GALVANOTAXIS OF PARAMECIA: A STUDY THROUGH THE USE OF BALANCED SQUARE WAVES OF FREQUENCY THRESHOLDS IN RELATION TO CURRENT DENSITIES

JOHN A. MILLER AND LEO S. GOLDSTON,
Department of Zoology and Entomology,
The Ohio State University,
Columbus, Ohio

INTRODUCTION

Parmecia have been widely used for behavioral studies in the fields of animal and cellular galvanotaxis. However, in any quantitative study of living material a major problem has been to maintain all phases of the environment excepting for the one variable being studied. In previous papers (Goldston and Miller, 1947; Miller and Goldston, 1947) the authors have demonstrated the use of balanced square waves as a means of electronic stimulation. To this type of stimulation the test organism responds only to the direction of electron flow.

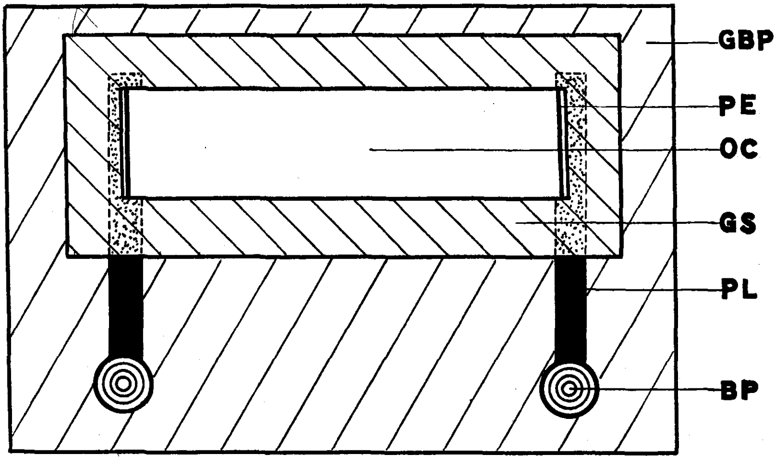

FIG. 1. Glass observation cell with platinum electrodes. GBP, glass back plate; PE, platinum electrode; OC, observation cell; GS, glass siding; PL, platinum leads; BP, binding posts.

We have reported in the above mentioned papers some galvanotropic responses of paramecia to balanced square waves. These studies were concerned with the relation of frequency to behavioral pattern when the current density "was sufficient to produce definite galvanotropism without any morphological distortion".

PROBLEM

In the behavior laboratory balanced square waves are applied as a means of electronic stimulation to a standard culture of *Paramecium caudatum.* This problem deals with the relation of thresholds to frequencies and current densities. At the threshold chosen the locomotor movements of the paramecia moving at right angle to the current appear normal, at the same time individuals whose long axis is parallel to current flow show an immediate avoiding reaction. This threshold is employed due to its minimum frequency grading, thus making mid frequency readings less variable.

APPARATUS

The methods for the production of balanced square waves applicable to behavioral studies were discussed by the authors in the previously cited papers.

For this study a glass cell of original design is utilized. (See Fig. 1.) This cell is rectangular; 28 mm long, 7.85 mm wide, and 1.58 mm deep. A full width platinum electrode is incorporated at both ends. In use, the paramecia culture fills the entire cell which is then covered with a coverslip.

