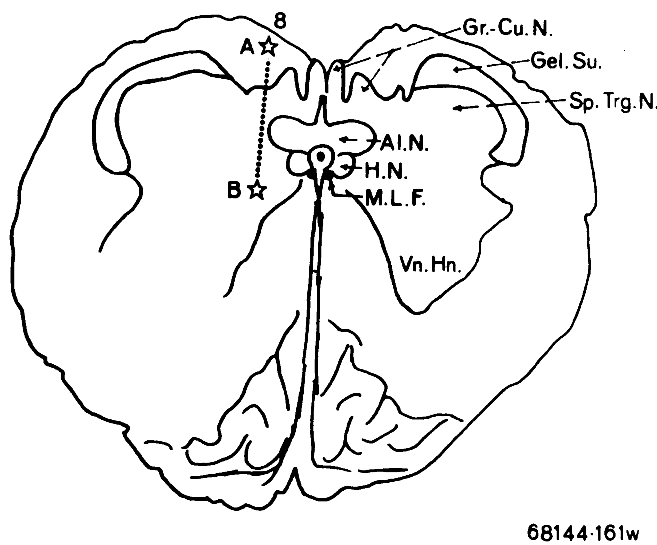

67143·584t



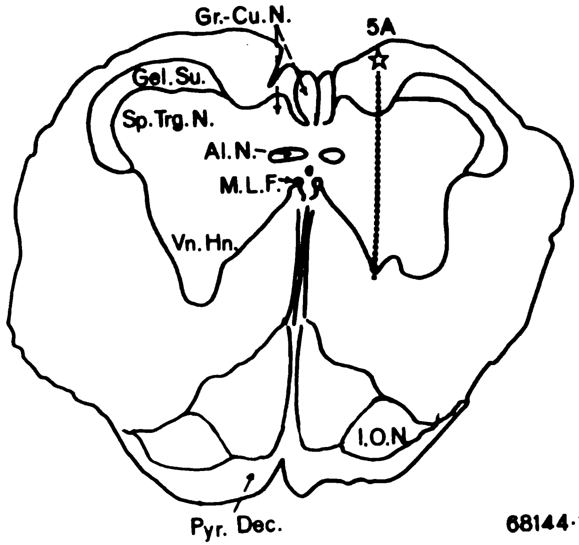

67143·686t



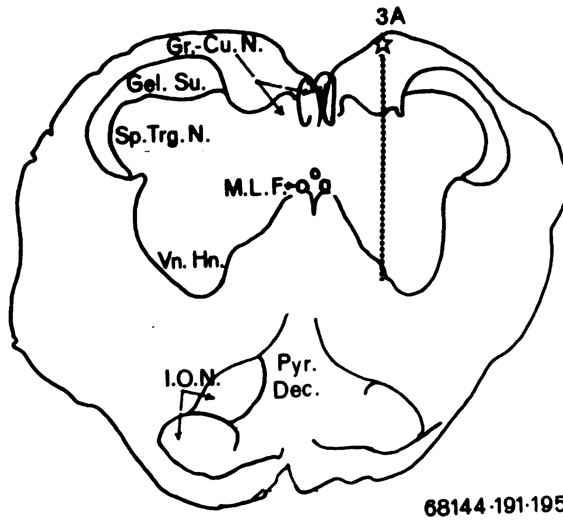

68144·79w



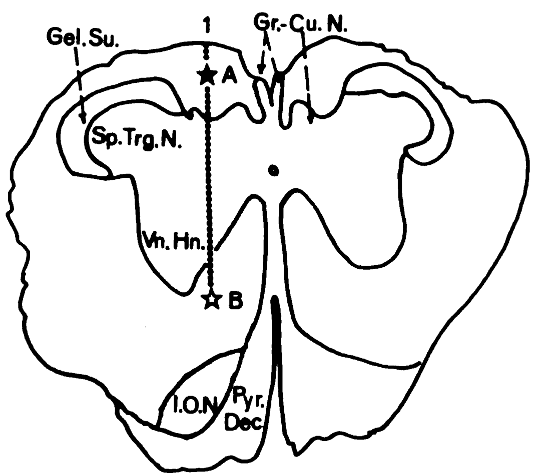

68144·161w



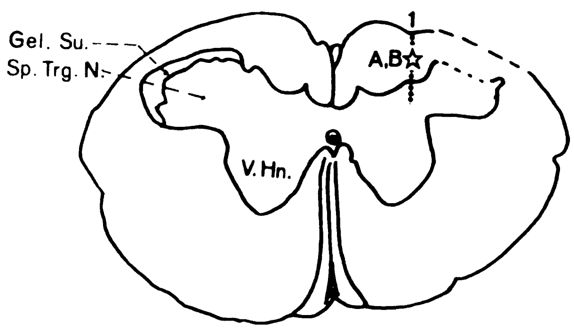

68144·181w



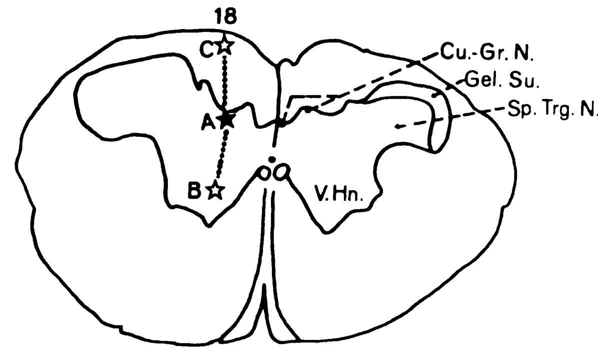

68144·191·195w



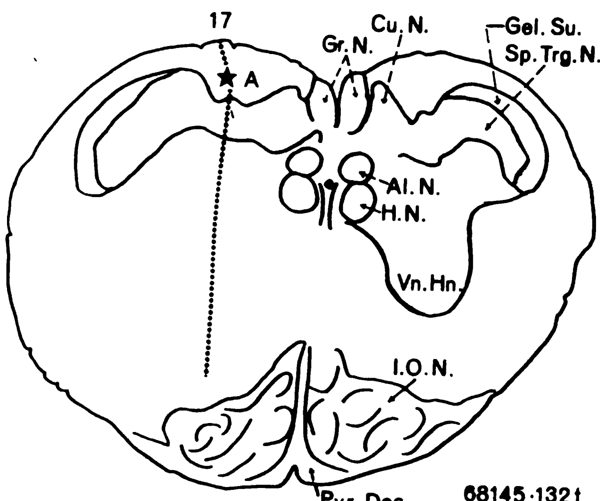

68144·221w



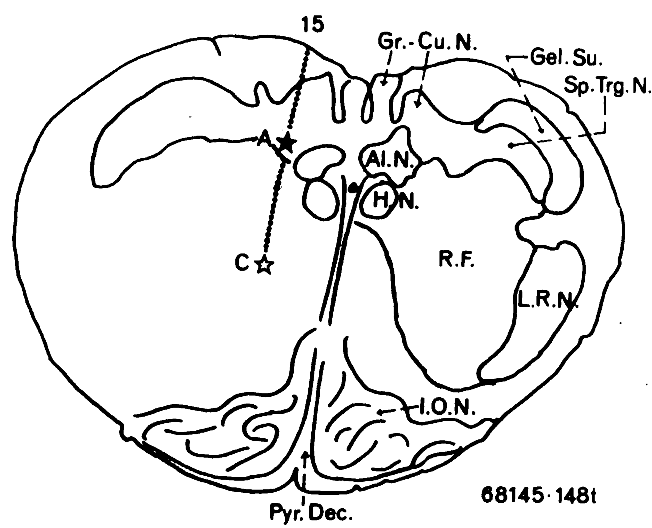

68145·20t



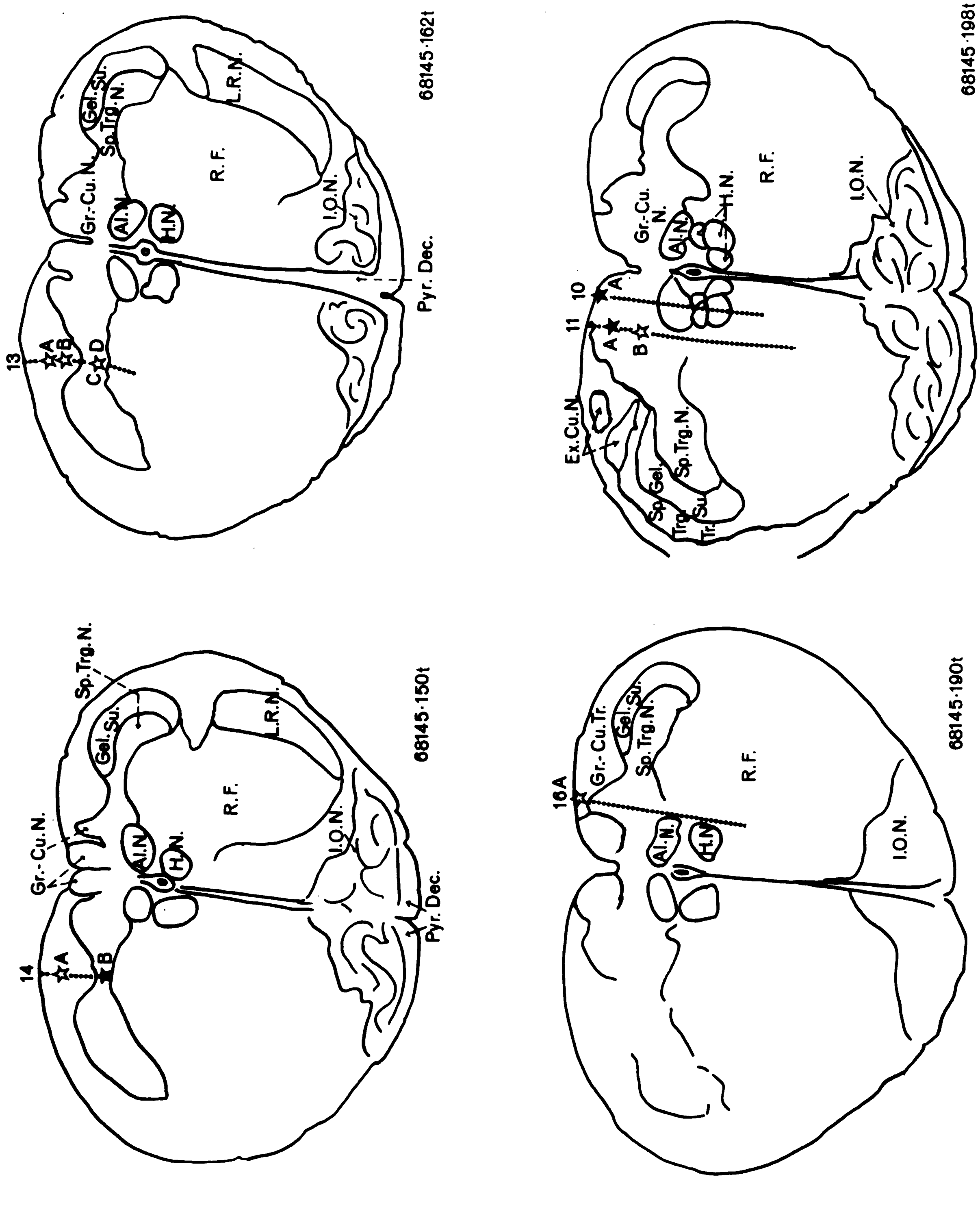

68145·28t

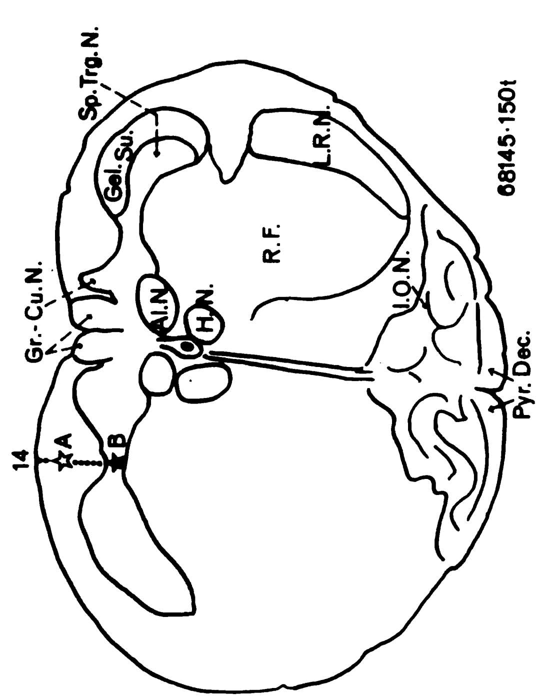


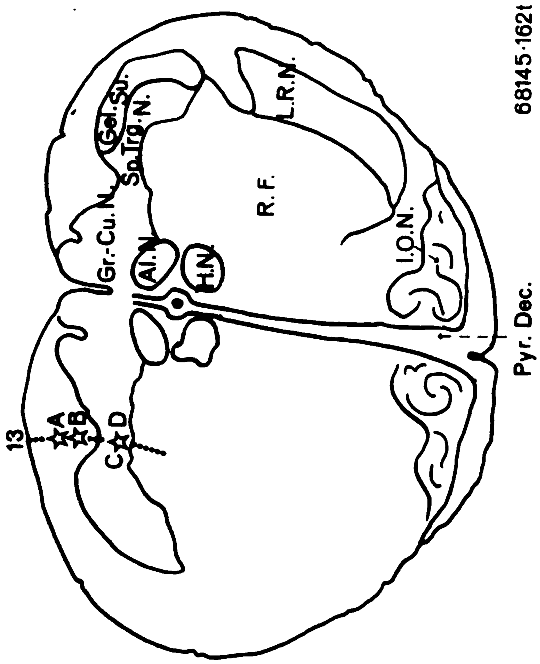

68145·132t

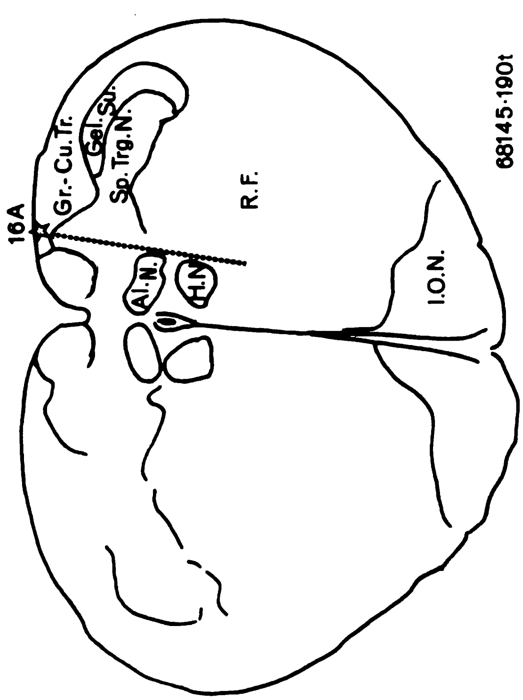


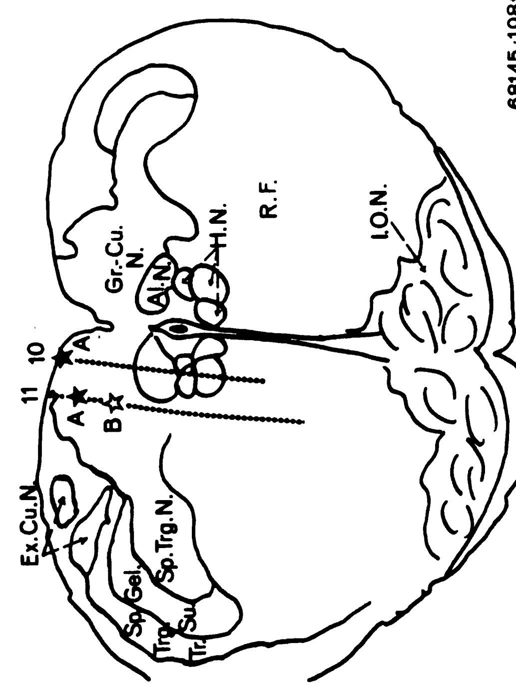

68145·148t

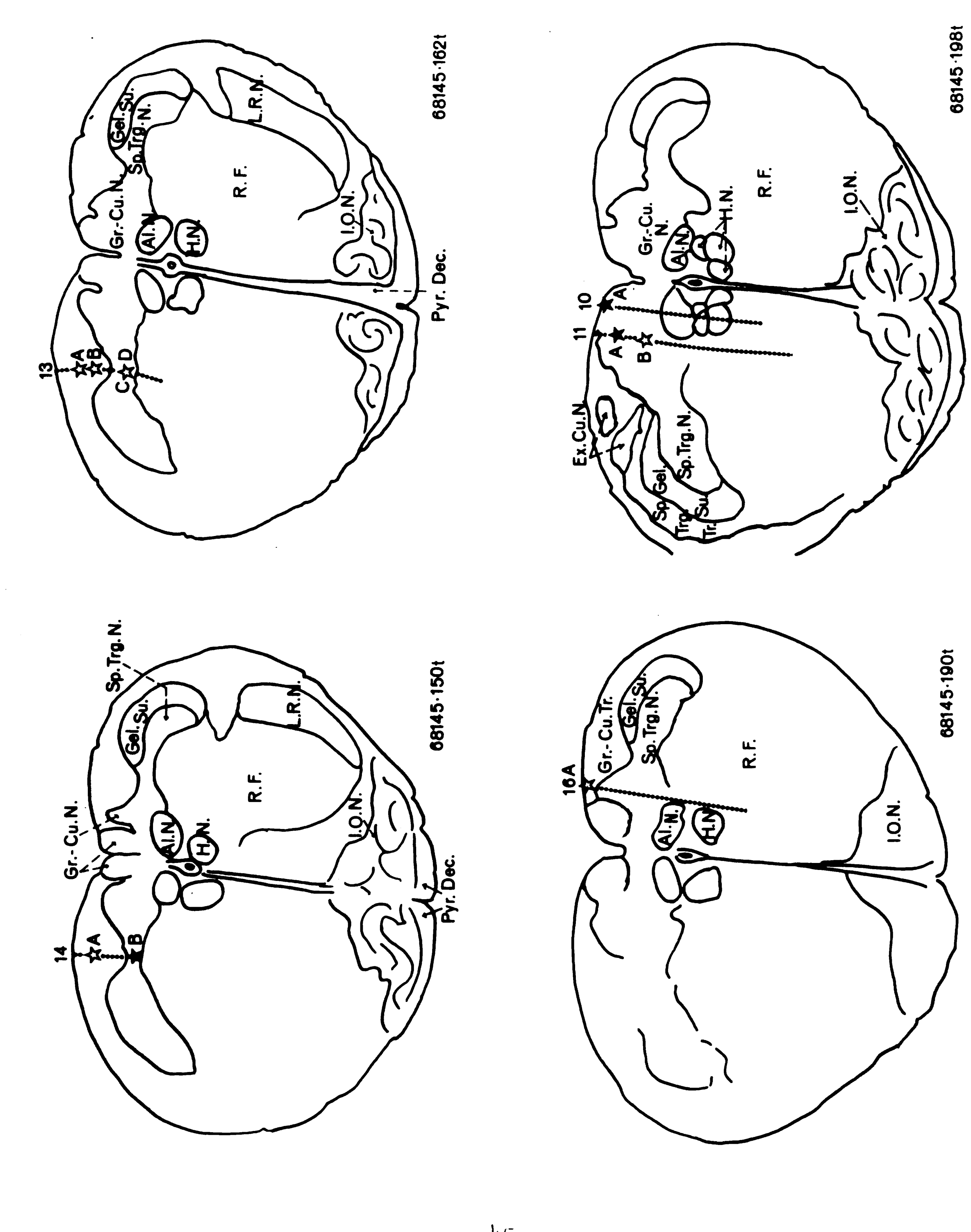

14
A
B
Gr.-Cu.N.
Al.N.
H.N.
Gel.Su.
Sp.Trg.N.
R.F.
L.R.N.
I.O.N.
Pyr. Dec.
68145·150t
13
A
B
C
D
Gr.-Cu.N.
Sp.Trg.N.
Gel.Su.
Al.N.
H.N.
R.F.
L.R.N.
I.O.N.
Pyr. Dec.
68145·162t
16A
Gr.-Cu.Tr.
Gel.Su.
Sp.Trg.N.
Al.N.
H.N.
R.F.
I.O.N.
68145·190t
10
11
A
A
B
Ex.Cu.N.
Sp.Gel.
Sp.Trg.N.
Trg.Su.
Tr.
Gr.-Cu.
N.
Al.N.
H.N.
R.F.
I.O.N.
68145·198t

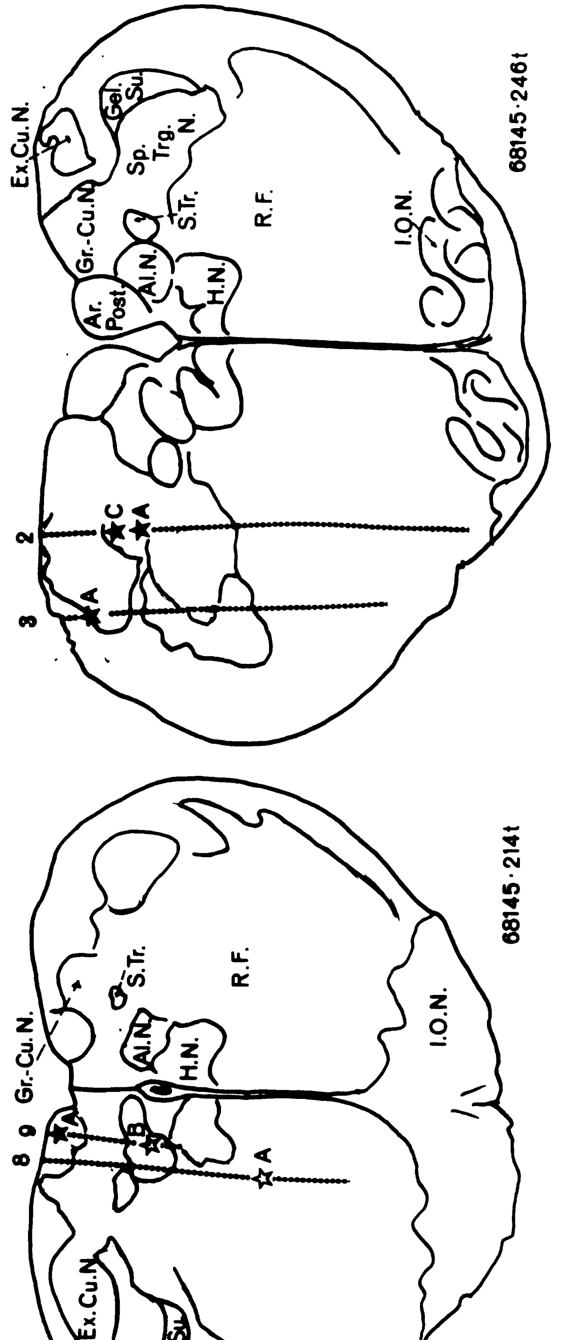

14
A
B
Gr.-Cu.N.
Sp.Trg.N.
Gel.Su.
Al.N.
H.N.
R.F.
L.R.N.
I.O.N.
Pyr. Dec.
68145·150t


13
A
B
C
D
Gr.-Cu.N.
Gel.Su.
Sp.Trg.N.
Al.N.
H.N.
R.F.
L.R.N.
I.O.N.
Pyr. Dec.
68145·162t


16A
Gr.-Cu.Tr.
Gel.Su.
Sp.Trg.N.
Al.N.
H.N.
R.F.
I.O.N.
68145·190t


Ex.Cu.N.
Sp.Gel.
Sp.Trg.N.
Trg.
Su.
Tr.
11
A
B
10
A
Gr.-Cu.
N.
Al.N.
H.N.
R.F.
I.O.N.
68145·198t


68145·150t
68145·162t
68145·190t
68145·198t
Gr.-Cu.N.
Sp.Trg.N.
Gel.Su.
Al.N.
H.N.
R.F.
L.R.N.
I.O.N.
Pyr. Dec.
Gr.-Cu.Tr.
Ex.Cu.N
Sp.Trg.N.


Ex.Cu.N.
Gel. Su.
Sp. Trg. N.
Gr.-Cu.N.
S.Tr.
R.F.
I.O.N.
Al.N.
H.N.
Ar. Post.
2
3
A
C
68145·246t
Gr.-Cu.N.
8
9
A
B
S.Tr.
R.F.
Al.N.
H.N.
I.O.N.
Ex.Cu.N.
Gel.Su.
Sp. Trg. N.
68145·214t

# Appendix D

## The Average Firing Rate Profiles

Figure D1: Notes on the average firing rate profiles.

67129-2L: This unit responded in a steady-state fashion to alterations in the position of the lower jaw. The "at rest" position is the free hanging or "neutral" position. The gap in the record at 500 plus seconds was caused by the time taken to change tape reels and not by a discontinuity in the spike sequence.

