AN ELECTROPHYSIOLOGICAL STUDY OF CHEMOSENSORY CHARACTERISTICS OF *DACUS OLEAE* (GMEL.)-ADULTS (TEPHRITIDAE, DIPTERA).

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Report on a research period
spent from april to november
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INTRODUCTION

As part of studies into the biology of the olive fruit fly, Dacus oleae Gmelin, this report considers the possibility of combining electrophysiological techniques with behavioural tests, both of which can yield better understanding of the relationship between D. oleae and the olive tree, Olea europea, its principal host-plant. More in particular the electrophysiological approach allows relatively rapid screening of potential sensory stimuli that may consecutively affect oviposition behaviour (Girolami et al.,1981).

In this study preliminary observations on electrophysiological characteristics are presented. Labellar and tarsal sensilla that respond to salts and some other compounds were mapped. Experiments were performed on environmental factors such as light and photoperiod, temperature and type or availability of food source, that affect the nutritional status and ageing of the adult flies, and thereby the electrophysiological responsiveness.

Knowledge on the way in which these variables affect chemosensory responses of phytophagous Dipterans is relatively scarce, certainly in comparision with the well-investigated saprophagous Diptera such as Phormia and Calliphora. Moreover, several particular difficulties have been encountered in electrophysiological recording from the small and specialised Tephritidae by several workers in this field (E. Staedler,R. Crnjar, pers. comm.;Stoffolano, 1973; Fujishiro et al., 1984).

SHORT BIOLOGY

The olive fruit fly, Dacus oleae Gmel. (further on designated as O.F.F), is found all over the Mediterranean area.
During the springtime, the O.F.F is normally occuring in the adult stage and occasionally, as pupa or larva, inside some olive-fruits that have remained on the trees, since the previous season.
In the spring and early summer the adult feeds on various types of food, e.g. on honeydew. In Italy, at the end of the summer, when the olive fruits have reached a certain minimum size, Dacus oleae adults invade into the olive orchards and the females oviposit into the ripening fruits.
Normally there are 2-8 generations per year on the olive tree and in the warmer region along the Mediterranean coast, these may overlap (Girolami,1978,1979).
The adult flies may well live up to 4-5 months of age.

MATERIALS AND METHODS

BREEDING

The O.F.F adults used were collected, at the end of June 1984, from olive orchards in Liguria, Italy and then reared upon fresh Ascolana olives (early variety) in the laboratory. Artificial diets were not used for larvae because Dacus oleae has a very delicate bacterial symbiosis (Girolami & Cavalloro, 1972; Girolami, 1973)
The adults were kept in a cold room at 15 degrees C (15/9 P/S) or at 15 degrees C, in an illuminated incubator with a photoperiod of 11:13 hrs P:S.

Separate groups (+/-100) of flies were isolated from the stock-group maintained at 15 degrees C and kept at different temperatures, according to experimental designs (10-15 degrees C; 20-25 degrees C; 27-30 degrees C).

All flies were offered 'normal fly food', consisting out of a mixture of sucrose, yeast and water (5:1:7) (Cavalloro & Girolami, 1968; Girolami, 1979).

This food-source was always available, apart from experiments in which starvation-times were applied to study their effects.

Flies were kept in 30x30x30 cm or 10x10x10 cm cages that consisted of metallic or plastic frames in which fine plastic nets were stretched.

Under these conditions, adult flies can reach an age of up to 4 months.

STIMULI

PURE COMPOUNDS

Sodium-chloride (NaCl) was employed as a standard physiological stimulus. Ammonium-chloride was used as a general microbial breaK-down product of proteins and may be considered as an ubiquitous indicator of nitrogenous compounds essential for fly-nutrition. Methylic and ethylic alcohols served mainly as solubilising agents for moderately polar plant compounds (e.g.phenols) and were applied in concentrations which may also be considered as normally occuring in O.F.F.-food sources.

TABLE

	COMPOUND	CONCENTRATION	SOLUTION'-CODE'
(1)	NaCl	0.001 M	' C '
(2)	NaCl	0.002 M	' L '
(3)	NaCl	0.100 M	' H '
(4)	NaCl + ethanol	0.001 M / 0.01%	' C1'
(5)	NaCl + ethanol	0.001 M / 0.05%	' C2'
(6)	NaCl + ethanol	0.001 M / 0.10%	' C3'
(7)	NaCl + methanol	0.002 M / 0.01%	' L1'
(8)	NaCl + methanol	0.002 M / 0.05%	' L2'
(9)	NaCl + methanol	0.002 M / 001%	' L3'
(10)	NaCl + methanol	0.100 M / 0.10%	' H1'
(11)	NH4CL	1.000 M	' N '

In the course of the investigations, solution (5) became the standard control.

NATURAL STIMULI

In order to obtain an impression of the reaction strength occurring upon natural stimuli,a 1:1,000 dilution in solution (5) of an olive-fruit extract (O.F.E.) obtained after a steam distillation procedure was applied (Girolami et al., 1983).

Out of 15 Kg (7500 pcs.) of still ripening olives 3 Kg of oil were obtained. This oil-fraction yielded at first distillation 50 micro-litres of steam-distillate extract (O.F.E.).

It can be calculated that the mesocarp of one olive yields approximately 65 nl of compounds thus extracted. The 1:1,000 dilution represents 15-times natural concentration , when assuming a homogeneous distribution in the mesocarp tissue. The more probable concentrated localisation of the trapped compounds leads us to conclude that O.F.E. concentrations applied were equal to natural (or lower).

ELECTROPHYSIOLOGY

The standard 'tip-recording'-technique, described by Hodgson, Lettvin & Roeder (1955) was applied to record chemosensory responses from labellar and tarsal-sensilla (L- and T-sensilla respectively).

In short, a 1 cm-long tungsten electrode connected directly to the amplifier-input was inserted into the thorax of the fly.

The indifferent electrode was the stimulus-containing micropipette connected to ground.

Duration of stimulation was between 3-8 seconds.

The biological signal was fed into a 1,000x, 300-3,000 Hz (-3dB-frequencies) biological amplifier designed at the

laboratory of Animal Physiology (cathode follower pre-amplifier (input-impedance 1 T Ohm) for impedance conversion and normal band-pass end-amplifier).

Signals were visualised on a Tektronix 922 oscilloscope and recorded on AKai GX-215D audio-tape recorder at 9.5 cm/sec. tape-speed. Signal to noise ratios ranged from 3 (minimum) to 10; stimulus-onsetartefacts showed a duration between 20 and 50 msec. Quantification of responses were performed according to convenience i.e. the number of spikes generated during the first 1000 msec after the onset of stimulation was the measure of response-intensity.

The recorded signal was printed afterwards on paper by means of a Siemens OscillominK-E (0-1,000 Hz) inK-jet recorder at 10 cm/sec paper speed and 3 volts-sensitivity-range for all recordings.

Calibration-bars are presented along with the recordings, indicating the absolute amplitude measured in m-volts (without amplification).

FLY HANDLING

Prior to electrophysiological recording, every fly was mounted on a blocK of plasticin and fixed by means of a metallic clip over the thorax.

Both anterior legs were positioned and then fixed.

Musculature of head and thorax were carefully crushed with a needle to abolish muscular activity that could disturb the contact between stimulus pipet and sensillum-tip or could otherwise interfere with the chemosensory activity to be recorded.

SHORT CLASSIFICATION OF SENSILLA

LABELLUM

On the labellar surface, 4 rows of setae were distinguished. Within every row, the hairs have approximately the same length. The method of classification used is comparable with that employed by Maes & Vedder (1978) for Calliphora vicina. The proximal row bears the longest hairs and the more distal (marginal) row the shortest (fig.1)
Only few hairs not belonging to any of the four rows, were noticed (cf.fig.1).

TARSUS

The anterior legs of the olive fruit fly were examined from a ventral view.
The sensilla stimulated were denoted with a number, letter and where necessary also a roman number was added to the former two (fig.2).

EXPERIMENTAL SET-UP AND RESULTS

CHARACTERISTICS OF THE ELECTROPHYSIOLOGICAL RESPONSES

Some particular features of the responses obtained in recording from L- and T-sensilla will be summarized in this

section as they are a necessary introduction to the sections to follow.

1. Non-operative sensilla

The criterion to decide whether a sensillum is operative or not, could be made in two ways that differ in their degree of certainty:

a. upon contact with the hairtip,a high noise level was recorded in which it was difficult to discriminate between high noise peaks, artifacts or actual spikes.Only an accurate, computerised analysis of wave-form and wave-duration could justify such a discrimination.

b. a normal noise level was registered on which no spikes were visible until a deliberate bending of the hair was performed using the stimulus-pipet, without loosing contact with the sensillum-tip. The bending-movement in this case resulted into a clear spike-train, most probably originating from the mechanoreceptor (example cf. TAVOLA I fig D). These cases yield a much more convincing proof of the non-responsiveness of the chemosensory neurones.

2. Permanently operative sensilla

In close connection to the previous subsection it must be mentioned that the tarsal sensilla A and B together with the sensilla 2AIII and 2BIII were in a significant higher number of instances operative than all other types of tarsal sensilla studied.As to the labellum, it was found that the setae of the marginal row (shortest hairs) showed a higher percentage of responsive sensilla than the middle or longest-hairs row, generally spoken. Also, in the latter two groupings of spikes were often found (example cf. TAVOLA 2).

Furthermore a high probability of responsiveness seems to be

correlated with larger spike-amplitudes recorded from the sensilla involved.

3. Occurence of high spike-frequencies independent of the onset of stimulation

In many stimulation-attempts of tarsal or labellar setae, after varying time-intervals high spike-frequencies with several spike-amplitudes (though mostly one is dominant) that show almost no adaptation during several seconds, were occurring in the recordings. It is important to mention in this respect that the same phenomenon was observed by contacting the intact cuticle of the pulvilla, with the stimulus-containing pipet or merely with a metal wire; on the pulvilla no setae are present (example).
Especially in the sensilla belonging to the marginal row of the labellum, these phenomena were very often encountered, which makes the quantifying of gustatory activity very unreliable (example). Most probably, these spike-trains are not at all produced by chemosensory cells but rather reflect muscular activities.

RESPONSIVE PERCENTAGES UNDER SEVERAL LIGHT-CONDITIONS

Four groups of flies of the same age were studied with respect to the responsiveness of L- and T-sensilla under different light regimes. The actual age of the adults during the electrophysiological tests ranged from 21-35 days.
Solution 1, 3 and O.F.E. were used as stimulants.

TABLE OF LIGHT-REGIME TREATMENTS

GROUP	ARTIFICIAL (*)	DAYLIGHT	P:S	TEMP C
1	+	-	11:13	15
2	+	-	15:9	15
3	+	+	15:9	15
4	-	+	14:10	23

(*) Artificial fluorescent TL-light, intensity +/- 1,000 Lux.

RESULTS

In all four groups of flies, low percentages of responsive sensilla were encountered. For every group, some 30 flies were tested and in every fly 10-12 sensillawere screened for responsiveness; mostly only one sensillum was operative on theaverage; these were not consistently thesame. Some flies, examined during the scotophase also showed poor responsiveness of T- and L-sensilla.

At the ages and temperatures studied, the different light regimes employed did not raise the percentage responsive sensilla.

EFFECT OF AGE SINCE ECLOSION OF ADULT O.F.F. FROM PUPAE

A pool of pupae close to eclosion were kept in an incubator at 10 deg. C; on week-days, 5 pupae per day were transferred to +/- 23 deg. C till, after a few hours, eclosion occurred. Then they were transferred to 15 deg. C 11/13 P:S. In this

way every week newly emerged flies were available.

RESULTS

Newly emerged Dacus oleae-adults, tested on day 1 (post-eclosion), showed a large (>90%) percentage of responsive L- and T-sensilla in reaction upon solutions 1, 3 and 11.
However, this percentage rapidly declines to about 50% on day 2, while on day 7 only 10% of the tested sensilla show reactions of chemosensory neurones.

EFFECT OF TEMPERATURE-REGIME

To study the effect of the rearing-temperature on responsiveness of L- and T-sensilla 1 week old flies, kept at 15 deg. C were transferred to 27-30 deg. C immediately prior to electrophysiological testing during at least 2 hours and at the most 24 hours.

RESULTS

The flies kept at 15 deg. C since eclosion normally showed a low percentage (+/- 10%) of responsive sensilla for both L+T, at more than 7 days of age.
However, it was very clear that after the warming treatment at 27-30 deg. C almost all sensilla responded to the stimuli tested.
Even after a period as short as 2 hours,the percentage responsive sensilla significantly increased.
Furthermore it was found (see next section) that the

responsiveness obtained after warming was retained during several weeks, even if the flies were kept since this 24 hours of warming (at 27-30 deg. C) at 15 deg. C again. This is in marked contrast to flies kept constantly at 15 deg. C which always showed poor responsiveness, except the very young ones (cf. section 'age').

EFFECT OF FOOD-SOURCE AT LOW TEMPERATURE

To judge the effect of food-source on the responsiveness of the O.F.F., 15 flies (7 days old) were kept 1 day at 30 deg. C to increase their responsiveness (see previous section). Three flies were tested to check if indeed this increase had come about.
The remaining 12 flies were split into two groups of 6 flies.
Both these groups were kept 6 days at 12 ^{o}C in an incubator; one group had available 'normal fly food', the other was fed with a 5% sucrose solution.

