Magnetic Detection of a Single Action Potential in *Chara corallina* Internodal Cells

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ABSTRACT The electrical activity that occurs in plants has not yet been detected magnetically. Magnetic detection of electrical activity in some animal as well as in human cells and organs, on the other hand, is an established research method. Our experiments demonstrate the propagation of a single action potential in the internodal cell of the green algae *Chara corallina*, measured magnetically. The propagation velocity and the intracellular current were determined.

INTRODUCTION

Action potentials in plants are believed to play a role in intracellular signaling, for example, stimulating plant movement by effecting cell turgor. They also play a role in growth, development, and in response to wounding (Tester, 1990; Wildon et al., 1992; Simons, 1992). There are both similarities and differences in the action potential behavior of some large plant single cells compared with large animal single cells. The most noticeable difference is the considerably longer duration of the action potential and a lower excitation propagation speed in plant cells. Also, the transfer of information, i.e., spreading of the action potential between two neighboring cells of *Chara corallina* placed up to 10 mm apart and immersed in artificial pond water, may proceed via the "jumping transmission" involving a traveling electric field (Ping at al., 1990), and it does not require intercellular coupling with low resistance as is usually the case in animal and plant cells.

Magnetic field measurements accompanying the spread of the action potential in single cells of *C. corallina* are interesting from several points of view: 1) It is a direct measurement method for determination of the intracellular current. 2) Together with models for signal transmission and the measured transmembrane potential they enable determination of signal propagation speed and the estimation of conductivities. 3) They can serve in testing different models of excitation propagation and serve as a suitable test for the multichannel superconducting quantum interference device (SQUID) (Zimmerman et al., 1970; Romani et al., 1982; Williamson et al., 1983) measuring system (Ahonen et al., 1991) which enables this type of measurement.

MATERIALS AND METHODS

A single *C. corallina* internodal cell was isolated from the stem and placed in artificial pond water (APW) for 1 or 2 days before being used in experiments. APW consisted of (mM): 0.1 KCl, 0.5 $CaCl_2$, 1 NaCl, 2 HEPES, pH = 6.8 (NaOH). All salts were from Sigma Chemical Co. (St. Louis, MO). One end of the internodal cell was mounted in a Plexiglas support (Fig. 1), which allowed electrical isolation of this end by means of a groove containing grease (Apiezon, London, UK; note the junction between the APW and KCl compartment on Fig. 1). The internodal cell (dotted structure in Fig. 1) was inserted in a horizontal Plexiglas tube with large holes in the wall. The size of each hole was ~20 mm × 5 mm and all together constituted more than 50% of the Plexiglas tube surface. Such large holes are necessary to provide undisturbed circulation of the return (volume) current. The Plexiglas support together with the *C. corallina* internodal cell was then submerged in a Petri dish of 220 mm inner diameter. The Plexiglas tube was 175 mm long. Recording of the action potential was similar to that described previously (Moriyasu et al., 1984; Clint and MacRobbie, 1987). Briefly, the Plexiglas support compartment was filled with 100 mM KCl. This compartment served as the reference point for electrical measurements by a voltage follower (V, WPI Instruments VF 1, New Haven, CT). Suprathreshold stimulation (S) was obtained by a pulse generator (3–8 V, 40–100 ms); the measurement setup for electric potential measurements had a band width of dc to 50 Hz. Ag/AgCl electrodes were used and the resting potential was determined as the potential difference between the APW and KCl compartments before stimulation. The junction and offset potentials between electrodes were cancelled by immersion of both recording electrodes in the KCl compartment. Internodal cells of 140–210 mm length and 1–2 mm diameter were selected. With a 120-s stimulation period, cells were responsive to several tens of stimulations.

The magnetic field was measured simultaneously at 37 points on a horizontal plane 50 mm above the internodal cell with a multichannel magnetometer system consisting of 37 dc-SQUID magnetometers, each with a 50-mm^2 pick-up coil and 0.016–250 Hz band width (Fig. 1) (Koch et al., 1991). Experiments were carried out at room temperature in the Berlin magnetically shielded room (Erné et al., 1981). Hewlett-Packard computers (HP 1000 series) were used for data acquisition and analysis.

