Applied electric field enhances DRG neurite growth: Influence of stimulation media, surface coating, and growth supplements

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Short running title: Electric field enhances neurite growth

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Abstract

Electrical therapies have been found to aid repair of nerve injuries and have been shown to increase and direct neurite outgrowth during stimulation. This enhanced neural growth existed even after the electric field (EF) or stimulation was removed, but the factors that may influence the enhanced growth, such as stimulation media or surface coating, have not been fully investigated. This study characterized neurite outgrowth and branching under various conditions: EF magnitude and application time, ECM surface coating, medium during EF application, and growth supplements. A uniform, low-magnitude EF (24 or 44 V/m) was applied to dissociated chick embryo dorsal root ganglia seeded on collagen or laminin coated surfaces. During the growth period, cells were either exposed to NGF or N2, and during stimulation cells were exposed to either unsupplemented media (Ca\(^{2+}\)) or PBS (no Ca\(^{2+}\)). Parallel controls for each experiment included cells exposed to the chamber with no stimulation and cells remaining outside the chamber. After brief electrical stimulation (10 min), neurite length was significantly increased 24 h after application for all conditions studied. Of particular interest, increased stimulation time (10 min to 100 min) further enhanced neurite length on laminin but not collagen surfaces. Neurite branching was not affected by stimulation on any surface, and no preferential growth of neurites was noted after stimulation. Overall, the results of this report suggest that short duration electric stimulation is sufficient to enhance neurite length in a variety of conditions. While further data is needed to fully elucidate a mechanism for this increased growth, this data suggests that one focus of those investigations should be the interaction between the growth cone and the substrata.

Keywords— DRG, ECM, electric field, neurite outgrowth, neurite branching
1. Introduction

Electrical therapies have been found to improve repair of nerve injuries *in vivo* [1, 2] and align and increase neurite outgrowth *in vitro* [3-6]. *In vitro* research has focused on the effects an electric field (EF) has on neurite behavior during stimulation; the presence of direct current EFs caused Xenopus and chick embryonic dorsal root ganglia (DRG) neurites to align themselves so as to face the cathode [3-6], accelerate growth to the cathode [3-6], and increase branching [5, 7]. Enhanced neural growth also has been demonstrated after EF application, although many of the parameters for stimulation, such as magnitude and application times have been varied. Additionally, magnitude and application time of EF have altered the outgrowth of neurites while the EF is present. For example, increasing EF magnitude subsequently increased neurite outgrowth rates toward the cathode but decreased rates toward the anode [3, 6, 8]. Similarly, during EF application, Xenopus neurite outgrowth rates and alignment to the electric field increased over the course of 4-5 h [4].

In contrast, the results *in vivo* have demonstrated that enhanced neural benefits derived from brief stimulation times were no different than those obtained from extended application times. For example, long (2 wk) or brief (1 h) pulsatile stimulation dramatically reduced the period of axonal regeneration in rats by 6 weeks (from 9 weeks to 3 weeks) with no clear difference between the two stimulation times [2]. Also, pulsed electromagnetic stimulation of rat sciatic nerves after a crush lesion enhanced neural regeneration rates with no difference between those stimulated for 1 h per day for 3 days compared to the same course for 4 h/day, 10 h/day, or 6 h on/off for 3 days [9]. Ultimately, if similar regeneration is noted for brief or extended stimulation times *in vivo*, overall application time may be reduced to minimize the exposure to EF and allow for potential use for tissue engineered applications.
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While extended stimulation in vitro may guide neurites after stimulation [10, 11], brief stimulation does not guide or orient neurites [12, 13]. Therefore, to utilize the benefits of EF while reducing the stimulation time, other guiding mechanisms may be required. Alternatively, neurite guidance could be obtained through extracellular matrix (ECM) coatings that provide orientation thru micropatterns [14, 15]. ECM provides signals that permit and control nerve regeneration in vivo. By binding neurites and nerve cells through specific cell-matrix receptors, ECM provides both chemical and physical guidance factors. Different surfaces coated with ECM proteins can affect neurite outgrowth through adhesive differences, which may result from different charges on the surface. Additionally, an electric field can affect the charge of the surface where the neurite binding is occurring, thus changing the extension rate [16]. The focus of this study was to characterize neurite outgrowth and branching after EF application on different ECM-coated surfaces. The effects of chemical and electrical factors on neurite outgrowth and branching after electrical stimulation were characterized by investigating the following circumstances: EF application time and magnitude, ECM surface coating, media during electric field application, and growth supplements.

