Direct-Current Electric Fields Induce Rapid and Directed Cathodal Galvanotaxis of Adult Subependymal Neural Precursor Cells

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Abstract—The mobilization of subependymal zone neural stem cells and their progeny (together termed neural precursor cells or NPCs) from their niche toward a lesioned region of the brain is imperative for the success of endogenous neurorepair paradigms. Following cortical injury, endogenous NPCs within the adult forebrain subependyma can be activated to proliferate and migrate toward the injury site where they differentiate into neural cells. This response can be augmented by the administration of growth factors and immunomodulatory agents to result in behavioural functional recovery following injury. With the goal of enhancing neural precursor migration to facilitate the repair process in a clinically-relevant manner we report that externally applied direct current electric fields induce rapid and directed cathodal migration (a process known as galvanotaxis) of pure populations of undifferentiated adult mouse subependyma-derived NPCs.

I. METHODS

Adult mouse subependymal tissue was isolated, enzymatically dissociated and cultured in the presence of epidermal growth factor (EGF, 20 ng/mL), basic fibroblast growth factor (bFGF, 10 ng/mL) and heparin (H, 2 ng/mL) [1-2]. Free-floating clonally derived colonies called neurospheres form after 7 days, consisting entirely of undifferentiated neural precursor cells (NPCs). Neurospheres were plated onto specially designed galvanotaxis chambers and incubated either in growth factor conditions (EGF + FGF + H, or EFH) for 17 hours, or differentiation conditions (1% fetal bovine serum, FBS) for 72 hours. Following this period, the galvanotaxis chambers were transferred onto the stage of a heated and CO₂-controlled live-cell imaging system. Time-lapse imaging microscopy was performed on the NPCs for 2–8 hours either in the presence or the absence of a direct-current electric field (dCEF, 250 mV/mm). Cells were fixed and standard immunocytochemistry was performed prior to or following time-lapse microscopy. Cells were analyzed for the presence of the NPC marker nestin, and the glial cell marker glial fibrillary acidic protein (GFAP). Cell migration was tracked and analyzed according to the velocity and directedness of migration, the latter of which is obtained by dividing the displacement of a cell along the direction of the dCEF vector by the total (x,y)-displacement between the initial and final positions of the cell.

II. RESULTS

In the absence of a dCEF, undifferentiated NPCs undergo random migration in all directions at a velocity of 0.23 ± 0.12 µm/min and mean directedness of 0.12 ± 0.07 (Fig. 1, Fig. 2A). Strikingly, in the presence of a dCEF undifferentiated NPCs exhibit a 4-fold increase in the velocity of migration (1.09 ± 0.15 µm/min) and a 9-fold increase in cathodal directedness (0.96 ± 0.01) (Fig. 1, Fig. 2B). Immunostaining verified that NPCs maintained nestin expression prior to and following dCEF exposure, confirming the undifferentiated state of the cells. Differentiated NPCs did not exhibit any cell body translocation in the presence of the dCEF. GFAP expression in differentiated cells was confirmed prior to and following dCEF exposure.

![Figure 1. Velocity (A) and directedness (B) of NPC migration in the presence and absence of a dCEF.](image1.png)

![Figure 2. Traces of individual undifferentiated NPC’s migration paths in the absence (A) and presence (B) of a dCEF.](image2.png)

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REFERENCES
