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Chick Embryonic Schwann Cells Migrate Anodally in Small Electrical Fields

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Abstract

Little is known about the cues that guide migrating neural crest derivatives to their targets. This lack of understanding is especially significant in the case of Schwann cells, which have been transplanted into the central nervous system in an effort to promote axonal myelination after injury or disease. We have investigated the response of Schwann cells, cultured from the peripheral nerves of E7/8 chick embryos, to applied electrical fields. We find that they respond by migrating to the anode, and show a significant anodal bias in directionality at 3 mV mm^{-1} . This is the smallest electrical field that has been shown to affect cellular movement or growth in culture, and the anodal direction is surprising given the known cathodal responses of neural crest cells. The effective fields are considerably smaller than endogenous electrical fields that have been measured in embryonic tissues.

Keywords

Schwann cells; directed motility; electrical fields; galvanotaxis

There is a need grounded in both basic neurobiology and in clinical efforts to repair damaged nerves for understanding the directional cues that are involved in the guidance of Schwann cells. During embryonic development, cells migrate from the neural crest to eventually form both the myelinating and non-myelinating glial cells necessary for proper function of the peripheral nerves. There is considerable information about the molecular signals that drive neural crest cells down the glial pathway (reviewed by Jessen and Mirsky (2005)). However, little is known about the gradients, diffusional and otherwise, that provide directional information to neural crest cells as they proceed to Schwann cell precursors and then to functional Schwann cells associated with peripheral nerves. This is also an issue during peripheral nerve regeneration. While this regeneration typically occurs spontaneously, it is often functionally incomplete and the atrophy of Schwann cells is implicated in this failure (Höke, 2006). Additionally, there is considerable interest in using cells from the neural crest-Schwann cell lineage for repair of myelin in the central nervous system (Woodhoo et al., 2007).

A potentially important, but often overlooked, directional cue is the steady electrical field (EF). EFs will arise whenever electrical currents flow through a conductive medium, which can occur either naturally or as a result of experimental intervention. In the case of natural EFs, the sources of the currents are usually ion-transporting epithelia, which become functional at the very early

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stages of embryonic development (Hotary and Robinson, 1990; Rajniecek et al., 1988). As a result of either developmentally programmed leaks or wounds to an epithelium, currents flow, and measured EFs of up to 100 mV mm^{-1} are produced in the tissues near the leak (reviewed by McCaig et al. (2005). EFs have also been used to enhance repair of spinal cord injuries in dogs (Borgens et al., 1999) and are being used in this regard in ongoing human clinical trials (Shapiro et al., 2005). However, the target of EFs generated in those experiments is unclear as the EF magnitudes used (0.5 mV mm^{-1}) were much smaller than those required to produce a directional effect on neurons in vitro, especially mammalian neurons (Robinson and Cormie, 2007). It is known that cultured neural crest cells from *Xenopus* and quail respond to applied EFs as small as 7 mV mm^{-1} by migrating toward the negative electrode (cathode) (Gruler and Nuccitelli, 1991; Stump and Robinson, 1983), so it is possible that Schwann cells may respond similarly.

In order to investigate the responses of chick embryonic Schwann cells to EFs, small pieces of peripheral nerves connecting sensory ganglia to the hind limb from chick embryos (E7 or E8) were removed. Two pieces were placed near the center of a laminin-coated coverslip that formed the bottom of a chamber specially designed for EF application (see Supplementary Fig. 1). The explanted tissue was covered with medium and incubated overnight at 37°C . Typically, six chambers were prepared for one set of experiments. The culture medium consisted of 100 mL of F12 (Invitrogen) buffered at pH 7.4 with HEPES and supplemented with 2 mM L-glutamine (Invitrogen), 10 μM NaPyruvate (Sigma), 8 mM glucose and 2 mL of B-27 Supplement (Invitrogen). Finally, 10 ng/mL of neuregulin 1 (R&D Systems, cat. no. 396-HB/CF) was added to complete the culture medium.

