HEALTH RESEARCH ABSTRACT SUBMISSIONS #35

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Title of Research *	ROCK Inhibitor H-1152 Disrupts Directional Growth of Neurites and Schwann Cells on Micropatterned Polymers
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Introduction & Purpose *

Sensorineural hearing loss occurs upon cochlear hair cell death, but through electrical stimulation of spiral ganglion neurons (SGN) from cochlear implants, hearing sensation can be restored. However, the distance between stimulating electrodes and SGNs remains a primary limitation to sound resolution. To overcome the distance, the electrical stimulus must be increased, which simultaneously decreases resolution due to broad stimulation of SGNs. One solution to this problem is to direct SGN growth toward the electrodes, which would allow for reduced current levels to provide adequate stimulation. Previous studies indicate that SGN and Schwann cell (SC) growth can be directed using micropatterned methacrylate polymers, although, a mechanism for how SGNs and SCs “read and respond to” surface topology remains unknown. The RhoA/ROCK pathway is known to influence various aspects of cell motility, playing roles in both actin and myosin contractility and focal adhesion maturation. In this study, SGNs and SCs grown on polymers were exposed to ROCK inhibitor H-1152 with the expectation that guidance will be hindered.

Experimental Design *

Postnatal days 3-5 rats were euthanized and temporal bones were harvested. Under an operating microscope, the otic capsule was dissected, followed by removal of the cochlear capsule and the spiral ligament. The organ of Corti was removed as well, and remnants of modiolar bone were cleaned from the spiral ganglion samples prior to collection and dissociation.
Dissociated SGN cultures were plated onto polymer surfaces coated with laminin (40 µg/mL). Cultures were maintained in DMEM/N2 and supplemented with 5% FBS, Neurotrophin-3 (50 ng/ml), and Brain Derived Neurotrophic Factor (50 ng/ml). ROCK inhibitor H-1152 was added to cultures 6 hours into incubation at various concentrations (0.1 µM, 1.0 µM, and 10 µM).
After 60 hours of incubation, cultures were fixed with 4% paraformaldehyde and stained with anti-neurofiliament 200 and anti-S100 to stain neurites and Schwann cells respectively, followed by addition of Alexa 546 and 488 conjugated secondary antibodies. Digital epifluorescent images were taken and analyzed using Image J. Neurite alignment was quantified via a ratio of total length to end-to-end pattern distance. Schwann cell alignment angles were quantified by measuring the angle of the long axis of the cell in respect to the pattern of the polymer. Kruskal-Wallis tests were used to compare differences in both neurite and Schwann cell alignment.

Results *

Neurites exposed to H-1152 aligned significantly less than the control group ($p < 0.001$) for all concentrations. There was no significant difference in neurite alignment between the three inhibitor concentrations ($p = 0.304$), nor was the

alignment significantly different between inhibitor groups and the unpatterned group ($p = 0.060$). Schwann cells exposed to ROCK inhibitor also aligned significantly less than the control ($p < 0.001$). There was no significant difference between alignment angles for H-1152 dosages ($p = 0.077$), or between unpatterned polymers and those exposed to H-1152 ($p = 0.174$).

Conclusions *	The ROCK inhibitor H-1152 hinders the ability of SGN neurites and Schwann cells to follow the topology of micropatterned polymers. These results suggest that surface topologic features recruit RhoA/ROCK signaling to direct neurite and Schwann cell alignment. Future endeavors will explore the effects of upstream regulators in the RhoA/ROCK pathway, such as Nogo, and p75, on neurite and Schwann cell alignment.

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3 Apr 2012
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