To effect a "stimulated" condition a 2 by 4 inch block of wood was inserted between the jaws and left in position. The unit's response to this can be seen in the "open" portion of the record. A slight adaptive trend was noted. The discontinuity before the onset of this sequence was due to the shorting of the recording input during the time the block was being placed.

To obtain the final portion of the record the block was removed without turning off the recording equipment. The jaw was allowed to hang free as in the first "at rest" position. The gradual increase in firing rate from a level somewhat depressed from the first "at rest" state was noted. After stimulation approximately 1000 seconds elapsed before the unit attained its original resting level.

No adaptation was observed in the first resting sequence because the recording of unit activity was not begun until the unit had been under observation for over an hour.

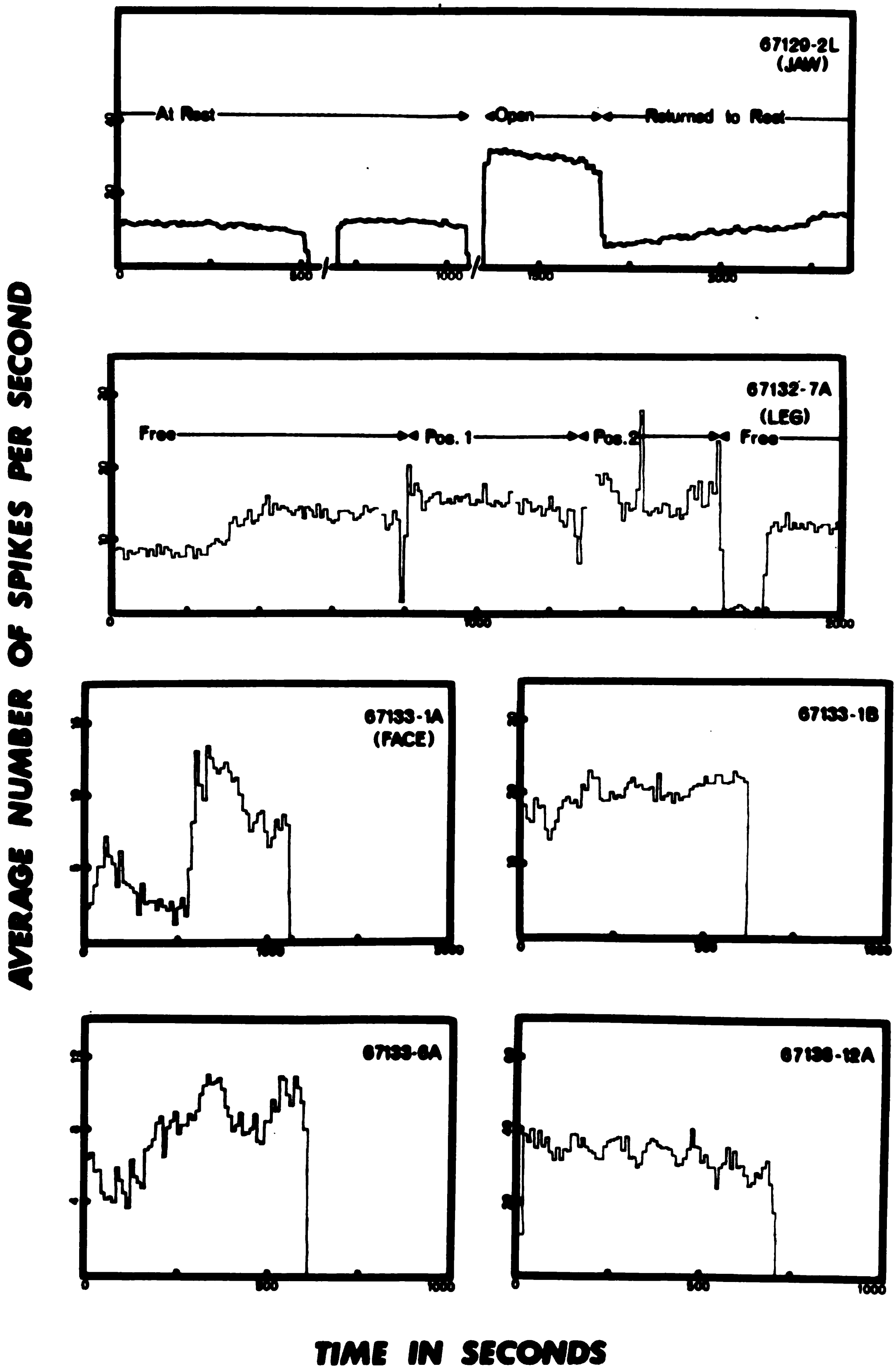
67132-7A: This is a drivable unit whose response to the chosen stimulus, while readily apparent was not as "nice" as the response of the unit immediately preceeding. The free position response was recorded from the unit while the leg lay undisturbed. The hoof was not in contact with any surface. The activity in the firing rate profile can be seen to alter firing rate entirely of its own accord, independently of any obvious stimulus.

Upon propping the leg upward by placing a stool under the hoof, the response portrayed in "Pos. 1" was obtained. A fast transient response was noted initially which subsided to a very gradually diminishing steady-state level subject to considerable minor irregularities in overall rate.

A similar transient response was noted when a stronger stimulus was applied (forcing the leg even higher). The resulting activity on the part of the driven unit was even more irregular exhibiting much more variation in firing rate even to the point of exceeding the initial transient response for brief periods.

Sudden removal of the stimulus resulted in approximately three minutes of "silence." The unit was almost totally inhibited. This period of activity was followed by a sudden rise to a steady-state level approximately the same as that observed for the initial free position.