RESULTS

Biomagnetic signals of all SQUID magnetometer channels, together with the electrophysiological signal (Fig. 2 *b*), were simultaneously recorded and monitored. The polarity reversal of the biomagnetic signals is clearly seen when comparing signals from magnetometers situated symmetrically with respect to the cell symmetry axis (Fig. 2 *a*), as well as from the isofield representation (Fig. 3). In Figure 3, three consecutive isofield representations are shown. They indicate a vertical component of magnetic field values. The time between the two representations is 300 ms. The dipolar distribution of the magnetic field suggests that the current generator behaves similar to that in animal cells, and the spatial coordinates of

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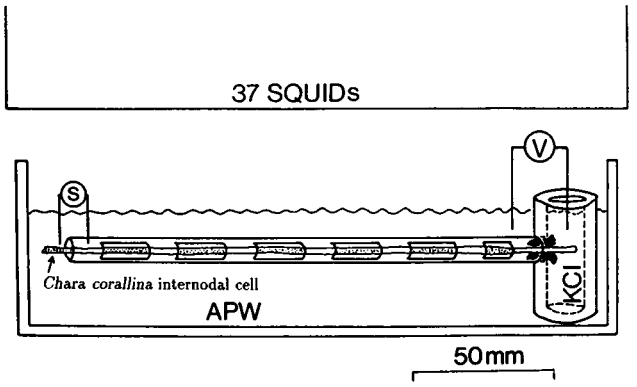

FIGURE 1 Schematic representation of the recording configuration. Magnetic recording of the action potential was carried out by 37 dc-SQUID magnetometers mounted in a cryostat filled with liquid helium and positioned above the *C. corallina* internodal cell. The distance between the plane containing SQUID magnetometers and the cell was 50 mm.

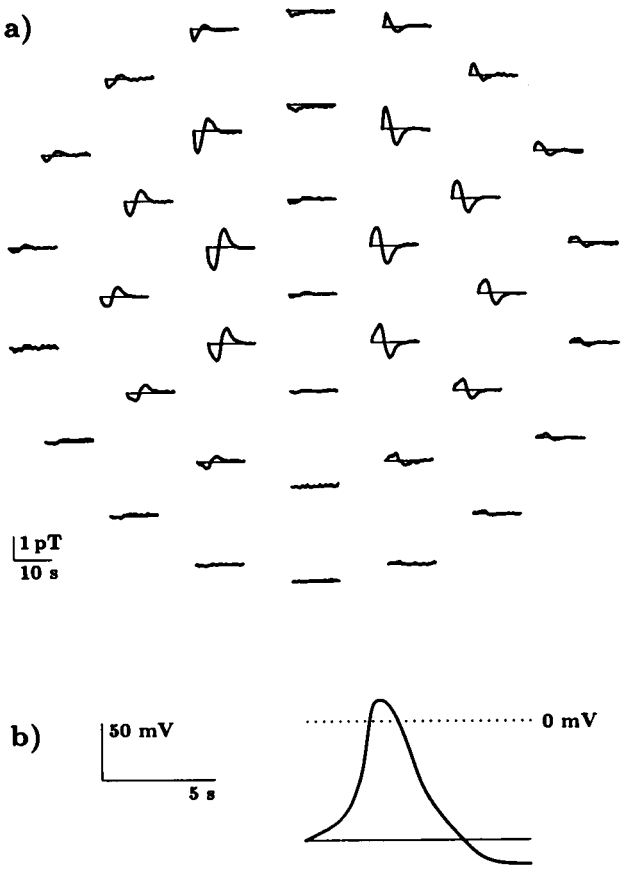

FIGURE 2 (*a*) The time evolution of magnetic field (vertical component) in 37 points in a plane 50 mm above the *C. corallina* internodal cell. The signal amplitude just above the cell, placed in the middle of the figure extending top to bottom, is practically 0, as is expected for the current distribution traveling along the cell. Horizontal lines denote the zero value of magnetic field. (*b*) The time evolution of an action potential.

the central part of the intracellular current distribution can be estimated. Close inspection of Fig. 3 also indicates the motion of excitation along the cell. The projection of the stimulus location corresponds to the point in the central upper part of each part of Fig. 3 (cf. Fig. 1). The excitation is spreading from top to bottom in each part. The time increases from left to right. By comparing the position of the magnetic field extrema, indicated by the + and – signs, this propagation can be followed as the shift in positions of both extrema in time. Evaluating the movement of the extrema per unit time, we obtain a speed of excitation propagation of 3–4 cm/s, which is in agreement with the value obtained from our electrophysiological measurements (data not shown; see also Findlay and Hoppe, 1976; Tester, 1990).

