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**NEUROPHYSIOLOGICAL STUDY OF VECTOR
RESPONSES TO REPELLENTS**

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Annual Report

Edward E. Davis

August 1978

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

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19. KEY WORDS (Continued)

20 ABSTRACT (Continued)

In Aedes aegypti, sensitivity to the insect repellents deet, 612, and indalone was related to the reported behavioral efficacy of the repellents. The sensilla trichodea (A2-I) appear also to be sensitive to oviposition site attractants, which raises doubts about whether these sensilla can encode repellent information. This issue is currently being investigated in greater detail. Other mosquito species have not been tested yet.

Examination of s. trichodea (A2-II) revealed that in all three species of mosquitoes--Ae. aegypti, Culex pipiens, and Anopheles albimanus--deet generally elicited no change in spike frequency, whereas 612 and indalone were excitatory. Oviposition site attractants elicited increases in spike frequency, which is similar to the reported specificity of a compound in attracting a given mosquito species.

Study of the grooved-peg sensilla (A3) in all three species showed that deet generally evoked no response whereas 612 and indalone tended to mimic the response of the cell to lactic acid (LA), whether LA excited or LA inhibited. When LA was presented to Ae. aegypti together with either deet or 612, the response of the LA-excited cell was attenuated compared to their response to LA alone. In the LA-inhibited cell, the combination of LA plus repellent was additive. In C. pipiens, a similar combination of LA plus repellent produced an attenuation of the LA-induced inhibition in that cell type and an additive effect in the LA-excited cell. Experiments to verify and quantify these responses are currently under way. The data presented here are preliminary, and no firm conclusions should be drawn from them.

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SUMMARY

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INTRODUCTION

When this contract was executed on 9 March 1978, we immediately began acquiring the equipment to be used for the research. Several items-- a Brush 2200 chart recorder, Leitz microscope, HP 365A Preamplifier, F. Haer Amplitude and Rate/interval Analyzers--were purchased with SRI capital equipment funds. An anti-vibration table, a quantitative odor delivery system with odor-switching and stimulus-marking circuits, and two microelectrode carrier devices, which are special items, were fabricated in our laboratory.

The equipment setup was completed, tested, and experiments were under way by 12 May 1978. We obtained eggs and/or adults of three species of mosquitoes from the Department of Tropical Medicine, LAIR, Presidio of San Francisco, to establish our own colonies. The three species were Aedes aegypti-Masaka, Culex pipiens, and Anopheles albimanus. We also have Ae. triseriatus obtained from Dr. Michael Bentley of the University of Maine at Orono.

RESULTS AND DISCUSSION

In the first series of experiments, we have been examining the qualitative electrophysiological responses of the antennal chemoreceptors of the three mosquito species to chemical repellents and other stimuli. The results of these experiments are presented below. Because the data are preliminary, we cannot yet draw any firm conclusions.

A2-Type Sensilla

Table 1 summarizes the results to date of testing A2-type sensilla. In the A2-I sensilla (long, blunt-tipped s. trichodea, Type I), all the repellents tested elicited an increase in the frequency of nerve impulses. Deet evoked the most consistent and vigorous response compared with 612

Table 1

RESPONSE OF A2-TYPE SENSILLA
TO VARIOUS CHEMICAL REPELLENTS

Sensilla and Species	Stimulus									Oviposition Site Attractant		
	DEET			612			Indalone					
	+	0	-	+	0	-	+	0	-	+	0	-
<u>A2-I</u>												
<u>Aedes aegypti-Masaka</u>	7	3		4	4		1	6		5	2	
<u>Anopheles albimanus</u>	Not tested											
<u>Culex pipiens</u>	Not tested											
<u>A2-II</u>												
<u>Ae. aegypti</u>	5	1		2	1		1	2		12		
<u>An. albimanus</u>	3	1		2	1		1	2		3		
<u>C. pipiens</u>	1	3		1	3		2	1		4		

and indalone. Some of the A2-I neurons were also found to be sensitive to oviposition site attractants at high stimulus intensities. This raises the question as to whether or not the A2-I sensilla can be considered as a "noxious" odor (repellent?) receptor as previously suggested. We will repeat these experiments at lower stimulus intensities to determine whether the responses observed were artifacts of the intensity level. If the A2-I neurons are not sensitive to low-level oviposition site attractants, then our nociceptor hypothesis will stand.

