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A Wireless Method for Stimulating *Characeae* Internodal Cells

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車軸藻節間細胞に対する非接触刺激法

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要　旨

車軸藻節間細胞を電気刺激するための，ピエゾ素子を用いる方法を開発した．この方法は電線の接続を要しない非接触のものであるので，遠心顕微鏡観察のもとで細胞を電気刺激することができた．またこの刺激方法は，オジギソウ，アメーバ，ラッパムシにも適用できた．

Introduction

Characeae cells have been used as a favorite material for studying cytoplasmic steaming (Kamiya 1986). We constructed a stroboscopic centrifuge microscope and have studied a behavior of the cytoplasm just after the cessation of cytoplasmic streaming under centrifugal acceleration (Kamitsubo *et al.* 1989). To stop the cytoplasmic streaming, one of the simplest methods is to apply various stimulations, including electrical one, to the cell. When the cell is stimulated, the cell generates an action potential and the streaming stops promptly (Tazawa and Kishimoto 1968, Barry 1969, Pickard 1969, Tsutsui *et al.* 1987). In an ordinary microscope study in which the specimen remains still, the electrical stimulus can be applied through a pair of stimulating electrodes, though the connecting wire may disturb the microscope observation in some extent. In the centrifuge microscope study, however, the above method cannot be used since the cell is rotating during the experiment. We developed a "wireless method" to electrically stimulate the cell in a non-contact manner.

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Materials and Methods

Nitella axilliformis was cultured in the laboratory. Internodal cells were isolated from the adjacent cells and kept in the culture medium for at least a day. The lengths of cells used were 2 mm to 30 mm. The algae were cultured at 25°C and the experiment was carried out at 20–25°C.

The electric stimulater was made up by modifying a piezoelectric lighter (SH-700, KNG Spark Plug Co., Nagoya) as follows: Originally the lighter was mainly composed of several parts; A, B, B′, C and D (Fig. la). By moving C with a finger like a trigger of a pistol, spark discharges are generated between two electrodes, B and B′; B′ is connected to a metal part (A) while B is insulated from A. D is the grip. C and D are made from plastic. The modifications were carried out as follows (Fig. 1b): A metal chain (Ch) was soldered to B, B′ was removed and the metal part (A) was insulated by taping it with a single layer of vinyl tape (V). Then the stimulater was supported on a laboratory stand by holding A.

An internode (cell) was placed on a thin polyacrylate plate (P; 100 mm × 100 mm × 1 mm) then a cover glass (C) was placed over the cell. A distance of 1 mm, which was required to insert a microelectrode into the cell, was created between the polyacrylate plate and the cover glass using spacers (SP). A thin aluminum foil strip (Al; about 3 mm width and 30 mm length) was inserted into the space beside the cell. One end of the foil remained outside the cuvette. Then the space remaining in the cuvette was filled with artificial pond water (APW; 0.1 mM each of KCl, NaCl and $CaCl_2$) (Fig. 2). The cell was electrically stimulated by applying a spark discharge to the aluminum foil strip. The distance between the metal chain and the strip was 1-3 mm. If necessary, the bathing medium, APW, was connected to the ground through Ag wire (Ag) inserted into the medium.

The generation of an action potential was checked by ordinary microcapillary electrode method. A glass microcapillary filled with saturated KCl was inserted into the cell. A reference electrode constructed with plastic tubing filled with 2% agar-APW was placed in APW. Each electrode was connected to a calomel electrode through a saturated KCl salt bridge. The potential difference between the electrodes (vacuolar potential, E_{vo}) was amplified through a differential amplifier constructed with FET-input operational amplifiers (TL084, Fujitsu) and recorded with a pen recorder (Yokogawa Electric Works).

Results and Discussion

A spark discharge was applied every ten minutes and the cell generated an action potential upon each stimulus (Fig. 3). Each action potential was followed by a cessation of streaming as was well established (Tazawa and Kishimoto 1968, Barry, 1969, Pickard, 1969, Kamiya 1986, Tsutsui *et al.* 1987, Tazawa and Shimmen 1987). Both the amplitude and the shape of the action potential were similar to those obtained by an ordinary electric stimulation. The gradual depolarization of resting E_{vo} did not mean a deterioration of the cell but reflected a loss of the electrogenic pump activity that occurred when the cell was kept under dim light (Kikuyama *et al.* 1979, Tazawa and Shimmen 1987). In fact, the resting E_{vo} hyperpolarized again when the cell was illuminated with an incandescent light after the 6th excitation. A seventh stimulus elicited an action potential that was almost identical to the first and the second. Judging from the resting potential, the shape of the action potential and the rate of streaming (data not shown), the cell was not damaged after the repeated stimuli.

