Physiological and Molecular Genetic Effects of Time-Varying Electromagnetic Fields on Human Neuronal Cells

English-language restatement of NASA/TP-2003-212054 · Thomas J. Goodwin · September 2003

Source: Physiological and Molecular Genetic Effects of Time-Varying Electromagnetic Fields on Human Neuronal Cells. NASA/TP-2003-212054. The charts below are newly drawn using the exact values in the study tables. This document presents selected textual and numerical findings, not the full 37-page original report.

Abstract

The investigation describes systems for culturing human neural progenitor cells in two- and three-dimensional configurations while applying a time-varying electromagnetic field (TVEMF). In two-dimensional cultures, cells grew longitudinally and formed tissue strands along electrodes. In three-dimensional cultures, cellular aggregates developed with organization resembling neural tissue. The report states that proliferation in both configurations was 2.5 to 4.0 times that of non-waveform controls. Gene-array analysis assessed the expression of more than 10,000 human genes.

Background

Earlier investigations used static electric fields, direct current, magnetic fields, and charged polymer materials in attempts to influence nerve growth. This study instead investigated a time-varying electromagnetic field near cultured neural cells. Three-dimensional cultures were grown in a rotating-wall vessel (RWV), which simulates certain physical aspects of microgravity.

Cells and culture conditions

Normal human neural progenitor (NHNP) cells from three donors were pooled to reduce variation between donors. Two-dimensional cultures used GTSF-2 medium with 10% fetal bovine serum, ciprofloxacin, and Fungizone. Cells were cultured in petri dishes either on the electrodes or without direct contact with the metal surface. Incubation was at 37°C with 6% CO■.

For three-dimensional culture, a 55-ml RWV was loaded with collagen-coated Cytodex-3 microcarriers (5 mg/ml) and NHNP cells at 2.5 × 10■ cells/ml. Cultures were maintained for 17–21 days or until tissue masses 3–5 mm in diameter formed.

Electromagnetic stimulation

A custom-built generator supplied a 1–6 mA alternating-current square wave at 10 Hz, with a variable duty cycle and pulse-width modulation. The report specifies low-level magnetic fields of approximately 10–200 milligauss. For three-dimensional cultures, an electromagnetic coil was wrapped around the RWV core.

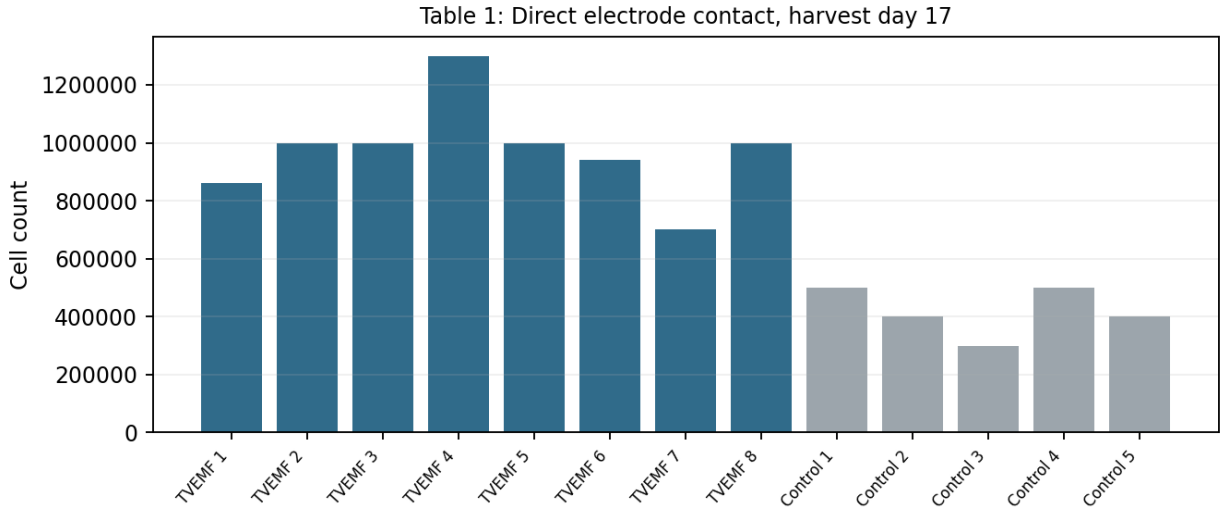
Experimental procedures and measurements

In the two-dimensional experiments, 2.5 × 10■ cells in 0.7 ml of medium were placed onto an electrode. After two days of incubation, experimental day 0 was defined, medium was added, and TVEMF exposure began. Medium was replenished on the specified experimental days, and cells were harvested after 17–21 days. In a parallel setup, electrodes were attached beneath the dishes without direct contact with cells.

