

N-METHYL-D-ASPARTATE (NMDA) RECEPTOR SUBUNIT COMPOSITIONS IN DORSAL ROOT GANGLIA

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Chronic pain accompanies arthritis, cancer, and irritable bowel syndrome and includes post-surgery and neuropathic pain. It is mediated by the release of Substance P (SP), an inflammatory neuropeptide, which is released by the activation of N-methyl-D-aspartate (NMDA) receptors. The subunit compositions of NMDA receptors varies between myelinated A-fibers and unmyelinated C-fibers. Determining the different subunit compositions in dorsal root ganglia (DRG) may lead to new drugs to treat chronic pain. Rats were anesthetized in order to remove the spinal cord and DRG. Free-floating sections were prepared with a cryostat, and NMDA receptor subunits were then localized by immunohistochemistry using fluorescence and confocal microscopy. NR2A/B subunits (found primarily in large, granular bodies in the cytoplasm) did not colocalize with the NR2C/D subunits (found in the plasma membrane, regions of the cytoplasm near the membrane, and along the axon). The NR1 subunit seemed to colocalize with both the NR2A/B and NR2C/D subunits. These results suggest that NR2A/B and NR2C/D subunits assemble separately with NR1 subunits to form different NMDA receptors present in different compartments in these cells.

MORPHOLGY AND ELECTROPHYSIOLOGY OF DEVELOPING HUMAN NEURONS OBTAINED FROM DYSPLASTIC CORTEX

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Cortical dysplasia is characterized by anatomical malformations of the cerebral cortex. It consists of disturbances in lamination patterns, alterations in neuronal size and appearance, and changes in neuronal orientation. The present study examined the electrophysiological and morphological properties of human cortical neurons obtained from children undergoing neurosurgery to alleviate intractable seizures due to the presence of cortical dysplasia. We compared the electrophysiological and morphological properties of dysplastic neurons with those of more normal-appearing cells. The dysmorphic and other abnormal cells from developing dysplastic cortex displayed abnormal electrophysiological properties as well as abnormal morphological appearance. Such changes may underlie seizure generation in these children.

**PHYLOGENETIC POSITIONING OF
PLANCTOMYCES MARIS,
MYXOCOECUS ANTHUS, AND
CHLOROFLEXUS AURANTIACUS
USING EF-G AND EF-TU**

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In the past, 16S ribosomal RNA sequences have been used to reconstruct the eubacterial tree of life. However, the use of 16S RNA for phylogenies led to reconstruction problems arising from artifacts such as long branch attraction (rapidly or slowly evolving organisms are grouped together despite their true evolutionary history). In order to reduce branch attraction problems, we have used the slowly evolving *tuf* and *fus* genes encoding the EF-Tu and EF-G proteins involved in peptide elongation during bacterial protein synthesis. To better understand the relationship of *Planctomyces* to other eubacteria, we cloned and sequenced *tuf* and *fus* from *Planctomyces mari*, a marine eubacteria, using the polymerase chain reaction. Primers were based on the most conserved regions of EF-Tu and EF-G determined from an alignment of available protein sequences. The gene sequences are being used to construct a more robust phylogeny of eubacteria.

**LOCATING ABNORMAL GENES
THAT ALLOW THE SUPERMAN
PHENOTYPE TO APPEAR WILD
TYPE IN ARABIDOPSIS**

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DNA methylation is essential to most organisms because it plays a crucial role in the regulation of gene expression. DNA methylation is the process of tagging certain cytosines with a methyl group. The placement and number of methyl tags appears to be an important signal in the folding of DNA that determines whether a particular gene is expressed. We are using *Arabidopsis thaliana* as a model because the plant can tolerate a disrupted methylation pathway. One phenotype in *A. thaliana* resulting from disruptions in the methylation pathway is Superman (SUP). SUP plants are characterized by their unfused carpels and abnormal amount of stamen. A mutant screen was performed on SUP plants to identify suppressors of the SUP phenotype: the SUP plants were treated with ethyl methanesulfonate (EMS) and screened for wild type appearance. Sixteen distinct mutants were found. The number and location of the abnormal genes that make the plants appear wild type are being determined.