Since an action potential is always followed by an instantaneous cessation of cytoplasmic streaming, the action potential generation was checked hereafter by observing whether the streaming stopped or not. In the experiment shown in Fig. 3, the APW was connected to the ground through the reference electrode in the APW, though the Ag wire (Ag in Fig. 2) was not used. On the other hand, an action potential was not evoked by the spark discharge when both the microcapillary and reference electrode were removed from APW, or APW was completely insulated from the ground.

The effect of the connection of APW to the ground was studied as follows. The equivalent electrical circuit of the wireless method is shown as Fig. 4. In Fig. 4, R_m and C_m are membrane resistance and membrane capacitance of the cell, respectively, R_s is electric resistance of the bathing medium, R_1 is a resistance between the metal chain and the aluminum strip and R_2 is a sum of resistances of vinyl tape, laboratory desk and others. We made the circuit open by keeping one end of Ag wire in the air (R_2, a sum of electric resistances of vinyl tape and others, was made infinite) while its another end was in APW. In this case, no action potential was evoked. If the bathing medium was connected directly to the ground (R_2 became finite) the cell generated an action potential upon each stimulus. We can repeatedly stimulate the cell without any damage judging from the rate of streaming. However, if the metal part of the lighter (A in Fig. 1) was taped with more than 2 layers of vinyl (R_2 was made much greater than the above case), an action potential was not evoked. When the Ag wire in the APW was connected to A through a 1000 MΩ resistor (R_2 was 1000 MΩ), the cell also generated an action potential upon a stimulus. But if the stimulus was repeated every 10 min, the rate of streaming gradually decreased and the chloroplasts rounded up, indicating that the cell was significantly damaged. After 3–5 applications of the stimulus, cytoplasmic streaming

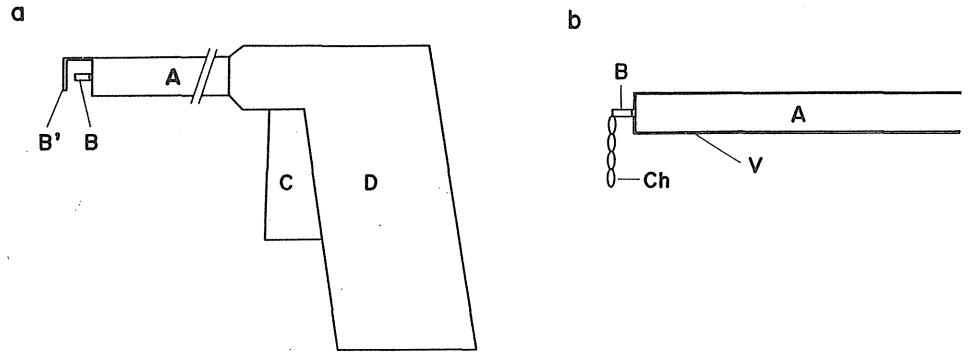

Fig. 1 An electric stimulater used for the wireless method. a. The original form of the piezoelectric lighter. D is a grip. By moving C like a trigger of a pistol, spark discharges take place between two electrodes, B and B′. B′ is connected to a metal part (A) while B is insulated from A. b. After a modification of the lighter. B′ was removed, a metal chain (Ch) was soldered to B, and A is insulated by taping it with vinyl tape.

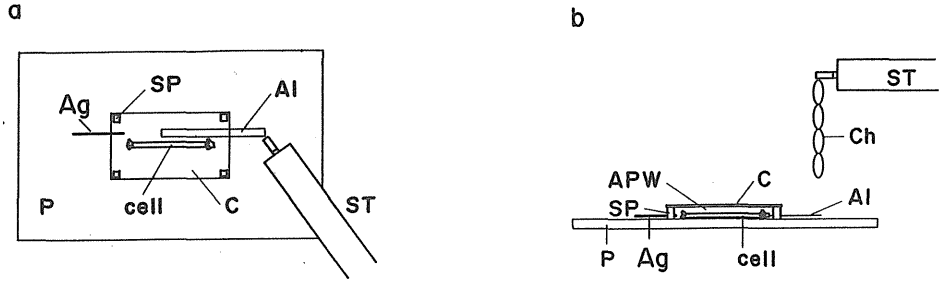

Fig. 2 Diagram of the cell chamber. a. Top view. b. Side view. A microscope, a microcapillary electrode and a reference electrode are omitted in the Figure. ST; stimulater, Ch; metal chain, Ag; Ag wire used to connect the bathing medium to the ground. For further explanation, see text.

