ABSENCE OF BIOMAGNETIC EFFECTS IN *NITELLA*

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ABSTRACT The ability of *N. flexilis* and *N. translucens* to survive exposure to magnetic fields up to 1.6 T for extended periods of time was tested. Internodal cells subjected to such fields for as long as 72 h showed no evidence of damage. The active response of internodal cells was studied using an especially constructed sample chamber which allowed the use of intracellular microelectrodes. Action potentials in longitudinal and transverse fields as large as 2.0 T appeared identical to those in zero field. These results are in sharp contrast to observations reported by Arajs et al. (1975, Proceedings of the International Conference on Magnetism and Magnetic Materials).

The study of possible biomagnetic effects has occupied the attention of a number of investigators over the years. The literature to 1963, as it pertains to plants, was reviewed by Audus and Whish (1964) who, in the same article, also present convincing evidence of magnetotropism due to an inhomogeneous magnetic field. However, as regards the influence of a homogeneous magnetic field on plant physiology the literature is replete with contradictory results (Audus and Whish, 1964).

Nitella is a fresh-water alga of the family Characeae, phylum Chlorophyta. The plant habit is that of erect filaments with branches of limited growth diverging approximately radially from nodal cells of the main filament. The internodal cells range from 2 to 10 cm in length and 0.5 to 1 mm diameter. These long, cylindrical single cells display bioelectric activity, reminiscent of nerve axons, which has been studied extensively by plant physiologists (Hope and Walker, 1975).

Sachava and Samokhvalova (1970) reported a statistically significant reduction of about 10% in the resting potential (RP) of *Nitella flexilis* upon exposure to magnetic fields between 0.1 and 0.34 T. Since in an earlier study (Blatt, 1974) of the resting and action potentials (AP) of *N. flexilis* we found that temperature changes had a much more profound influence on the AP than on the RP, we decided to study the influence of a magnetic field on the AP in this system. While we were engaged in this work Arajs et al. (1975) reported on the influence of a magnetic field on *N. clavata.* They found that transverse and especially longitudinal fields had a very substantial effect in reducing the AP and, most surprising, that internodal cells of *N. clavata* failed to survive exposure to fields of 1 T for periods exceeding 24 h. The purpose of this note is to describe our experiments and report results which are at variance with those of Arajs et al.

We constructed two identical sample chambers of acrylic plastic which could be per-

fused with artificial pond water (APW) at a controlled rate. Three internodal cells of *N. flexilis* were placed in each chamber. Cyclosis was used as an indication of viability, and cells were selected in which cyclosis proceeded at 60–70 μm/s at 20°C. One sample chamber was then placed between the pole faces of a large electromagnet, the other next to the magnet. Both chambers were continually perfused with APW from the same reservoir at a rate of about 20 ml/h. Temperature was monitored and controlled by inserting nearly matched thermistors—Veco T43A53 (Victory Engineering Corp., Springfield, N.J.), with resistances at 20°C of 28,010 and 27,950 Ω, and dR/dT = −1,200 Ω/°C at 20°C—in each chamber. The chamber external to the magnet was placed on a heat sink whose temperature could be controlled electronically to better than ±0.1°C by means of a Peltier unit of local construction. During the course of a run the sample temperatures would generally rise from about 24°C to about 28°C. Approximately identical illumination was provided by two incandescent lamps placed equidistant from the chambers.

In the first of three runs the magnetic field was maintained at 1.0 T for 48 h. At the end of the exposure the cells were examined and cyclosis was measured. In all cells cyclosis proceeded at normal rate and we could observe no visible deterioration of the cells. Using six new cells we exposed three to a field of 1.6 T for 72 h. One of the cells had lysed at the end of the time, but this was in the chamber outside the magnet and failure to survive was obviously the result of accidental mechanical damage inflicted when the cell was placed in the chamber. In the third run six cells of *N. translucens* were used, the field was again maintained at 1.6 T for 72 h. Once again we found no evidence of damage due to the magnetic field. All cells used in these experiments were examined daily for a week after exposure to the field and no aftereffect was apparent.