2. Materials and Methods

2.1 Substrate Preparation

Glass coverslips (25 mm) were cleaned with Alconox detergent, stored in absolute ethanol, and acid etched with 9:1 H$_2$SO$_4$: H$_2$O$_2$. The underside of the coverslip was marked with a diamond tipped pen to indicate alignment to the field. The coverslips were dried in a stream of filtered air and stored in a desiccator at 25°C until ECM coating.

Laminin (mouse EHS at 50 µg/mL) or collagen (rat type I at 150 µg/mL) was adsorbed to the coverslips overnight at 2-8°C. The following day, the coverslips were washed in phosphate buffered
Electric field enhances neurite growth saline (PBS), sterilized by absolute alcohol treatment for 30 min, and washed 2x in PBS. The coverslips were stored at 2-8°C for up to 3 weeks before use.

2.2 Cell Culture

Dorsal root ganglia (DRG) were harvested from E9-10 chicks (Specific Pathogen Free, Charles River Laboratories) and dissociated in 1X trypsin for 20 min. Cells were then washed in media and counted via trypan blue exclusion. For the serum-free portion of the study, dissociated DRG were seeded onto ECM-coated coverslips at $2.1 \times 10^4$ cells/cm$^2$ in minimum essential medium (MEM, Sigma-Aldrich) containing 1% N-2 supplement (Invitrogen), 1% penicillin-streptomycin (Sigma-Aldrich), and 200 mM L-glutamine (Invitrogen). Otherwise, the dissociated DRG were seeded onto ECM-coated coverslips at $7.5 \times 10^3$ cells/cm$^2$ in MEM containing 10% fetal bovine serum (FBS, Invitrogen) with 10 ng/ml nerve growth factor (NGF, Chemicon). The seeding densities utilized were deemed optimal for image analysis in preliminary experiments. All DRG were incubated for 24 h at 5% CO$_2$ and 37°C to allow for cell adhesion and initial neurite growth.

2.3 Electrical Stimulation

Chambers to conduct the electrical stimulation have been described previously [13]. Essentially, two platinum sheets served as electrodes within a milled polyethylene rectangular chamber. The stimulation was applied via constant voltage power supply. The electric field was measured as the voltage applied divided by the distance between the plates (11 cm). The chambers were tested to determine the voltages that could be applied as high current caused dramatic pH changes in the media. All electric fields applied in the study had little effect on pH for the duration of stimulation, therefore, position in the chamber was not considered to affect the study [13].

Electrical stimulations were performed 24 or 44 V/m, 250 or 500 µA respectively, for 10 min or 100 min to cells seeded on collagen or laminin coverslips. These field strengths were within
Electric field enhances neurite growth physiological ranges [17]. To stimulate, coverslips were placed into a chamber containing unsupplemented MEM, Leibovitz media (L-15, Sigma-Aldrich), or PBS. Table 1 contains a complete list of tests performed. Sham-exposed samples were treated identically (i.e., placed in the media-filled chamber) except no field was applied. Additionally, control slips were incubated in media-filled Petri dishes for the same time duration as used during stimulation. After stimulation, seeded coverslips were transferred back to an incubator at 37°C and 5% CO₂ and given the same culture media used initially.

Cells were fixed in methanol (-20°C) approximately 22–24 h after stimulation completion (100 min and 10 min, respectively). After fixation, cells were stained with Hoescht nuclei stain (H33258, Sigma-Aldrich) at 100 ng/coverslip to assure no overlap of other neural or Schwann cells with neurite extensions.

2.4 Controls for Stimulation

To examine possible effects of the stimulated medium on neurite growth, PBS was stimulated for 10 min at 24 V/m in an otherwise empty chamber. Cells were seeded on laminin coated slips as described above (NGF-containing medium) and after 24 hr of culture, the stimulated PBS was then placed on coverslips for 10 min, mimicking stimulation. The cells were then resupplemented with complete medium and incubated for an additional 24 hrs. To investigate if protein surfaces were a factor for enhanced growth, laminin coated coverslips were stimulated in L15 for 10 min or 100 min at 24 V/m. After stimulation, the surfaces were seeded with dissociated cells and cultured in NGF supplemented medium for 48 hours prior to fixation.