DISCUSSION

We report the magnetic detection (to our knowledge the first such experiment) of an action potential elicited in a single plant cell, the internodal cell of *C. corallina*. We used the highly sensitive 37-channel dc-SQUID magnetometer system and magnetic shielding. This measurement setup was essential for our experiments, since an increase in signal-to-noise ratio by time averaging could hardly be effective with the attainable small number of repetitions. The applied measuring technique gives us the simultaneous time evolu-

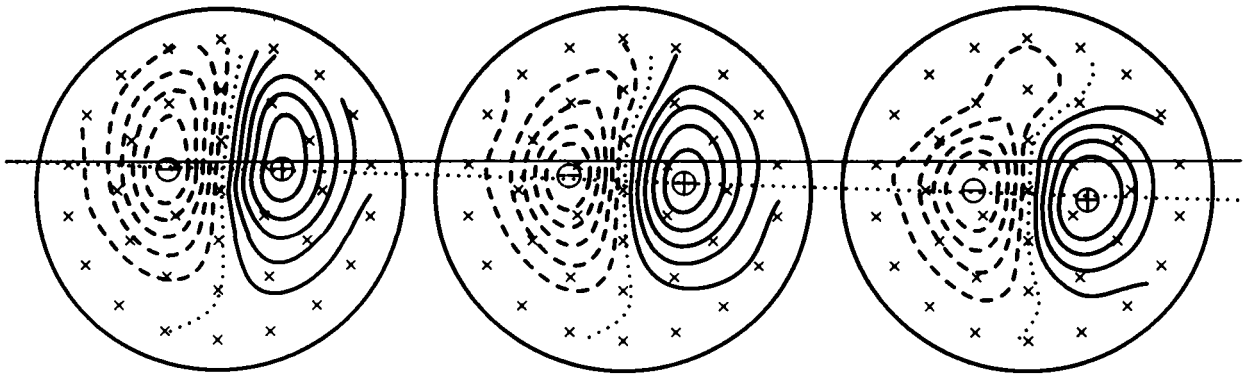

FIGURE 3 The isofield line representation at three particular times, from left to right at 1300, 1600, and 1900 ms after the stimulus. The solid line (a reference) and the dotted line lead the eyes to follow the displaced extrema as explained in the text. The crosses indicate the points where magnetic field was measured. They are arranged in three concentric circles with radii of 3.5, 7, and 10.5 cm. The two neighboring isofield lines are 150 fT apart.

tion of magnetic field at 37 points above the studied cell. Variability from one stimulus to the next is thus excluded. The measured magnetic field directly reflects the behavior of cellular currents. The intracellular current and its spatial distribution can be calculated in a similar way to that of Woosley et al. (Woosley et al., 1985), provided that cylindrical symmetry is valid. For our experiment it can be estimated that the border radius for the validity of cylindrical symmetry reaches $r = \sim 5$ mm around the longitudinal axis of the measured cell. At $r = 5$ mm, the ratio of the total and return current $B_{itot}/B_{ret} \approx 10$. The extracellular σ_{ex} and the intracellular σ_i conductivity were estimated from ionic concentration to be 0.025 $\Omega^{-1}m^{-1}$ and 1.2 $\Omega^{-1}m^{-1}$, respectively. For the ratio of the extracellular to intracellular resistances we get $R_{ex}/R_{in} \approx 140$, taking into account the cell radius of 0.5 mm and the length of the depolarized region as 50 mm. We can therefore assume that the *C. corallina* internodal cell acts as a current source, sending current (when stimulated) into surrounding APW. At larger distances from the cell axis, the cylindrical symmetry no longer holds in our experiment and a numerical calculation of return current is necessary. An estimation can be obtained by drawing the equipotential surfaces and considering the boundary conditions. The geometry of the Petri dish with the APW is such that the contribution of return currents to the magnetic field seen by our magnetometers is small. The magnetometer system detects, namely, the vertical components of magnetic fields caused by all currents which are flowing in the vicinity of the pick-up coils. Taking 35 mm/s as the signal propagation speed and ~1.5 s as the rise time (between 10% and 90% of peak amplitude) for the depolarization (Fig. 2 *b*), we get for the length of the depolarized area 50 mm. An estimation of the peak value of the intracellular current can also be obtained by the method of minimum norm estimation (Hämäläinen and Ilmoniemi, 1984). In this way we obtain a value of about 1 μA for the peak value of the intracellular current. All calculations and details will be published elsewhere.