After overnight incubation at 37°C , the construction of the chambers for EF application was completed. The chambers were designed to produce a uniform EF in the medium containing the cells, as well as to restrict the cross-sectional area of medium in order to minimize the current (and thus the Joule heating) required to produce the desired EF. This allows good access for imaging, and provides for protection from electrode products (Robinson, 1989). The chamber design also protected the cells from possible EF-induced gradients of growth factors and other medium components (see Supplementary Fig. 1). The ambient temperature during EF application was $28\text{--}30^\circ\text{C}$, except for one set of experiments that was done at 37°C .

Cells were observed on an inverted microscope using phase contrast 4x or 10x objectives. Digital images were acquired every 5 min. Areas for imaging were selected from regions in which cells were well dispersed so that the trajectories of individual cells could be analyzed; only one region was imaged from each chamber. Image stacks were analyzed using MetaMorph (Molecular Devices) and the locations of identified cells determined in each frame. At each time point, the position of each cell with respect to its starting position was recorded. The angle, ϕ , between a line defined by the origin and the final position of each cell and the direction of the EF was measured (with 0° being toward the anode), and a cosine was calculated for this angle. Thus, a positive cosine indicates movement toward the anode. The cosines of the n cells within a data set were averaged to give the mean cosine, $\langle \cos \phi \rangle = (\sum_i \cos \phi_i) / n$. Significant deviation of the angles from random movement was assessed by the Rayleigh test for randomness of direction (Batschelet, 1981), with the critical level, P , preassigned as 0.05. This approach was used because the angles, and their cosines, do not constitute a normal distribution. The instantaneous speed of migration was calculated by measuring the distance moved in five minutes and the mean speed was calculated from the instantaneous measurements. Mean speeds are presented as $\pm$ standard error of the mean.

After overnight incubation, most peripheral nerve explants yielded abundant Schwann cells that migrated actively from the explants. In order to confirm that these cells were, in fact, Schwann cells, we prepared the cells for immunostaining for Po, an abundant protein in

peripheral myelin that has been shown to be an early marker for the Schwann cell lineage (Bhattacharyya et al., 1991). Nearly 100% of the cells were immunopositive for this protein (Fig. 1), indicating that there was minimal contamination with fibroblasts or other cell types in these cultures. This result was seen all six cultures that were immunostained for Po, so we are confident that cultures exposed to EFs were composed of Schwann cells.

The mean speed of migration of cells not exposed to EF was $0.67 \pm 0.04 \mu\text{m/hr}$ ($n=62$). When the trajectory of the Schwann cells was mapped for 3 hr, we found that the movement was random (Fig. 2). We then applied EFs of various magnitudes and durations. At 100 mV mm^{-1} , the cells showed strong movement toward the anode, and after 1 hr in the field, showed significant asymmetry and a mean cosine of 0.58. The mean cosine increased to 0.72 after a two hour exposure to the EF. Fig. 3 shows the movement of five Schwann cells during a two hour exposure to an EF of 100 mV mm^{-1} . The mean speed of movement was not affected by the EF ($0.66 \pm 0.14 \mu\text{m/hr}$, $n=96$). When the cells were imaged at 37°C , instead of $28\text{--}30^\circ\text{C}$, both the speed and the mean cosine after 2 hr of 100 mV mm^{-1} EF exposure increased slightly ($0.70 \pm 0.09 \mu\text{m/hr}$ and 0.76, respectively).

As the EF magnitude was reduced, we observed significant asymmetries in migration toward the anode at 30 mV mm^{-1} and 10 mV mm^{-1} at all tested exposure times (Fig. 2). At 3 mV mm^{-1} , significant asymmetry was not detected after 2 hr EF exposure, but significant asymmetry was measured after 3 hr and 5 hr exposures. No significant asymmetries were seen at 1 mV mm^{-1} at any EF exposure time up to 5 hr.