2.5 Imaging and Data Analysis

DRG neurites, 25 maximum per coverslip, were imaged 48 h after seeding and at least 3 stimulation trials were performed per variable. All cells were imaged using light (phase-contrast) and
Electric field enhances neurite growth fluorescent microscopy and were captured utilizing multi-channel processing, which allows the images to be super-imposed on one another.

Images were analyzed for neurite outgrowth and branching where the longest neurite per cell was measured to assess the neurites. Taking the longest neurite per cell rather than all or a selection of neurites from cells alleviated some error since lengths would differ from the same cell, and most cells only had a single neurite while others had multiple, shorter neurites. Additionally, neurites with paths that intersected or ended with other neurites or cells, denoted via nuclear staining, were not included. Neurite length was assessed by tracing a path from the start of the neurite at the cell body to the tip of the neurite through the appropriate branches. Neurite branching was a count of how many times the neurite divided into two or more paths. A branch was indicated when the neurite width did not decrease in diameter to the original neurite. The average of branching per 100 µm of neurite length was calculated. For one set of experiments involving electric stimulation, the neurite orientation to the field was measured by considering the angle of the cell body center (referenced to the cathode) to the neurite tip, where angles ranged from 0° (cathode directed) to 180° (anode directed) with respect to orientation in the electric field. It was found that electric stimulation did not cause a change in orientation of neurite growth compared to the control (unstimulated), similar to what was found previously for whole DRG after brief stimulation [13]. Therefore, neurite orientation was not measured for additional experiments.

Statistical analysis was performed for comparison between neurite outgrowth, orientation, and branching for DRG using single factor ANOVA (Microsoft Excel) where p < 0.05 was considered significant. At least 85 DRG per group and variable were imaged. The mean ± standard error of the mean (SE) were reported in the results and figures.

3. Results
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Simple exposure to the electrical stimulation chamber design did not alter DRG neurite growth or branching. Table 2 represents the average lengths for 24 V/m and 44 V/m samples and controls; Table 3 represents the average branching for 24 V/m and 44 V/m samples and controls. Statistical comparison between the sham and the control revealed no significant difference for neurite length or branching for the two in any of the experiments. For example, sham DRG grown in media containing N-2 on laminin surfaces exhibited neurite outgrowth of 141 ± 13 µm and control DRG neurite outgrowth was 139 ± 12 µm. Therefore, all controls that follow represent sham data for uniformity.

3.1 Effect of Stimulation Medium

The stimulation medium was altered during the course of the experiments depending upon the test conditions. PBS was utilized initially as a non-Ca\(^{2+}\) containing stimulation medium and to compare to previous experiments with whole DRG [13]. MEM was utilized as a Ca\(^{2+}\) containing medium for comparison. However, the pH of MEM was variable if the time of the stimulation was longer and the atmosphere was not CO\(_2\) controlled. Therefore, L15 was utilized to help buffer the pH without the use of CO\(_2\) and to include Ca\(^{2+}\) still (as opposed to PBS). Additionally, the pH remained more balanced over the time course of the stimulation (unpublished data). Overall, results demonstrated that regardless of stimulation medium, stimulation resulted in a statistically increased neurite length.

Tests were performed with NGF+serum supplemented growth medium to examine if stimulation in MEM correlated to stimulation in L15 prior to changing medium. Neurite growth on laminin surfaces after 10 min stimulation at 24 V/m demonstrated no significant differences, with lengths of 167 ± 9 µm in MEM (44% increase over control) and 147 ± 7 µm in L15 (27% increase over control). Similarly, the branching was not significantly different than controls (0.40 ± 0.06 branch/100 µm (MEM) and 0.37 ± 0.05 branch/100 µm (L15)). On collagen surfaces, L15 stimulation medium was compared to PBS at 44
Electric field enhances neurite growth V/m with no statistical differences in lengths (181 ± 10 µm (L15; 44% increase over control) and 181 ± 8 µm (PBS; 44% increase over control)) or branching (0.32 ± 0.06 branch/100 µm (L15) and 0.30 ± 0.05 branch/100 µm (PBS)). As stimulation medium did not alter the enhanced growth after stimulation at the different voltages, further results were compared across the different stimulation media.