# AVERAGE FIRING RATE PROFILES

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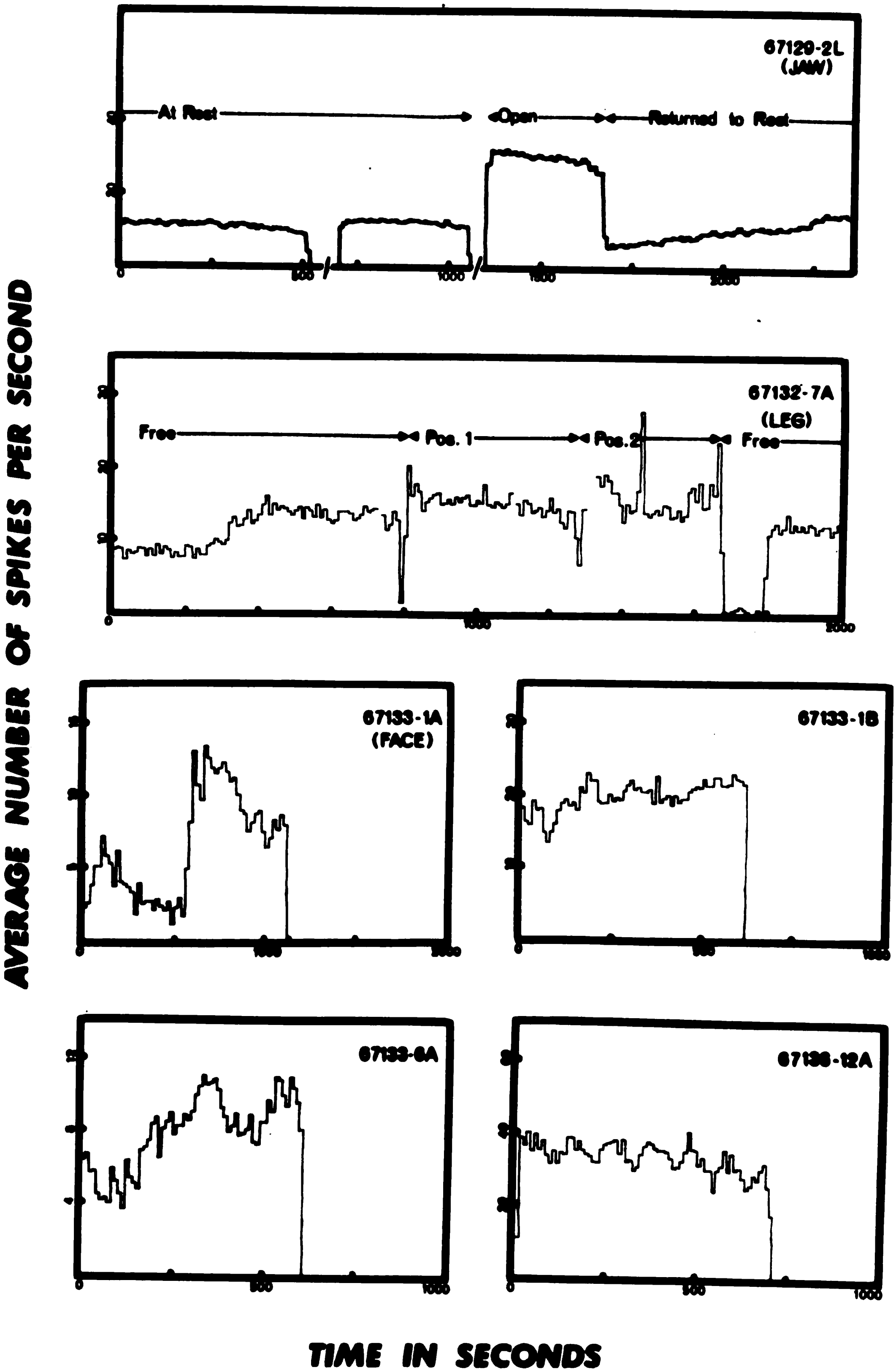


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# AVERAGE FIRING RATE PROFILES

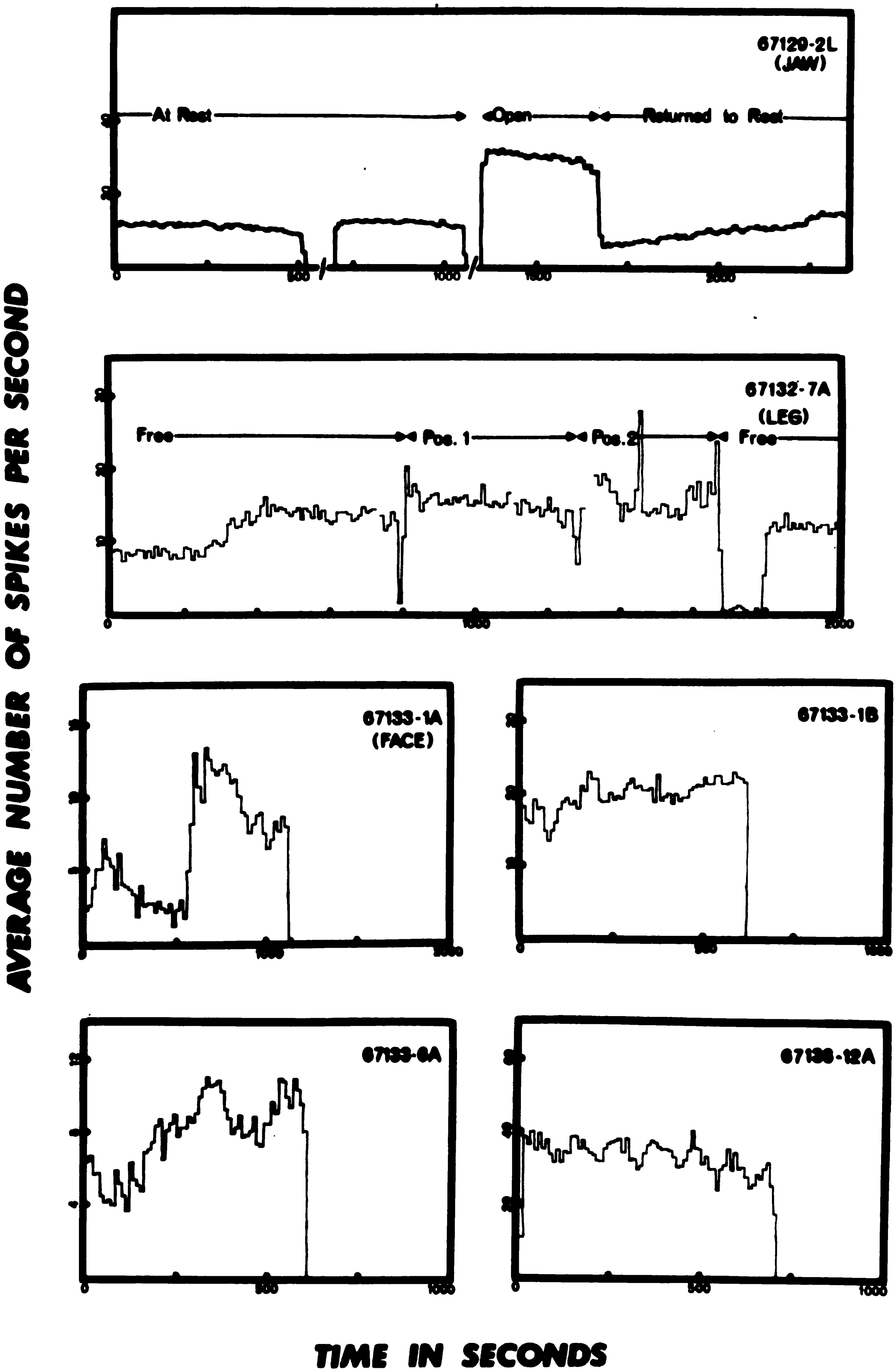



Figure D1.

Figure D2: Notes on the average firing rate profiles.

67141-3B: The discontinuity in the record just before the 3000 second point was due to a tape reel change. The unit continued to fire during the change, but the spike activity was lost.

67141-10B-1: This unit was recorded continuously for over 4000 seconds. The gap in the center of the record represents a period of absolute silence on the part of the unit.

# AVERAGE FIRING RATE PROFILES

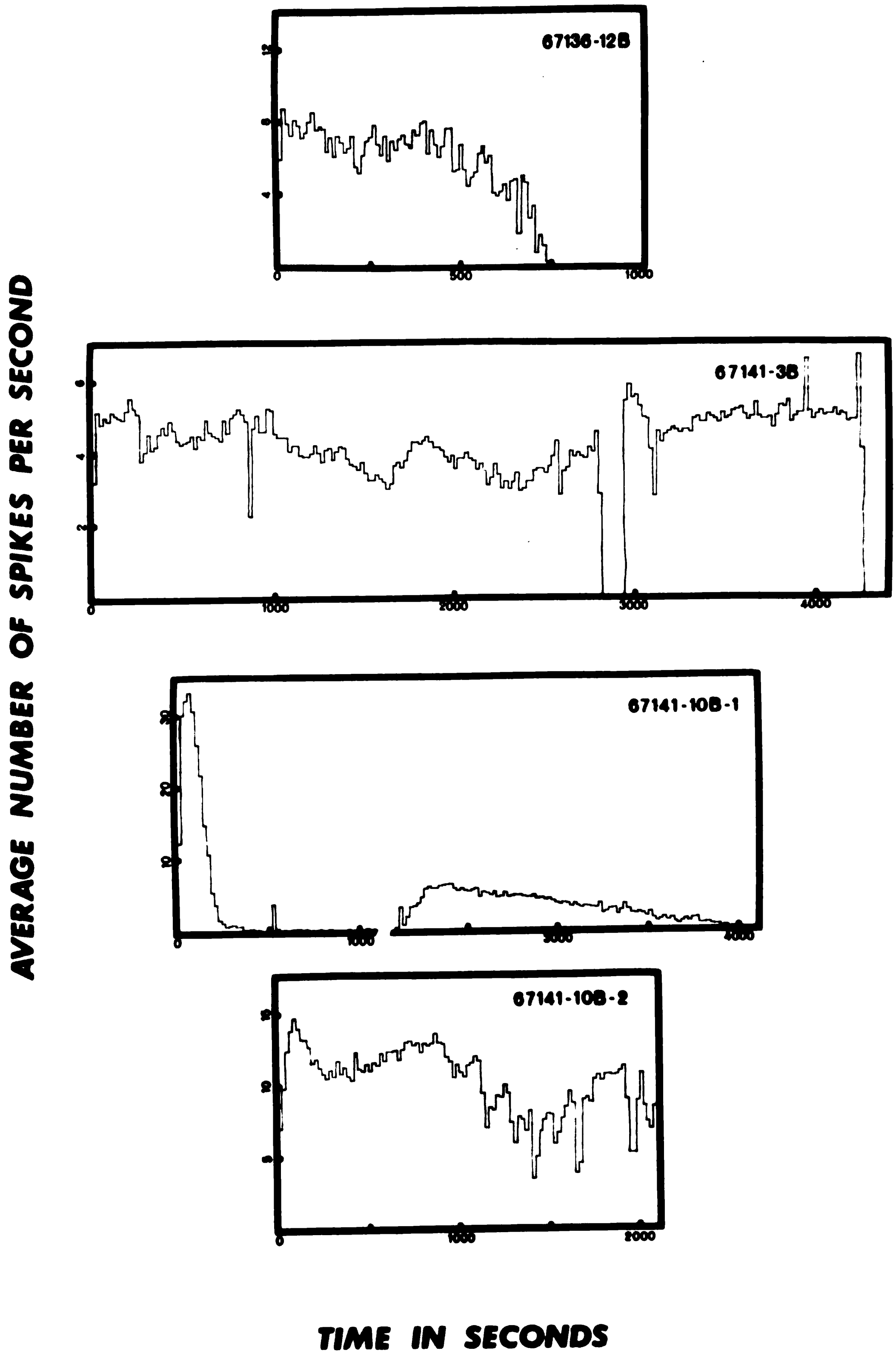



Figure D2.

Figure D3: Notes on the average firing rate profiles.

67141-14A: The break in the record is due to a tape change. The unit continued to fire.

67143-3B: Again the gaps in the record do not represent unit silence but are due to time lost while changing tape reels.

67143-4C: This unit's behavior is probably a good example of an irritability response followed by a "settling down" to a steady level.

# AVERAGE FIRING RATE PROFILES

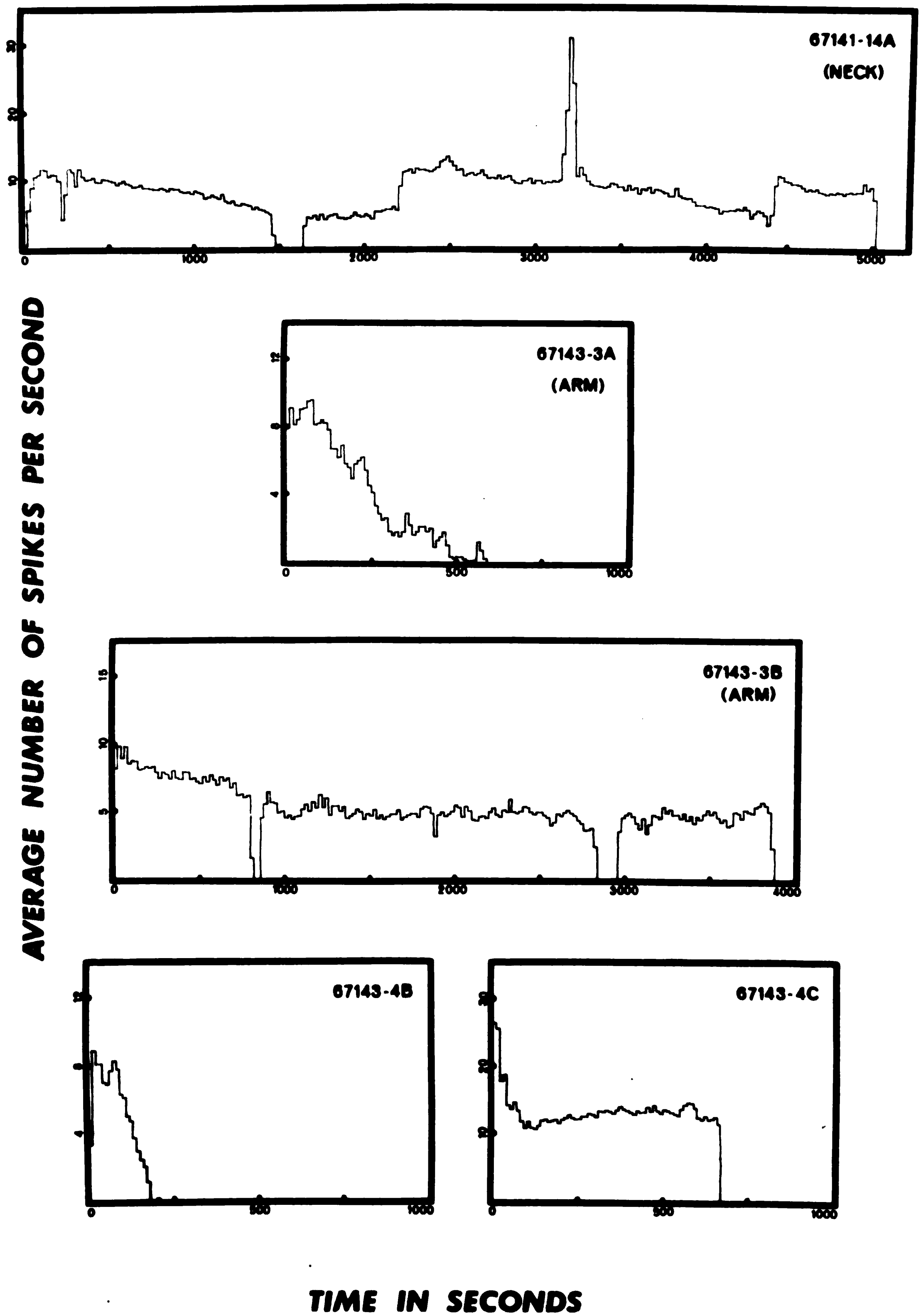



Figure D3.

Figure D4: Notes on the average firing rate profiles.

68144-11A: The gap in the record does not indicate unit silence.

