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An improved electroantennogram apparatus with a new automatic sample injection system

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ABSTRACT. An improved electroantennogram (EAG) apparatus with a new automatic sample injection system, based on a cyclic timer connected to a 12 V D.C. electrovalve, is described. The device is fully automatic and allows insertion of constant cyclic air puffs of the test compounds to the antenna at predetermined intervals.

Key words. Electroantennogram apparatus, insect sex pheromones, cyclic timer, injection system.

Introduction

Laboratory analysis of compounds which modify behaviour in insects has been effectively carried out with the electroantennogram technique (EAG) developed by Schneider (1957). The technique has been used successfully for the identification of sex pheromone components in many species of insects (e.g. Roelofs & Comeau, 1971; Roelofs, 1984; Nagai, 1981) as well as inhibitors and synergists thereof (Kamm & McDonough, 1980). The combination of a gas chromatograph coupled to the EAG has also been extremely useful in the detection of these behaviourally active compounds (see Struble & Arn, 1984, for review).

The original EAG setup described by Schneider (1957) has been simplified over the years, for example with the replacement of the oscilloscope by a voltmeter to measure the antennal response (Bjostad & Roelofs, 1980). In this communication we describe an improved EAG apparatus (Fig. 1) with an automatic system for injecting samples.

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Description of the apparatus

The injection system is based on a cyclic timer connected to a normally closed 12 V D.C. electrovalve (Fig. 2). The timer can be regulated to open the electrovalve during a chosen period to allow the air, produced by a 1 atm pressure membrane pump (A, in Fig. 1) to pass through a disposable pipette containing a small filter paper (*c.* 0.8 cm^2) on which 10 μg of the test compound has been absorbed. The ambient air is filtered through activated charcoal and fed into another independent stream of air, which flows continuously over the antenna. This air line is supplied by a second pump (B, in Fig. 1) and regulated by a flowmeter to give 0.1–2 litres/min. It is essential that the insertion of the air line carrying the test sample should be as near to the antenna as possible to avoid significant response variations of the antennal receptors in the same test.

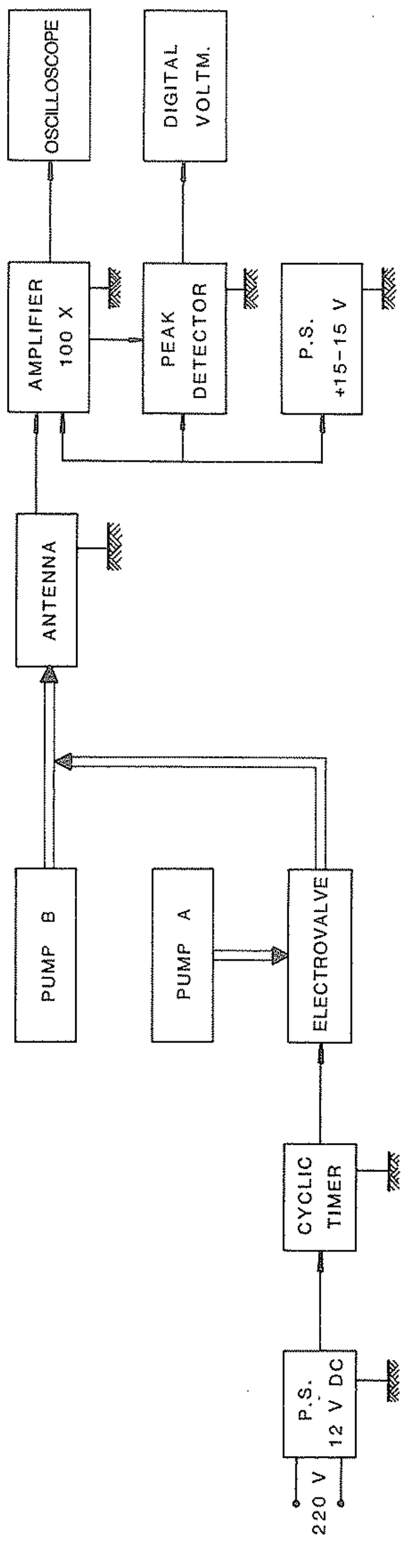

FIG. 1. Diagram of the EAG apparatus built-up in our laboratory.

