

**2887-Pos Board B579****Extracellular Matrix Elasticity Determines Stem Cell Fate through Stretch-Activated Ion Channels**Medha M. Pathak<sup>1</sup>, Jamison L. Nourse<sup>2,3</sup>, Truc Tran<sup>1</sup>, Janahan Arulmoli<sup>3,4</sup>, Lisa A. Flanagan<sup>2,3</sup>, Francesco Tombola<sup>1</sup>.<sup>1</sup>Dept. of Physiology & Biophysics, UC Irvine, Irvine, CA, USA, <sup>2</sup>Dept. of Neurology, UC Irvine, Irvine, CA, USA, <sup>3</sup>Sue & Bill Gross Stem Cell Research Center, UC Irvine, Irvine, CA, USA, <sup>4</sup>Dept. of Bioengineering, UC Irvine, Irvine, CA, USA.

Extracellular matrix elasticity is a well-established determinant of lineage specification for many stem cell types. In neural stem cells, substrate stiffness controls glial versus neuronal specification. While matrix elasticity is known to be transduced at focal adhesion zones, the molecular mechanisms that direct mechanosensitive fate pathways are not well understood. Here we use a combination of electrophysiology, live cell microscopy and molecular techniques to uncover a missing player in mechanosensitive lineage commitment. We find that human neural stem/progenitor cells (hNSPCs) express stretch-activated ion channels (SACs), whose activity triggers spontaneous, transient  $Ca^{2+}$  signals that are modulated by substrate stiffness. Pharmacological inhibition of SAC activity suppresses neuronal differentiation while promoting astrocyte formation, indicating that SACs are important for lineage choice. We will present results from an RNAi approach aimed at determining the molecular identity of the hNSPC SAC channel.

**2888-Pos Board B580****Effect of General Anesthetics and Alcohols on Prestin (SLC26A5) Function**

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Prestin is a membrane protein essential to the electromotility of outer-hair cells (OHC) in the cochlea. This protein with piezoelectric properties has shown sensitivity to changes in cell membrane physical properties. The presence of molecules which impact the lateral pressure or the fluidity of the membrane -such as cholesterol, NSAIDs or lipophilic ions- can alter the electrophysiological properties of prestin as well as the electromotility. In an OHC, the nonlinear capacitance (NLC) and the electromotility are both conferred by prestin and coupled. This allows monitoring the NLC as a surrogate measure of prestin's function.

Here we describe the impact of general anesthetics and alcohols on prestin, and aim to use this protein as a model system for studying membrane-protein interaction through changes in the NLC. We tested the effect on prestin of alcohols with various C-chains (C2 to C10) and some general anesthetics (GA: propofol, isoflurane, halothane, chloroform, etomidate and xylazine). All the alcohols tested trigger a dose-dependent shift in the characteristic voltage at half-maximal charge transfer ( $V_{1/2}$ ), with the sensitivity increasing as the alcohols' carbon-chain is longer. The direction of the shift changes with concentration, the lower concentration cause a negative shift while higher concentrations shift  $V_{1/2}$  closer to 0mV. These shifts are correlated with changes in the linear capacitance of the cell. All of the GA we tested caused a dose-dependent increase in charge density at sub-millimolar concentrations as well as a shift of  $V_{1/2}$  toward hyperpolarized voltages. The sensitivity of prestin to these molecules seems to follow the Meyer/Overton correlation.

The shift in the NLC as well as the increase in charge density can modify the function of the OHC at resting potential and alter cochlear amplification.

**2889-Pos Board B581****Theoretical and Experimental Framework of Neurite Response to Chemical Gradients in 3D Matrices**Parthasarathy Srinivasan<sup>1</sup>, Ioannis K. Zervantonakis<sup>2</sup>, Chandrasekhar R. Kothapalli<sup>3</sup>.

<sup>1</sup>Mathematics, Cleveland State University, Cleveland, OH, USA, <sup>2</sup>Cell Biology, Harvard Medical School, Boston, MA, USA, <sup>3</sup>Chemical and Biomedical Engineering, Cleveland State University, Cleveland, OH, USA. During the development of nervous system, various attractive and repulsive signals in the surrounding extracellular matrix (ECM) environment guide the growing neurites along specific directions to reach their intended targets. Neuronal motility is controlled by extracellular signal-sensing via the growth cone at the neurite tip, including chemoattractive and repulsive cues. We quantitatively investigate this response using a combination of mathematical modeling and in vitro experiments, and determine the role of guidance cues and ECM on neurite outgrowth and turning. A microfluidic system was used to show that cortical neurite outgrowth and turning under chemogradients (IGF-1 or BDNF) within 3D scaffolds is highly regulated by the source concentration of the guidance cue and the physical characteristics of the scaffold. A partial differential equation model of neurite outgrowth has been proposed that may be used as a predictive tool. The parameters for the chemotaxis

term in the model are determined from experimental data. Resulting model simulations demonstrate how neurite outgrowth was critically influenced by the experimental variables, which was further supported by experimental data on cell-surface-receptor expressions. We demonstrate that our model results are in excellent agreement with experimental findings.