HOW SPONTANEOUS ACTIVITY AFFECTS BONE DENSITY IN MICE

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The objective of this study is to determine the impact of spontaneous activity on bone mineral density (BMD) in mice. F1 progeny from two inbred parental strains (Balb/c and CE) have been mated in order to produce Balb/c x CE intercross (F2 generation) mice that contain different proportions of the parental chromosomes. The two inbred strains were selected because of their difference in spontaneous running activity. Activity and BMD are being measured in the progeny. Activity is being measured by two different tests. The first test involves placing mice in cages containing running wheels. The distance each mouse runs in 24 hours is measured by a counter on the wheel. The second test is a spontaneous locomotor activity test that involves placing mice in a large plastic cage and monitoring their movements for an hour via infrared beams. BMD is being measured by computerized tomography scans. Preliminary data suggest that highly active mice generally have greater BMD than more sedentary mice. Genetic analysis is to be performed to determine which genes influence bone density.

COCAINE INDUCES APOPTOSIS AND RESTRUCTURING OF THE ACTIN CYTOSKELETON IN CORONARY AND BRAIN ENDOTHELIA

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Cocaine abuse is a widespread in the United States. Cocaine is considered to be a cofactor in HIV-1 cardiomyopathy, encephalitis and cerebral vasculitis, but the mechanisms of cocaine's effects on endothelia are not understood. We examined the effects of cocaine on apoptosis and the actin cytoskeleton in cultures of brain and coronary artery endothelial cells (CAEC). Cocaine induced endothelial cell apoptosis in a dose-responsive fashion (10^{-4} to 10^{-6} M). Cocaine also induced actin cytoskeleton remodeling with development of stress fibers and, in some endothelial strains, disruption of tight junctions. These effects may lead to increased vascular permeability and invasion of the heart and brain by HIV-1.

DETERMINATION OF CELLULAR SUB-LOCALIZATION OF TRAF3 AND T2-2

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TRAF's (Tumor Necrosis Factor Receptor-Associated Factors) are important signal mediators for the tumor necrosis family of receptors, such as CD40. CD40 serves a critical function in the T cell dependent and B cell mediated immune responses. TRAF's 2, 3, 5 and 6 have been shown to interact with the CD40 receptor. Overexpression of TRAF's 2, 5 and 6 has been shown to lead to signal activation while the overexpression of TRAF 3 does not. In cellular fractionation experiments, TRAF's 2, 5 and 6 have been found in both detergent insoluble and soluble fractions, while TRAF 3 has been found primarily in soluble fractions. Various proteins interact with TRAF3, among them is T2-2 that allows for the activation of the JNK (c-Jun N-terminal Kinase) pathway. T2-2 affects the subcellular localization of TRAF3 through its recruitment to the detergent insoluble fraction, which is correlated with increased JNK and NF- κ B activation. Immunofluorescence is being used to determine the exact locations of TRAF3 and T2-2 as well as which regions are important for downstream signaling.

NUMERICAL SIMULATIONS OF LOBED FUEL INJECTOR GEOMETRIES

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A lobed fuel injector is a device that creates strong streamwise vorticity during fuel injection into surrounding (coflowing) oxidizer. The vorticity generation allows for the improvement of the mixing of reactants and permits the formation of high rates of fluid mechanical straining at reactant interfaces, which act to delay ignition in a controlled fashion. Optimization of the lobed injector for use in an aircraft engine fuel injection system can lead to reduced aircraft emissions such as NO and NO₂ (otherwise known as NO_x) which contribute to depletion of the ozone layer, and CO, which is a well-known environmental pollutant. Our work focuses on the numerical simulation of various lobed injector geometries, for finite length and infinite length (wall bounded) configurations, in a high-speed air stream. We hope to identify geometrical features that can be utilized to optimize the mixing as a means of controlling the reaction processes.

DEEP-SEA FEATHER STARS FROM THE BAHAMA ISLANDS

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The ecology of deep-sea feather stars, and animals in general, has been little studied due to the limits that deep-sea diving imposes. This project describes the feeding behaviors and preferred habitats of twelve feather star (starfish, sand dollars, and relatives) species from the Bahama Islands observed *in situ* from the Johnson-Sea-Link submersibles. Still photographs and videos were analyzed to determine whether each species of feather star displayed a specific feeding posture and habitat preference. Specimens from the Smithsonian collections were examined to confirm the identifications in the videos and photographs. It was determined that each species of feather star arranges its arms in a specific way to feed and prefers certain habitats. Feather stars arrange their arms in three ways: parabolic (arms arranged like a bowl), fans, and radial filtration fans (arms arranged like spokes of a bicycle wheel). A direct correlation was found between feeding posture and preferred habitat. Most of these behaviors are also exhibited by shallow-water feather stars.