The timer is fed by a 12 V power supply and contains five controls, S_1, S_2, S_3, P_1 and P_2. The push-button S_1 opens the electrovalve, which can be finely adjusted by the horizontal potentiometer P_1 (10 kΩ) (cycle A) for a predetermined period (for example 300 ms). After this time, the electrovalve returns to its normally closed position and a second cycle, B, of variable duration, is automatically switched on to allow the antennal receptors to recover (40 s, for example). The recovery period is regulated by the horizontal potentiometer P_2 (10 kΩ) and when it is over a new 300 ms cycle is automatically initiated (Fig. 3). The device can be operated in two modes. The complete sequence can be reversed by activating S_2, and the push-button, S_3, allows the current cycle to be interrupted leaving the circuit in a standby position.

Since a power transistor TR_2 (2N 3055) drives the 12 V D.C. electrovalve, it should be mounted on a heat radiator, whereas the signal transistor TR_1 (BC 108C) can be set up on the body of the printed circuit without cooling. Integrated circuits IC_1 and IC_2 (LM 555 in both cases) are programmed for cycles A and B, respectively, and when they are activated the corresponding diodes LED D_1 and D_2 (20 mA) are switched on to let the operator know which cycle is in operation at any time. The other diode, D_3 (1N 4007), protects the power transistor TR_3 from the transient pulse produced when the electrovalve closes.

Continuous cycling can be prevented by removing capacitor C_3 which then allows only one complete cycle, A and B, without returning to A. Alternatively, the order of the operation can be reversed by removing capacitor C_2, i.e. cycle A following cycle B.

With the aid of a stereoscopic zoom microscope, the last two or three segments of the antenna are excised and the cut end placed inside the capillary tube, which contains the Ag/AgCl recording electrode. The electrical contact between the antenna and the recording electrode is made by a 1% Ringer solution. A Faraday cage of 76×70×60 cm of aluminium wire protects the system from extraneous electrical signals. It is essential that the ground of the cage as well as that of the power supply, indifferent electrode, amplifier, cyclic timer and oscilloscope, be the same and, if possible, that of the building. It is also important to avoid any extraneous agent, apart from the continuous air