3.2 Effect of Growth Supplements

The effect of growth supplements was examined to determine if the growth factor during culture was necessary for enhanced growth after EF application. Dissociated DRG on both substrates exhibited statistically increased neurite length when stimulated at 24 V/m for 10 min compared to their respective controls; this result held regardless of the growth supplement (Figure 1). Without any stimulation, neurite lengths for cells grown in N2 supplement (180 ± 13 µm on collagen and 141 ± 13 µm on laminin) were longer than those in NGF (119 ± 5 µm on collagen and 116 ± 6 µm on laminin). For N2 supplemented medium, neurites were 220 ± 14 µm on collagen and 208 ± 21 µm on laminin after stimulation, an increase of 22% and 47%, respectively, over controls. With NGF supplemented growth medium, neurites were 174 ± 7 µm on collagen and 167 ± 9 µm on laminin after stimulation, increasing
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46% and 44% over controls, respectively. To directly compare conditions, neurites were stimulated in MEM on laminin and cultured in N2 or NGF, resulting in neurites that were 47% or 44% longer than controls, respectively. In N2 supplemented media, unstimulated controls on collagen-coated surfaces had neurites that were significantly longer than those on laminin-coated surfaces; the controls in NGF were similar regardless of surface coating. Branching was similar between each test and control except for N2 growth supplement on collagen surfaces, where the stimulated samples had 0.23 ± 0.04 branch/100 µm and controls had 0.13 ± 0.03 branch/100 µm. Overall, results ranged from 0.13 – 0.28 branch/100 µm for N2 and 0.13 - 0.49 branch/100 µm for NGF.

Figure 1: Growth supplements during cell culture periods did not alter the enhanced growth seen after stimulation. Extremes of serum+NGF and N2(no NGF) were examined, and the stimulated samples had statistically increased growth over controls.

Field was 24 V/m for 10 min.
3.3 Effect of Stimulation Parameters

Three different stimulation parameters were studied to examine how differences during the stimulation may affect the resulting neurite growth; these parameters were temperature, field strength, and stimulation time. Most stimulations took place at room temperature. However, as the time was increased to 100 min, it was deemed more important to keep the temperature at 37°C. Two field strengths were examined: 24 V/m and 44 V/m. These two field strengths were the same conditions utilized in a previous study to examine whole DRG neurite outgrowth, and neither field strength altered the stimulation media pH significantly (unpublished data). Finally, duration of stimulation was examined as a factor in neurite growth. Two times were examined, 10 min and 100 min (10x longer). Temperature as a factor was examined on laminin coated slips for a 10 min, 24 V/m stimulation. As noted above, neurite lengths were statistically increased over their respective controls for both room temperature (RT) and 37°C by 27% and 32%, respectively, but no difference was noted between the lengths at RT compared to 37°C for either the stimulated or the controls (Figure 2). This result was expected as the time outside of 37°C was only 10 min. Therefore, further results are compared to 10 min stimulations regardless of the temperature. When comparing the increase in average neurite length of cells exposed to different field strengths for 10 min, no differences on collagen were noted with 46% (173 ± 7 µm, 24 V/m) and 43% (181 ± 10 µm, 44 V/m) increases over controls (Table 2). Field strength also did not produce any statistical changes in branching (Table 3). However, duration of stimulation produced interesting results, with no statistical differences when examining lengths on collagen (173 ± 7 µm (46% increase), 10 min and 177 ± 8 µm (33% increase), 100 min) but lengths statistically increased when
Electric field enhances neurite growth cultured on laminin (154 ± 8 µm (32% increase), 10 min and 201 ± 11 µm (54% increase), 100 min) (Figure 2). Neither case had branching that was statistically different than their respective controls.

3.4 Effect of Stimulated Surfaces or Medium

Two studies were conducted to examine the potential effects of the stimulation on the environment of the cells rather than the cells themselves. PBS was stimulated and placed on cultures for 10 min; no statistical differences were found on neurite length or branching compared to controls.