RESULTS

No large differences were found between the groups; the suspection that normal fly food may obstruct sensillar pores by pollution with sticky material was not confirmed.
In both groups a good responsiveness (high percentage responding setae) was found, as expected after warming up at 27-30 deg. C.

EFFECTS OF STARVATION

To investigate the effect of starvation, an experiment was performed with a total of 30 flies.

These flies were less than 7 days of age because at this age the animals still perform a rather high rate of food-intake, probably related to maturation of developing ovaria in females.

Two groups were made of 15 flies each: both groups were starved with access only to water during 24-48 hours. The first group was kept at 30 C,the second one at 15 deg. C.

RESULTS

In normally fed flies kept at 13-15 deg. C, tested in all previous experiments, a low percentage of responsive sensilla was encountered in all cases (this refers to observations on more than 40 individuals). The deprivation-treatment in the 15 deg C-group resulted into a significant increase in responsiveness, while in the 30 deg C-group a somewhat higher responsiveness than the already high fraction of operative sensilla was observed.

QUANTIFICATION OF THE ELECTROPHYSIOLOGICAL RESPONSES IN INDIVIDUALS WITH A HIGH FRACTION OF OPERATIVE SENSILLA

In this section some quantitative data will be presented on the sensitivity of the chemosensory neurones in response to four types of stimuli. Four types of tarsal sensilla are taken into consideration for this aim. Data on labellar sensilla, though available, are not included because the latter could not be defined individually. A graphical representation is given in figure . Corresponding

statistical data are given in table FIG 9 . The recordings themselves can be judged in a separate addendum. A short description of the results is given in the corresponding section of the discussion.

TABLE (p. 14)

Quantification of electrophysiological responses: four stimuli on four sensilla

sensillum	solution	control				H				N				O.F.E.			
	X	SD	SEM	n1	n2	X	SD	SEM	n1	n2	X	SD	SEM	n1	n2	X	SD	SEM	n1	n2
A/B	48	15,7	5,9	7	9	41,4	11,4	3,8	9	12	81,3 7,0	11,7 2,2	6,7 1,2	3 3	3 3	18,6	9,5	4,2	5	5
1A/1B	20,7	3,1	1,8	3	3	19,1	7,3	2.4	9	10	22,4 22	10,3 10,4	4,6 4,6	5 5	5 5	n.d.				
2AI/2BI	16,3	5,9	3,4	3	3	26,2	11,5	2,7	18	29	40,7	7,1	3,2	5	6	n.d.				
2AIII/2BIII	28,4	6,9	2,4	8	8	n.d.					43	13	12,7	2	3	26,5	9,3	4,7	4	5

DISCUSSION

Relatively few electrophysiological studies are available on Tephritids as Rhagoletis (Crnjar & Prokopy, 1982; Liscia et al., 1982), Ceratitis (Gothilf et al., 1971) and Dacus (Angioy et al., 1978a and b). This is in contrast with the notion that Diptera as a group are among the best investigated animals at the sensory level (Dethier, 1976). However, the vast majority of studies were performed on the non-phytophagous Phormia, Calliphora, Sarcophaga and Musca. Chemoreception of phytophagous Diptera was reviewed by Schoonhoven (1983).Therefore the discussion of this subject is limited to the specific context of this study. A second important theme in this report is the discovery of changes in sensitivity as correlated with environmental factors. By now, sensitivity changes in insect chemoreceptors have become rather well documented. Recently, the literature on this subject, including possible causal factors involved and regulation mechanisms, was reviewed by Blaney et al. (1985, in press).For this reason, we will only briefly discuss the findings on Dacus presented here in their relation with relevant literature data and focus on the remarkable effects of temperature and starvation.

THE EFFECTS OF LIGHT REGIME

As already noticed, in the four groups kept under different light regimes no difference in the very low percentage of operative sensilla encountered was found.

To our knowledge, only Omand and Zabara (1981) found a clear

effect of sustained darkness on the number of operative sensilla and the sensitivity of the chemosensory cells of Musca. No other instances of short-term effects of light regime on chemoreceptor responsiveness are known.

AGE EFFECTS

In Dacus oleae, only very young flies (age one day post eclosion), when kept at temperatures below 25 deg C, show a high percentage of operative sensilla (> 90 %). A rather steep decline of this fraction is occuring during the first seven days post eclosion, when only about 10 % of responsive sensilla was found (i.e. in animals kept at 15 deg C). Rees (1970) found a comparable decline in Phormia although in a slower rate (< 50 % operative sensilla after 25 days).
In an other Tephritid dipteran, Rhagoletis cerasi, the same phenomenon is observed. The rapid decrease in sensitivity, expressed as the percentage of sensilla that respond out of the total number of sensilla tested, can be described with a hyperbolic curve, indicating a steep drop in responsiveness during the first two days (E.Staedler, pers. comm.).
It is difficult to understand why the responsiveness is declining so rapidly after eclosion while Dacus can reach an age of upto five months.

TEMPERATURE

As to the effect of temperature on the responsiveness of L-and T-sensilla, it was discovered that Dacus oleae-adults, that were kept at temperatures higher than 25 deg C (till 30

deg C) for only two hours or more (till 24 hours) showed a large increase in the fraction of operative sensilla. Furthermore, this increase was stable for at least two weeks, even if the animals were kept at lower temperatures since this "warming up"-treatment (mostly at 15 deg C). As far as we know such a drastic effect of temperature regime on such a short term has never been reported in the literature.

Several possible explanations can be put forward with regard to this temperature-related phenomenon.

It can be suggested that the electrical resistance of the hair is influenced by a temperature-effect on the tormogen-cell. High electrical resistance would then prevent the recording of sensory responses. Another resistance-increasing factor may be found on the formation of a protective wax-layer at the hair (pore) surface. This wax-layer could then be damaged by a higher temperature (F.Hanson, pers . comm.).

Temperature also affects the general activity level and locomotion, while feeding- and cleaning-behaviour are more intense at higher temperatures. However, cleaning of sensilla of flies kept at 15 °C which showed a poor degree of responsiveness, did not improve the latter.

From behavioural observations under natural conditions it is known that the activity of Dacus oleae is to a high degree correlated with temperature, even over small ranges of variation (Girolami, 1978; 1979). Starting at 7 deg C, Dacus-adults show slow locomotory movements and drinking behaviour. At 15 deg C, the temperature most frequently applied in this study to retard ageing processes, vertical flight behaviour, copulation and oviposition are observed. Optimum temperatures can be indicated to be around 23-25 deg

C. Above 30 deg C, Dacus has a very high need for water-intake, as it cannot live much longer than few hours without (Girolami, 1979). Indeed, the effect of temperatures higher than +/-25 C on the rise in the percentage responsive ('operative') L+T sensilla was striking and reproducible.

However, it is difficult to explain that flies exposed once to +/-25-30 deg C remained more responsive,even when transfered back to 15 C again.

It may indicate that temperature is not the only factor involved, but acts as a trigger for a process that is responsible for the increase in chemosensory sensitivity in a more direct way.

Perhaps diapause-effects (Stoffolano, 1973), broken by higher temperatures are involved. Preliminary results on protein patterns obtained with electrophoretic methods indicate that the temperature-history results into clear differences in these patterns in the female head-homogenates, even after short periods (15 days) of chilling at 15 deg C (Moretto,E. et al., unpublished results).

FOOD SOURCE

No significant differences were found in the number of operative sensilla between groups provided with different food sources ('normal fly food' vs. sucrose only) during 6 days at 12 deg C. Stoffolano (1973) reported on effects of food type on the sensitivity of Phormia chemoreceptors and gives the only example of such an effect known to us. Apart from a direct effect of food source, some cases have been documented in which the timing of feeding activity seemed correlated with corresponding changes in sensitivity of

chemoreceptors, which are thought to be functional in relation to internal maturation processes (Pappas & Fraenkel,1977,1978; Davis & Takahashi, 1980; Bowdan,1982).

STARVATION

When given access only to water, a surprising increase in responsiveness was found in flies kept constantly at 15 deg C for 8-9 days of age post eclosion; such a high fraction of operative sensilla was never encountered in fed flies (the normal situation) of the same age kept at the same temperature. However, the effect of starvation was not as strong as that of "warming". Also in the 30 deg C group, starvation seemed to increase both the fraction of responsive sensilla and the sensitivity of the cells. So it was qualitatively established that there seems to be a reinforcement of the higher temperature effect and starvation. A comparable influence of starvation was registered for Musca by Omand and Zabara (1981). Since a higher temperature stimulates flight- and walking-behaviour, resulting in a higer need for nutrients to replenish the lowered blood sugar level, this may lead to a higher responsive fraction of L+T-sensilla in Dacus oleae.

It is known from field observations that Dacus oleae-adults,living in environments with a low humidity, are continuously in need for water to prevent the danger of dessiccation. Therefore, at temperatures higher that 25 C, a high sensitivity of water receptors may be functionally important.

QUANTIFICATION OF THE ELECTROPHYSIOLOGICAL RESPONSE

As pointed out earlier, a severe selection was made prior to presentation of quantitative electrophysiological data; only data on 4 solutions and four, exclusively tarsal, sensillar types are given.

Still some conclusions may be drawn from the summarizing graphical presentation of fig. .

1. It seems that the different tarsal sensilla studied react in different ways to the four solutions tested (at least in several cases), as can be judged in a comparative view of the vertical rows of bars.

2. Though no systematic concentration-series were applied, it can be said that:

a. no significant difference in reaction-intensity upon stimulation with 1 mM NaCl or the mixture 1 mM NaCl + 0.05 % ethanol (i.e. the standard control) was observed

b. only for sensillum 2AI or 2BI a higher response was obtained in answer to 100 mM NaCl as compared to 1 mM NaCl + 0.05 % ethanol. We think that it is justified to consider these responses as belonging to the category of 'water responses'.

3. In most cases, unicellular reactions were obtained. Only 1 M NH_4Cl caused in tarsal sensilla A or B and 1A or 1B clearly a bicellular response. Caution must however accompany this observation, because discrimination of activity originating in different cells cannot unequivocally be based upon the criterion of difference in spike-amplitudes as was used here and in the majority of the insect-chemosensory publications. Indeed, especially in the small sensilla of these Tephritid flies special problems can be expected to

occur, as has been proven for Drosophila in this respect by Fujishiro et al. (1984).

4. In the tonic phase of the reaction, lasting from 0 - 500 msec after the onset of stimulation in most insect chemosensilla, also in recordings from Dacus-sensilla a fast adaptation occurred. This is exemplified in figures TAV. 3 d,e TAV 4 I.II.° TAV 22 ! TAV. 5.

5. For only 2 sensillum-types (A/B and 2AIII/2BIII) comparison of stimulating effectiveness of control-solution with O.F.E. is possible. While in sensillum A/B a consistent inhibition occurs with respect to the proper control (solution 5), in sensillum 2AIII or 2BIII no differences between the 2 stimulating solutions were registered. It may be noted that sensilla 2AIII /2BIII are, as far as position is concerned, comparable with the oviposition deterrent pheromone-sensitive'D'-hairs of Rhagoletis pomonella, as described by Crnjar and Prokopy (1982).

(TAV. 6)

THE NATURE OF THE ELECTROPHYSIOLOGICAL RESPONSE-REPRISE

Naturally a profound influence on the quantification of the chemosensory responses is exerted by the qualitative nature of the recordings obtained. Features of this qualitative nature are i.a. duration of the stimulus-artifact, signal-to-noise ratio, unicellular vs. multineural, the unwanted presence of spikes originating from cells other than chemosensory neurones.

We have reasons to believe that many of the recordings obtained during our experiments are difficult to interprete and thereby unsuitable for quantification because of the presence of high-frequency, non-adapting spike-activity most probably generated by mechanosensory neurones or muscle

contractions. These can also be recognized by their different shape (more unipolar).

It is in this respect that we have our reservations with regard to the recordings presented by Angioy et al. (1978a and b). Their recordings do not show the onset of stimulation, no adaptation can be seen, even after 30-60 seconds of continuous contact high spike-frequencies are found, many responses are multi-neural and the S/R-ratio is rather low (ranging from 2 to 4). Their findings (the only ones on Dacus known from the literature previous to this report) are in several respects in contradiction with our results.

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ACKNOWLEDGEMENTS

At the end of this report I would like to express my gratitude towards all the people who made my stay in Wageningen a very pleasant experience.

First I thank Prof. L. Schoonhoven for raising the grant from the IAC , for offering the opportunity to become acquainted with electrophysiological techniques and for his interest and advices during the research.

Also Prof. V. Girolami for his confidence in me and for his activities in providing practical facilities from out of Italy.

I thank Joop van Loon for his continuous assistance during the work, for his exercise in giving structure to the report, valuable criticisms during its preparation and his help in translating it into English.

Job Klijnstra I thank for guiding me during my first steps in Holland and for his interest and advices during the period.

Willem Bijlsma was always willing to advise me in improving the equipment and to discuss with me about all kinds of electronic matters.

Peter Roessingh helped me skillfully in using the EAG-technique and was always willing to give advice.

Gabriella Tamino assisted me in many respects during my Dutch life and was of help in preparing the report.

Michel de Leeuw and Thea van Bemmel took their parts in

making my stay as nice as possible.