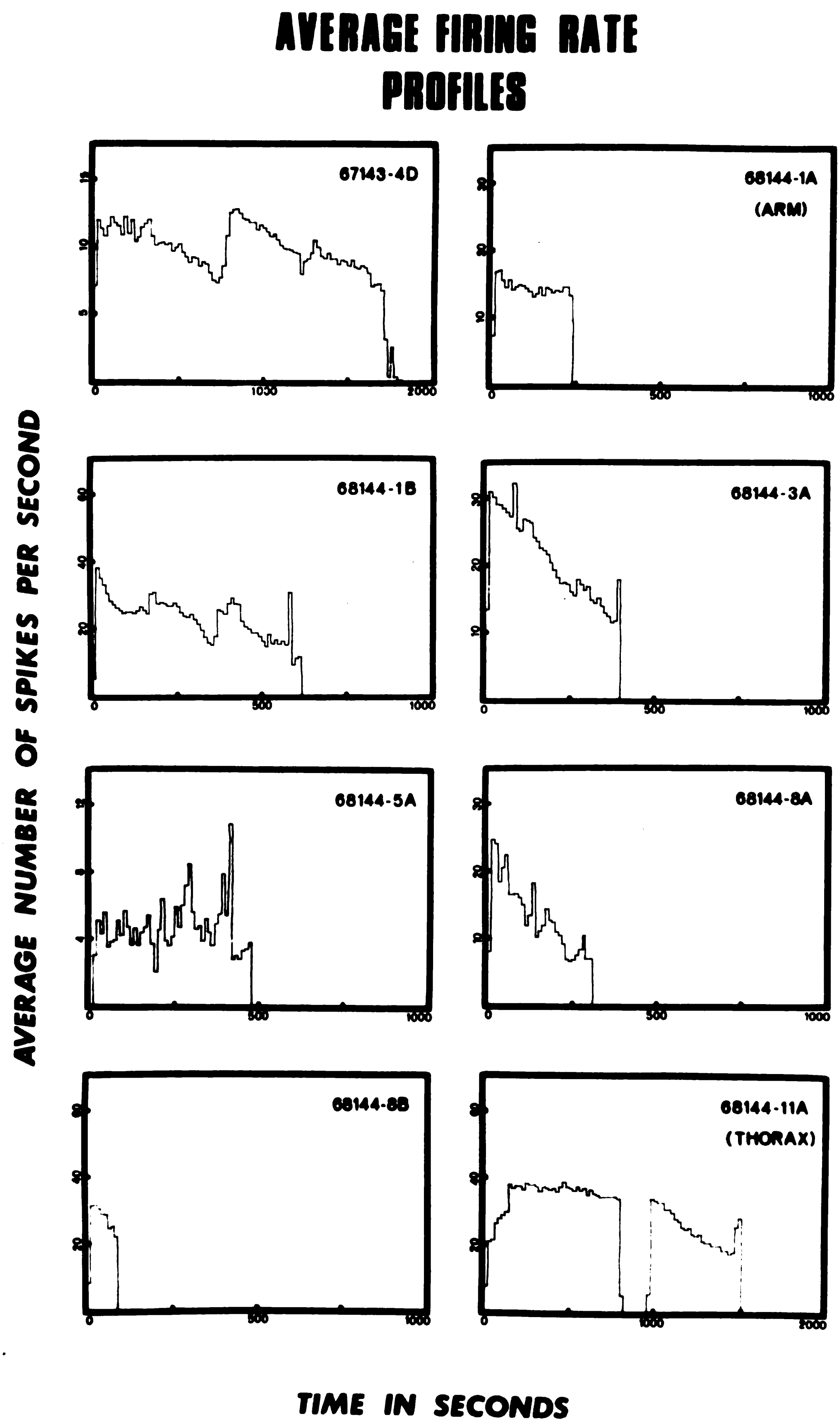

AVERAGE FIRING RATE
PROFILES
AVERAGE NUMBER OF SPIKES PER SECOND
TIME IN SECONDS
67143-4D
68144-1A
(ARM)
68144-1B
68144-3A
68144-5A
68144-8A
68144-8B
68144-11A
(THORAX)
0
1000
2000
500

Figure D4.

Figure D5: Notes on the average firing rate profiles.

68145-3A-1: This unit was treated in much the same way as unit 67132-7A. Position i. is the activity resulting from a free hanging arm. This is followed by position ii. which involved flexing the arm such that the elbow formed an acute angle. This was followed by a partial relaxation of the pressure which allowed the elbow to form an obtuse angle (position iii.).

A return to the free position (iv.) was followed by an obtuse flexion (v.) which, in turn was followed by an acute flexion (vi.). Finally the limb was allowed to return to its resting state (vii.).

It is apparent that the average rate profile does not afford a direct means of determining the limb position. The presumably equivalent free positions (i., iv., and vii.) display considerable variability in their effect upon the unit.

The two acute positions (ii. and vi.) show a higher overall rate than any of the other positions. The unit, in response to this degree of flexion, would appear not to show the gradual rate increase exhibited by the obtuse positions (iii. and iv.).

The gaps in the record are in all cases due to the cessation of recording during stimulus changes.

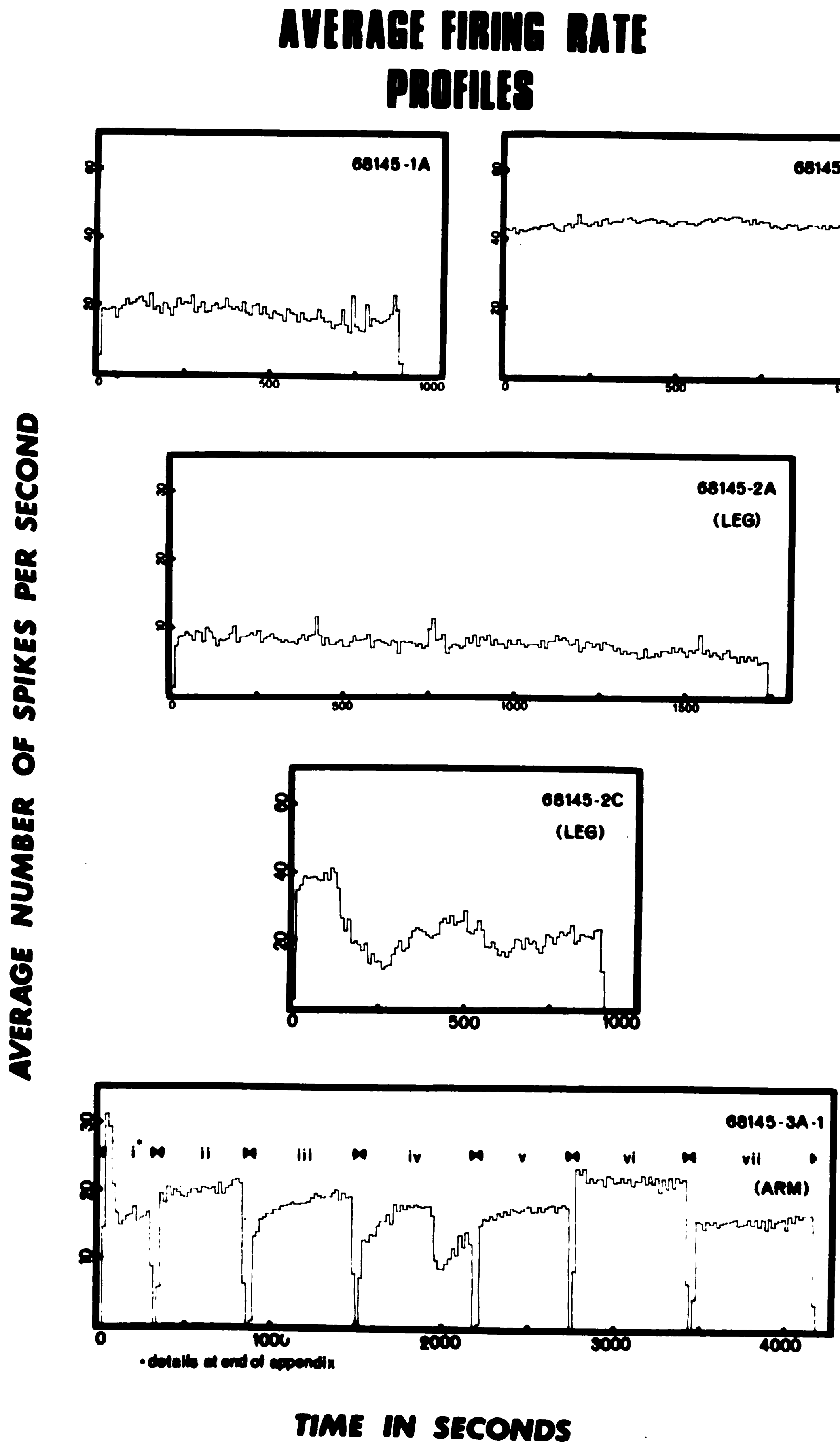

AVERAGE FIRING RATE
PROFILES
68145-1A
68145-1B
68145-2A
(LEG)
68145-2C
(LEG)
68145-3A-1
(ARM)
i*
ii
iii
iv
v
vi
vii
*details at end of appendix
AVERAGE NUMBER OF SPIKES PER SECOND
TIME IN SECONDS

Figure D5.

Figure D6: Notes on the average firing rate profiles.

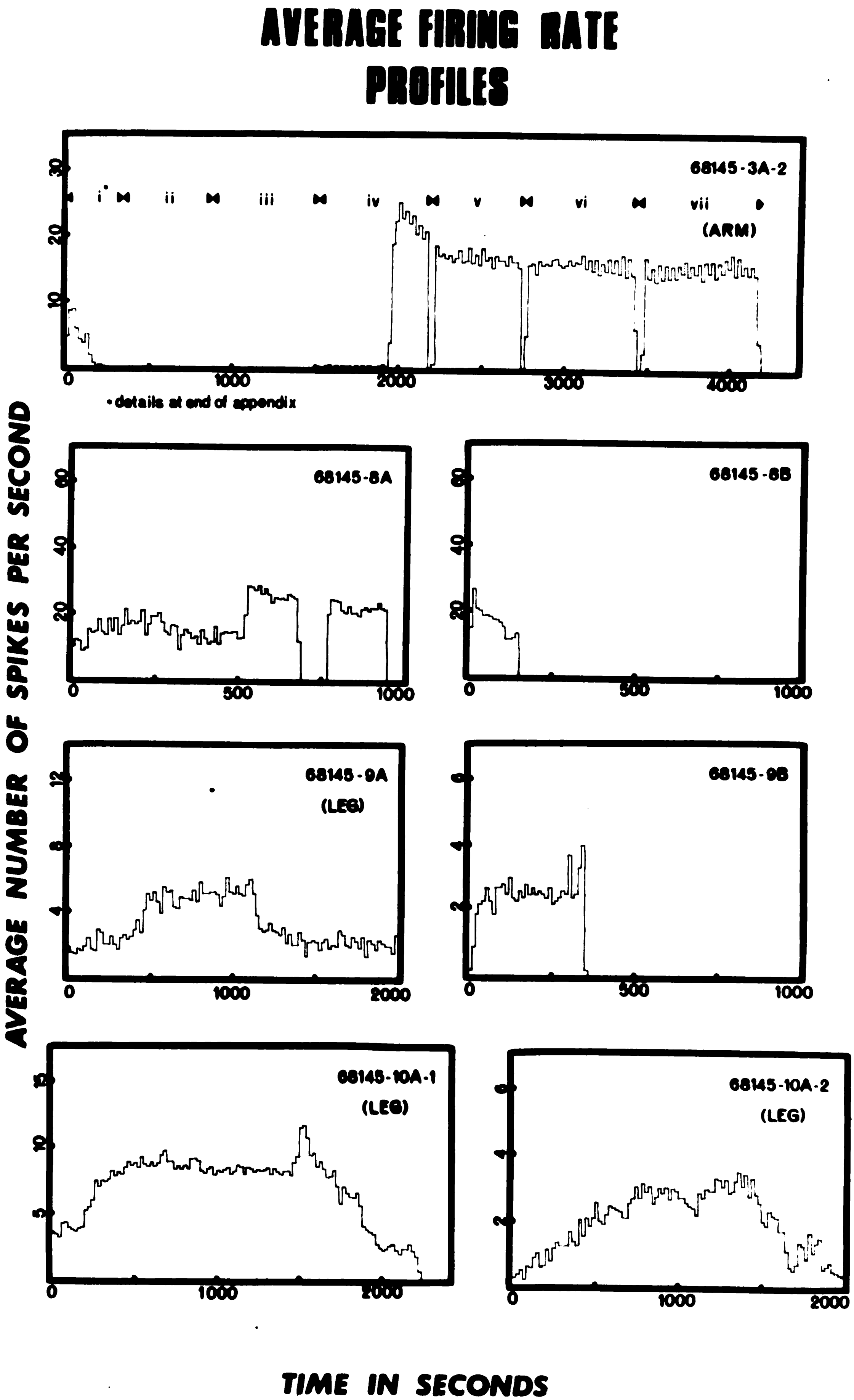
68145-3A-2: This unit was recorded simultaneously with unit 68145-3A-1 and, consequently, was subjected to identical external stimulus conditions.

It would appear that this unit, though anatomically closely associated with unit 68145-3A-1, is rather disinterested in the particular stimuli chosen to activate its neighbor. About the only obvious correlation is seen in position iv. where 68145-3A-1 takes a dip in firing rate and 68145-3A-2 reactivates at the same time.

There are then correlations which are (perhaps) related to the interactions of these two units but not necessarily directly coupling both units to the stimulus, but rather, possibly demonstrating a differential interest on the part of the two units to varying aspects of the same stimulus.

The record gaps are due to the halting of recording during stimulus changes.

68145-8A: The gap at approximately 750 seconds is due to a tape change. The unit continued to fire.



AVERAGE FIRING RATE PROFILES
68145-3A-2
(ARM)
i ii iii iv v vi vii
• details at end of appendix
68145-8A
68145-8B
68145-9A
(LEG)
68145-9B
68145-10A-1
(LEG)
68145-10A-2
(LEG)
AVERAGE NUMBER OF SPIKES PER SECOND
TIME IN SECONDS


Figure D6.

Figure D6: Notes on the average firing rate profiles.

68145-3A-2: This unit was recorded simultaneously with unit 68145-3A-1 and, consequently, was subjected to identical external stimulus conditions.

It would appear that this unit, though anatomically closely associated with unit 68145-3A-1, is rather disinterested in the particular stimuli chosen to activate its neighbor. About the only obvious correlation is seen in position iv. where 68145-3A-1 takes a dip in firing rate and 68145-3A-2 reactivates at the same time.

There are then correlations which are (perhaps) related to the interactions of these two units but not necessarily directly coupling both units to the stimulus, but rather, possibly demonstrating a differential interest on the part of the two units to varying aspects of the same stimulus.

The record gaps are due to the halting of recording during stimulus changes.

68145-8A: The gap at approximately 750 seconds is due to a tape change. The unit continued to fire.

# AVERAGE FIRING RATE PROFILES

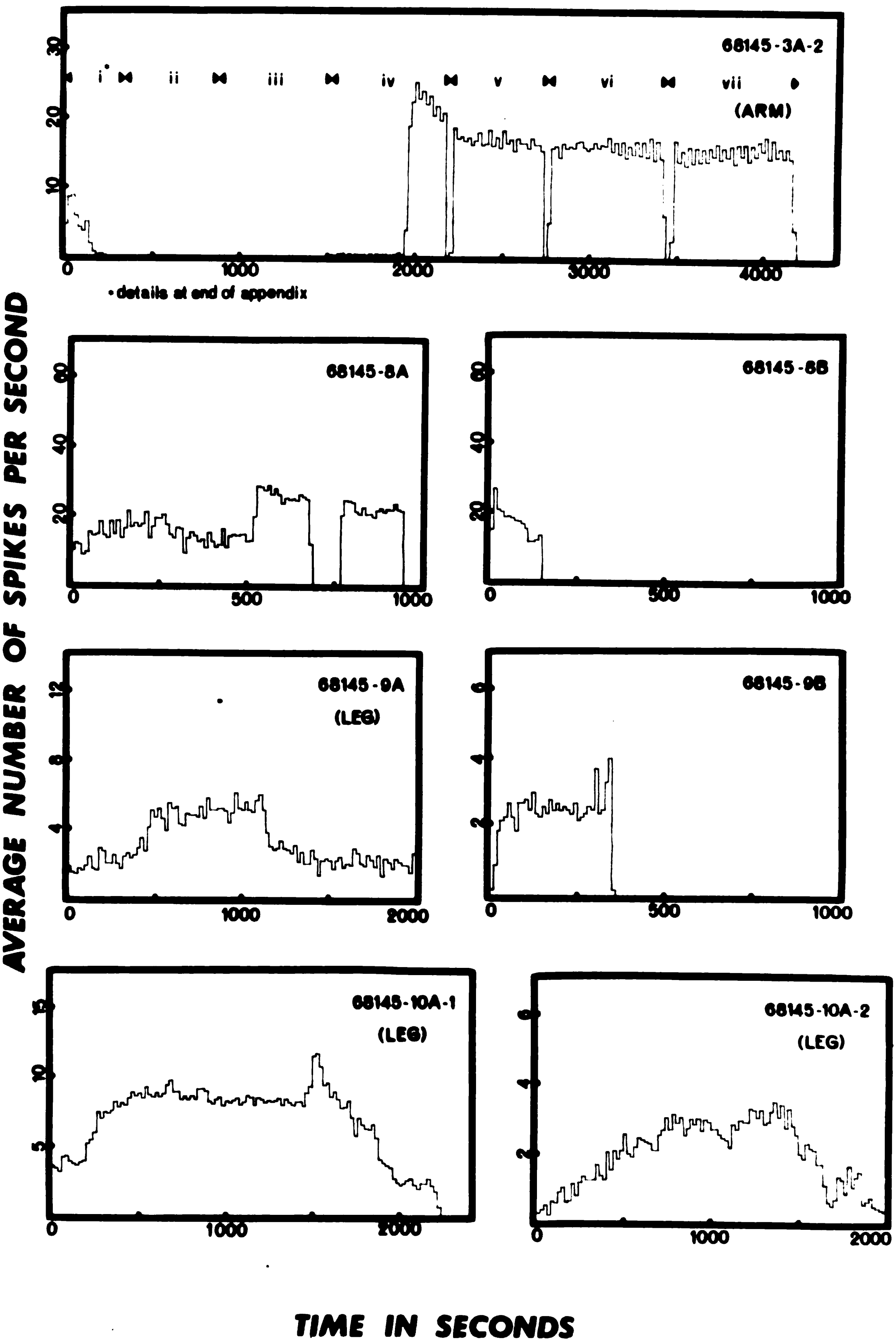



Figure D6.

