Effects of Various EMFs on Nerve Regeneration *In Vitro.*

Ewa Herbst[1], Philip Resig[2], Scott Ranney[2], Betty F. Sisken[2]
[1] Herbst Research, Inc., P.O. Box 89, Edgewater NJ 07020-0089, USA
[2] Center for Biomedical Engineering, University of Kentucky, Lexington KY 40506-0070, USA

Abstract: *Three electromagnetic fields (EMFs) with a peak value of 0.3 mT but with differently designed shapes and timing parameters were applied to a chick embryo dorsal root ganglia (DRG) model in vitro. One of the three fields resulted in a significant (p=0.0002) increase in the neurite length. No effect on neurite number was observed. We are continuing a systematic approach to signal optimization in the in vitro nerve regeneration model.*

METHODS

Dorsal root ganglia (DRG) dissected from 8-1/2- to 9-day chick embryos were explanted to 60-mm culture dishes coated with rat tail collagen and cultured [1]. Six dishes per group with 12 DRG per dish were used in each experiment; one group served as a sham control and one as an EMF exposed group. The NGF concentrations used were 0 ng/ml, 2 ng/ml, and 50 ng/ml [2]. The cultures were fed with neurobasal and N2 supplement (Gibco Co., NY) and cultured for 48 hours at 37°C and 95% air, 5% $C0_2$, before fixation with phosphate-buffered formalin.

All six EMF treated culture dishes were placed on one shelf, centrally located in a horizontal 30 cm x 30 cm Helmholtz coil housed in the bottom chamber of a two-chamber incubator. The other six dishes (controls) were placed in the top incubator with no EMF. Three different EMFs were tested in this experimental set-up for 2 hrs/day for 2 days. Each experiment was repeated seven to eight times. The peak magnetic flux density was constant for all three fields and equal to 0.3 mT, but their shapes and time characteristics were different.

ASSESSMENT OF NEURITE OUTGROWTH

Pictures of each DRG were taken and color prints were made. DRG neurite length (i.e, the distance measured in a straight line from the main body of the DRG to the tip of each neurite) and the number of neurites per DRG were determined by manual measurements [3].

Mean neurite length and mean number of neurites was computed for each location (inner and outer DRG) in each dish and a series of linear mixed models was fitted to these data. Statistical significance for all main effects and all interaction effects was set at 0.05. Post hoc comparison of mean response for statistically significant effects was based on Fisher's least significant difference procedure using Procedure Means and Procedure Mixed in SAS.

RESULTS

There is a significant effect due to the interaction of treatment and NGF concentration on the neurite length, but not on the neurite number, for one of the three fields tested. For NGF concentration of 2 ng/ml the mean neurite length response to one of the three EMFs is significantly larger than the mean response for control (P = 0.0002). There is no difference between treatment and control at the remaining two NGF concentrations for the same EMF, nor for the other two fields at the same NGF concentration.

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