Mr. Scalera, collaborator of the Human Physiology Department of the University of Modena, Italy, is thanked for making available to me the scanning photographs of Dacus-tarsi.

Finally, I express my gratitude to the International Agricultural Centre in Wageningen for their financial support and their hospitality.

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FIG. 1 - drawing of labellum

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FIG. 5 - SEM-photo: detail tarsomeres 3, 4 and 5

FIG. 6 - SEM- photo: detail hairs 'A' and 'B'

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FIG. 8 - SEM-photo: detail 4th tarsomere: side view

FIG. 9 - 23 : ELECTROPHYSIOLOGY

FIG. 9 - Quantification of responses: graphical presentation

FIG.10 - 23 - Time course of firing rate: adaptation-curves.

	sensillum	solution	sensillum	solution
FIG. 10 -	tarsal A	C	id.	O.F.E.
FIG. 11 -	" A	H	id.	H 1
FIG. 12 -	" A	N	id.	H
FIG. 13 -	" A	H	id.	N
FIG. 14 -	" A	C	B	C
FIG. 15 -	" 1A	H	id.	N
FIG. 16 -	" 1A	N	id.	N
FIG. 17 -	" 2A	C	id.	N
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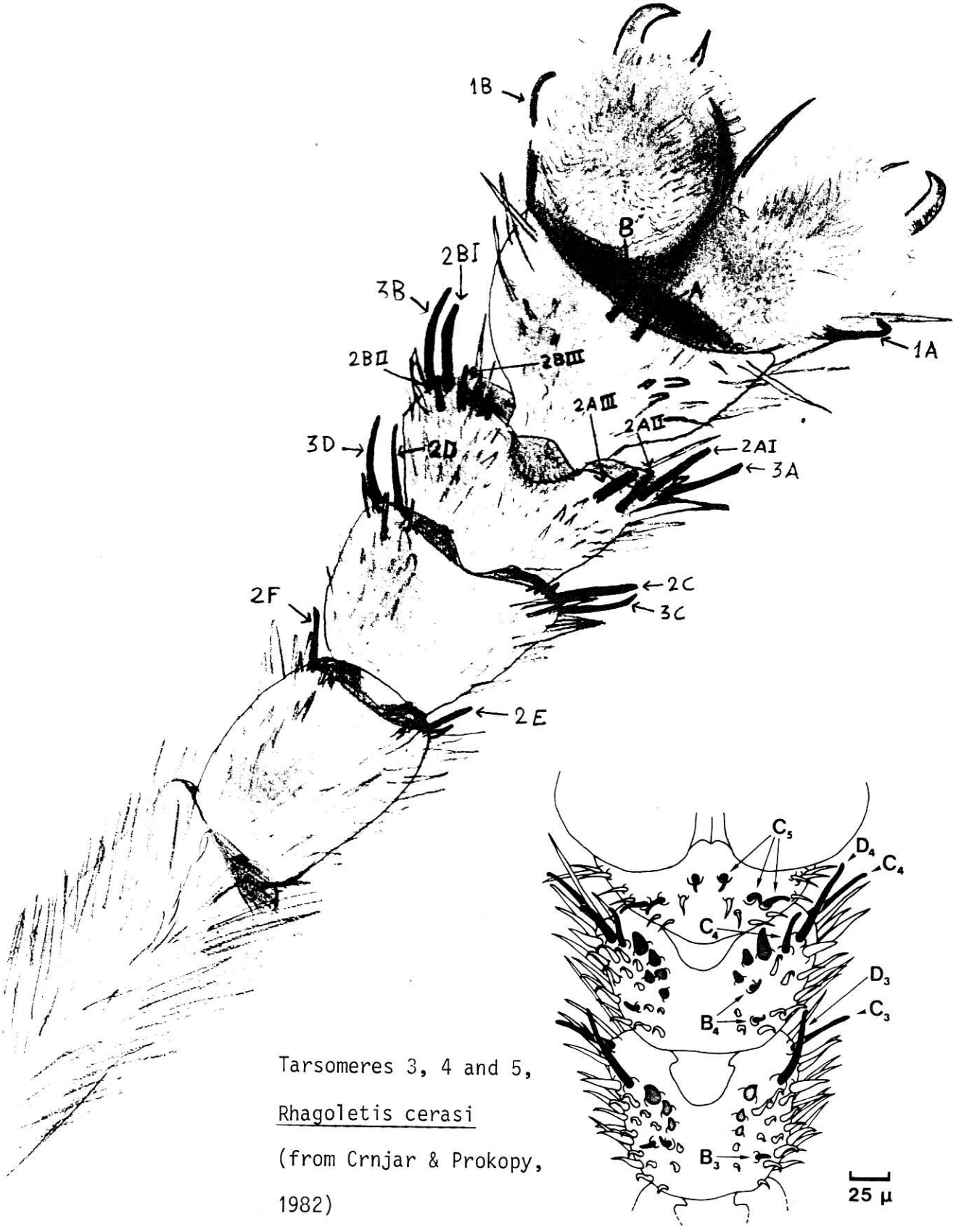
FIG. 20 -	" LP	H	MA	H
FIG. 21 -	" LP	H	LP	H
FIG. 22 -	" M	C	LM	O.F.E.
FIG. 23 -	" MP	C	MP	O.F.E.

FIGURE 2

SCHEMATIC DRAWING OF THE ANTERIOR LEFT LEG OF A DACUS OLEAE MALE, SERVING AS A MAP TO THE TYPES OF SENSILLA DISCRIMINATED.



Tarsomeres 3, 4 and 5,

Rhagoletis cerasi

(from Crnjar & Prokopy, 1982)

FIGURE 1

SCHEMATIC DRAWING OF THE LABELLUM OF DACUS OLEAE , SERVING AS A MAP TO THE TYPES OF SENSILLA STUDIED .

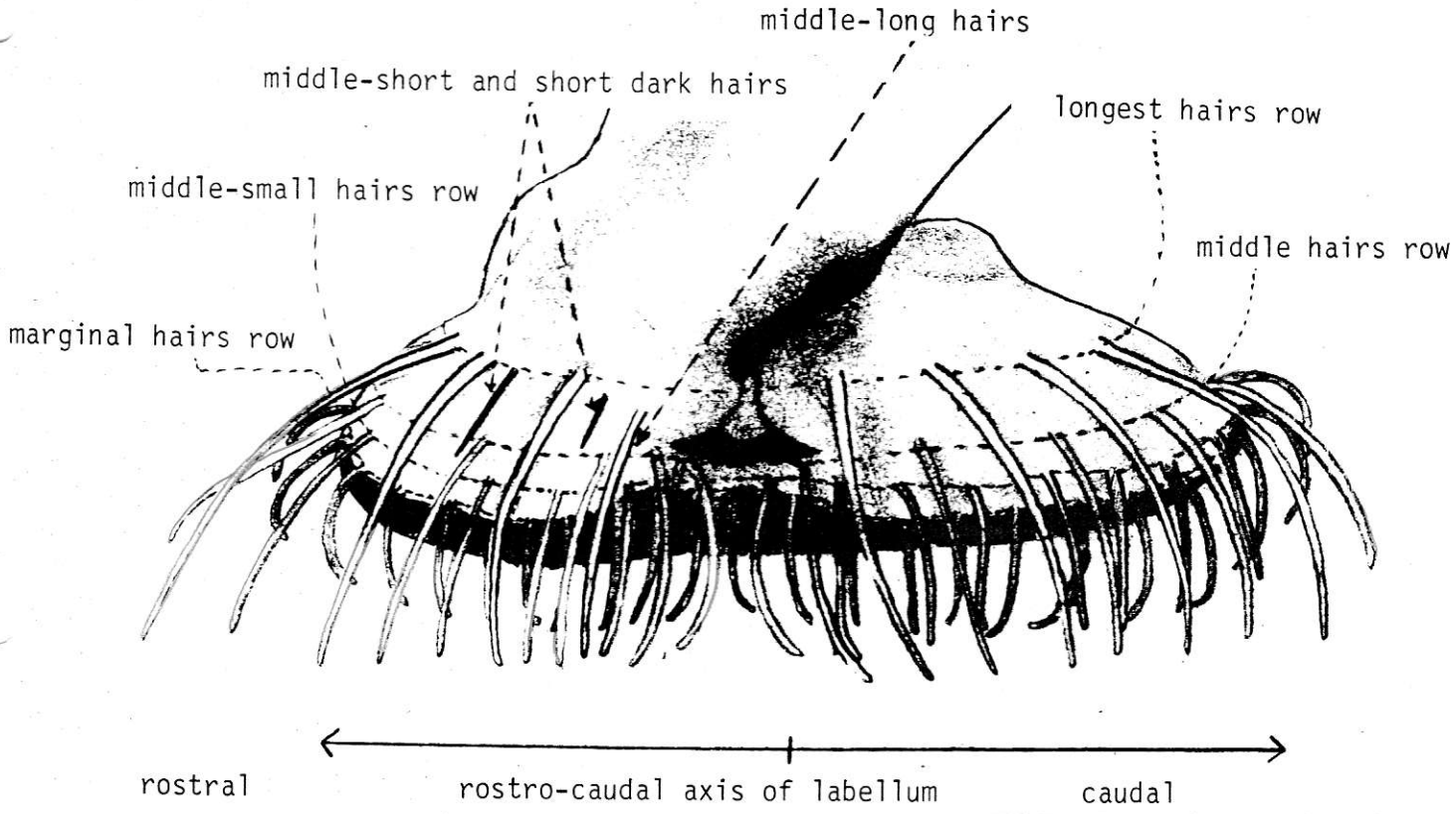
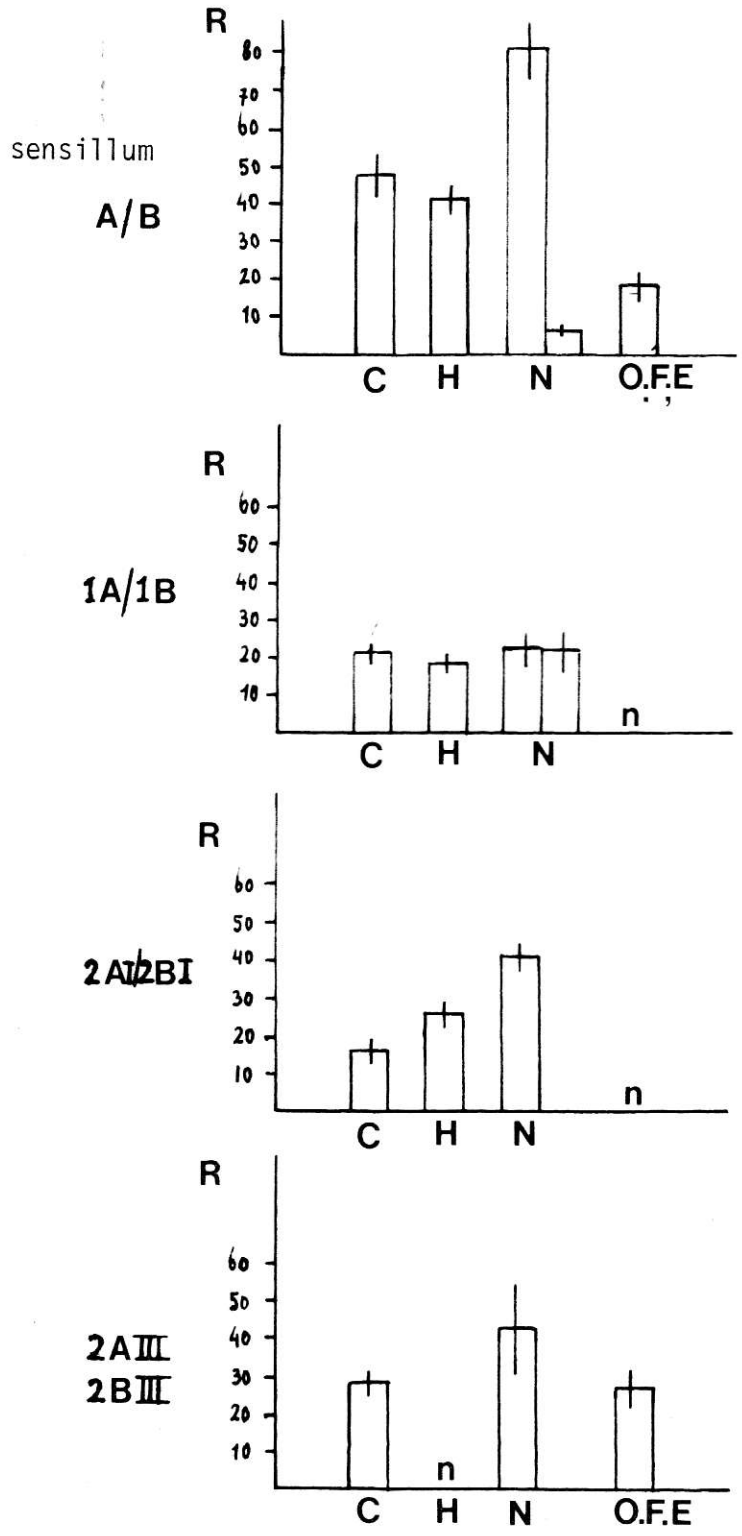