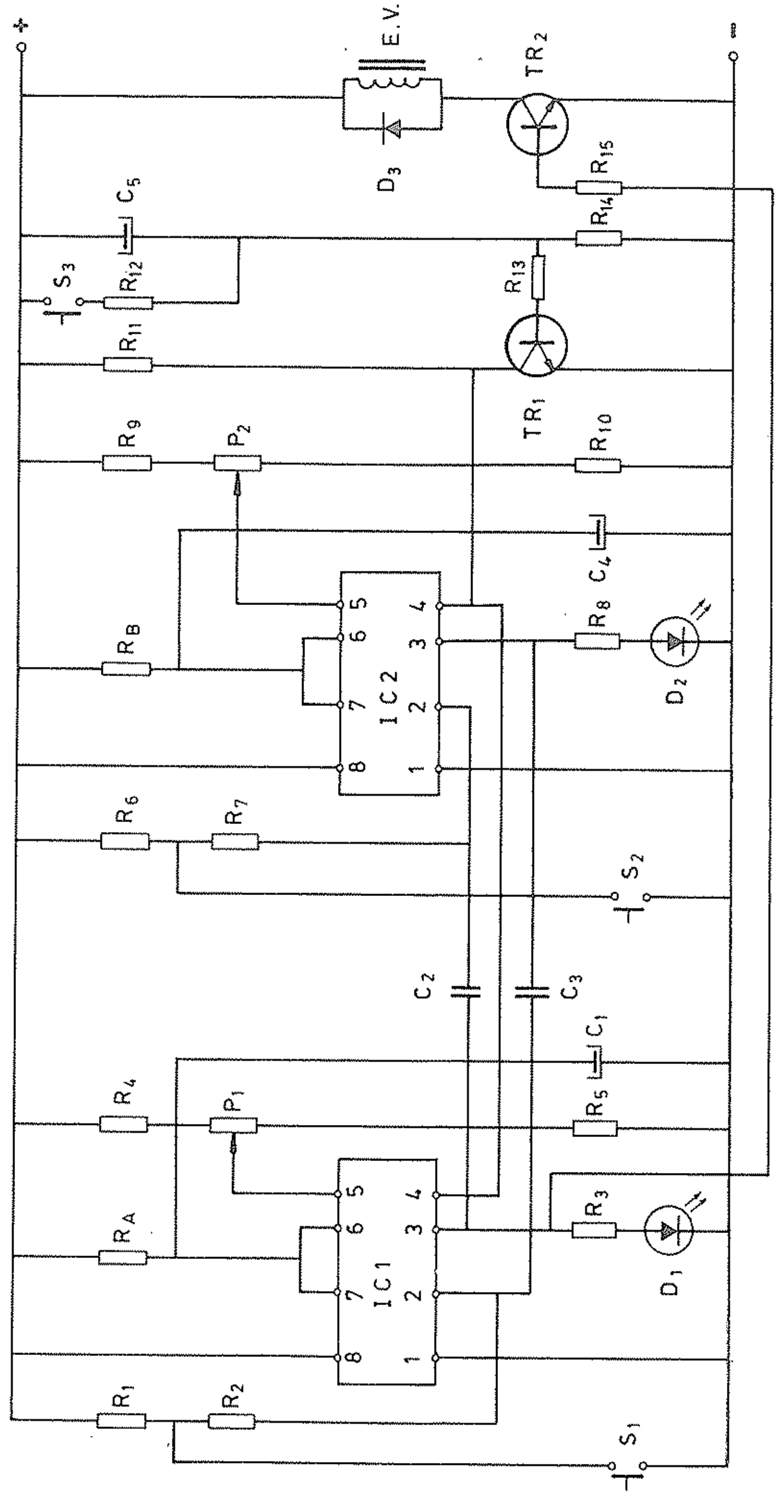

FIG. 2. Electric circuit drawing of the cyclic timer and electrovalve.

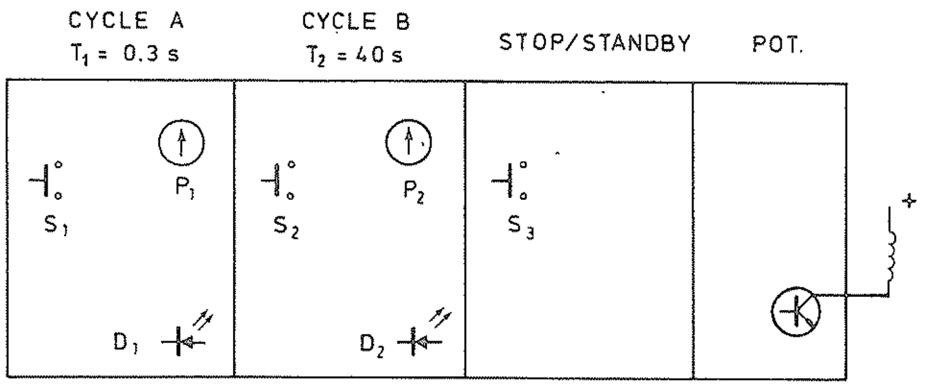

FIG. 3. Block diagram of the cyclic timer.

flow to the antenna, that affects the physical stability of the solution surface and, hence, the electrical contact between the insect head and the saline solution; otherwise, oscillations of the baseline signal may occur.

The EAG apparatus is completed with the digital voltmeter of Bjostad & Roelofs (1980). The input electrode is connected to a high impedance (10^{12} Ω) amplifier fed by an independent power supply (+15 V to −15 V). The amplified signal (×100) is carried to a peak detector, which finds the maximum level of input voltage. The peak detector output is simultaneously coupled to a storage oscilloscope (Tektronix model 5111) and to a liquid crystal voltmeter YF 1030. In this way it is possible to read accurately the maximum EAG displayed by the voltmeter and, at the same time, to observe on the oscilloscope the shape of the EAG response.

Discussion

The fully automatic injection system described herein allows a rapid and iterative way of recording EAGs by constantly puffing the sample over the antenna at predetermined intervals. Because it also permits the minimum antennal receptors recovery time to be measured quickly, the apparatus allows a large number of samples to be tested on the same excised antenna. These advantages, together with the high accuracy readouts obtained with the voltmeter, might be specially important in structure–activity relationships studies (Priesner, 1979).

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Parts list

Resistors* (Ω)

R_1=1 M	R_9=1 K
R_2= 56 K	R_{10}=1 K
R_3= 470	R_{11}=15 K
R_4=1 K	R_{12}=100
R_5=1 K	R_{13}=270 K
R_6=1 M	R_{14}=1 K
R_7=56 K	R_{15}=100
R_8=470	R_A=10 M
	R_B=1 M

Capacitors† (μF)

C_1=4
C_2=0.1
C_3=0.1
C_4=2.2
C_5=50

*5% tolerance, 1/8 W potency except R_5 (1 W).
†16 V voltage work.

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