There are two interesting features in these results. First, the Schwann cells are more sensitive to EFs than any cell yet studied. A number of cell types have been reported to exhibit directional growth or migration to fields in the range of $5\text{--}10 \text{ mV mm}^{-1}$, but none have been shown to have statistically significant responses to uniform EFs of less than 5 mV mm^{-1} . Second, the anodal direction of the response is surprising. Neural crest cells from both amphibians and avians have been shown to move cathodally in applied EFs, and to do so with great sensitivity. This suggests that the EF-sensing mechanism changes dramatically as the neural crest cells progress along their developmental pathway toward Schwann cells. Anodal galvanotaxis is not unknown; for example, a line of human breast cancer cells are reported to move anodally and to have a sensitivity to EFs that is related to the metastatic potential of the cells (Pu et al., 2007). However, another kind of transformed cells, prostate cancer cells, move cathodally (Djamgoz et al., 2001). Another neural crest derivative, melanocytes from both *Xenopus* and humans, has been reported to be unresponsive to applied EFs (Grahn et al., 2003; Stump and Robinson, 1983)

The directional response of the Schwann cells to an EF of 3 mV mm^{-1} is quite robust, as the average cosine reaches 0.31 after five hours in the field. It is possible that the cells would show significant responses to smaller fields, given a longer exposure time. It is also possible that the cells may have greater sensitivity to EFs in the in situ environment. In any case, the magnitude of the effective EF is well below the value of the EFs in both *Xenopus* and chick embryos, where endogenous fields of $20\text{--}40 \text{ mV mm}^{-1}$ due to epithelia-driven currents through developmentally programmed leaks have been measured (Hotary and Robinson, 1990; Hotary and Robinson, 1992; Hotary and Robinson, 1994). While the details of the EF distribution in the embryos is not known, the Schwann cells may experience and respond to EFs as they migrate.

It is also reasonable to consider generating EFs of a few mV mm^{-1} by means of indwelling current sources in whole animals. In this way, it might be possible to direct transplanted Schwann cells to appropriate regions in either the central nervous system or in peripheral nerves. Our results suggest that glial cells may be a more likely target than neurons for

exogenous electrical guidance as many vertebrate neurons, especially mammalian neurons, are not sensitive to applied EFs or are inhibited in their growth, albeit sometimes asymmetrically (Robinson and Cormie, 2007).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Shown in the figure are transmitted light (A and C) and immunofluorescence (B and D) images of cells cultured from pieces of peripheral nerve. A and B show the same field of view of cells fixed and stained only with the fluorescent secondary antibody. C and D show the same field of view of cells that were first stained with the Po antibody, followed by staining with the fluorescent secondary antibody. All cells in C and D are immunopositive for Po. The primary antibody (Bhattacharyya et al., 1991) was supplied by the Developmental Studies Hybridoma Bank, cat. no. 1E8.

Figure 2.

Summary of the directional responses of chick Schwann cells to applied EFs. The average cosines of cells exposed to different EFs for different times are shown. The number of cells included in each average ranged from 37 to 121 and were derived from at least three independent replicates. The numbers at the tops of the bars indicate the time in hours that the cells were exposed to the fields. Asterisks indicate that the angles, ϕ , that the cells' trajectories made with respect to the field vector were statistically significantly asymmetrical as determined by the Rayleigh test with the critical level preassigned as 0.05. The experiments indicated by the solid bars were done at an ambient temperature of 28–30°C while the ones represented by the cross-hatched bar were done at 37°C.

Figure 3. Shown in the figure is an overlay of the initial (cells in blue) and final (cells in tan) images from a two hour exposure to an EF of 100 mV mm^{-1} . The five cells that were present in both images are indicated initially by green numbers and finally by red numbers. The identification of the cells in the final frame was made by following the cells from the initial frame with intervening images made every five minutes. All five cells show marked anodal movement. The scale bar at the upper left indicates $25 \mu\text{m}$.