FIG. 9 - QUANTIFICATION OF ELECTROPHYSIOLOGICAL RESPONSES

RESPONSE INTENSITIES (R) UPON STIMULATION WITH FOUR SOLUTIONS

(C, H,N, and O.F.E.) AND IN FOUR TYPES OF TARSAL SENSILLA



LEGENDA :

SOLUTION CODES:

C = CONTROL

(NaCl 1 mM/ethanol 10 mM)

H = NaCl 100 mM

N = NH_4Cl 1000 mM

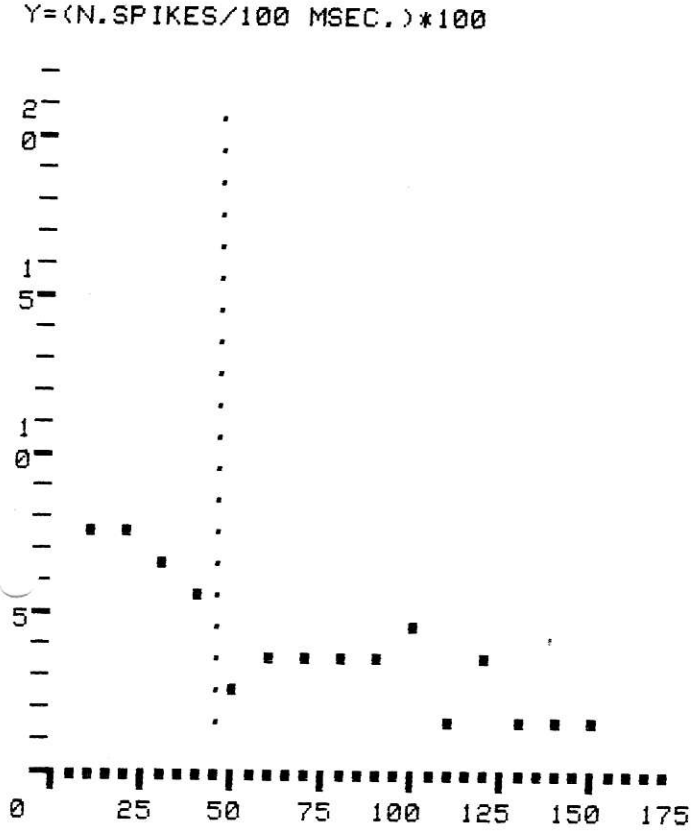
O.F.E. = OLIVE FRUIT EXTRACT

R = RESPONSE INTENSITY

(i.e. the number of action potentials generated from 0 - 1000 msec)

n = not done

FIG. 15



DATE : 21/9/'84

SEQUENTIAL CODE: 7

AREA STIMULATED: TARSAL

HAIRS CODE : 1A

STIMULANT : 0.1 M NACL

FIRST FOUR POINTS :

MEAN-VALUE = 6.25

VARIANCE = .687500007

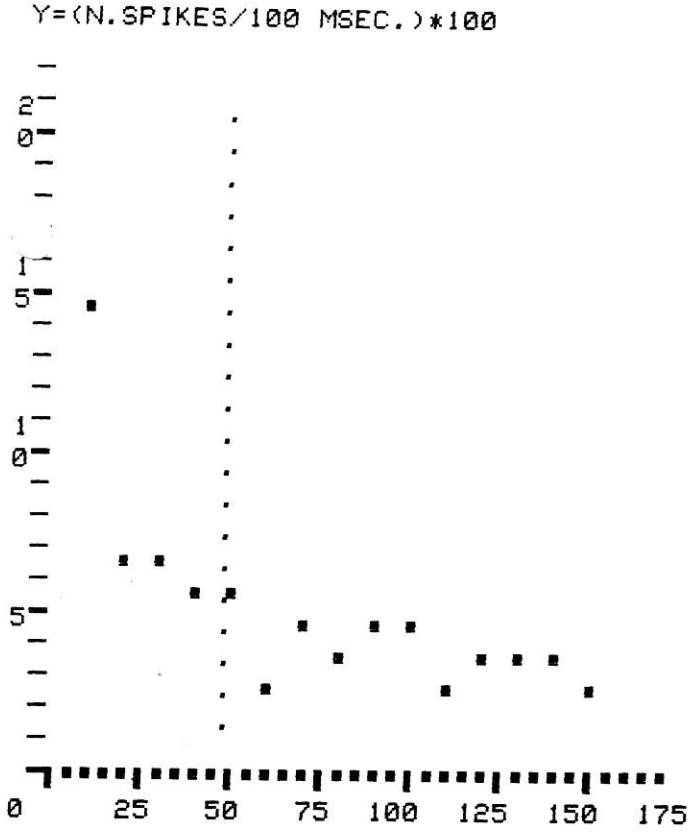
STANDARD DEV.= .829156207

NEXT ELEVEN POINTS:

MEAN-VALUE = 2.27272727

VARIANCE = 1.10743802

STANDARD DEV.= 1.05234881



DATE : 21/9/'84

SEQUENTIAL CODE: 10

AREA STIMULATED: TARSAL

HAIRS CODE : SAME ABOVE

STIMULANT : 1 M NH4CL

FIRST FOUR POINTS :

MEAN-VALUE = 7.75

VARIANCE = 13.1875

STANDARD DEV.= 3.63145976

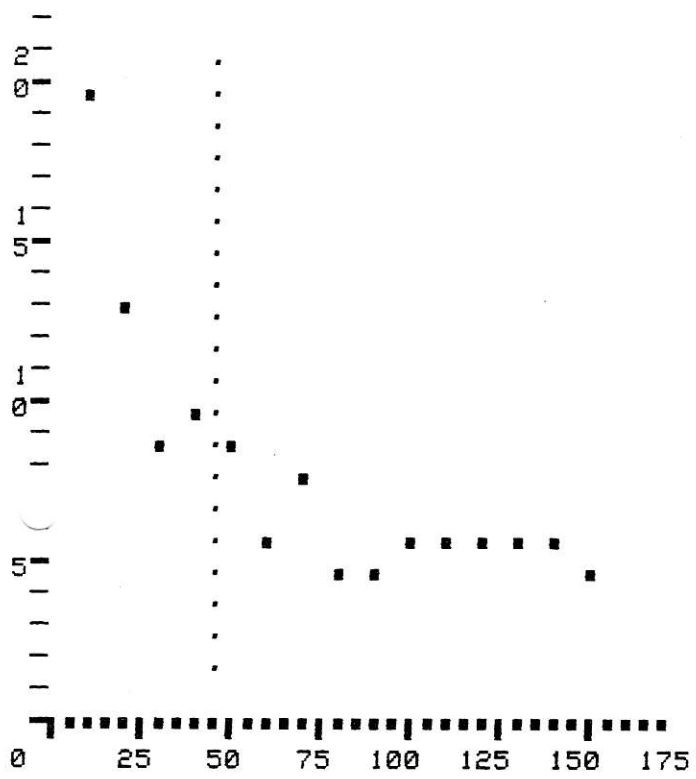
NEXT ELEVEN POINTS:

MEAN-VALUE = 3.18181818

VARIANCE = .87603306

STANDARD DEV.= .935966378

FIG. 14



DATE : 30/9/'84

SEQUENTIAL CODE: 39

AREA STIMULATED: TARSAL

HAIRS CODE : AREA A

STIMULANT : CONTROL

FIRST FOUR POINTS :

MEAN-VALUE = 12

VARIANCE = 18.4999999

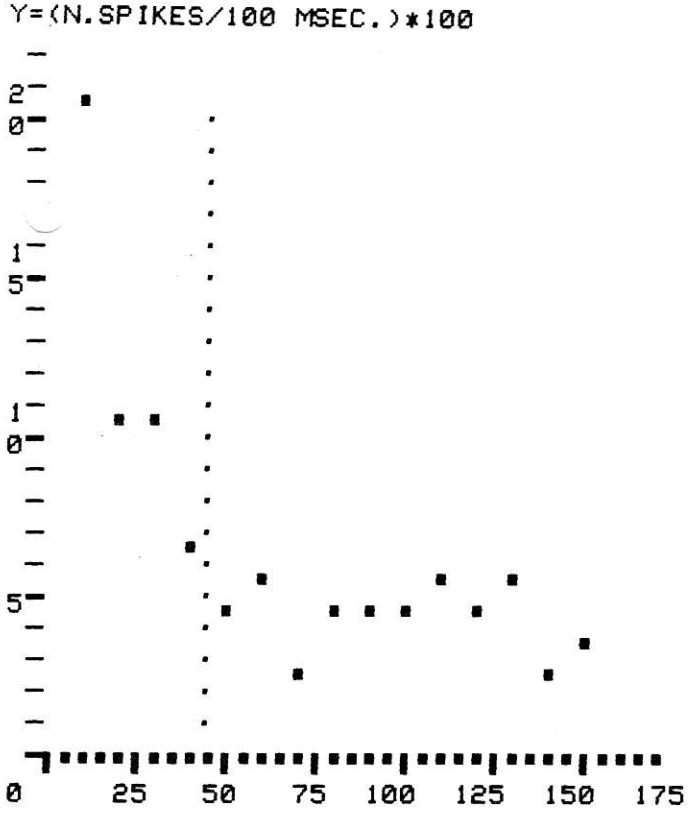
STANDARD DEV.= 4.30116263

NEXT ELEVEN POINTS:

MEAN-VALUE = 5.18181818

VARIANCE = 1.42148758

STANDARD DEV.= 1.19226154



DATE : 30/9/'84

SEQUENTIAL CODE: 40

AREA STIMULATED: TARSAL

HAIRS CODE : AREA B

STIMULANT : CONTROL

FIRST FOUR POINTS :

MEAN-VALUE = 11.5

VARIANCE = 26.7500001

STANDARD DEV.= 5.17204022

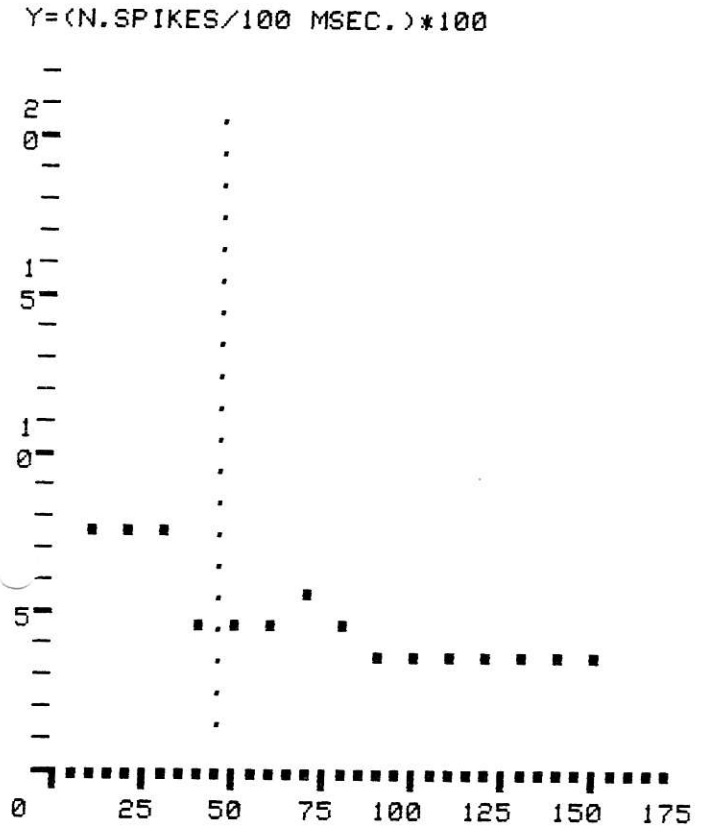
NEXT ELEVEN POINTS:

MEAN-VALUE = 3.81818182

VARIANCE = 1.05785123

STANDARD DEV.= 1.02851895

FIG. 13



DATE : 29/9/'84

SEQUENTIAL CODE: 2

AREA STIMULATED: TARSAL

HAIRS CODE : CENTR.AREA

STIMULANT : 0.1 M NACL

FIRST FOUR POINTS :

MEAN-VALUE = 6.25

VARIANCE = 1.68750001

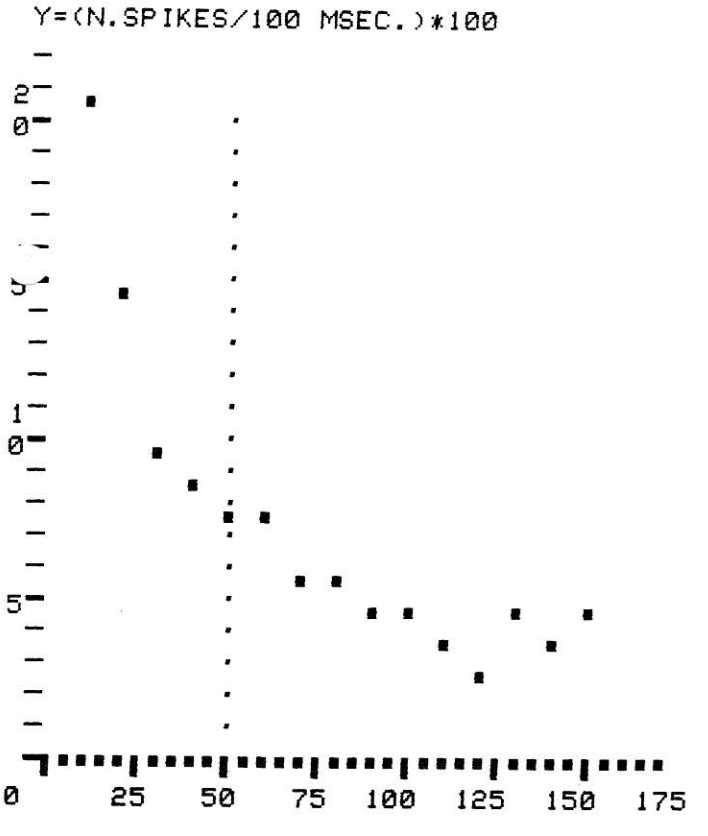
STANDARD DEV.= 1.29903811

NEXT ELEVEN POINTS:

MEAN-VALUE = 3.45454545

VARIANCE = .429752057

STANDARD DEV.= .655554771



DATE : 29/9/'84

SEQUENTIAL CODE: 8

AREA STIMULATED: TARSAL

HAIRS CODE : SAME ABOVE

STIMULANT : 1 M NH4CL

FIRST FOUR POINTS :

MEAN-VALUE = 12.75

VARIANCE = 22.6875001

STANDARD DEV.= 4.76313973

NEXT ELEVEN POINTS:

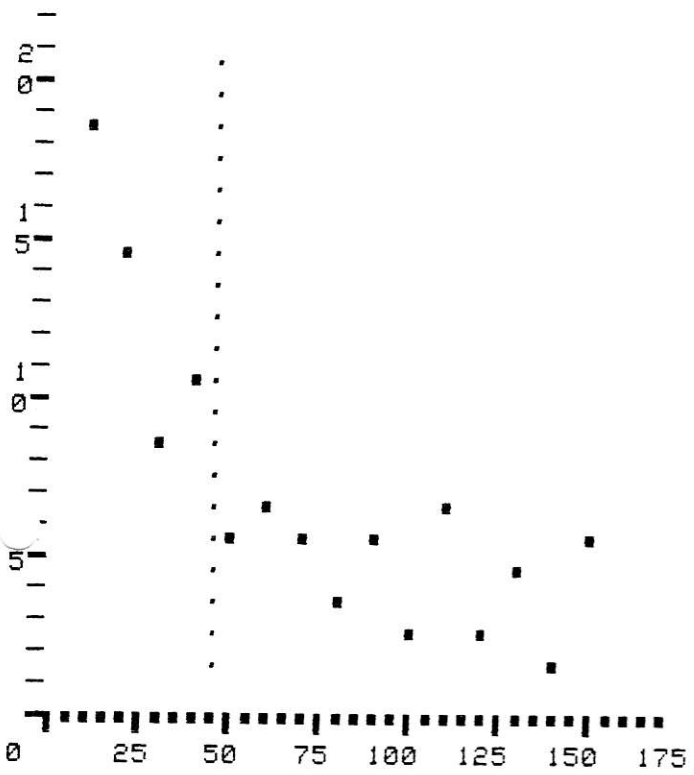
MEAN-VALUE = 4.36363637

VARIANCE = 2.23140495

STANDARD DEV.= 1.49378879

FIG. 12

Y=(N.SPIKES/100 MSEC.)*100



X=1/10 SEC.

DATE : 4/9/'84

SEQUENTIAL CODE: 9

AREA STIMULATED: TARSAL

HAIRS CODE : AREA A

STIMULANT : 1 M NH4CL

FIRST FOUR POINTS :

MEAN-VALUE = 12.5

VARIANCE = 14.7500001

STANDARD DEV.= 3.84057288

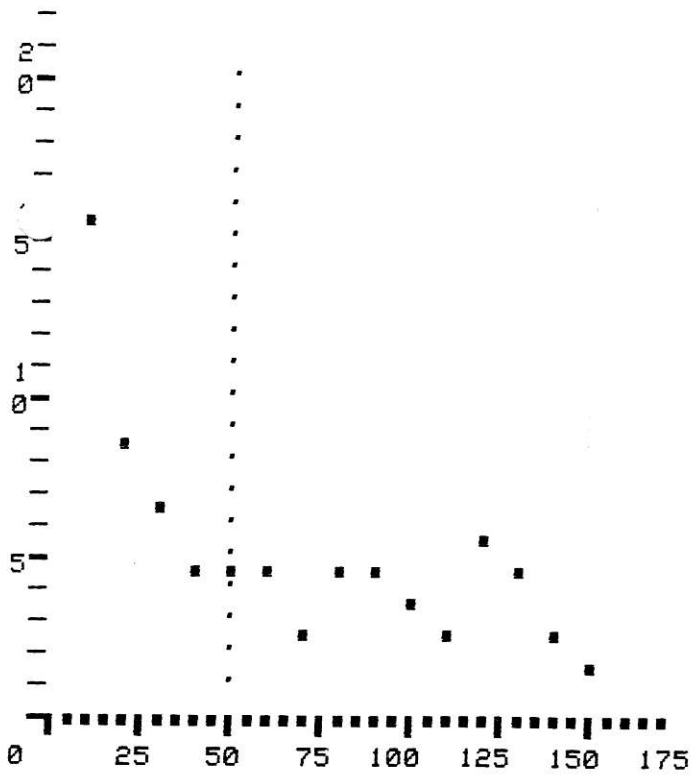
NEXT ELEVEN POINTS:

MEAN-VALUE = 4

VARIANCE = 2.72727272

STANDARD DEV.= 1.65144564

Y=(N.SPIKES/100 MSEC.)*100



X=1/10 SEC.

DATE : 4/9/'84

SEQUENTIAL CODE: 14

AREA STIMULATED: TARSAL

HAIRS CODE : SAME ABOVE

STIMULANT : SOL.3 = 0.1 M NaCl

FIRST FOUR POINTS :

MEAN-VALUE = 8.25

VARIANCE = 17.1875

STANDARD DEV.= 4.14578099

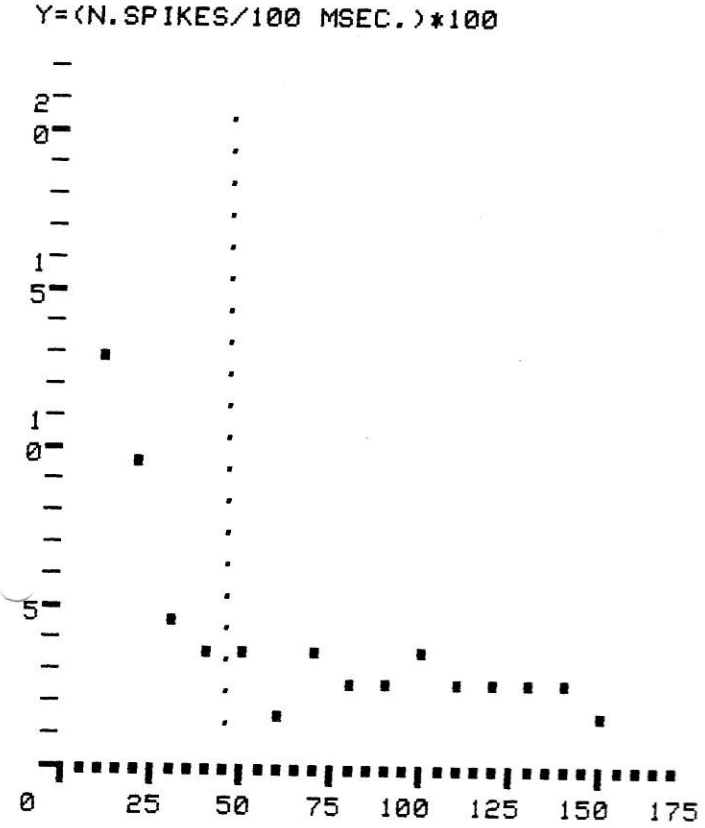
NEXT ELEVEN POINTS:

MEAN-VALUE = 3.18181818

VARIANCE = 1.4214876

STANDARD DEV.= 1.19226155

FIG. 11



DATE : 11/9/'84

SEQUENTIAL CODE: 4

AREA STIMULATED: TARSAL

HAIRS CODE : AREA A

STIMULANT : SOL.H

FIRST FOUR POINTS :

MEAN-VALUE = 7

VARIANCE = 13.5

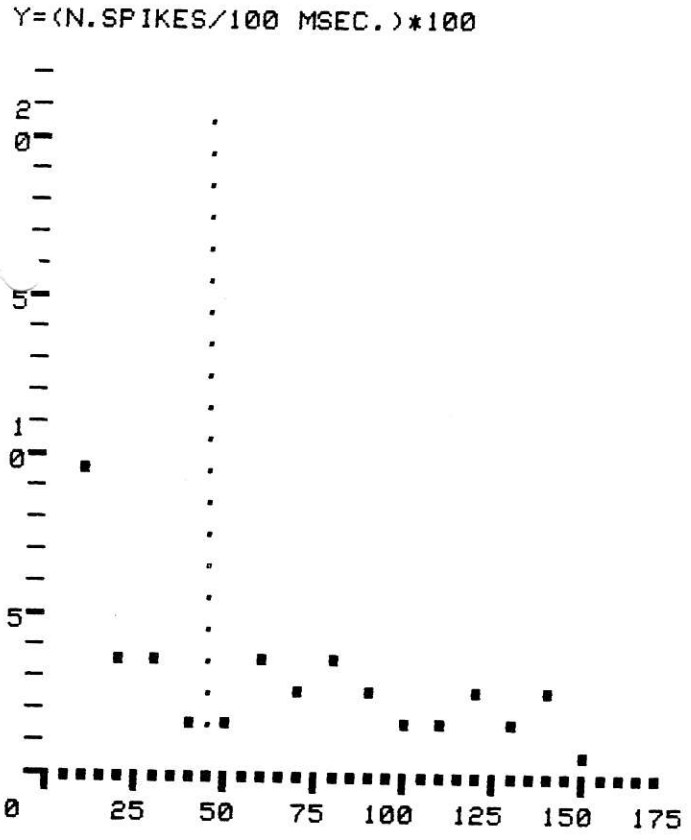
STANDARD DEV.= 3.67423461

NEXT ELEVEN POINTS:

MEAN-VALUE = 2.09090909

VARIANCE = .44628099

STANDARD DEV.= .668042656



DATE : 11/9/'84

SEQUENTIAL CODE: 5

AREA STIMULATED: TARSAL

HAIRS CODE : AREA B

STIMULANT : SOL.H1

FIRST FOUR POINTS :

MEAN-VALUE = 4

VARIANCE = 9.00000002

STANDARD DEV.= 3

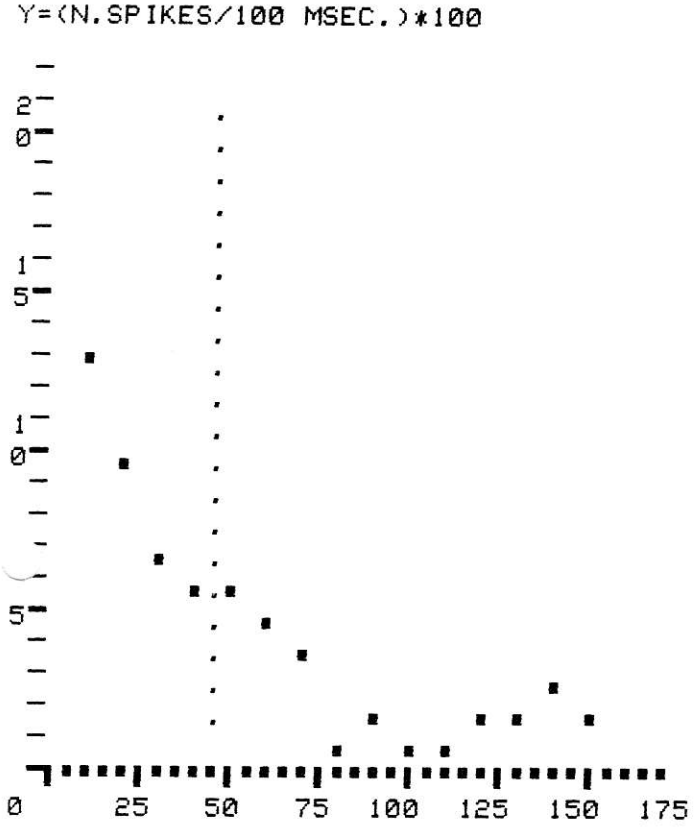
NEXT ELEVEN POINTS:

MEAN-VALUE = 1.63636364

VARIANCE = .776859501

STANDARD DEV.= .881396336

FIG.10



DATE : 7/9/'84

SEQUENTIAL CODE: 2

AREA STIMULATED: TARSAL

HAIRS CODE : AREA A CENTR.