The jumping transmission suggested by Ping et al. (1990) has been analyzed using magnetic measurement data. To generate an action potential in *C. corallina* cells, the resting membrane must be depolarized by a few 10 mV. The induced signal is several orders of magnitude too small to start the depolarization of a neighbor nonstimulated cell. It follows that other mechanisms have to be considered to explain the jumping transmission. They might include electric or magnetic field receptors within the membrane macromolecular structure as the first step in the whole mechanism of jumping transmission.

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REFERENCES

Ahonen, A. I., M. S. Hämäläinen, M. J. Kajola, J. E. T. Knuutila, O. V. Lounasmaa, J. T. Simola, C. D. Tesche, and V. A. Vilkman. 1991. Multichannel SQUID systems for brain research. *IEEE Trans. Magn.* 27: 2786–2792.

Clint, G. M., and E. A. C. MacRobbie. 1987. Sodium efflux from perfused giant algal cells. *Planta (Berl.)* 171:247–253.

Erné, S. N., H. D. Hahlbohm, H. Scheer, and Z. Trontelj. 1981. Berlin magnetically shielded room (BMSR). *In* Biomagnetism. S. N. Erné, H. D. Hahlbohm, and H. Lübbig, editors. Walter de Gruyter, Berlin. 79–89.

Findlay, G. P., and A. B. Hoppe. 1976. Electrical properties of Plants. *In* Transport in Plants II. U. Luettge and M. G. Pitman, editors. Springer Verlag, Berlin. 70–92.

Hämäläinen, M. S., and R. J. Ilmoniemi. 1984. Interpreting measured magnetic fields of the brain: estimates of current distributions. Report TKK-F-A559, Helsinki University of Technology. 1–35.

Koch, H., R. Cantor, S. N. Erné, K. P. Matthies, M. Peters, T. Ryhänen, H. J. Scheer, and H. D. Hahlbohm. 1991. A 37 channel dc SQUID magnetometer system. *IEEE Trans. Magn.* 27:2793–2796.

Moriyasu, Y., T. Shimmen, and M. Tazawa. 1984. Electric characteristics of the vacuolar membrane of *Chara* in relation to pHv regulation. *Cell Struct. Funct.* 9:235–246.

Ping, Z., T. Mimura, and M. Tazawa. 1990. Jumping transmission of action potential between separately placed internodal cells of *Chara corallina*. *Plant Cell Physiol.* 31:299–302.

Romani, G. L., S. J. Williamson, and L. Kaufman. 1982. Biomagnetic instrumentation. *Rev. Sci. Instr.* 53:1815–1845.

Simons, P. 1992. The secret feelings of plants. *New Scientist* 17:29–33.

Tester, M. 1990. Tansley review no. 21: plant ion channels: whole-cell and single-channels studies. *New Phytol.* 114:305–340.

Wildon, D. C., J. F. Thain, P. E. H. Minchin, I. R. Gubb, A. J. Reilly, Y. D. Skipper, and D. J. Bowles. 1992. Electrical signalling and systematic proteinase inhibitor induction in the wounded plant. *Nature* 360:62–65.

Williamson, S. J., G. L. Romani, L. Kaufman, and I. Modena, eds. 1983. Biomagnetism: An Interdisciplinary Approach. Plenum Press, New York and London.

Woosley, J. K., B. J. Roth, and J. P. Wikswo Jr. 1985. The magnetic field of a single axon: a volume conductor model. *Math. Biosci.* 76:1–36.

Zimmerman, J. E., P. Thiene, and J. T. Harding. 1970. Design and operation of stable RF-biased superconducting point-contact devices and a note on the operation of perfectly clean metal contacts. *J. Appl. Phys.* 41: 1572–1580.