# AVERAGE FIRING RATE PROFILES

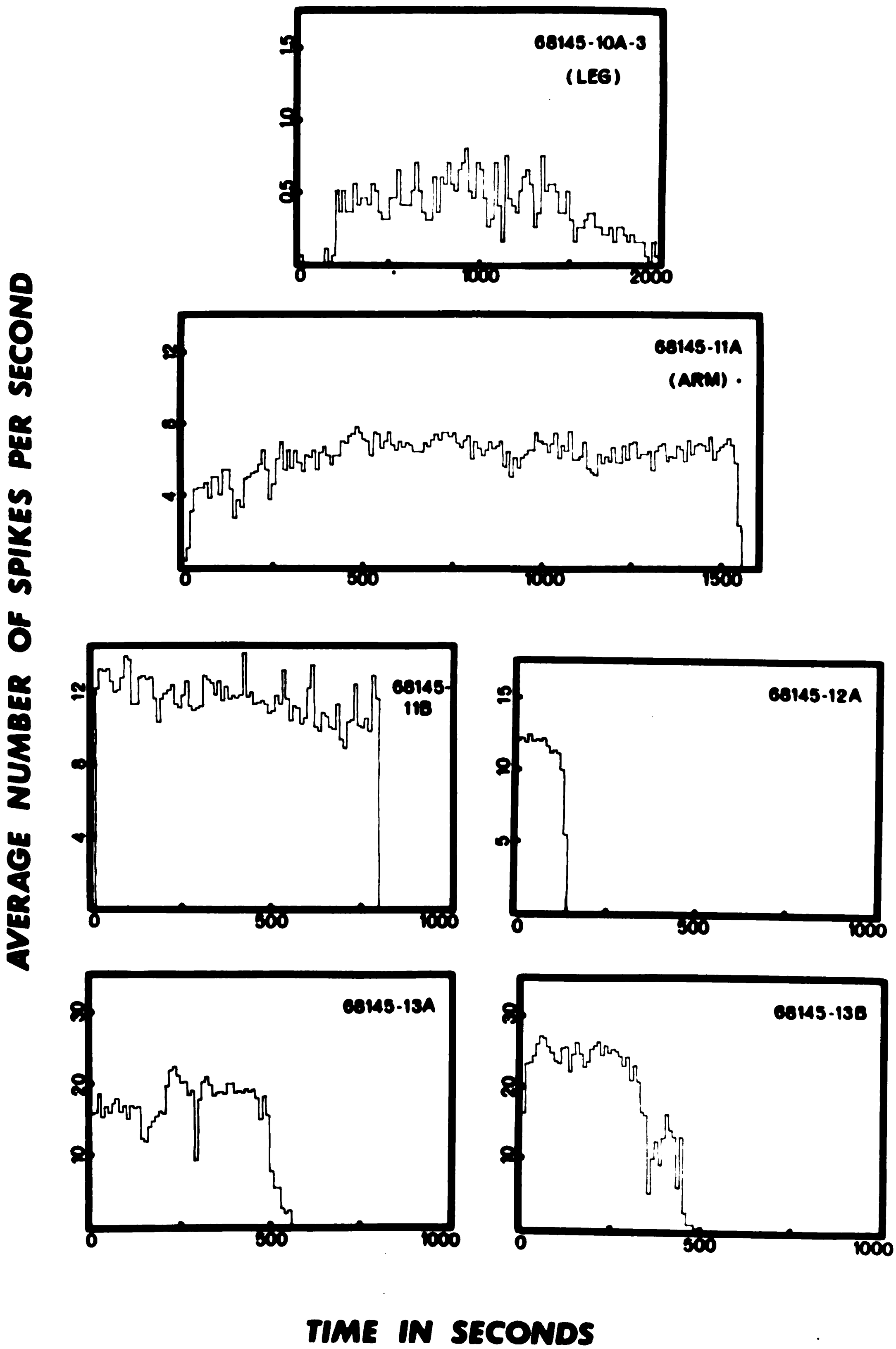



Figure D7.

Figure D8: Notes on the average firing rate profiles.

68145-14B-2: The high peak at 900 seconds is due to an accidental stimulation of the unit's response area on the body surface. This unit was typical of the somatic responding units which responded to appropriate mechanical stimulation with a furious burst of activity that usually was very quick to adapt back to the maintained level.

68145-15A: The gap is again due to a change of tape reels and not to unit shut down.

# AVERAGE FIRING RATE PROFILES

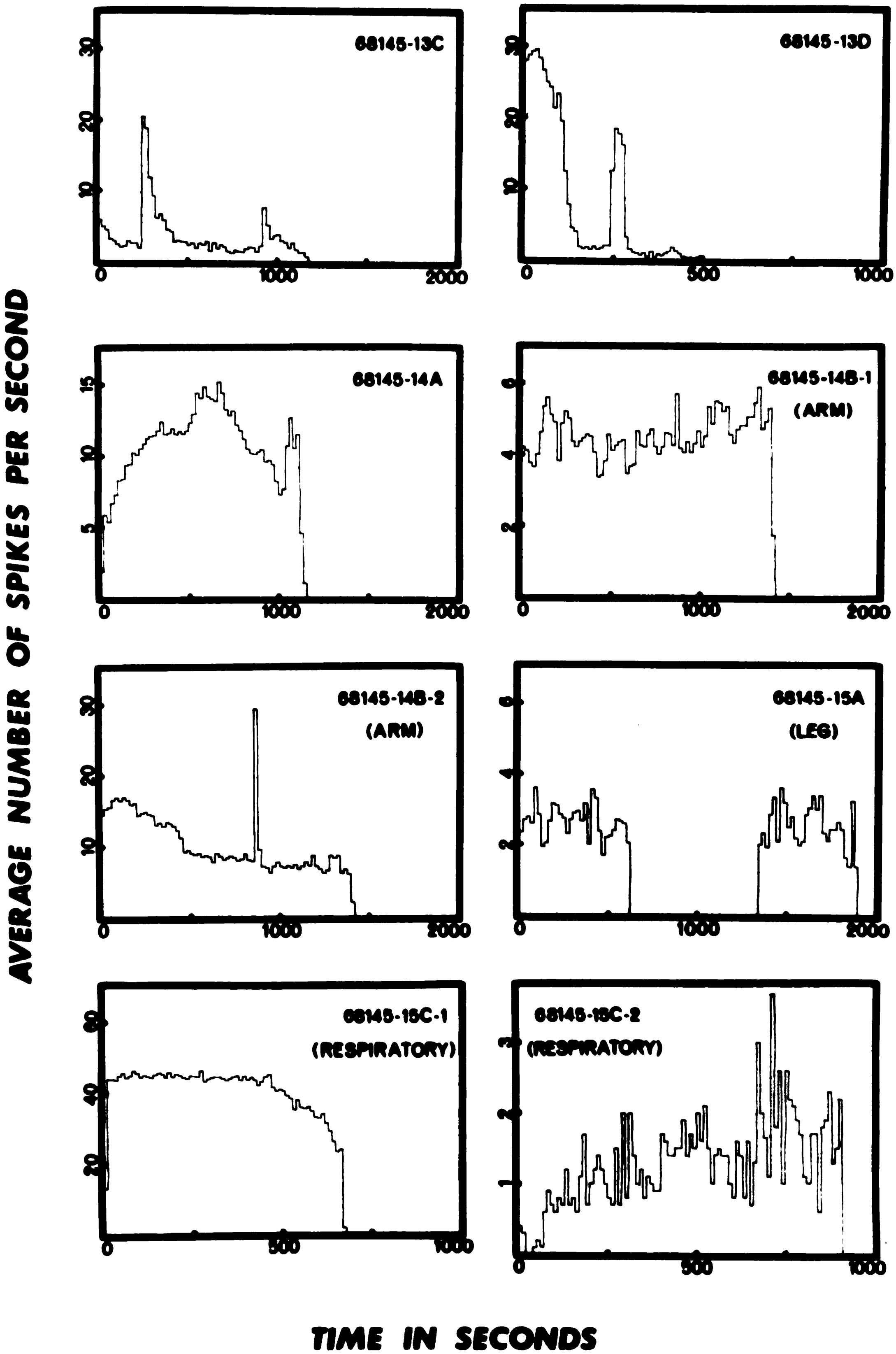



Figure D8.

Figure D9: Notes on the average firing rate profiles.

68145-15C-3: This unit was the lowest in amplitude of the 4 units obtained from this recording locus. It frequently was buried in rather large electrical artifacts (arising from a source external to the sheep) which made the presentation of a continuous record difficult. Hence, only a relatively clean sample is presented here. It should be noted that such artifacts were easily removed during the accumulation of the interspike interval histogram and, thus, do not constitute a serious source of error in the plotting of the spike interval distribution. Technical vagaries, however, made suppression of these artifacts a difficult matter while the average firing rate profile was being computed.

68145-18A: The dotted discontinuities represent electrical artifacts derived from an external source which, for reasons set forth in the discussion of unit 68145-15C-1 above, were not removable. These artifacts do not affect the accuracy of the interspike interval histogram calculated from these data, however.

# AVERAGE FIRING RATE PROFILES

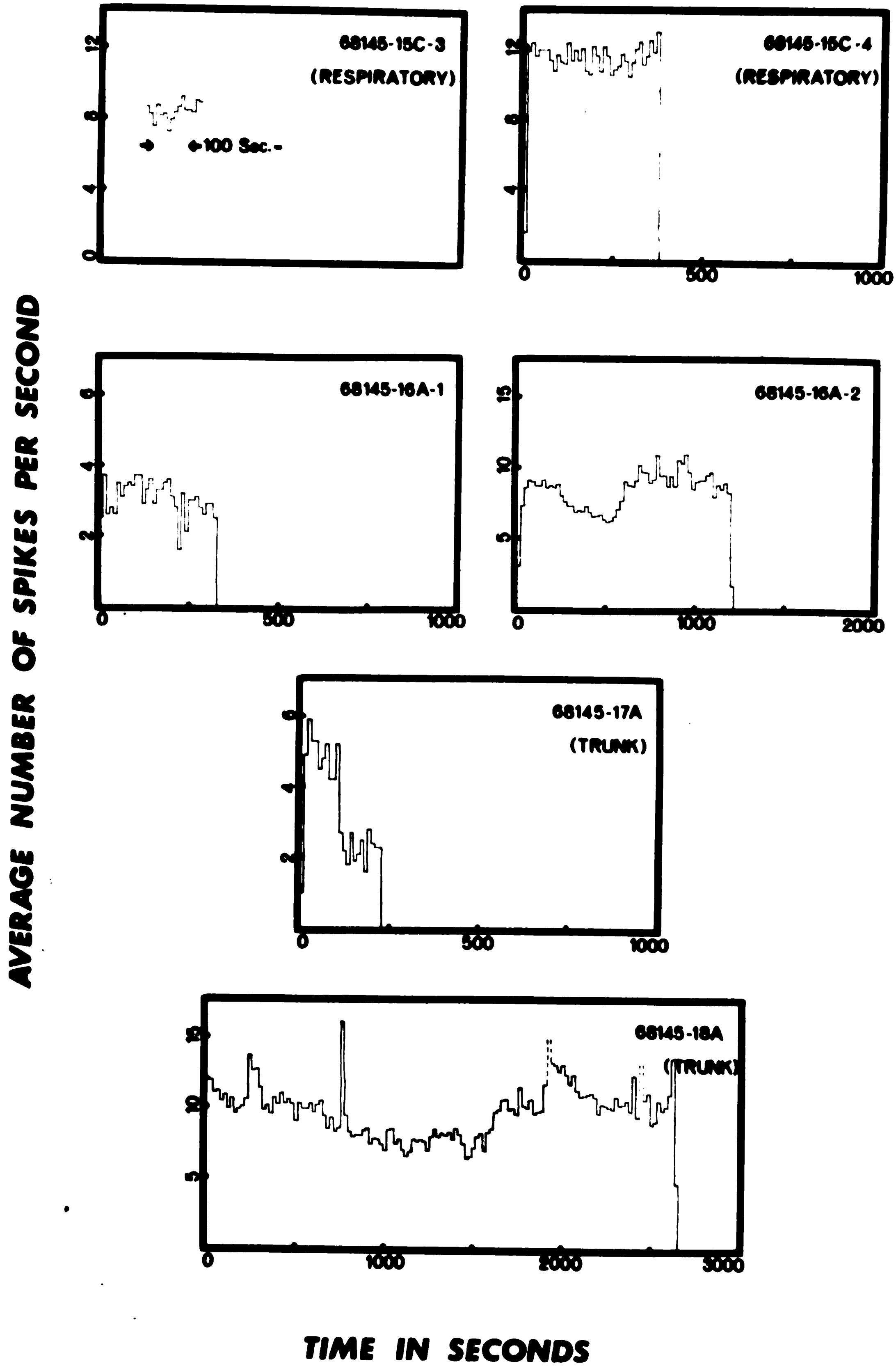



Figure D9.