STIMULANT : CONTROL

FIRST FOUR POINTS :

MEAN-VALUE = 8

VARIANCE = 7.49999997

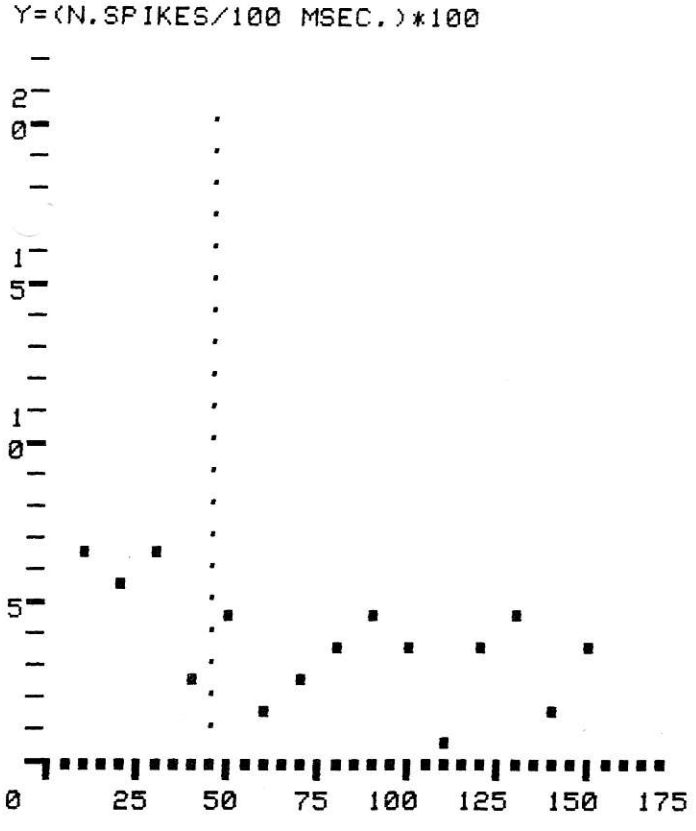
STANDARD DEV.= 2.73861278

NEXT ELEVEN POINTS:

MEAN-VALUE = 1.63636364

VARIANCE = 2.59504132

STANDARD DEV.= 1.6109132



DATE : 7/9/'84

SEQUENTIAL CODE: 3

AREA STIMULATED: TARSAL

HAIRS CODE : SAME ABOVE

STIMULANT : OLIVE EXTRACT

FIRST FOUR POINTS :

MEAN-VALUE = 4.75

VARIANCE = 2.68749998

STANDARD DEV.= 1.63935963

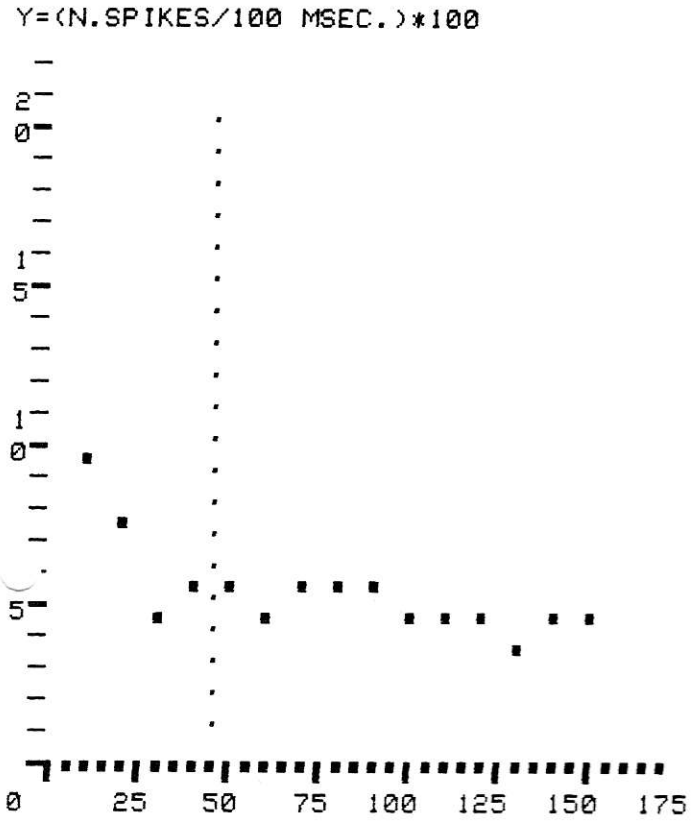
NEXT ELEVEN POINTS:

MEAN-VALUE = 2.54545455

VARIANCE = 1.70247933

STANDARD DEV.= 1.30479091

FIG. 21



DATE : 30/9/'84

SEQUENTIAL CODE: 4

AREA STIMULATED: LABELLAR

HAIRS CODE : LONGEST POST.

STIMULANT : 0.1 M NACL

FIRST FOUR POINTS :

MEAN-VALUE = 6.25

VARIANCE = 3.68750002

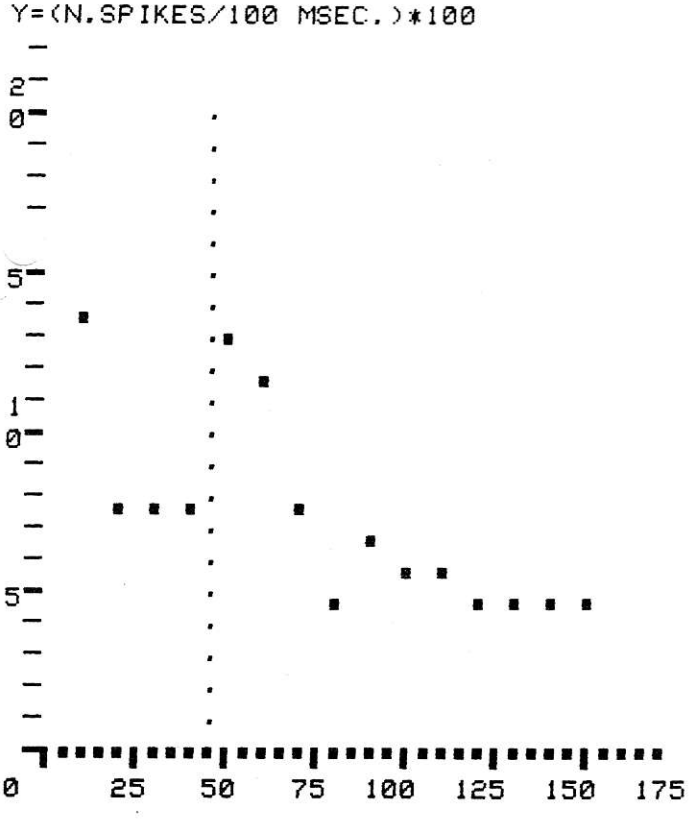
STANDARD DEV.= 1.92028644

NEXT ELEVEN POINTS:

MEAN-VALUE = 4.27272727

VARIANCE = .380165279

STANDARD DEV.= .616575445



DATE : 30/9/'84

SEQUENTIAL CODE: 5

AREA STIMULATED: LABELLAR

HAIRS CODE : LONGEST POST.

STIMULANT : 0.1 M NACL

FIRST FOUR POINTS :

MEAN-VALUE = 8.5

VARIANCE = 6.75000003

STANDARD DEV.= 2.59807622

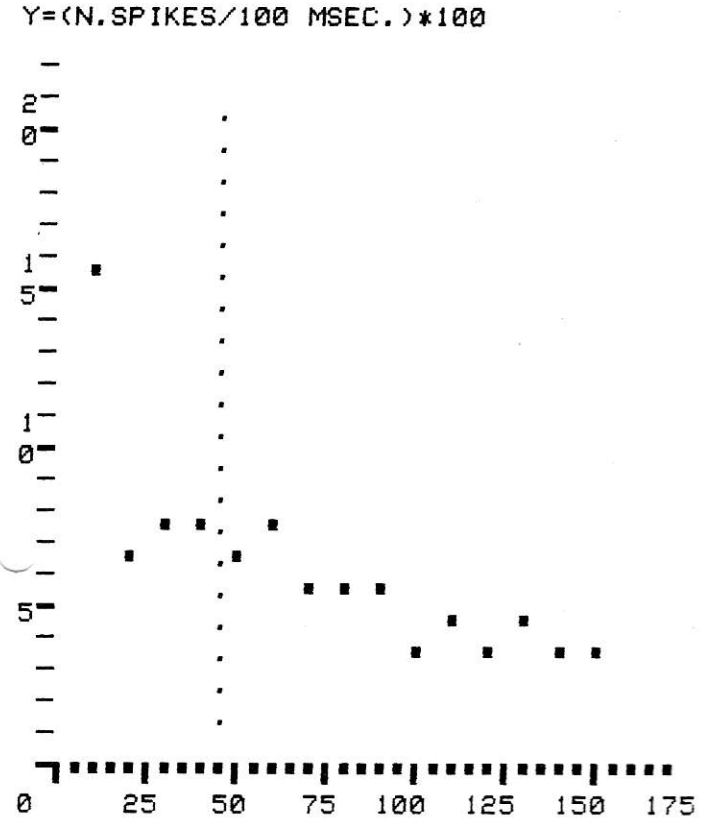
NEXT ELEVEN POINTS:

MEAN-VALUE = 6

VARIANCE = 7.63636361

STANDARD DEV.= 2.76339712

FIG. 20



DATE : 29/9/'84

SEQUENTIAL CODE: 9

AREA STIMULATED: LABELLAR

HAIRS CODE : LONGEST POST.

STIMULANT : 0.1 M NACL

FIRST FOUR POINTS :

MEAN-VALUE = 8.75

VARIANCE = 13.1874999

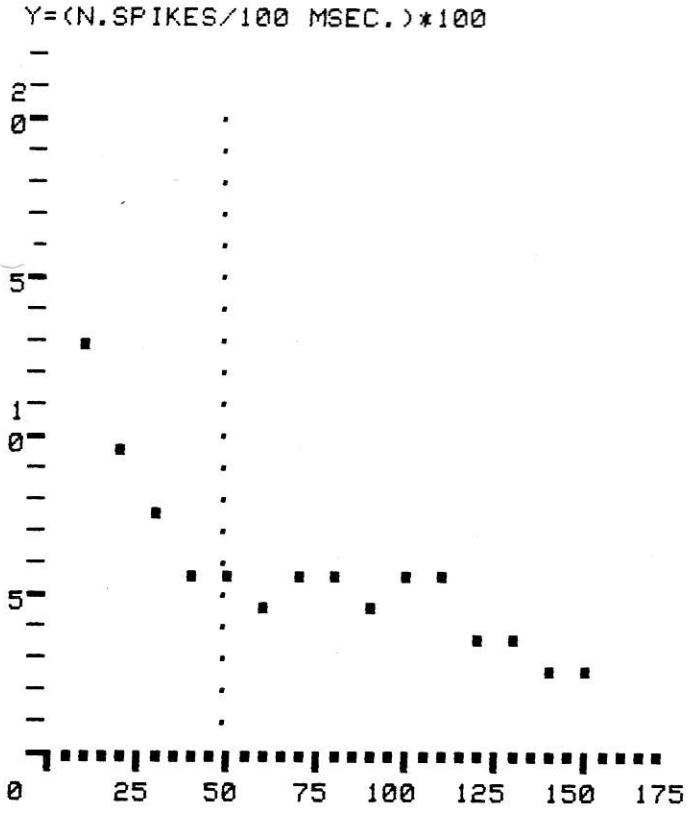
STANDARD DEV.= 3.63145975

NEXT ELEVEN POINTS:

MEAN-VALUE = 4.36363637

VARIANCE = 1.68595037

STANDARD DEV.= 1.29844152



DATE : 29/9/'84

SEQUENTIAL CODE: 11

AREA STIMULATED: LABELLAR

HAIRS CODE : MIDDLE ANT.

STIMULANT : 0.1 M NACL

FIRST FOUR POINTS :

MEAN-VALUE = 8.25

VARIANCE = 6.68749994

STANDARD DEV.= 2.5860201

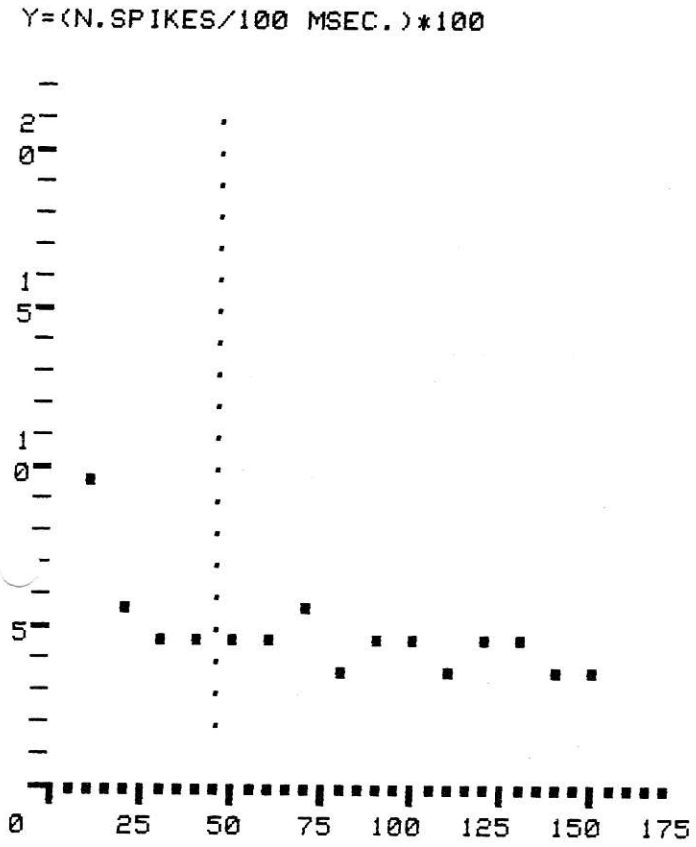
NEXT ELEVEN POINTS:

MEAN-VALUE = 3.90909091

VARIANCE = 1.35537189

STANDARD DEV.= 1.1642044

FIG. 19



DATE : 7/9/'84

SEQUENTIAL CODE: 1

AREA STIMULATED: LABELLAR

HAIRS CODE : LONGEST ANT.