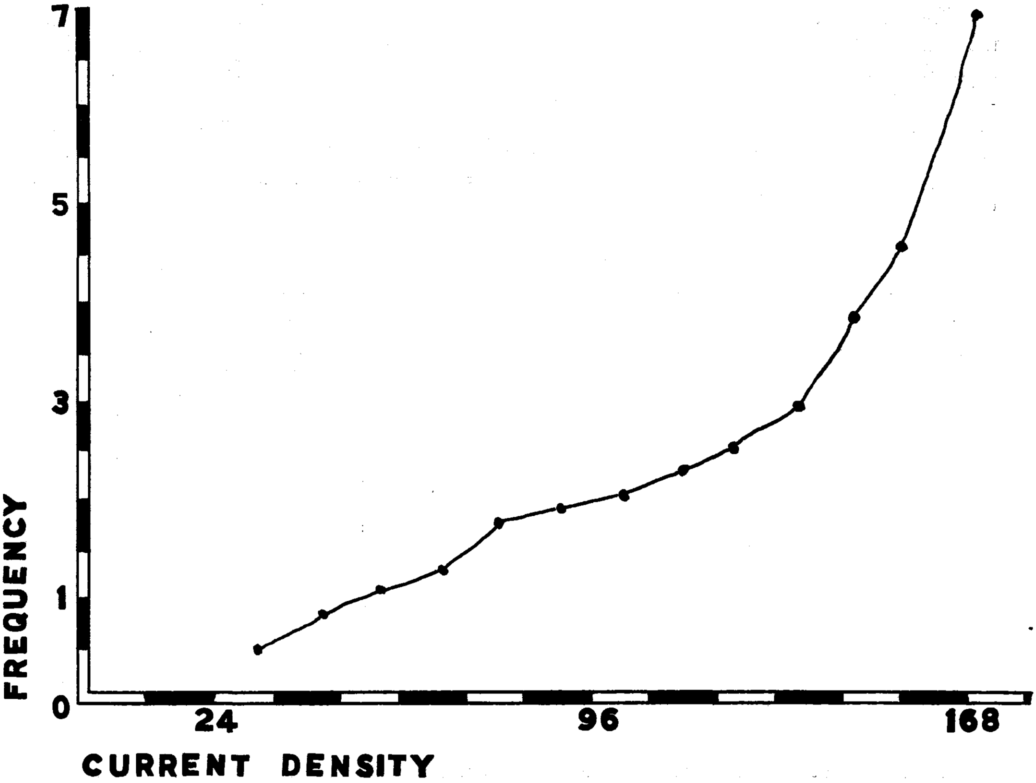

FIG. 2. Showing frequency in kilocycles per second and current density in microamperes per square millimeter necessary to produce responses discussed in text.

Frequency, balance, wave shape, and voltage (which is in direct proportion to current flow and current density) are checked at every step through the use of the direct deflection plates of an oscillograph. A calibrated audio frequency oscillator is employed to verify the frequency output of the square wave generator. Current levels are determined through the use of a microammeter for the various voltages.

The apparatus as described produces a form of electronic stimulation which can be quantitatively measured as to frequency, current, wave form, and voltage. The cell herein described further provides a volume of media the cross sectional area of which remains constant. With this apparatus it is possible to produce definite current densities at various frequencies. The current density is the microamperes passing in one direction through each square millimeter of the cross sectional area of the cell during one half the square wave cycle.

APPLICATION

Paramecium caudatum from the same culture source are subjected to the above mentioned form of stimulation. Frequent changes are made of the sample being tested. However, it is significant that with identical stimulation the reaction of the used sample is at no time different from that of the fresh sample. Room temperature of approximately 25°C prevailed throughout the testing period.

The amount of current passing through the observation cell is measured in microamperes. Current changes are made in units of 40 microamperes ranging from 420 to 2100uA (See Table I, column 1). The current density is then determined for each of the thirteen variables (See Table I, column 2). Within the range of each current density studied the optimum frequency to which the paramecia respond as noted previously, is determined and recorded in Table I, column 3.

Figure 2 illustrates the relationship between the two variables which interact to produce the studied response.

TABLE I

Microamperes Passing through Observation Cell	Current Density (uA/mm^2)	Mid Frequency for Threshold Noted. (c.p.s.)
420	33.9	600
560	45.2	800
700	56.5	1100
840	67.7	1300
980	79.0	1800
1120	90.3	2000
1260	101.6	2200
1400	112.9	2400
1540	124.2	2600
1680	135.5	2900
1820	146.8	3800
1960	158.1	4500
2100	169.4	7000

SUMMARY

Electronic stimulation in a variety of forms has been employed in behavioral studies. In this paper the authors describe the effect of balanced square waves at varying frequencies and current densities upon *Paramecium caudatum*. In this experiment a specific reaction is selected as a constant the variables being frequency and current density. The table and graph clearly illustrate that the frequency threshold for the selected reaction is determined by actual current density passing through the culture. In other words the same behavioral expression can be induced at different frequencies through the variance of the actual current passing through the organism.

In summary the responses of an organism to polarity interchange, in an electronic field, are determined not only by the frequency but also by the current density.

LITERATURE CITED

Goldston, L. S., and **J. A. Miller.** 1947. Production of balanced square waves for electronic stimulation, Ohio Journal of Science, 47(2): 59–61.

Miller, J. A., and **L. S. Goldston.** 1947. Galvanotropic responses of paramecia to balanced square waves, Ohio Journal of Science, 47(3): 127–129.