# AVERAGE FIRING RATE PROFILES

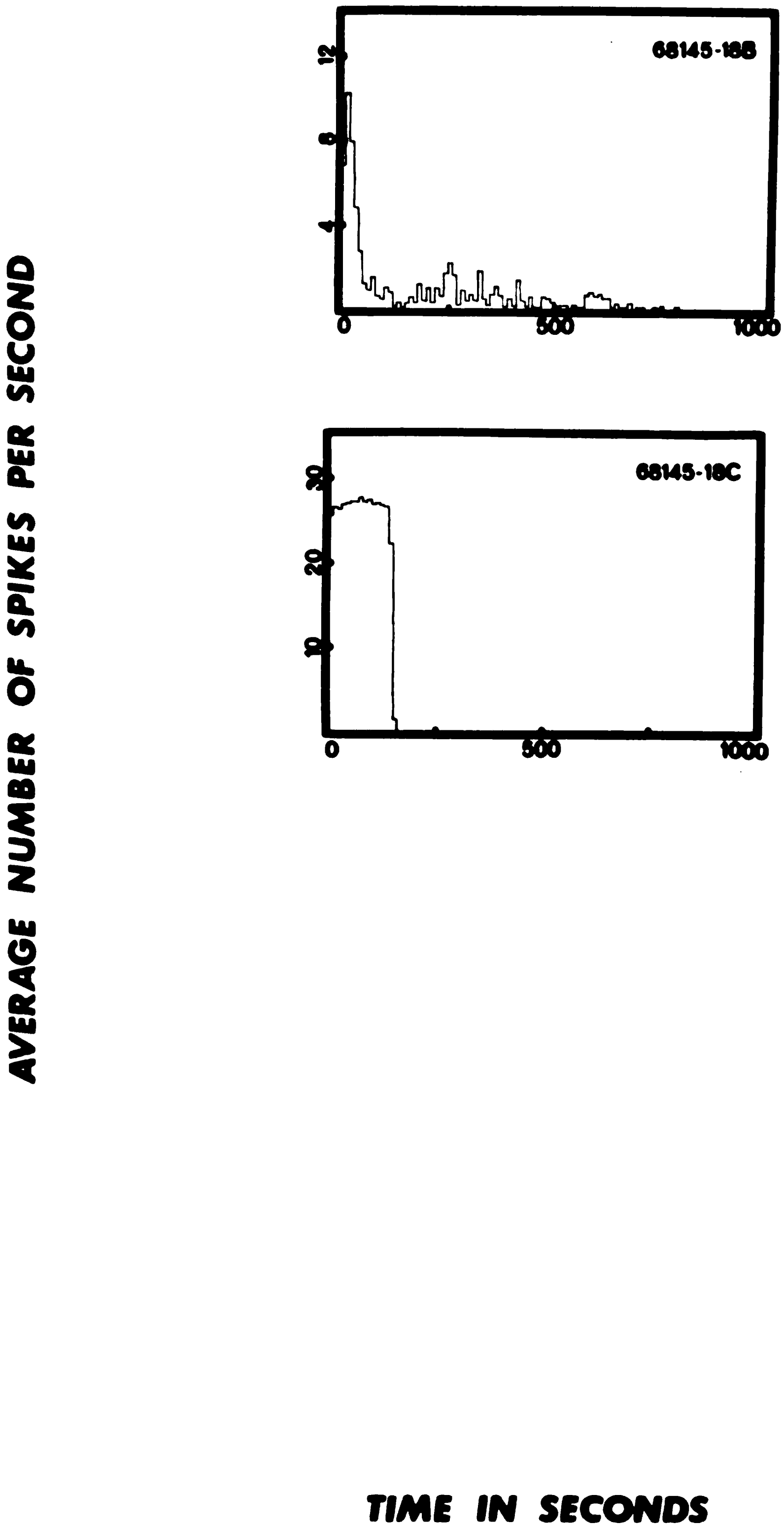



Figure D10.

# Appendix E

## The Interspike Interval Histograms

Figure E1: Notes on the interspike interval histograms.

67129-2L: See notes on this unit in Appendix D, Figure D1.

67132-7A: See above.

# INTERSPIKE INTERVAL HISTOGRAMS

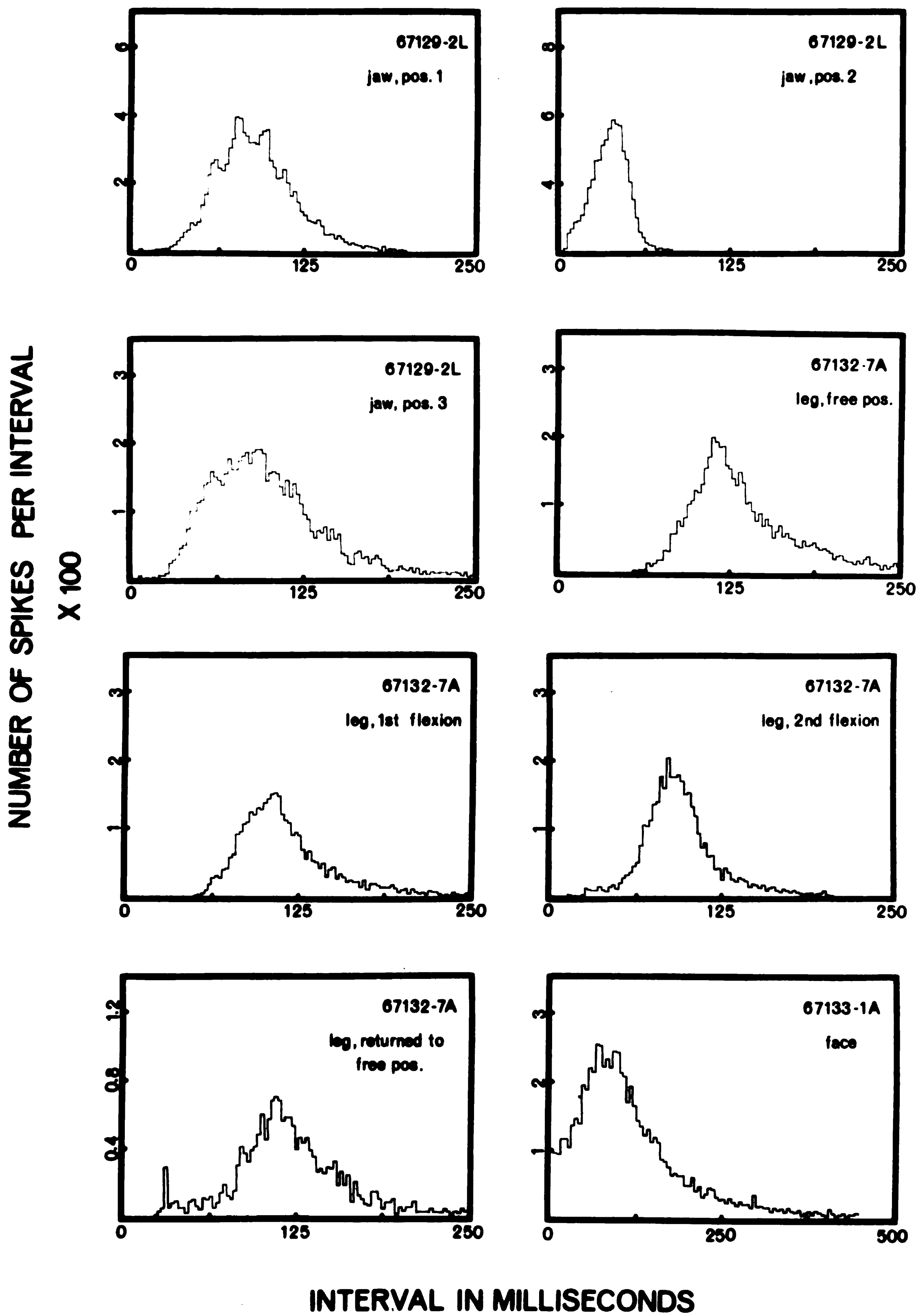



Figure E1.

Figure E2: Notes on the interspike interval histograms.

67141-10B-2: The intervals are continuous in sections i. through iii. The vertical scale is multiplied by a factor of 10x in section ii. and by 100x in section iii.

67141-14A: 10x vertical scale multiplication at 250 msec.

# INTERSPIKE INTERVAL HISTOGRAMS

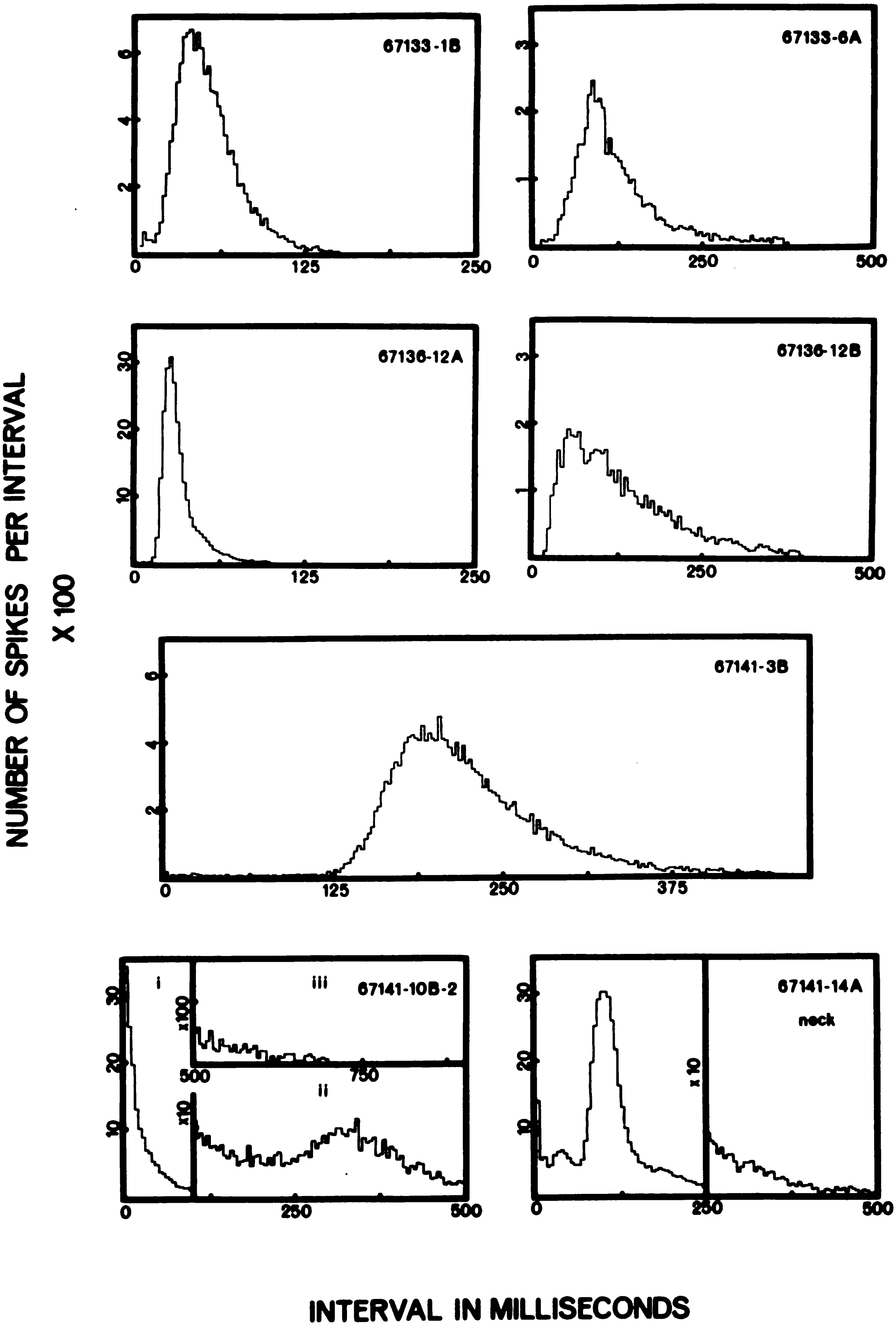



Figure E2.

Figure E3: Notes in the interspike interval histograms.

67143-3A: 10x vertical scale multiplication at 400 msec. Note harmonic nature of maxima.

68144-3A: See footnote 4, Appendix B. The extreme symmetry of this unit's interval histogram may indicate that an unaccounted for stimulus was being applied during the recording session.

# INTERSPIKE INTERVAL HISTOGRAMS

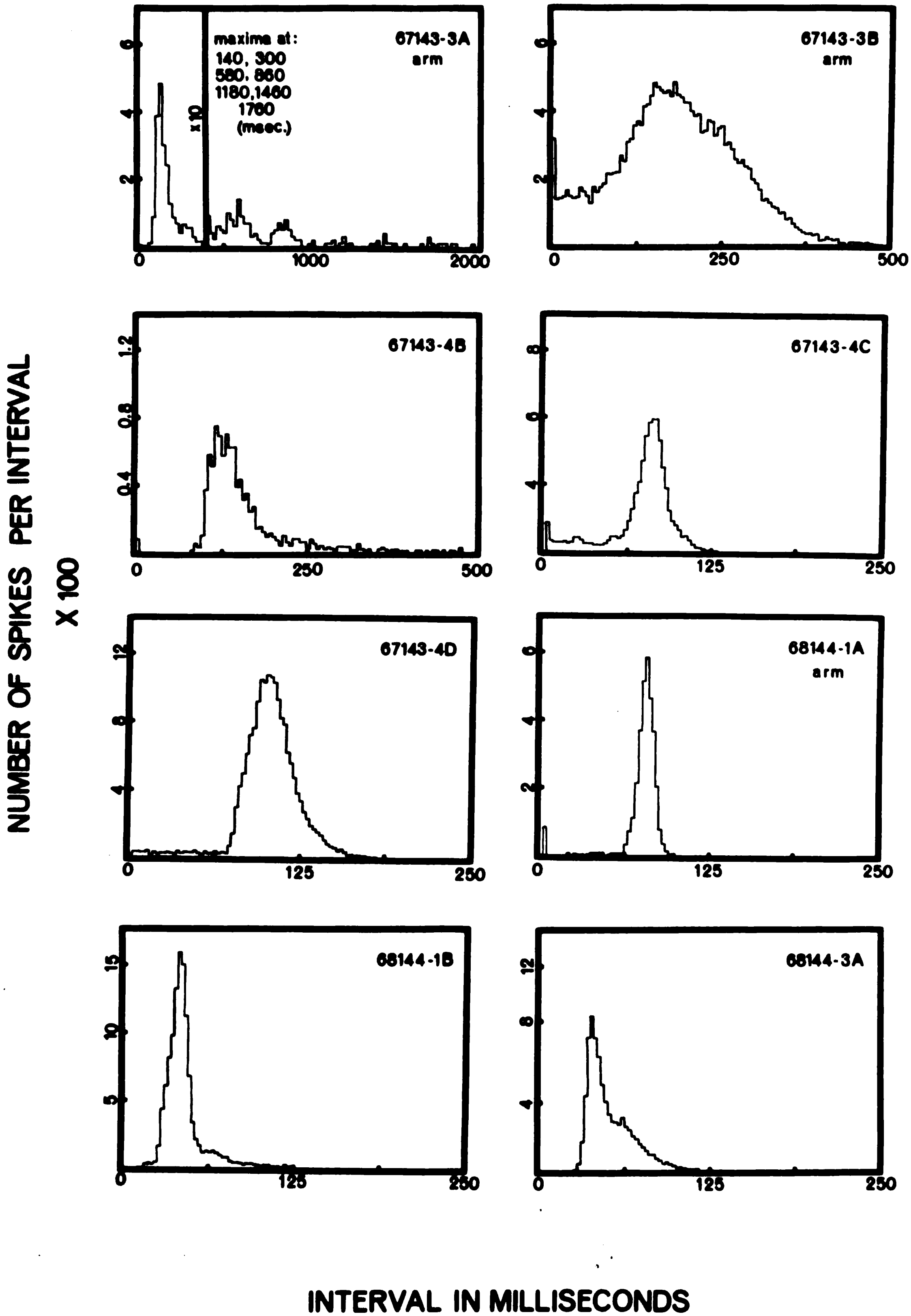



Figure E3.

Figure E4: Notes on the interspike interval histograms.

68144-5A: No scale expansion in inset.

68144-8A: 2.5x scale expansion in inset. Note absence of tail in this region.