Figure 2: Variations in field strength, temperature and time were all investigated to determine if any of these factors would alter the enhanced growth of DRG seen after electrical stimulation. All samples demonstrated statistically enhanced growth over controls, but the samples on similar surfaces were not different from each other. The only exception was for DRG neurites on laminin surfaces; increasing the time of stimulation from 10 min to 100 min significantly increased the length.
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However, while not statistically different, the average length of the samples was decreased compared to the control (124 ± 8 μm vs. 149 ± 10 μm). Similarly, the stimulation of laminin-coated surfaces for 10 min or 100 min followed by cell seeding had no statistical effect on neurite length or branching, with average lengths of samples 88% (10 min) and 92% (100 min) of controls.

3.5 Percentage of cells bearing neurites

Since DRG exposed to EF for 100 min on either surface coating exhibited increased neurite outgrowth, the percentage of cells bearing neurites was quantified. DRG grown on collagen-coated surfaces expressed a decreased percentage of cells bearing neurites than those grown on laminin whether or not they were exposed to EF (Table 4). Additionally, DRG on collagen-coated surfaces after exposure had increased neurite expression compared to the sham, from 13% to 23%, while those on laminin-coated surfaces did not show any differences in neurite expression after stimulation, with 27% expression when unstimulated and 31% when stimulated.

4. Discussion

A majority of the research in electrical stimulation of nerve cells has investigated its effects on neurite and growth cone guidance, branching, and outgrowth during the applied stimulation [3, 6, 18]. While results differ, most studies sought to investigate effects or mechanisms that were altered during long term stimulations. While long duration stimulations are possible in vitro, in vivo situations require permanent implants for long term EF application [19]. For tissue engineering applications, short term stimulation may be a reasonable alternative if the effects of long term stimulation can be mimicked. Therefore, this study sought to understand the conditions important to enhancing nerve growth after short term EF application. Growth medium, stimulation medium, stimulation factors, and culture
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substrata were all examined for their ability to alter neurite behavior after EF application. The results indicate that neurite outgrowth was increased after brief electrical stimulation under all the circumstances, but enhanced neurite branching and guidance was not observed.

4.1 Growth Media

The components of growth medium have been demonstrated to have an effect on the behavior of neural cells. NGF, for example, has been shown to initially increase the rate of adult rat dissociated DRG outgrowth, but after 3 days, no difference in growth rates was noted with or without NGF [20]. Additionally, rat or chick DRG neurites grew in NGF and NGF-free environments similarly, as long as DRG were young [21] or cultured on 2D substrata [22]. However, using antisera to block NGF, purified chick E8-10 DRG did not survive or sprout neurites, indicating that the supporting cells, even if few are present, may release enough factors for cell survival and neurite growth [23], although the amount of NGF in the culture media was not measured. Serum has also been demonstrated to influence cell behavior in culture, but supplements, such as N1 or N2 neural serum replacements, are typically sufficient to culture nerve cells short term [24, 25]. For the present study, two extreme conditions were examined: full serum+NGF versus N2 (no NGF). Both conditions resulted in neural growth in controls, and enhanced neural growth in stimulation conditions, suggesting that supplementing the cultures with serum and NGF were not necessary for enhanced growth over controls. While these conditions by themselves don’t offer a mechanism for the enhanced growth, the results help to eliminate some possibilities for a defined pathway that drives the enhanced growth. For example, if serum-deposition or availability was required for the enhanced growth, the cultures without serum would not demonstrate similar growth increases after stimulation. Similarly, if NGF regulation was altered via stimulation, differences in outgrowth may have been noted after stimulation between the two cultures. Interestingly, serum conditions have been shown to better support Schwann cell culture than serum-free replacements.
Electric field enhances neurite growth [26], so the serum-free condition may also indicate that Schwann cell behavior in both cultures was similarly affected (or unaffected) by electrical stimulation. As differences in percentage increase were limited for the two extreme conditions, the serum+NGF condition was chosen for the remainder of the studies as it would be the most likely encountered for tissue engineered applications.

4.2 Stimulation Conditions

Temperature, voltage, stimulation medium, and stimulation time were varied during the studies to examine their effects. For the short stimulation time (10 min), the temperature was not expected to have any impact on the enhanced growth. However, as longer stimulations (100 min) were performed at physiological temperatures to mimic in vivo conditions, the effect of the change in temperature (RT versus 37˚C) was examined for the 10 min stimulation to allow for a comparison between the two stimulation times. As expected, the temperature had no effect on the enhanced growth after stimulation.