**2890-Pos Board B582****Single-Molecule Analysis of LFA-1/ICAM-1 Binding in Lymphocyte**

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Leukocyte integrin LFA-1 is activated through inside-out signaling by Rap1 upon chemokine and TCR stimulation, thereby regulating lymphocyte migration and arrest via immunological synapse (IS) formation. However, the precise mechanisms of the spatio-temporal regulation of LFA-1 binding to ICAM-1 and the role of Rap1 signaling during these processes are not well understood. To address this issue, we established live imaging of LFA-1 and ICAM-1 at the single-molecule levels on supported planar lipid bilayer to measure distribution and binding kinetics of LFA-1 and ICAM-1. To visualize LFA-1 we attached halotag to LFA-1 tail. We also labeled ICAM-1 using TMR derived dyes at low concentrations and incorporated into lipid bilayers. LFA-1 and ICAM-1 was visualized and tracked at the single-molecule level using TIRFM. In chemokine-stimulated T cell migration, randomly diffusing ICAM-1 was frequently captured in attached area and were relatively mobile with short life-time. We set up the conditions of the immunological synapse formation of OT-II T cells on supported planar membrane presenting OVA-peptide MHC with ICAM-1. We confirmed that primary OT-II T cells exhibited typical IS with clear cSMAC formed by TCR/pMHC surround by pSMAC formed by LFA-1/ICAM-1. The single-molecule analysis revealed that long-lived immobilized ICAM-1 in the distinct areas of pSMAC. In contrast, OT-II T cells deficient for Rap1 effector Mst1 exhibited inefficient attachment with aberrant IS. The immobilized ICAM-1 populations were also diminished. These results showed the binding kinetics of LFA-1 and ICAM-1 was distinct between chemokine and TCR and involvement of Mst1 in inside-out signaling leading to immunological synapse. Further studies are currently under way to examine the role of LFA-1 tails and Rap1 signaling.

**2891-Pos Board B583****Bayesian Analysis Distinguishes Brownian Motion from Motor-Driven Transport within Organelle Trajectories**George Holzwarth<sup>1</sup>, Amanda M. Smelser<sup>2</sup>, Matthew J. Martin<sup>1</sup>.<sup>1</sup>Physics, Wake Forest University, Winston-Salem, NC, USA, <sup>2</sup>Biochemistry, Wake Forest University, Winston-Salem, NC, USA.

Many organelles and vesicles move in a start-stop manner in live cells when observed by optical microscopy at 10 to 100 frames/s. One explanation for start-stop behavior is that the vesicles switch between a driven state in which they are being actively pulled by motor proteins, and a Brownian state in which they obey the laws of diffusion in the cytoplasm. To test this idea, we have carried out a hidden Markov, variational Bayesian Expectation Maximization, Gaussian mixture model ("Bayes") analysis. Either vesicle velocity  $v(t)$  or the direction of travel  $\theta(t)$  was used to "train" the model. When tested with simulated tracks, Bayes reliably determined the number of states  $K_{best}$  corresponding to the number of distinct physical processes required to describe the data. The mean and variance of the velocity or direction were also found reliably for each state in the simulated dataset. The assignment of individual frames to particular states showed few false positives or false negatives as long as the vesicle remained in each state for 6 or more adjacent frames. Once each frame was assigned to a state by Bayes, the mean-squared displacement (msd) for each state was computed, displaying the distinctive t-dependence of its physical origin, e.g. motor-driven, Brownian, etc. Individual tracks of fluorescently labeled peroxisomes in HME cells and unlabeled vesicles in PC12 cells were analyzed in the same manner to separate driven from Brownian bouts in an objective manner.

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**2892-Pos Board B584****Mismatch Repair Protein Mobility in Human Cancer Cells**Keith D. Bonin<sup>1</sup>, Justin Sigley<sup>1</sup>, Martin Guthold<sup>1</sup>, Karin Scarpinato<sup>2</sup>, John Jarzen<sup>2</sup>.<sup>1</sup>Physics, Wake Forest University, Winston-Salem, NC, USA, <sup>2</sup>Biology, Georgia Southern University, Statesboro, GA, USA.

We will report our measurements on the mobility of the mismatch repair protein MSH2 in cells from a cell line that has normal, immortal, and tumorigenic cells. Additionally we have measured the mobility of the protein in metastatic