2469-Pos Board B439

Quantitative Investigation of FGF-2-Modified Nanofibrillar Prosthetic for Neural Cell System Re-establishment

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Recent advances in regenerative medicine have improved understanding of the materials properties of nanofibrillar scaffolds used to aid cell system re-establishment. A picture is emerging that a successful scaffold has a defined set of physical properties including mechanical properties, topographical properties, and growth factor inclusion, and that the weighting of each may vary depending on the cell system and its functionality. In the present work, results from investigations of a slowly biodegradable nanofiber prosthetic for neural cell system re-establishment are presented. Preliminary data from in-vivo investigations (rat model) indicate that a nanofiber prosthetic device of FGF-2-modified nanofibers contributes to system re-establishment including aligned guidance for regenerating axons across an injury gap, and angiogenesis. Research by Meiners' group also demonstrated that FGF-2 retains biological activity significantly longer when immobilized on nanofibers than when presented as a soluble molecule [1]. The present investigations use atomic force microscopy operated in a new mode, Scanning Probe Recognition Microscopy (SPRM) [2], developed by Ayres' group, to quantitatively investigate properties of FGF-2-modified nanofibers. The SPRM system is given the ability to auto-track on regions of interest through incorporation of recognition-based tip control realized using algorithms and techniques from computer vision, pattern recognition and signal processing fields. Statistically meaningful numbers of reliable data points are extracted using an automatic procedure that maintains uniformity of experimental conditions. Properties under quantitative SPRM investigation include nanofiber stiffness and surface roughness, nanofiber curvature, nanofiber mesh density and porosity, and growth factor presentation and distribution. Each of these factors has been demonstrated to have global effects on cell morphology, function, proliferation, morphogenesis, migration, and differentiation.

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[2] Y. Fan et al., Int. J. Nanomedicine 2 (2007) 651-661.

2470-Pos Board B440

Multi-detection Device For Studying Neuronal Cell Networks

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We are developing new analytical devices in order to analyse molecules involved in neuronal cell signalling in individual cells and networks of cells. The unique chip design will allow us to characterize the pattern of synaptic connections between neurons by stimulation with directed microflows to specific individual cells and subsequent detection of cell activity by fluorescence imaging or detection of release (exocytosis) by electrochemical imaging with electrode arrays. We will present the fabrication scheme for this device and preliminary results obtained by culturing pheochromocytoma (PC12) cells onto the chip. The location of the cells will be determined by microcontact printing of surface adhesion proteins (e.g. laminin or poly-L lysine) on the surface of the device. Upon stimulation, intracellular calcium levels in PC12 cells increase and they release dopamine by exocytosis. The former can be monitored with ratiometric fluorescence imaging and the latter can be quantified by electrochemistry and both are non-invasive.

These combined measurements represent an important technical development and will help bridge the gap between single cell measurements and those at neuronal systems in organisms. Once characterized, they will be applied to understand patterns of cell signalling and to develop an *in vitro* model of degenerative diseases like Parkinson's disease.

2471-Pos Board B441

Observations of Sensory Neuron Behaviors on Substrates with Various Stiffnesses through Living Cell Imaging

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With the development of materials science and fabrication techniques, we can fabricate an elastic contexture similar to the physiological condition of living organisms to address how cultured cells behave when grown on materials of physiologically realistic elastic moduli. In our previous studies, we found that neurons cultured on a substrate with a low stiffness coated with fibronectin exhibited a higher excitability responding to a stretching force compared with those cultured on the same substrate coated with poly-L-lysine. Neurons cultured on fibronectin-coated soft substrate also altered the morphology of microtubules. Herein, we further attempt to address how culture substrates with various stiffnesses influence the glia cell-neuron interaction and neurite outgrowth of cultured sensory neurons. To examine these effects on dorsal root ganglion (DRG) neurons, we first prepared substrates for neuron culture by using polydimethylsiloxane (PDMS) that is composed of a base and a curing agent with a ratio of 35:1. The elastic modulus of this soft PDMS is around 88 kPa. CD1 mice at 8-12 weeks old were used for DRG primary culture. Through living cell imaging, we found that the neurite outgrowth velocity of DRG neurons cultured on a coverslip was faster than cultured on PDMS matrix. In addition, the glial cells did not attach and spread well on a soft matrix compared with cells on a coverslip. When cycloheximide was applied to inhibit the synthesis and secretion of extracellular matrix molecules from glial cells, DRG neurons hardly survived after culturing for 24 hours. In conclusion, extracellular matrix signalling and substrate stiffness have profound effect on the velocity of neurite outgrowth and the survival of both neurons and glia cells.

2472-Pos Board B442

Natural-born Pacemakers

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The pre-Botzinger complex is a group of about 100 neurons in the brain stem that produce perdiodic bursting. The complex regulates breathing rythms. Individually, these neurons do not produce bursts, while the bursting is not regulated by pacemaker neurons. We report on a numerical and analytical study of periodic bursting by an array of coupled identical neurons showing the spontaneous emergence of clusters of neurons that act like pacemakers. The bursting is periodically interrupted by ''dephasing events'' that are encountered in in-vivo and in-vitro studies, which are compared with our results.