We also measured the AP of *Nitella* in transverse and longitudinal fields. Arajs et al. used extracellular contacts (cotton-covered metal wires on which the cell was supported) to stimulate and sense the AP. In our experiments we employed intracellular glass microelectrodes, one for introducing the stimulating current pulses, the other to detect the AP. Since it would be extremely difficult to locate micromanipulators in the immediate vicinity of the magnet pole faces and virtually impossible to place a dissecting microscope in this region, we constructed a sample chamber into which several small, crude, but adequate micromanipulators were incorporated. This construction had the great advantage that the chamber, with sample cell in position, could be placed on the microscope stage and the microelectrodes inserted into the cell while outside the magnet. Thereafter, with microelectrodes firmly fixed in position, the entire unit could then be relocated between the pole faces of the magnet. The sample chamber was constructed to permit the study of excitable response in both transverse and longitudinal configurations.

After penetration by the microelectrodes, the cell was stimulated at room temperature at 15-min intervals for 1 h and the AP was recorded using a Brush recorder and also displayed on a storage oscilloscope. The sample chamber was then positioned between the pole faces of the unenergized magnet and the cell stimulated for another

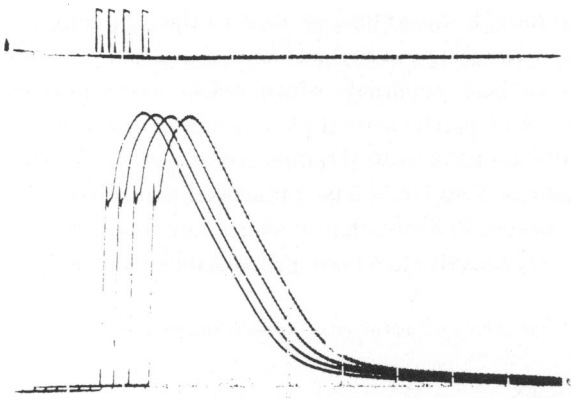

FIGURE 1 Response (lower trace) of *N. translucens* to near threshold stimulus pulses of 0.4 μA and 50 ms duration (upper trace). Increased delay was employed to separate responses at 0, 0.5, 1.0, and 2.0 T. The small ripple on the lower trace at the higher fields is due to pickup of line-frequency ripple in the magnet current. Abscissa: 0.5 s/div. Ordinates: upper trace, 0.5 μA/div; lower trace, 20 mV/div.

hour to verify that the small remanent field ($\sim 3 \times 10^{-3}$ T) had no effect on the response. The magnet was then energized to 0.5, 1.0, 1.5, and 2.0 T for 90 min at each field value. Before changing the field, the magnet was turned off for 60 min. Throughout the 10 h of the experiment the cell was stimulated at 15-min intervals.

We made three runs in the transverse and two in the longitudinal configuration, using *N. flexilis* in two transverse and one longitudinal, and *N. translucens* in the other runs. We could detect only small random fluctuations of less than 5%; application and subsequent removal of the magnetic field had no observable effect on the AP nor, apparently, on the threshold for excitation. We normally used stimulating pulses of 50 ms duration and maintained the current at 1.25 times the threshold current at zero field. At the end of each experiment the stimulus pulse was reduced to near threshold and response was checked for that stimulus at 0, 0.5, 1.0, and 2.0 T. Normal response was observed at each field value (Fig. 1). Following the experiment the cells were examined for normal response for 2 days and we observed no aftereffects. A final run, using *N. flexilis,* in which a cell was stimulated continuously for 15 h at 15-min intervals gave results identical to those observed on cells subjected to magnetic fields.

Our results, therefore, fail to support the findings of Arajs et al. Cells subjected to fields of 1.6 T for as long as 72 h showed no evidence of damage, nor could we detect any influence of transverse or longitudinal fields on the excitable response, using fields up to 2.0 T.

While we cannot offer any explanation for the discrepancy between our results and those of Arajs et al., there are various possibilities that come to mind. For example, when external electrodes are employed to stimulate the cell, only a fraction of the current between the electrodes actually transverses the membrane. It is then quite likely that a magnetic field which distorts the current flow will modify the actual stim-

ulating current even though the voltage applied to the stimulating electrodes is kept constant. Similarly, when using external probes to detect the AP, a magnetic field may well cause changes in these potentials which reflect electromagnetic effects on the "external" circuitry. As regards survival of cells in a magnetic field, as far as we can discern Arajs et al. did not use a control sample, so that extraneous factors not directly attributable to a magnetic field could have influenced their observations. Finally, it is possible, though we believe unlikely, that in sharp contrast to *N. flexilis* and *N. translucens, N. clavata* is very sensitive to a homogeneous magnetic field.

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