# INTERSPIKE INTERVAL HISTOGRAMS

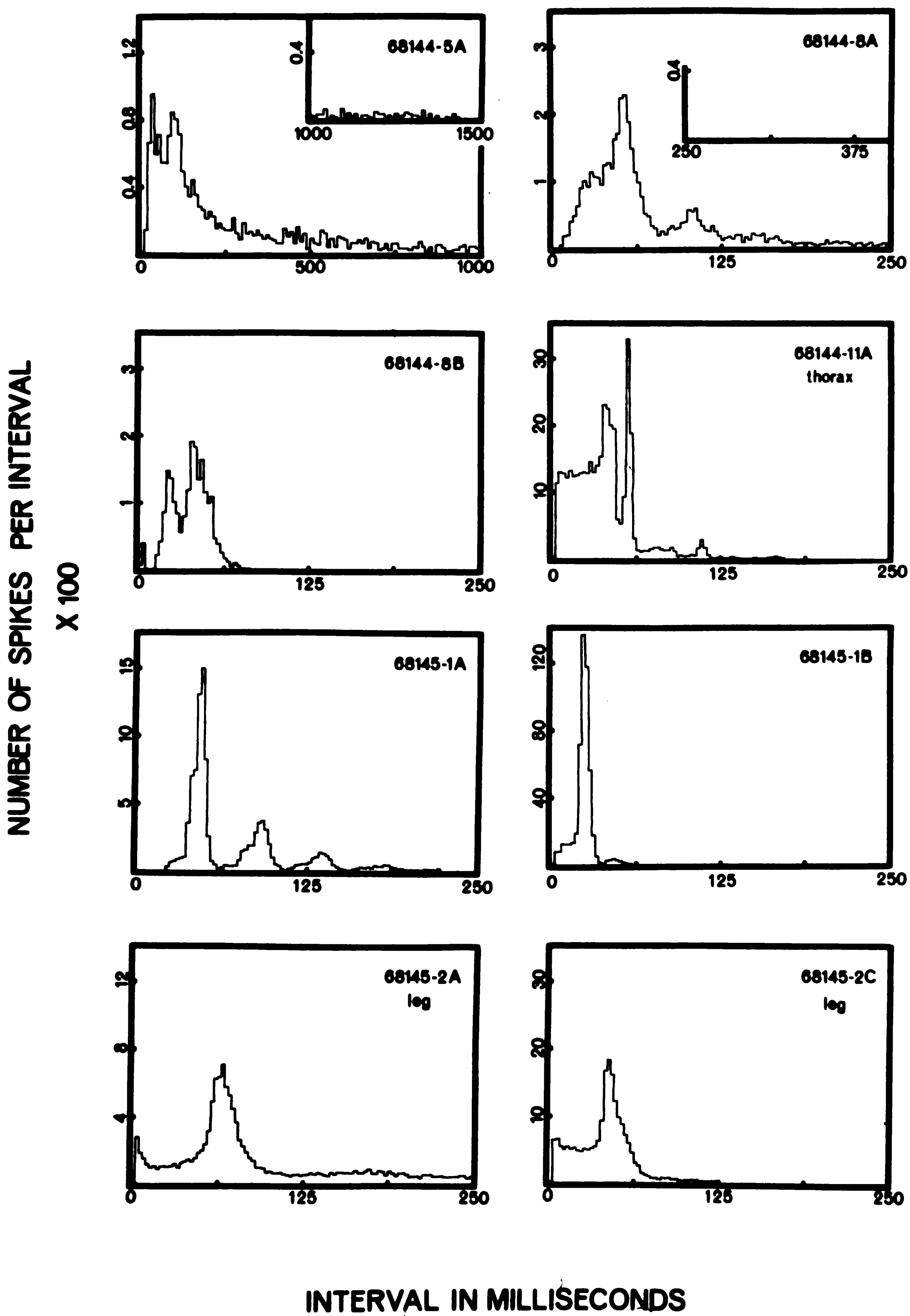



Figure E4.

Figure E5: Notes on the interspike interval histograms.

68145-3A-1: This unit was being driven while the interspike interval histogram accumulated. Note in particular the dimunition of the histogram tail and the increase in peak symmetry as the tension is increased. For further details concerning this unit and unit 68145-3A-2 see Appendix D, fugures D5 and D6.

68145-8A: 100x vertical scale expansion at 250 msec. Note harmonic nature of all but first maxima.

68145-9A: 10x vertical scale expansion from 400 msec. on.

# INTERSPIKE INTERVAL HISTOGRAMS

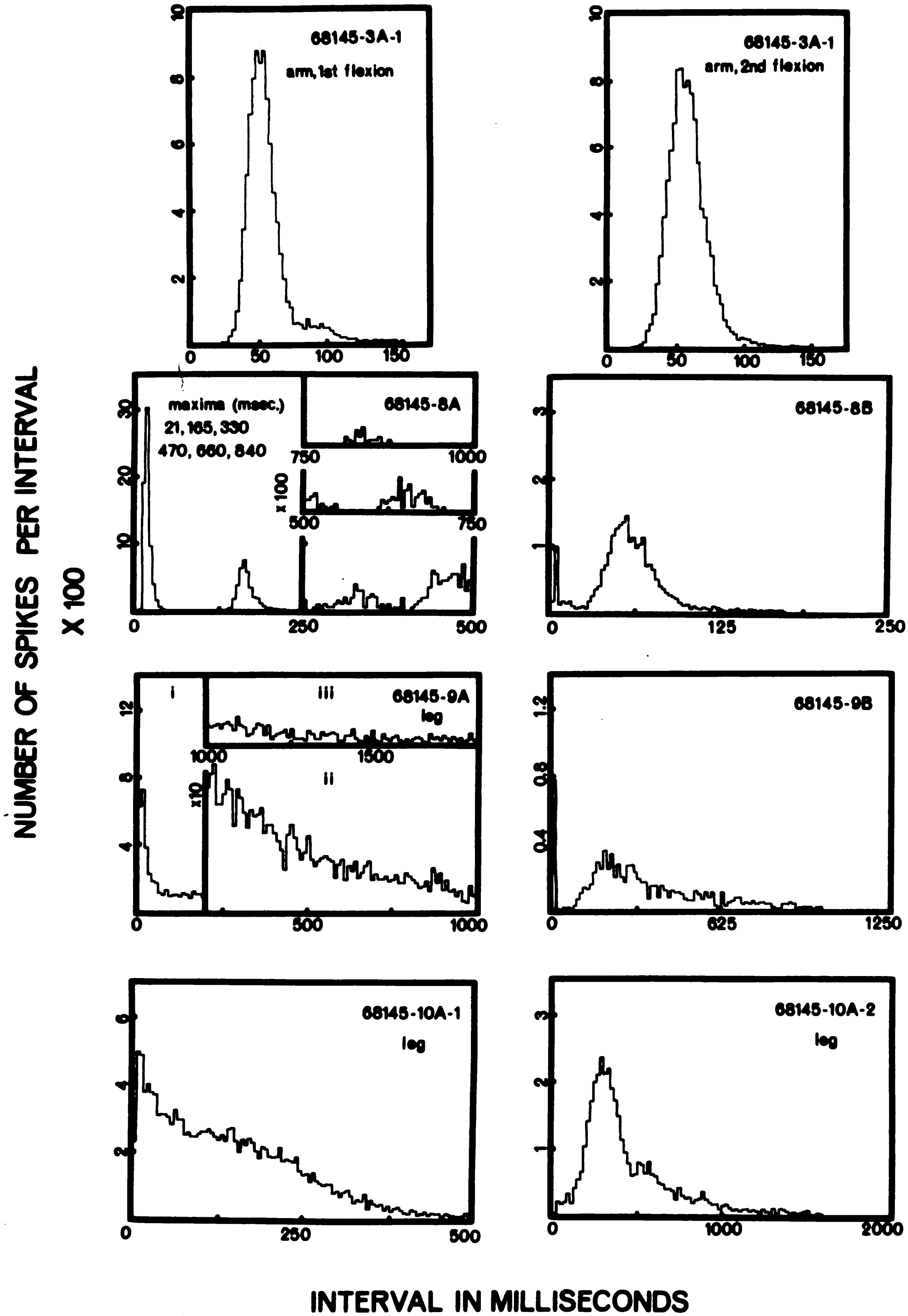



Figure E5.

Figure E6: Notes on the interspike interval histograms.

68145-13B: 100x vertical scale expansion at 200 msec. Note harmonic nature of peaks.

68145-13C: 10x vertical scale expansion at 400 msec.

# INTERSPIKE INTERVAL HISTOGRAMS

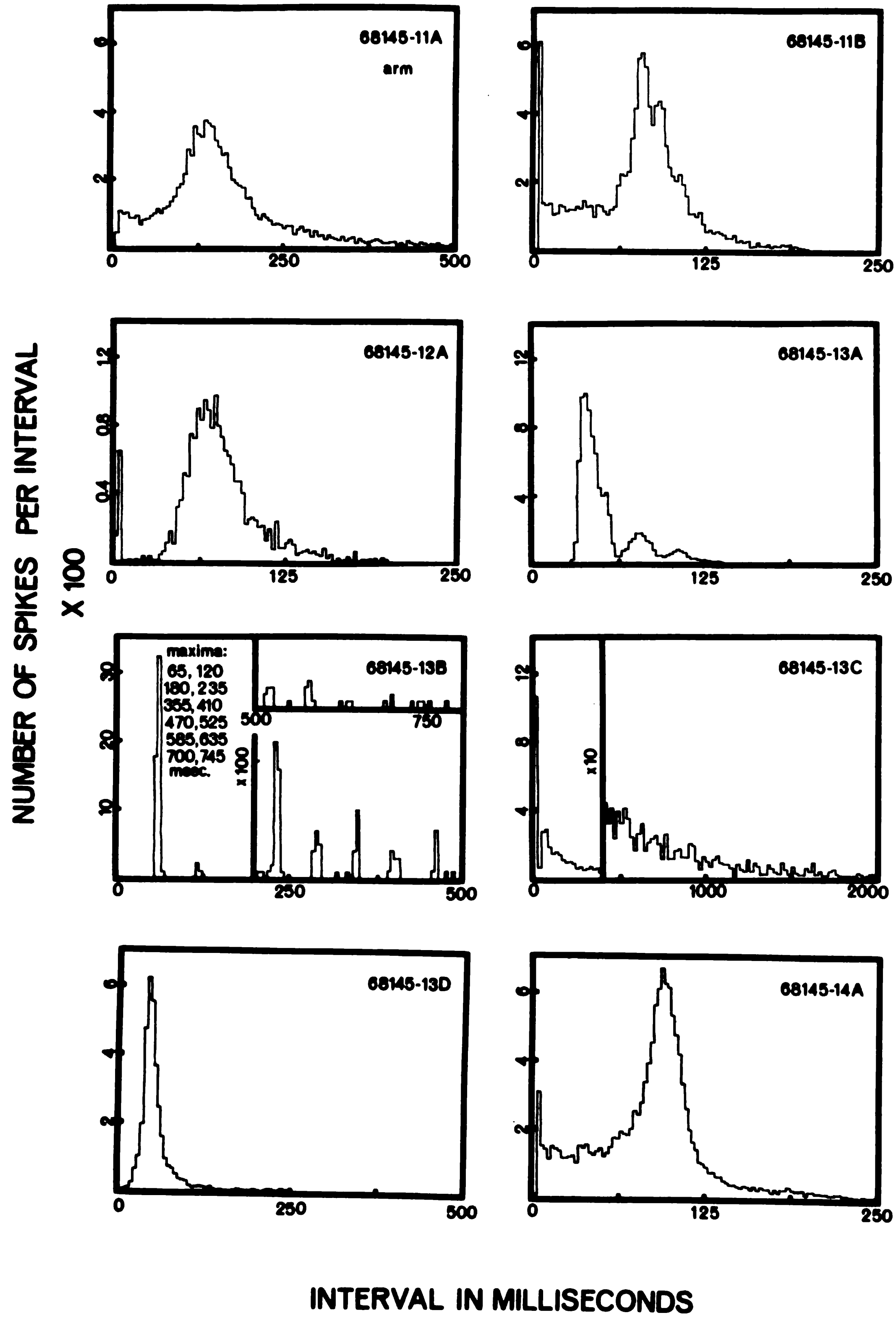



Figure D6.

Figure E7: Notes on the interspike interval histograms.

68145-15A: 10x vertical scale expansion at 50 msec.

68145-15C-1: 10x vertical scale expansion at 100 msec.

68145-15C-3: 10x vertical scale expansion at 200 msec.

68145-15C-4: 10x vertical scale expansion at 50 msec.

# INTERSPIKE INTERVAL HISTOGRAMS

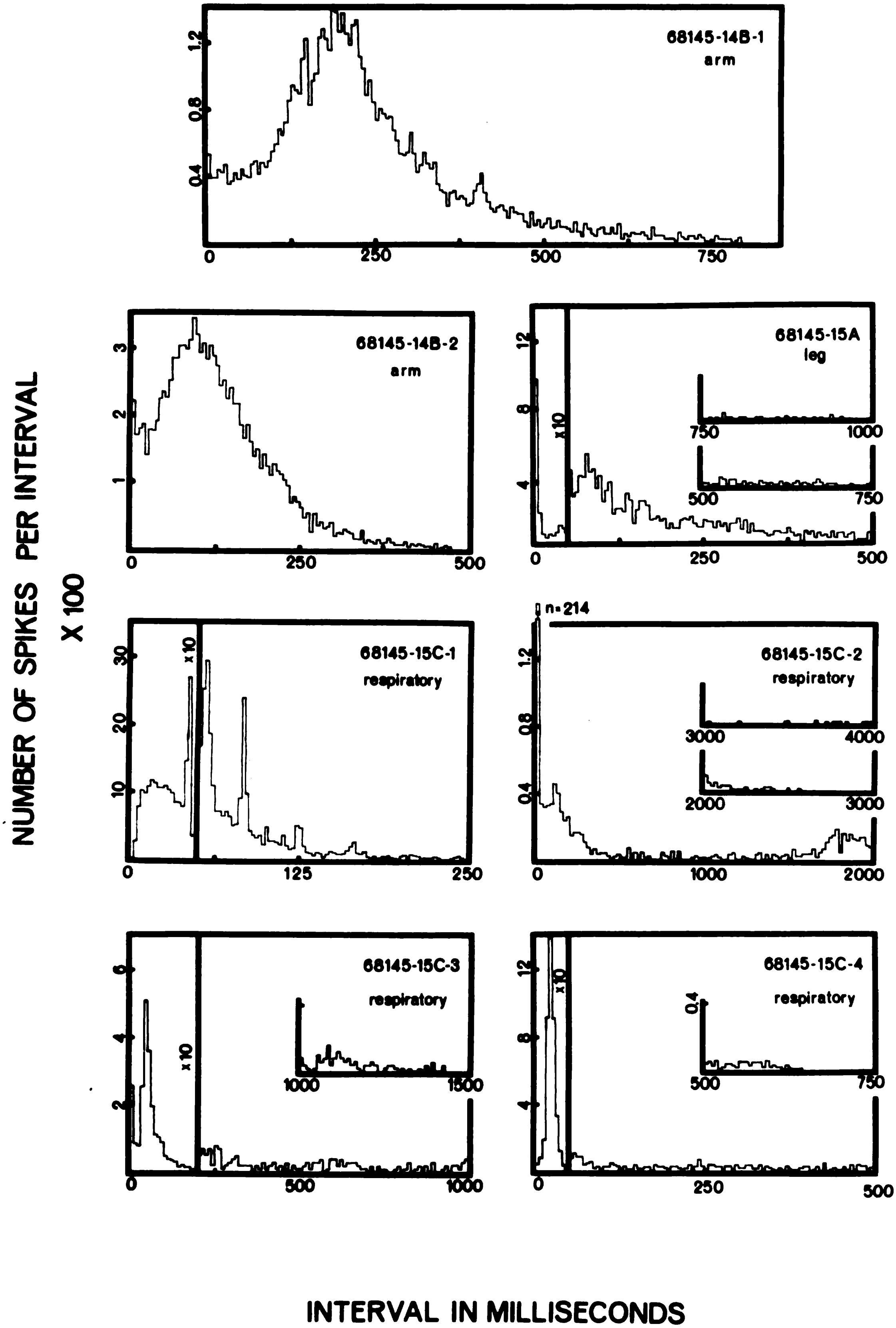



Figure E7.

Figure E8: Notes on the interspike interval histograms

68145-16A-2: 10x vertical scale expansion at 200 msec.

68145-18B: 10x vertical scale expansion at 1200 msec.

68145-18C: Note the extreme regularity of firing and the harmonic nature of the multiple peaks. 10x scale expansion at 50 msec.