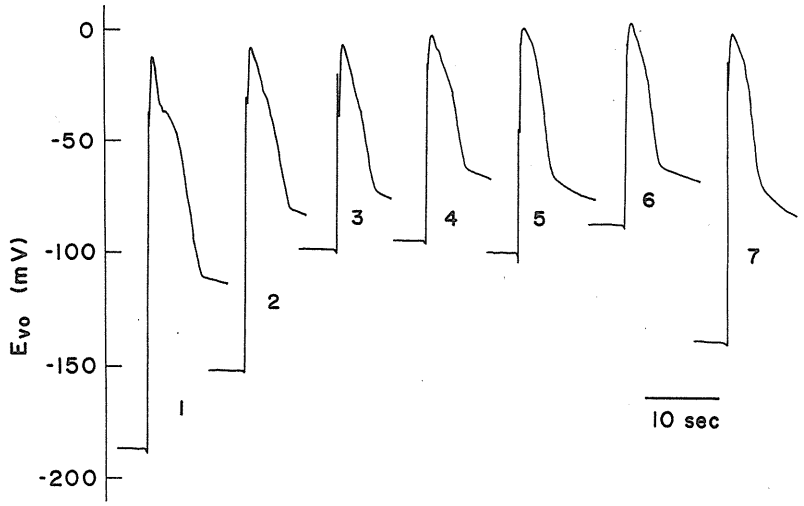

Fig. 3 Action potential generation checked by an ordinary microelectrode method. The cell generated action potentials upon each stimulus which was applied every 10 min. Figures in each trace indicate the order of excitation. The resting E_{vo} was about −185 mV before the first excitation. It gradually depolarized to about −85 mV before the 6th excitation because of a loss of the electrogenic pump activity. By illuminating the cell after the 6th excitation, the resting E vo again hyperpolarized.

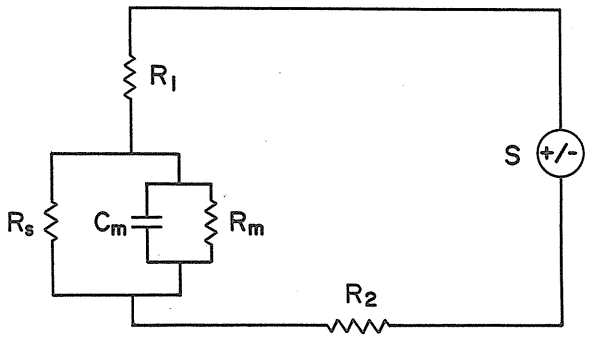

Fig. 4 An equivalent circuit of the wireless stimulation method. An internode is shown as a membrane resistance (R_m) connected to a membrane capacitance (C_m) in parallel. R_s is a resistance of the bathing medium. The air gap between the aluminum foil strip and a metal chain is shown not as a capacitor but as a resistor, R_1, because the insulation of the air is broken during the spark discharge. Resistances of the vinyl tape, the laboratory desk and others are shown as R_2. S is the piezoelectric lighter used for a stimulater.

irreversibly stopped.

These results indicate the followings. If the resistance (R_2) is too large, the stimulating electric current becomes so small that the cell cannot generate an action potential. If R_2 is small (1000 MΩ or less), electric current is too large and the cell is damaged. If R_2 is kept within a certain range, the amplitude of the electric current remains in a suitable range and the cell can be repeatedly stimulated without any harm.

If a polyacrylate plate was used to place the cell, it was necessary to connect the bathing medium to the ground, as shown above, because the electric resistance of the plate is too high. However, if a glass slide was used, no connection between the bathing medium and the ground was required for stimulating the cell with wireless method. This may be accounted for by the lower electric resistance between the glass slide and the ground because of the hydrophilic property of glass.

The wireless method may not be applied to electro-physiological studies because it is very difficult to control both the amplitude and duration of the stimulus which are very important parameters in the electrophysiology. However, this simple and non-contact manner for stimulation can be applied to various studies other than electrophysiology. The behavior of cytoplasm just after the cessation of cytoplasmic streaming was studied under centrifuge microscope (Kamitsubo and Kikuyama 1989) and under ordinary light microscope with oil immersion optics.

The wireless method was also effectively applied in a non-contact manner to induce the movement of *Mimosa* leaves, the temporal cessation of movement in *Amoeba* and the instantaneous contraction of *Stentor*.

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