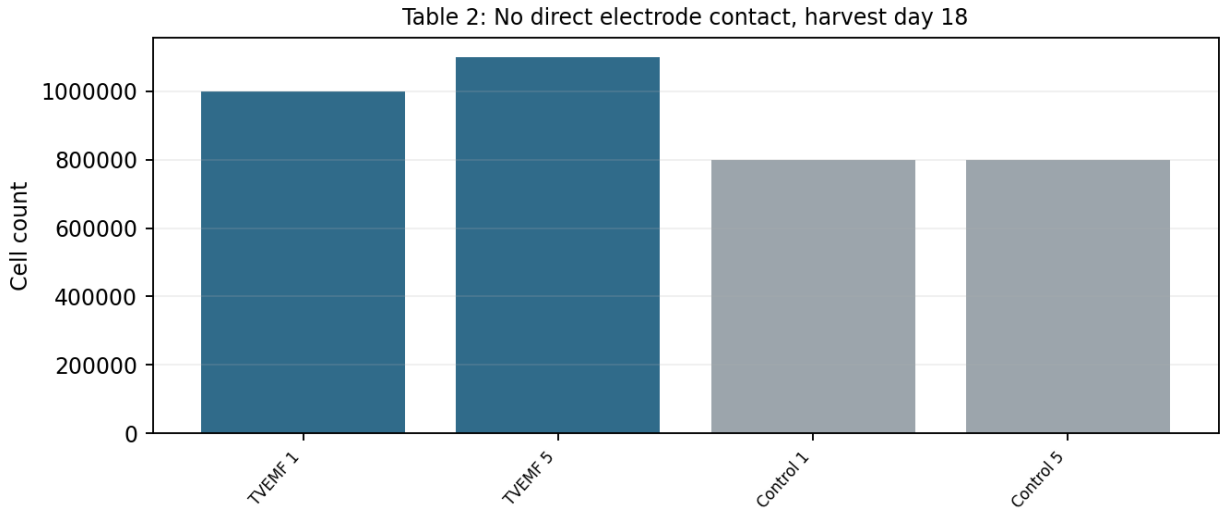
In RWV cultures, pH, dissolved oxygen, dissolved carbon dioxide, glucose, viability, and lactate dehydrogenase were monitored. Light microscopy, scanning electron microscopy, and gene-expression analysis were used. Experimental and control groups were tested in triplicate or quadruplicate, depending on material availability.

Results: two-dimensional cultures

According to the report, TVEMF-exposed cells showed faster growth and altered morphology compared with controls, whether or not cells directly contacted electrodes. Exposed cells displayed preferential alignment, whereas controls showed a more random growth pattern. Stimulation persisted for up to 72 hours after field removal with direct electrode contact. Without direct contact, accelerated growth was reported as late as 168 hours after exposure ended. Growth returned to normal rates without further stimulation.



Every individual value comes from Table 1, report page 7. Viability: 98%.



Every individual value comes from Table 2, report page 8. Viability: 100%.

Results: three-dimensional cultures

TVEMF-exposed RWV cultures showed changes in growth, orientation, and gene expression similar to those observed in two-dimensional cultures. The report describes little or no difference in the measured metabolic

parameters between exposed and control cultures. Glucose consumption and lactate production were nearly identical. Oxygen consumption and carbon dioxide levels were slightly higher in the TVEMF group in the later stages of the experiment.

Gene-expression analysis

Gene expression was assessed after 17–21 days of culture using an array containing more than 12,000 unique human genes. The report describes down-regulation of approximately 175 genes associated with maturation and regulation and up-regulation of approximately 150 genes associated with growth and cellular proliferation. Table 5 reports changes from −1.4 to −2.7 on the report's log scale; Table 6 reports changes from 1.7 to 8.4 on that scale.

Microscopic observations

Figures 4 and 5 show a rim of growth around tissue previously exposed to TVEMF, described as the "corona" effect. Figure 6 shows control tissue that attached but did not proliferate during the same 24-hour interval. Figures 12 and 13 compare cellular organization and structures in control and exposed cultures.

Discussion and conclusions — restated from the report

The report concludes that, under the described culture conditions, time-varying electromagnetic fields were associated with increased proliferation, altered cell morphology, and altered gene expression in human neural progenitor cells. The author describes the models as potential systems for investigating regulation of neural tissue growth.

Basis for the charts

The newly drawn charts use the individual measurements in Tables 1 and 2, rather than digitized or estimated points from the original line graphs in Figures 7–11. The report does not provide underlying numerical tables for those line graphs, so they have not been redrawn with purportedly exact data.