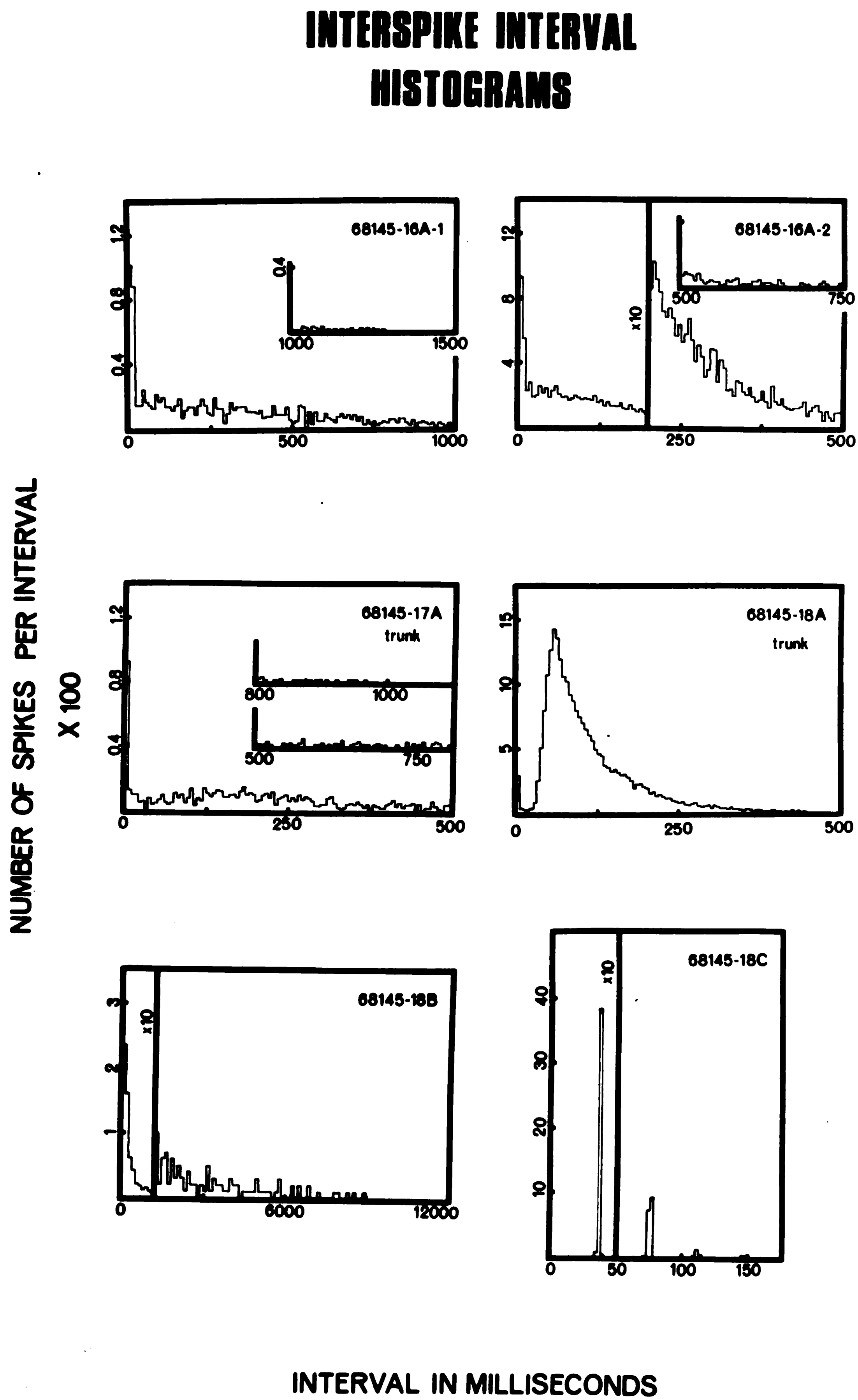

INTERSPIKE INTERVAL
HISTOGRAMS
NUMBER OF SPIKES PER INTERVAL
X 100
68145-16A-1
68145-16A-2
x10
68145-17A
trunk
68145-18A
trunk
68145-18B
x10
68145-18C
x10
INTERVAL IN MILLISECONDS

Figure E8.

Appendix F

## Further Details of Methods

A. The Animal

The subjects. The subjects were eight domestic sheep *Ovis aries*, of either Suffolk or a Suffolk-Dorset cross breed. All the subjects were female ranging in age from one to eight years and in weight from 100 to 225 pounds.

Anesthesia. Food deprivation was in effect for 24 hours prior to each experiment. Approximately one hour before anesthetization, 100 to 250 milligrams of atropine sulfate were administered interperitoneally to prevent excess secretion. Seven of the animals in this series were anesthetized initially and maintained on a "dial Urethane" mixture. The anesthetic recipe will be found in Appendix G following. Anesthetic concentration was so arranged as to require approximately 1.0 milliliter of "Dial" per pound of animal when injected interperitoneally. One animal was initially anesthetized with Sernylan (Parke, Davis and Co.) administered intervenously. This animal (67133) was later maintained on a nitrous-oxide and halothane gas mixture.

Surgical Preparation. When the animal lost consciousness - from 15 to 45 minutes after injection for the "Dial" animals and almost instantly for the Sernylan animal - it was transported quickly to the operating area and tracheotomized. Particular attention was paid to maintenance of the airway. Removal of all blood and mucous by aspiration followed by cauterization of all exposed wound surfaces proved beneficial in this regard. A glass cannula was inserted into the exposed trachea and sutured in place. The neck wound was then clamped shut. From this point on both oxygen and mechanical respiration were available in case of respiratory failure. Such failure was not uncommon within the first few hours after anesthetization. Generally, a few minutes of mechanical assistance were all that were needed to bring the animal back to unaided breathing.

Tracheotomy completed, the animal was hoisted onto supporting rails and the head positioned properly for eventual fastening into a head holder. A midline incision from the eyebrows to the mid-shoulder was made. Working caudally, the skull was exposed over a broad area laterally and back to the occipital ridge. At this point the head was positioned in such a fashion as to allow maximum access to the as-yet-to-be-exposed medulla. Three-eighths inch brass rods were affixed to the skull with wood screws and dental acrylic. The head holder accepted the opposite ends of the rods, thus providing a firm support.

The cranium caudal to the occiput and the first two cervical vertebrae were exposed from the dorsal aspect. Wide areas of muscle were retracted from the wound opening to provide maximum working area. Cauterization and hemostatic clamps served to check excess bleeding and also provided good visualization. Additional brass rods were fixed to $C_2$ and occasionally the lateral aspect of the occiput to provide the required rigidity. Medullary exposure was accomplished by removal of the portions of the skull case overlying the caudal portion of the cerebellum and the dorsal portion of the first and the rostral half of the dorsal aspect of the second cervical vertebrae. Excessive bleeding was stemmed with gelatin sponges and bone wax. Care was taken to avoid dural puncture or the application of undue pressure to the neural tissue underlying the exposure. Exposure completed, a dam of dental acrylic was built around the entire opening. The dura was carefully removed from both the medulla and the caudal portion of the cerebellum which was then aspirated away until the obex was exposed. The exposure was then flushed with 0.9% saline solution several times, drained and the dam filled with mineral oil. At this juncture the preparation was ready for recording.

Supportive measures. Normal supportive measures generally included periodic IP injections of normal saline solution to combat dehydration. Anesthetic and atropine were provided as needed during the course of the experiments which often lasted from 24 to 36 hours. Body temperature was monitored and maintained between 34 and 37°C. by means of infra-red lamps. Periodic drainage of the trachea helped maintain the airway. Blood seepage occasioned the drainage, flushing and replacement of the mineral oil over the exposed medulla numerous times during the course of an experiment.

B. The Recording Session

Photography. Upon completion of the preparative procedures, a photograph was taken of the preparation and the exposure. Developed and printed before the recording session began, such a print served as a convenient means of locating topographical landmarks such as blood vessels, sulci and the like with respect to the electrode punctures. These punctures were marked on the photograph as they were made. Later they served as an aid in track tracing, giving the relative positions of the punctures with respect to one another. The receptive fields of those units which responded to mechanical stimulation of the body were mapped out and identified as to puncture on the whole body pictures.

The recording electrodes. The electrodes were either glass insulated tungsten microelectrodes similar to those used by Baldwin, et al. (1965) or they were lacquered tungsten similar to the type used by Hubel (1957). In either case the best tip size was found to be on the order of 30 to 40 microns of exposed metal beyond the insulation. Such a tip size typically gave a DC resistance of less than one megohm, thereby allowing a Tektronix type 122 AC preamplifier to be used without a cathode follower input.

The recording equipment. Other recording equipment consisted of Tektronix types 502A or 565 oscilloscopes for visual monitoring. The preamplifier also drove a Grass type AM-5 audio monitor. The vertical output signal from the oscilloscope fed one channel of a high fidelity audio tape recorder (Magnecord type 1028); the second channel being reserved for voice commentary and time marking. The neural activity obtained was always taped in this manner. Occasionally an interesting unit would be buried in considerable background activity or be plagued with a widely varying baseline. In these cases the signal was led through a 1.0 kHz filter which effectively removed most activity with risetimes less than that possessed by most nerve spikes. In general this was avoided where possible as it was felt that such signal processing could be accomplished to better advantage upon playback of the signal.

Recording procedures. The electrode punctures were ideally arranged in rows running medio-laterally and from rostral to caudal. In such an arrangement prior punctures would interfere minimally with the neural inputs of the punctures yet to follow. The puncture rows and columns were generally spaced one-half millimeter to one millimeter apart. In practice this seldom turned out to be the case. The pial sheath at the level of the exposed obex is such that all but the hardiest electrodes come to grief when a puncture in this region is attempted. Hence, most punctures in this area were gladly accepted wherever they happened to fall. Elsewhere on the medullary surface, blood vessels had to be avoided, as did the midline. Extreme lateral portions were not available either as the electrodes usually will not enter the tissue unless the approach is at a near normal angle. The coverage obtained in these experiments was unquestionably weighted in favor of the regions of the dorsal column, the descending fibers of the fifth nerve and their respective nuclei. The rostral medulla was ignored completely. The more ventral portions of the medullary areas considered also suffered from lack of equal representation with respect to the dorsal half.

Individual punctures were conducted in the following manner. The medullary surface having been broached - not always an easy matter as has been noted above - the surface position was noted on the vernier of the microdrive apparatus controlling the electrode movement. The tip was lowered until a single unit was discernable from the background. An attempt at amplitude maximization was made. This usually served as an indication as to whether the unit was responding primarily to the electrode or to, hopefully, something else. A wait of a minute or two gave an idea as to the permanency of the unit. If the amplitude and frequency remained reasonably constant over this period, it was assumed that the unit in question was not responding, as least primarily, to the pressure of the electrode. At this point mechanical stimulation of the body surfaces was begun to determine whether the unit could be so driven and thus attributed to the somatic sensory system.

The recording periods ranged from a minimum of 1.5 minutes to over an hour. The length of time that a unit was recorded depended upon a number of factors such as the frequency of the unit (that is, how rapidly the spikes accumulated), the unit's viability and, to a certain extent, the durability of the experimenter. "Good" units would usually grow weaker or stronger in amplitude as the electrode was advanced or retracted slightly, but the unit's frequency would remain unaffected by electrode movement. Once a "good" unit was obtained, it was not bothered by further movement of the electrode nor was the animal touched at all until the desired data had been collected.

However, certain units responded with varying discharge patterns to changes in limb and jaw position. This positions could be varied to elicit a new firing rate or pattern in the unit being recorded. Once the somatic or non-somatic nature of a unit had been determined, the animal was left alone with the exception of units 67129-2L, 67132-7A, 68145-3A-1 and 68145-3A-2. Further details on these "driven" units will be found in appendices D and E.

C. Data Processing

Playback procedures. Preliminary to the actual construction of either interspike interval histograms or average firing rate profiles, the taped data were played back through both the audio monitor and an oscilloscope. Qualitative judgments were then made as to the retrievableness and subsequent reliability of the signals. Where needed, sharp low frequency filtering and/or rectification of the spike train under examination made the creation of standard pulses much easier. It was not infrequent that two or more spikes would be present simultaneously. Our equipment usually permitted the retrieval of the data when two spikes were present. Three spikes usually resulted in retrieval of the average firing rate profiles but prevented an accurate construction of the interspike interval histogram of the smallest amplitude spike. For instance, two spikes of similar positive amplitude might have dissimilar negative amplitudes. Rectification of the positive portion of the signal and inversion of the negative portion would allow the amplitude gate to select one or the other of the signals. For these purposes both a voltage amplitude gate and a voltage "window" were used. The window was useful when the spike train of interest was of less amplitude than some interfering train. Both gating devices put out standard pulses which were then replayed into the oscilloscope and visually compared with the original neural signal. A one to one correspondence of shaped pulse to original spike was required before further processing took place.

A CAT 400B computer was programmed to construct either interspike interval histograms or to count the spikes falling within a measured time interval. Close visual and aural monitoring of the signal while it was undergoing processing allowed some compensation for amplitude changes to be made. Large artifacts could sometimes be removed from the record by "riding" the input gate control if it was know when to expect these interfering signals.

<u>Construction of the average firing rate profiles</u>. The data presented in this thesis which is referred to as "average firing rate profiles" were obtained from the CAT 400B computer under the following conditions. An external square wave generator operating at 0.1 Hz/sec. advanced the CAT address every 10.0 seconds. Any pulse arriving at the CAT memory input during a given ten second interval would be stored

consecutively until the address was advanced to the next channel. In this way it was possible to obtain the number of spikes per ten seconds for periods extending to 4000 seconds. The ten second bin width was used throughout these experiments. Thus, all the profiles are directly comparable, though some plotted profiles are "doubled" into 20 second bins to conserve plotting space.

Computation of the interspike interval histograms. Interspike interval histograms could be computed directly with the CAT in its H or histogram mode. The entire 400 channel address bank would be swept at some predetermined rate such that the full scale sweep time was substantially longer than the majority of the interspike intervals. Each spike would initiate a sweep; its successor would terminate it, entering that portion of the sweep which had been completed into the appropriate channel's memory, and starting the process over again. For most of the units in this study a sweep time of 1.0 second was used, giving a bin width of 2.5 milliseconds for each of the 400 channels. A few slower firing units required somewhat longer sweep periods.

Data presentation. The processed data were printed out on a teletype printer. Analogue output into an X-Y plotter was also possible and was used for on the spot checks of progress during computation. The data presented in graphic form in this study were plotted by hand from the numerical teletype data for reasons of accuracy and format. The plotted data are presented in appendices D and E.

D. Histology

At the termination of the recording session, an electrode was driven into the medulla at a location sufficiently far from the recording sites so as not to be mistaken later for a true puncture. This electrode was released from the driving mechanism and its shaft clipped off just above the point of entry into the medulla. The purpose of this was to provide a slicing plane reference for later microtoming so that the punctures would be in the plane of the cut. This accomplished, the animal was released from the head holder given a fresh dose of anesthetic and lowered to the floor, chest up. An intercardial perfusion of 0.9% saline followed by 10% formalin in 0.9% saline served to fix the tissue. The head was removed, partially skinned and placed in a bucket of fixative to await removal of the medulla. The carcass was incinerated.

After the medulla was removed from the head, it was cleaned of excess dura and epipia and dehydrated in successive stages preparatory to celloidin embedding. The blocked medulla was sliced at 30 microns with alternate sections being Nissl (thionin) stained. Alternate sections of the remaining half of the slices received either a Weil (hematoxylin) or a Sanides Heidenhain (hematoxylin) stain for myelinated fibers.

Generally the thionin sections were sufficient for electrode track localization. The tracks appeared as a greenish-yellow streak where gliosis had occured in response to the presence of the electrode. Finer anatomical detail was often discernable with the other stains. Used conjointly, these stains made track localization and unit identification possible as a matter of routine.

Appendix C gives the tracings of the electrode tracks with puncture locations of relevant units for the six animals in which these techniques were used. Individual unit locations within a puncture were roughly located on the basis of response to stimulation, if any, and relative depth as recorded from the microdrive verneir. Placing an electrolytic lesion at some known depth in the puncture sometimes allowed the precise location of a particular unit. Other units in the puncture could then be approximately located by using a simple proportional relationship. Differential tissue shrinkage and other factors make these methods rather imprecise, however.

Appendix G

The Recipe for Dial-Urethane

1. Dissolve 29.5 grams of dial (5,5-diallybarbituric acid, Gane's Chemical works, 611 Broad St., Carlstadt, N.J.) in 150 cc. of distilled water together with approximately 35 pellets of sodium hydroxide. Warm slightly until fully dissolved.

2. Dissolve 94.6 grams of urethane (Sargent, USP grade) in 300 cc. of distilled water.

3. Mix 1. and 2.

4. Dosages:
    a. initially: 1.0 cc. per pound of animal (sheep)
    b. maintenance: 0.25 cc. per pound of animal as needed.