2473-Pos Board B443

Morphological Amplification of Action Potentials in Axonal Varicosities

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Action potentials, the regenerative electrical waves traveling along axons, are the electrical trigger of synaptic neurotransmitter release. Aside from propagation failures at branch points, axon geometry is presumed to be immaterial to action potential propagation. Our numerical simulations, in contrast, indicate that in-line varicosities can significantly modulate action potentials, and over large distances. A single in-line varicosity can severely depress action potential amplitudes upstream from it while, within the varicosity, depolarization is amplified. Amplification within varicosities varies in a non-trivial manner with varicosity size, and is most pronounced for varicosities close in dimensions to Herring bodies, the secretory specializations of neurohypophysial axons. Enhancement in secretory terminals is equally significant. Amplification is dominated by geometrical factors, but does vary with the kinetics of voltage-gated ion channels.

2474-Pos Board B444

Morphology of Neurofilament Protrusions: Sequence-Based Modeling of Neurofilament Brush

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Neurofilaments are essential cytoskeletal filaments that impart mechanical integrity to nerve cells and regulate the cross-sectional area of axons. They are assembled from three distinct molecular weight proteins (NF-L, NF-M, NF-H) bound to each other laterally forming 10 nm diameter filamentous rods along with side-arm extensions. These protrusions contain an abundance of charged amino acid residues. The charged side-arms are considered to mediate the interactions between neighboring filaments, regulating interfilament spacing, and hence axonal caliber. The precise mechanism by which neurofilament protrusions regulate axonal diameter remains unsettled. In particular, the role of individual proteins has remained to be a matter of debate. Computer modeling is instrumental in demonstrating the details about the structural arrangement of individual protrusions. The present study employs Monte Carlo Simulations of neurofilament to reveal the role played by individual side-arms. The simulations are conducted under different phosphorylation state by making use of physically motivated 3D model of neurofilament brush. The model consists of a neurofilament backbone along with side-arm extensions that are distributed according to the stoichiometry of the three subunits. The side-arms are modeled at amino acid resolution with each amino acid represented by a hard sphere along with the corresponding charge valence. The phosphorylation states are specified through charges assigned to the serine amino acids of the Lys-Ser-Pro (KSP) repeat motifs of the side-arms. The equilibrium structure of the neurofilament brush has been studied via the model that maintained the proper charge distributions and grafting density of neurofilament side arms. It has been found that in spite of extensive phosphorylation sites present on NF-H, the tails of the medium sized neurofilament subunit (NF-M) is more elongated than NF-H tails. This suggests that NF-M protrusions are more critical in regulating neurofilament spacings and axonal caliber.

2475-Pos Board B445

Efficient Coding in the Olfactory Receptor Neuron Signaling Pathway

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The efficiency of neuronal coding has been studied extensively within the context of spike trains. Significantly less attention has been paid towards coding efficiency in biological signaling pathways. This study applies Shannon information theory to the olfactory receptor neuron signaling pathway to determine under what conditions the olfactory system can code most efficiently. We explore which types of odor stimuli the vertebrate olfactory system is most proficient at encoding by analyzing simulated data from a computational model of the pathway. We focus on odor stimuli of constant length but of varying concentration. This study concludes that the olfactory system's ability to encode such stimuli decreases significantly when presented with odor pulses of length greater than one second. We further explore the roles of particular signaling molecules in contributing to this decrease in coding efficiently. Finally, we perform a parameter sensitivity analysis on our information-theoretical calculations to identify the mechanisms responsible for information bottlenecks. We found that variations in upstream mechanism rate coefficients such as the G-protein activation rate have a significant effect on the transmission of information over stimuli longer than one second. In addition, parameter variations of the calcium extrusion rate through the sodium-calcium exchanger had a significant effect on information transfer over all pulse lengths.

2476-Pos Board B446

Reptitive Firing In Neurons - Analysing The Interaction Between Channel Density And Kinetics In Membrane Models

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More than sixty years after Alan Hodgkin presented his classification of firing patterns in the axons of the crab Carcinus maenas, the underlying mechanisms of the firing patterns are still only fragmentarily understood. Two main types have been discerned in neurons and dynamical membranes models. Type 1 shows a continuous frequency-stimulation current (f-I) relationship and thus an arbitrarily low frequency at threshold current, while Type 2 shows a discontinuous f-I relationship and a minimum frequency. Type 1 obtains rhythmicity via a saddle-node bifurcation, thus requiring three stationary potentials at subthreshold stimulation current. Type 2 obtains rhythmicity via a Hopf or double-orbit bifurcation. In a previous investigation of a hippocampal neuron model we showed that the membrane density of critical ion channels could regulate the bifurcation type and consequently the threshold dynamics. In the present study we extend our previous analysis to other quantitatively well-described excitable membranes. These studies show that not merely the channel density, but the overall structure of the phase space around the stationary potentials determine the onset frequency. We show, by means of techniques from nonlinear dynamical system theory, that this phase space is altered both by changes in channel density and channel kinetics. Understanding these interactions is an important step towards understanding global oscillatory activity in brain networks.