As opposed to the change in temperature, altering the voltage was expected to alter the growth behavior, as described in previous work. For example, turning of xenopus neurites toward the cathode increases with increased applied voltage [3, 5, 8], especially in fields of the range examined here. No changes in growth were noted here for the different fields suggesting that either the differences in EF weren’t large enough to cause a change in neurite behavior or possibly that the field was not applied for sufficient time to emphasize the differences. Many of the previous studies noting behavioral changes with changing electrical fields were applied for much longer than even the longest time here. For example, the turning studies mentioned above had application times of 6 hr [3] or 5 hr [5, 8].

Interestingly, the increased duration of stimulation had no effect on neurite growth on collagen surfaces. This result suggested that the duration of exposure to the EF was not a factor in enhancing the neurite growth. Others have found varying results, depending on the time applied and the cell type. For example, Xenopus neurites have increasing growth rates over controls for exposure times up to 5 hr [5,
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Several studies have investigated changes in stimulation protocols in vivo and found little or no benefits to longer stimulation times. When comparing 1 hr and 2 week stimulation protocols, no differences were noted for rat motoneurons [2] and the 1 hr protocol demonstrated enhanced numbers of rat sensory neurons over 2 weeks [28]. Our results support these in vivo studies and demonstrate the possibility of decreasing the duration of ES even further.

While neurites on collagen substrata had no differences in outgrowth for the two stimulation times investigated, the neurites grown on laminin were statistically longer after 100 min stimulation than 10 min stimulation. Collagen and laminin are typical surfaces for DRG neurite growth, and their behavior on these surfaces has been previously studied [23, 29, 30]. The surfaces did not appear to have an effect on the outgrowth of the neurites in any of the stimulation experiments, except the duration of the stimulation. One possibility for the differences between lengths on collagen and laminin after 100 min stimulation may have been the number of cells expressing neurites on the laminin surface (27-31%) compared to the collagen surface (13-23%). However, 100 min stimulation was not found to statistically alter the number of cells bearing neurites. In previous work, Rogers et al studied effects of DRG neurites after 24 hrs on laminin and fibronectin, demonstrating that chick DRG neurite length was not significantly different when cultures expressed different percentages of cells bearing neurites [31]. For example, DRG cultured on 10 µg/ml fibronectin had an average of 15% of cells with neurites and an average length of 366 µm and when cultured on 100 µg/ml fibronectin, 34% of cells expressed with an average length of 347 µm [31]. Therefore, taken with previous work, it is unlikely the differences in cells bearing neurites would cause changes in length.

As increased time led to further enhanced growth only on laminin surfaces, it was hypothesized that the surface itself may be modified during the stimulation. As the stimulation of laminin surfaces prior to cell seeding did not alter the growth of the neurites compared to the controls, the statistical
changes noted between the 10 min and 100 min stimulation suggested that the cell has been altered by the stimulation. The interaction of the growth cone with the laminin surface is governed through integrin receptors and other studies have suggested that integrin regulation would alter the adhesive interactions, and therefore the growth, of the neurite with the surface [32, 33]. Therefore, a possible mechanism for increased growth after stimulation may be altered regulation of integrin receptors at the growth cone. This possible mechanism deserves consideration for future investigations.

5. Conclusion

Culture and stimulation conditions were compared for their ability to alter the effect of an applied EF on chick DRG outgrowth. For all conditions examined, neurite lengths were statistically increased after 10 min stimulation. These experiments included conditions where Ca\(^{2+}\) was excluded during stimulation and where cells were cultured with and without NGF and serum. After 100 min stimulation, neurites were again statistically longer than controls, however, on collagen surfaces neurites were not longer than after 10 min stimulation. In contrast, on laminin surfaces, neurites were statistically longer after 100 min stimulation than 10 min. No differences were noted in cells expressing neurites on laminin surfaces after stimulation and surfaces that were stimulated prior to cell seeding did not alter cell growth. Further research is needed to determine whether this effect on laminin is due to alterations in regulation of integrin binding or signaling.

Acknowledgments

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References


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Table 1: Various test conditions performed for this study, including the number of neurites measured for each sample and its respective sham and/or control. NP means not performed.