STIMULANT : CONTROL

FIRST FOUR POINTS :

MEAN-VALUE = 5.5

VARIANCE = 4.24999999

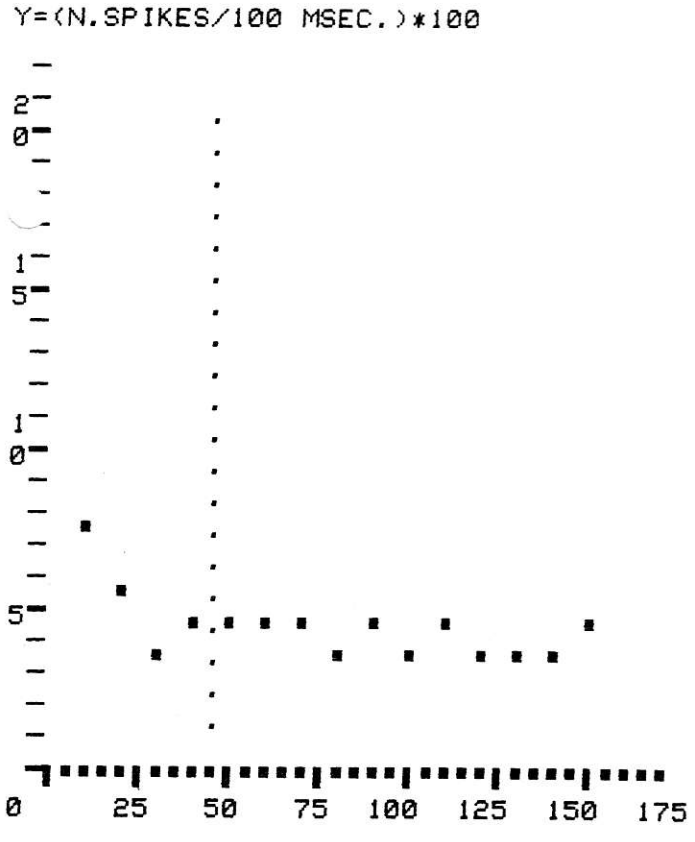
STANDARD DEV.= 2.06155281

NEXT ELEVEN POINTS:

MEAN-VALUE = 3.72727273

VARIANCE = .380165284

STANDARD DEV.= .616575449



DATE : 7/9/'84

SEQUENTIAL CODE: 5

AREA STIMULATED: LABELLAR

HAIRS CODE : SAME ABOVE

STIMULANT : OLIVE EXTRACT

FIRST FOUR POINTS :

MEAN-VALUE = 4.75

VARIANCE = 2.18750001

STANDARD DEV.= 1.47901995

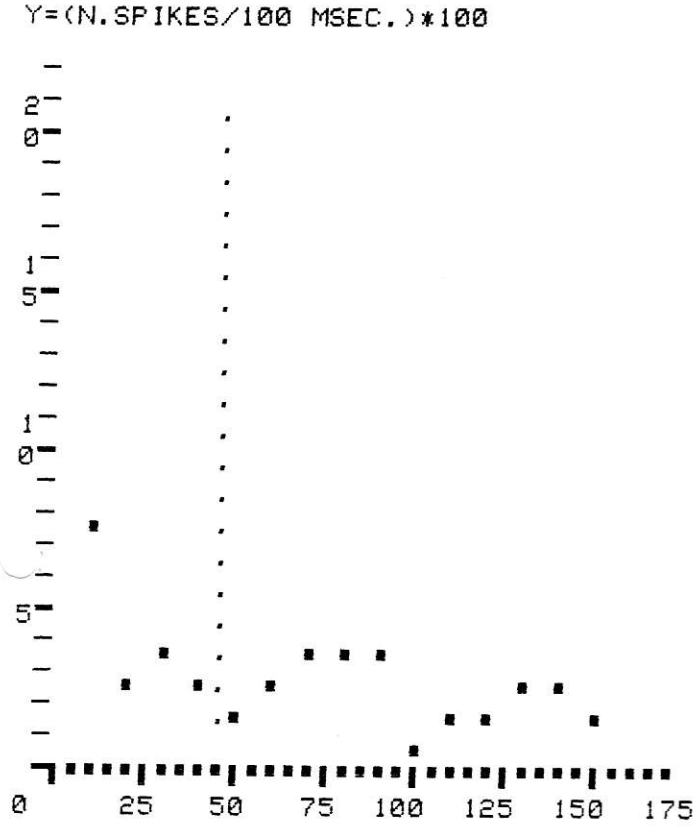
NEXT ELEVEN POINTS:

MEAN-VALUE = 3.54545455

VARIANCE = .24793387

STANDARD DEV.= .497929583

FIG. 18



DATE : 4/9/'84

SEQUENTIAL CODE: 23

AREA STIMULATED: TARSAL

HAIRS CODE : 2A

STIMULANT : 0.1 M NACL

FIRST FOUR POINTS :

MEAN-VALUE = 3.5

VARIANCE = 4.25000001

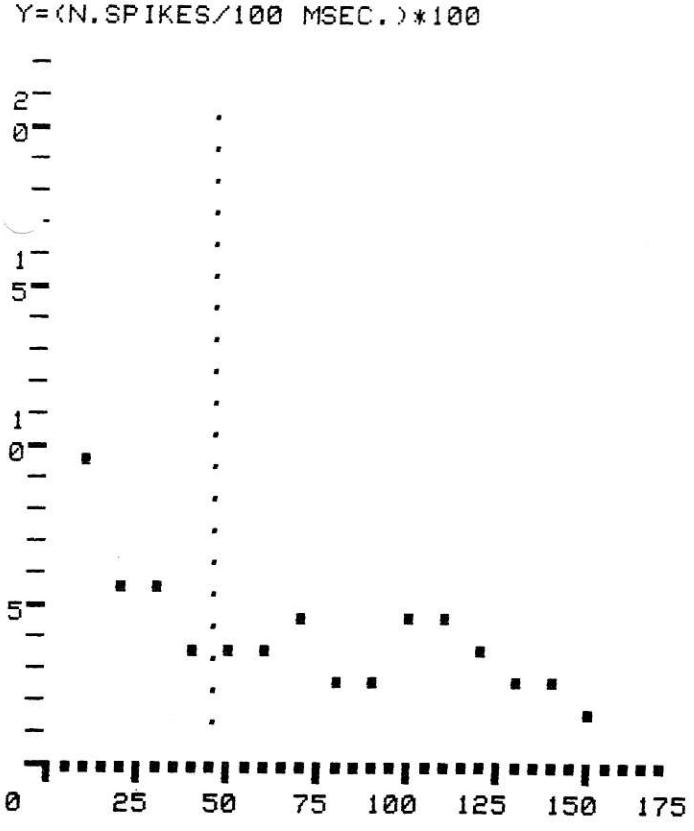
STANDARD DEV.= 2.06155282

NEXT ELEVEN POINTS:

MEAN-VALUE = 1.72727273

VARIANCE = .925619831

STANDARD DEV.= .962091384



DATE : 4/9/'84

SEQUENTIAL CODE: 5

AREA STIMULATED: TARSAL

HAIRS CODE : 2B

STIMULANT : 1 M NH4CL

FIRST FOUR POINTS :

MEAN-VALUE = 5.5

VARIANCE = 4.74999999

STANDARD DEV.= 2.17944947

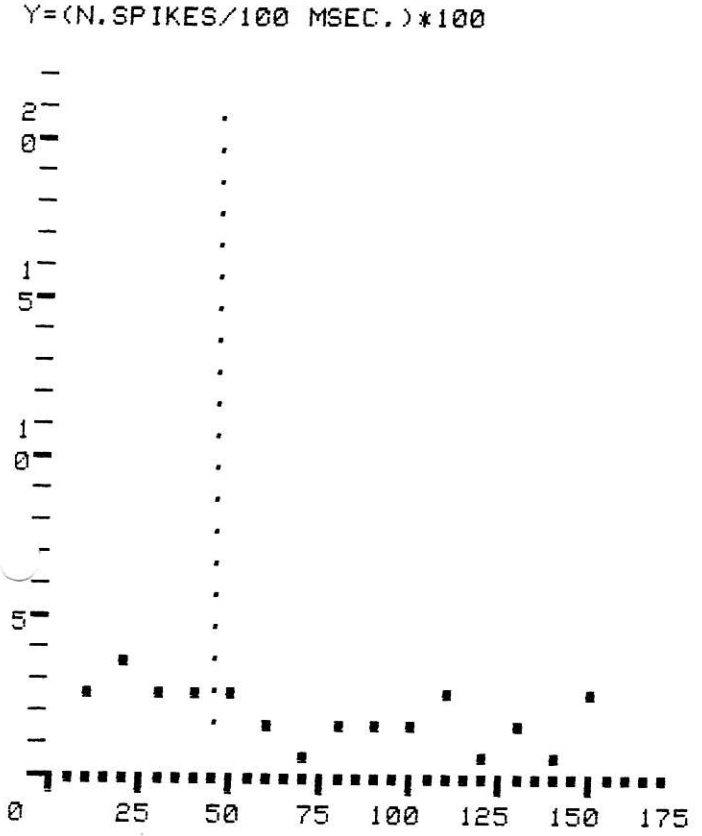
NEXT ELEVEN POINTS:

MEAN-VALUE = 2.72727273

VARIANCE = .925619827

STANDARD DEV.= .962091382

FIG. 17



DATE : 4/9/'84

SEQUENTIAL CODE: 1

AREA STIMULATED: TARSAL

HAIRS CODE : 2A

STIMULANT : 0.001 M NACL

FIRST FOUR POINTS :

MEAN-VALUE = 2.25

VARIANCE = .187499993

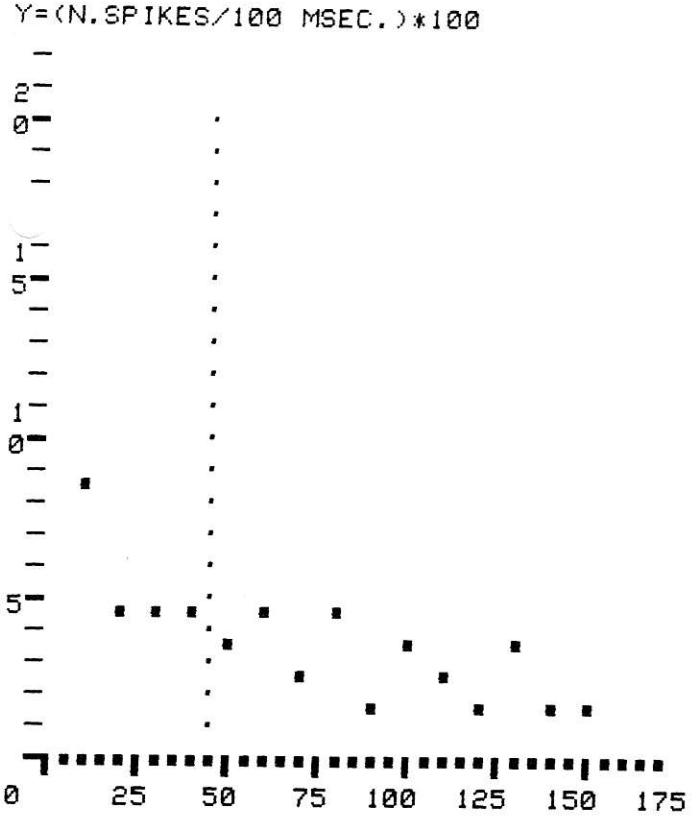
STANDARD DEV.= .433012693

NEXT ELEVEN POINTS:

MEAN-VALUE = 1

VARIANCE = .545454545

STANDARD DEV.= .738548946



DATE : 4/9/'84

SEQUENTIAL CODE: 13

AREA STIMULATED: TARSAL

HAIRS CODE : SAME ABOVE

STIMULANT : 1 M NH4CL

FIRST FOUR POINTS :

MEAN-VALUE = 5

VARIANCE = 2.99999999

STANDARD DEV.= 1.73205081

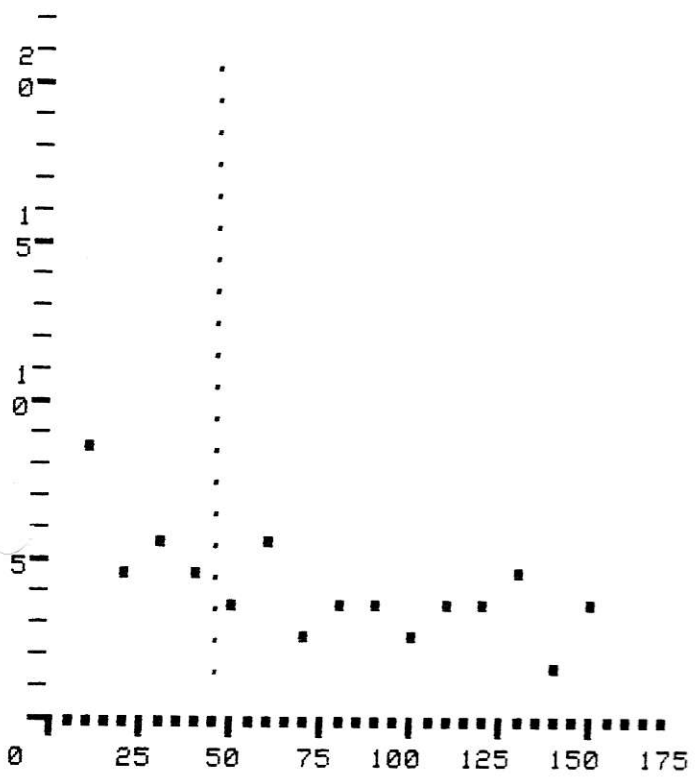
NEXT ELEVEN POINTS:

MEAN-VALUE = 2.27272727

VARIANCE = 1.2892562

STANDARD DEV.= 1.13545418

FIG. 16



DATE : 4/9/'84

SEQUENTIAL CODE: 7

AREA STIMULATED: TARSAL

HAIRS CODE : 1B

STIMULANT : 1 M NH4CL

FIRST FOUR POINTS :

MEAN-VALUE = 5.25

VARIANCE = 2.68749998

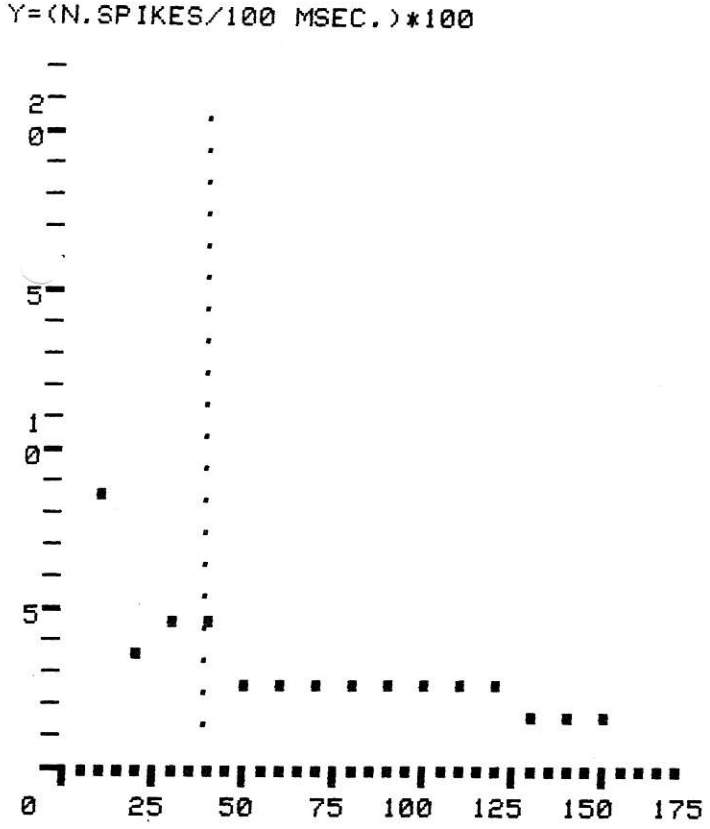
STANDARD DEV.= 1.63935963

NEXT ELEVEN POINTS:

MEAN-VALUE = 2.90909091

VARIANCE = .991735531

STANDARD DEV.= .995859193



DATE : 4/9/'84

SEQUENTIAL CODE: 10

AREA STIMULATED: TARSAL

HAIRS CODE : SAME ABOVE

STIMULANT : 1 M NH4CL

FIRST FOUR POINTS :

MEAN-VALUE = 4.75

VARIANCE = 3.6875

STANDARD DEV.= 1.92028644

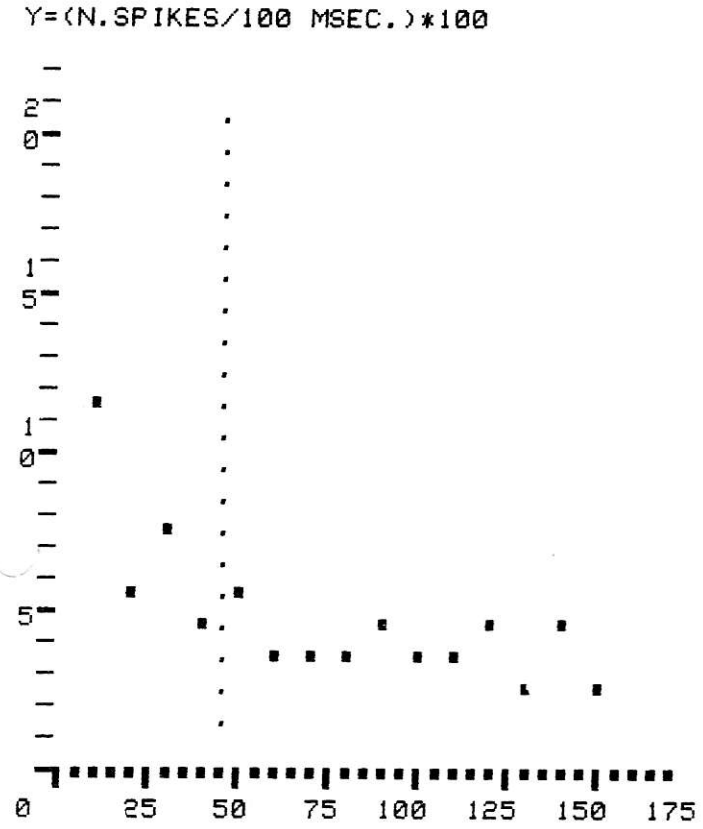
NEXT ELEVEN POINTS:

MEAN-VALUE = 1.72727273

VARIANCE = .198347105

STANDARD DEV.= .445361769

FIG. 23



DATE : 7/9/'84

SEQUENTIAL CODE: 2

AREA STIMULATED: LABELLAR

HAIRS CODE : MIDDLE POST.

STIMULANT : CONTROL

FIRST FOUR POINTS :

MEAN-VALUE = 6.75

VARIANCE = 7.1875

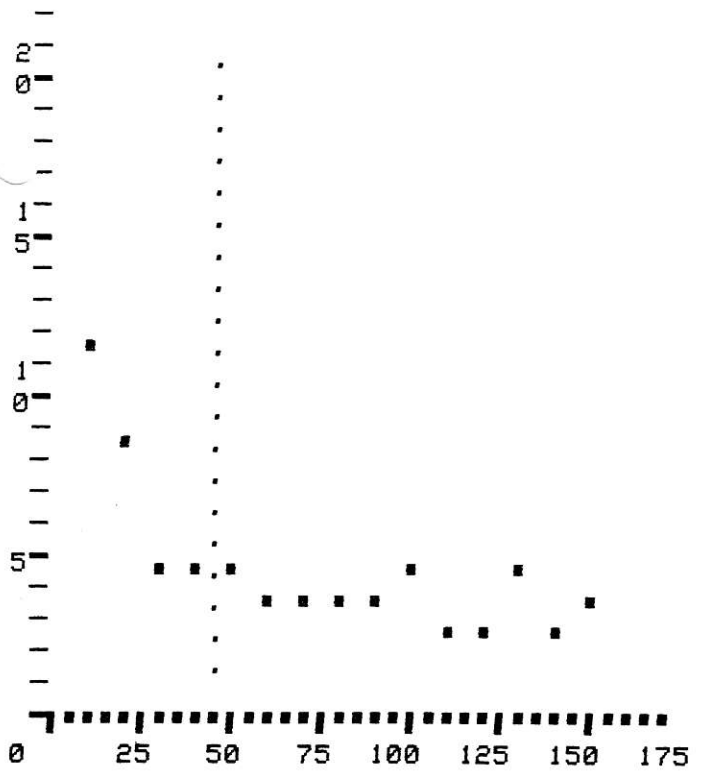
STANDARD DEV.= 2.68095132

NEXT ELEVEN POINTS:

MEAN-VALUE = 3.27272727

VARIANCE = .743801645

STANDARD DEV.= .862439358



DATE : 7/9/'84

SEQUENTIAL CODE: 6

AREA STIMULATED: LABELLAR

HAIRS CODE : SAME ABOVE

STIMULANT : OLIVE EXTRACT

FIRST FOUR POINTS :

MEAN-VALUE = 6.75

VARIANCE = 8.68749998

STANDARD DEV.= 2.94745653

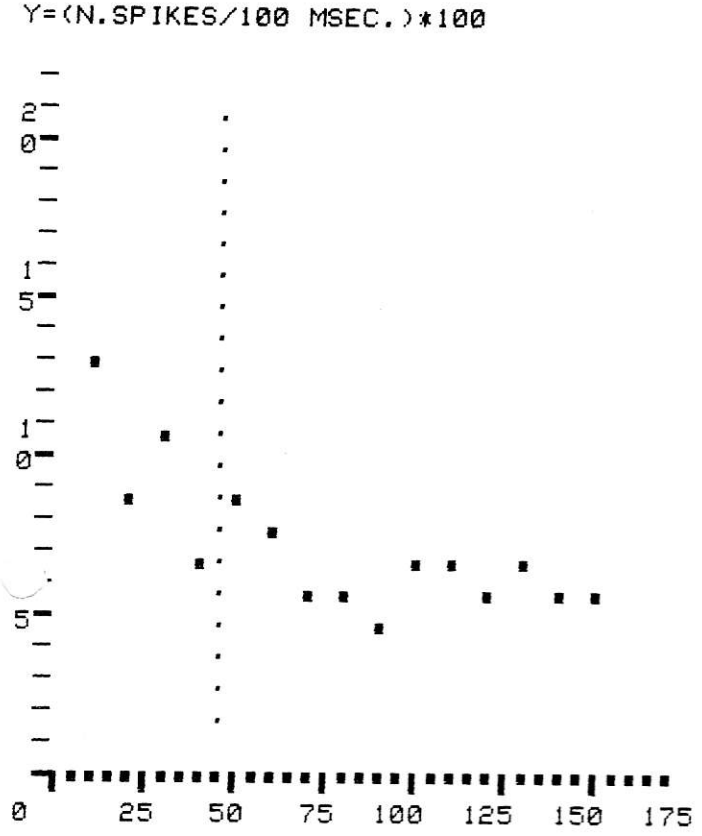
NEXT ELEVEN POINTS:

MEAN-VALUE = 3

VARIANCE = .54545454

STANDARD DEV.= .738548943

FIG. 22



DATE : 7/9/'84

SEQUENTIAL CODE: 9

AREA STIMULATED: LABELLAR

HAIRS CODE : MIDDLE

STIMULANT : CONTROL

FIRST FOUR POINTS :

MEAN-VALUE = 9

VARIANCE = 4.99999988

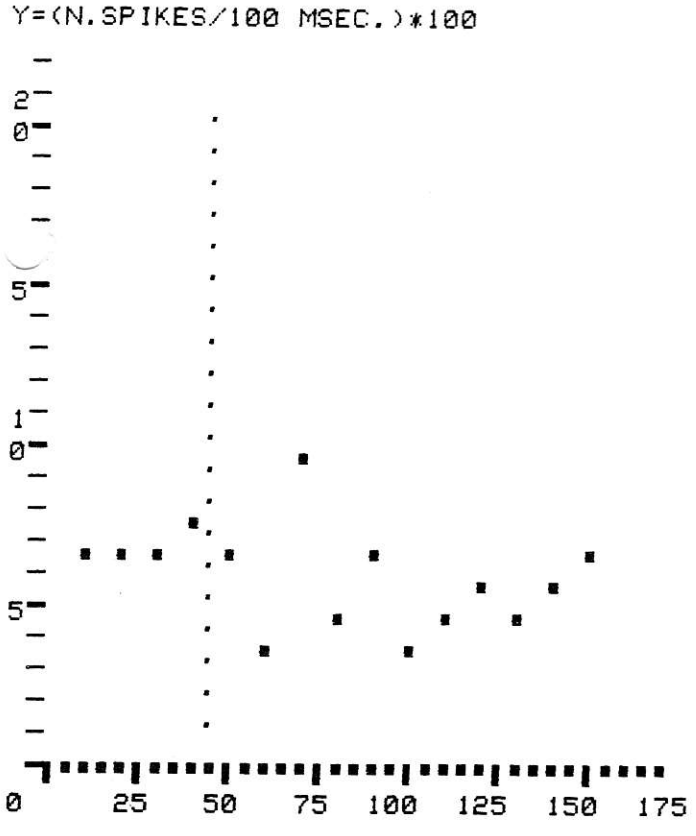
STANDARD DEV.= 2.23606795

NEXT ELEVEN POINTS:

MEAN-VALUE = 5.63636364

VARIANCE = 1.14049587

STANDARD DEV.= 1.06794002



DATE : 7/9/'84

SEQUENTIAL CODE: 13

AREA STIMULATED: LABELLAR

HAIRS CODE : SAME ABOVE

STIMULANT : OLIVE EXTRACT

FIRST FOUR POINTS :

MEAN-VALUE = 6.25

VARIANCE = .187499985

STANDARD DEV.= .433012685

NEXT ELEVEN POINTS:

MEAN-VALUE = 5

VARIANCE = 2.7272727

STANDARD DEV.= 1.65144564