2477-Pos Board B447

How Can BK Channels Increase Excitability of Central Neurons and Decrease Excitability of Nodose Neurons?

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K^+ currents are generally known to hyperpolarize cells and to inhibit neuronal excitability. Thus, in nodose neurons, BK channel inhibition *increases* excitability (Snitsarev et al. J.Physiol. 582, 177). In central neurons, however, *increased* BK activity leads to *increased* excitability (Brenner et al. Nat.Neurosci. 8, 1752). To gain an insight into this opposing physiological effect of BK channel, we used Simulink (Mathworks) to perform mathematical modeling of action potential (AP) generation: http://www.mathworks.com/matlabcentral/fileexchange/loadFile.do?objectId=18812&objectType=file.

In response to simulated depolarization with 0.04 nA current injection, the simulation generated 4 APs adapting within 200 ms. Doubling BK conductance from 0.0065 to 0.013 microS resulted in 12 APs adapting within 600 ms, and halving BK conductance to 0.00325 microS resulted in 3 APs adapting in less than 200 ms. Thus, contrary to our expectations, an increase in excitability resulting from increased BK current was obtained in the nodose neuron model system. This behavior is reminiscent of central neurons. Other K^+ conductances and their effects on excitability in the nodose neuron model were also tested. In line with current experimental and theoretical knowledge, an increase in A-, K-, or D-current resulted in expected decrease in excitability (Schild et al. J. Neurophysiol. 71, 2338). To reconcile the experimental data from nodose neurons, central neurons and mathematical models of these neurons, we are introducing into our models recently discovered endogenous inhibition of BK current by a toxin-like domain of acid-sensing ion channels (Petroff et al. PNAS 105, 3140) and its competition with experimentally added scorpion toxins.

Mathematical models of neuronal excitability, and especially involvement of K^+ channels, may help our understanding of altered excitability of central neurons in epilepsy, neurodegenerative and psychiatric diseases, and decreased excitability of baroreceptor nodose neurons in hypertension and heart failure.

2478-Pos Board B448

New Metrics of Intrinsic Axonal Excitability from a Computational Model of Demyelination

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In white matter, oligodendrocytes tightly wrap axons at regular intervals to form the myelin sheath, the primary attribute of which is conduction velocity acceleration. Axonal demyelination diseases represent a devastating group of neurological disorders that affect more than 2 million people annually worldwide. The process of unraveling the periodic insulation causes axon conduction dysfunction in many diseases of the central nervous system (CNS), as in multiple sclerosis (MS) and infectious encephalomyelitis, or the peripheral nervous system (PNS) as in Guillain-Barré or Charcot-Marie-Tooth syndromes. Although the etiology of these diseases in most cases is thought to be immunological, the mechanisms of the diverse neurological symptoms are just as poorly understood. These confounding symptoms can present intermittently, resolving and returning in a way that is desynchronized from re-myelination. Symptoms include spasticity, dysfunction of somatic sensation, motor control, impairment of vision and other modalities. But these multiple neuropathies cannot be understood by conduction velocity changes alone. Physiological features are accompanied by anatomical and cellular perturbations in affected neurons that include changes in voltage-gated ion channel densities.

Here we present a compartmental model of a partially demyelinated axon using the NEURON simulator (http://www.neuron.yale.edu/neuron/) that sheds light on the function of normal, healthy axons as well as those undergoing demyelination. The model suggests a simple set of rules that could explain the wide range of intermittent symptoms observed during demyelination. The rules that govern these destabilized excitability patterns are critically dependent on ion channel densities and the anatomical parameters of the axon. Support: HHMI, NIH R01-MH079076.

2479-Pos Board B449

Light-dark Cycle Memory In The Mammalian Circadian Clock

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The mammalian circadian oscillator, or superchiasmatic nucleus (SCN) contains several thousand clock neurons in its ventrolateral (VL) part, many of which are spontaneous oscillators with periods that range from 22 to 28 hours. In complete darkness this network synchronizes through the exchange of action potentials which release the neuropeptide VIP, striking a compromise, free-running period (FRP) that is close to 24 hours long. We lock Siberian hamsters to various light-dark cycles and then track their activity into the dark to show that they retain a memory of the particular cycle to which they were entrained before returning to their own FRP. Then using model clock neurons (1) we model the VL SCN network and show that strong rythmicity of the VIP oscillation can account for both synchronization in darkness and the light-dark cycle memory which we observe. Additionally, light is known to initiate a MAP kinase cascade that induces transcription of both per and mkp1 phosphatase. We show that the phosphatase-kinase interaction can account for the dead zone in the mammalian Phase Response Curve. Finally, we hypothesize that the SCN acts like a lock-in amplifier to reject noise and to entrain the light edges of the circadian day.