Only A2-I sensilla of Ae. aegypti-Masaka have been tested to date. On the antennae of An. albimanus, the A2-type sensilla are present, but we have not been able to differentiate between them based on their external morphology. We hope that the Tropical Medicine Department at LAIR will be able to aid us in this respect. If not, we will have to increase our sample size until we can recognize both types.

In the A2-II sensilla (short, tapered, sharp-tipped s. trichodea), 612 and indalone elicited an increase in spike frequency in at least four of the five sensilla tested. Deet typically did not evoke a response. All the A2-II sensilla in all three species of mosquitoes were sensitive to oviposition site attractants. The sensitivity of C. pipiens to 2-butoxyethanol (BOE) was greater than that of the other two species and was greater than to the other stimuli. This agrees with the report in the literature that BOE is a behavioral oviposition attractant for Culex.

A3 (Grooved-Peg) Sensilla

Of the neurons associated with the 24 grooved-peg sensilla examined, 12 were excited and 13 were inhibited by lactic acid (LA). In one sensillum, we were able to record the responses of both types of neurons simultaneously. Table 2 shows the responses of these neurons to chemical repellents. In all three species of mosquitoes, deet tended to evoke either no response or a slight decrease in the spontaneous spike frequency. Indalone and 612, on the other hand, tended to mimic the response of the neuron to lactic acid. When LA was presented together with a repellent, two different response patterns were observed between Ae. aegypti and C. pipiens. (Combined stimuli of LA and repellents have not as yet been presented to the grooved-peg sensilla in An. albimanus.) Considering first the grooved-peg sensilla of Ae. aegypti, we observed one LA-excited neuron when LA was given in the presence of deet; an increase in spike frequency was observed that was either equal to or less than that produced by LA alone. This suggests that deet attenuates the response of this type of neuron to LA. In contrast, LA plus deet in the three LA-inhibited neurons tested elicited a greater degree of spike frequency reduction than LA alone. In C. pipiens, the two LA-inhibited neurons tested responded to combined repellent and LA in the same manner as the LA-excited neurons of Ae. aegypti. When LA was given in combination with a repellent (either deet or 612), the decrease in spike frequency was attenuated, or was less than that caused by LA alone. The degree of attenuation induced by the repellents was proportional to the level of repellent presented. In addition, we noted that less deet than 612 was needed to produce a given level of reduction of the LA-response.

Table 2

RESPONSE OF A3-TYPE SENSILLA
TO VARIOUS CHEMICAL REPELLENTS

<u>Species and Stimulus</u>	<u>Response to Lactic Acid</u>					
	<u>Excited</u>			<u>Inhibited</u>		
	<u>+</u>	<u>0</u>	<u>-</u>	<u>+</u>	<u>0</u>	<u>-</u>
<u>Aedes aegypti</u> -Masaka	(N = 10)			(N = 6)		
deet	1	4	1	2	1	
612	3				3	
Indalone	1	1	2	1	2	
<u>Anopheles albimanus</u> *	(N = 1)			(N = 4)		
deet		1		3		
612			1		2	
Indalone			1		2	
<u>Culex pipiens</u>	(N = 1)			(N = 3)		
deet			1	3		
612	1			1	1	
Indalone			1	1	1	

* Of the two neurons present in the grooved-peg sensilla of this mosquito, the one with the larger amplitude spike was sensitive to both deet and air. Its activity, therefore, is probably not a response due to the chemical stimulation per se. The one cell excited by LA was the smaller of the two spikes and was not sensitive to air. It was considered to be an olfactory response.

PLANS FOR THE FUTURE

We have added a biologist (Ph.D. level) to our laboratory staff on 14 August 1978. This will enhance our overall capabilities to better meet the goals of this research.

The remainder of the year will be spent on the following tasks:

- Completion of the qualitative examination of the responses of antennal chemoreceptors of Ae. aegypti-Masaka, C. pipiens, and An. albimanus and initiation of similar studies with C. tarsalis, An. quadrimaculatus, and Ae. taeniorhynchus.
- Examination of the responses of the A2-I sensilla to low-intensity stimulation with oviposition site attractants to determine whether this sensillum type can discriminate and encode for chemical repellent substances.
- Collaboration with the Department of Tropical Medicine, LAIR, in an effort to differentiate between A2-type sensilla on An. albimanus based on the morphological characteristics of the sensilla.
- Quantification of the responses of the lactic acid-sensitive neurons in the grooved-peg sensilla to lactic acid in the presence of repellent substances and comparison of the effectiveness of different repellent compounds in eliciting the responses observed.

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