<table>
<thead>
<tr>
<th>EF (V m⁻¹)</th>
<th>Time (min)</th>
<th>Surface</th>
<th>Growth Media</th>
<th>Temperature (°C)</th>
<th>Stimulation Media</th>
<th>N value (sample:sham:control)</th>
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</thead>
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<td>MEM</td>
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<td>L15</td>
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<td></td>
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<td>25</td>
<td>MEM</td>
<td>103:114:138</td>
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<td></td>
<td></td>
<td></td>
<td>NGF</td>
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<td>MEM</td>
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Table 2: DRG neurite outgrowth for exposure to 24 & 44 V/m; all data at RT unless otherwise noted. Data reported as mean ± SE; NP means not performed. * indicates p < 0.05 when compared to controls.

<table>
<thead>
<tr>
<th>EF (V m⁻¹)</th>
<th>Surface</th>
<th>Growth Supplement</th>
<th>Stimulation media</th>
<th>Time (min)</th>
<th>Stimulated</th>
<th>Sham</th>
<th>Control</th>
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<td>L-15</td>
<td>10</td>
<td>181 ± 10*</td>
<td>126 ± 7</td>
<td>NP</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PBS</td>
<td>10</td>
<td>181 ± 8*</td>
<td>129 ± 6</td>
<td>NP</td>
<td></td>
</tr>
</tbody>
</table>
Table 3: DRG neurite branching per 100 µm for exposure to 24 & 44 V/m fields; all data at RT unless otherwise noted. Data reported as mean ± SE; NP means not performed. * indicates p < 0.05 when compared to sham, but not control.

<table>
<thead>
<tr>
<th>EF (V m⁻¹)</th>
<th>Surface</th>
<th>Growth Supplement</th>
<th>Stimulation media</th>
<th>Time (min)</th>
<th>Stimulated</th>
<th>Sham</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>Laminin</td>
<td>NGF</td>
<td>MEM</td>
<td>10</td>
<td>0.40 ± 0.06</td>
<td>0.35 ± 0.07</td>
<td>0.33 ± 0.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>L-15</td>
<td>10</td>
<td>0.37 ± 0.05</td>
<td>0.49 ± 0.08</td>
<td>NP</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>L-15 (37°C)</td>
<td>10</td>
<td>0.26 ± 0.05</td>
<td>0.21 ± 0.05</td>
<td>NP</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>L-15 (37°C)</td>
<td>100</td>
<td>0.14 ± 0.03</td>
<td>0.15 ± 0.04</td>
<td>0.18 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>N-2</td>
<td>MEM</td>
<td>10</td>
<td>0.22 ± 0.04</td>
<td>0.23 ± 0.06</td>
<td>0.28 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>44</td>
<td>Collagen</td>
<td>NGF</td>
<td>L-15</td>
<td>10</td>
<td>0.42 ± 0.07</td>
<td>0.46 ± 0.07</td>
<td>NP</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>L-15 (37°C)</td>
<td>100</td>
<td>0.38 ± 0.06</td>
<td>0.48 ± 0.07</td>
<td>0.40 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>N-2</td>
<td>MEM</td>
<td>10</td>
<td>0.23 ± 0.04 *</td>
<td>0.13 ± 0.03</td>
<td>0.25 ± 0.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NGF</td>
<td>L-15</td>
<td>10</td>
<td>0.32 ± 0.07</td>
<td>0.39 ± 0.08</td>
<td>NP</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PBS</td>
<td>10</td>
<td>0.30 ± 0.05</td>
<td>0.29 ± 0.06</td>
<td>NP</td>
<td></td>
</tr>
</tbody>
</table>
Table 4: Percentage of DRG with neurites after exposure to 24 V/m electric field for 100 min. Data reported as mean ± SE; * indicates p < 0.05 when compared to sham.

<table>
<thead>
<tr>
<th>Surface</th>
<th>Sample Number</th>
<th>Stimulated (%)</th>
<th>Sham (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laminin</td>
<td>55</td>
<td>31 ± 2</td>
<td>27 ± 2</td>
</tr>
<tr>
<td>Collagen</td>
<td>61</td>
<td>23 ± 2*</td>
<td>13 ± 1</td>
</tr>
</tbody>
</table>