

16th Annual Life Sciences Undergraduate Research Symposium



Photo: Billy Brazelton (#12)



Photo: Elizabeth Vinson-Lonsdorf

**Wednesday, May 1, 2002
Earle Brown Center**

**University of Minnesota
St. Paul Campus**

Oral Presentations

1:30 - 2:00 Rm 155

Poster Session

2:00 - 4:30 Rm 135

**Dinner & Presentation by
Robert Elde, Dean, CBS**

5:00 - 7:30 Shingle Creek

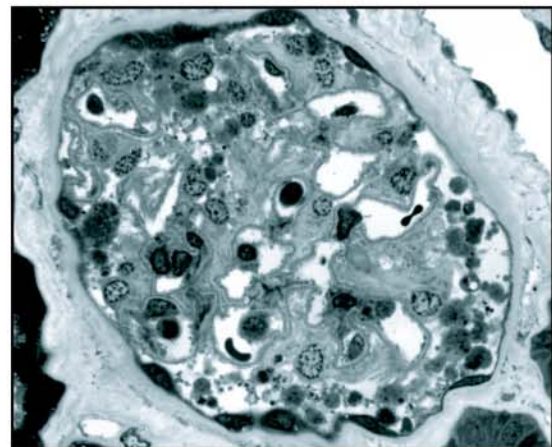
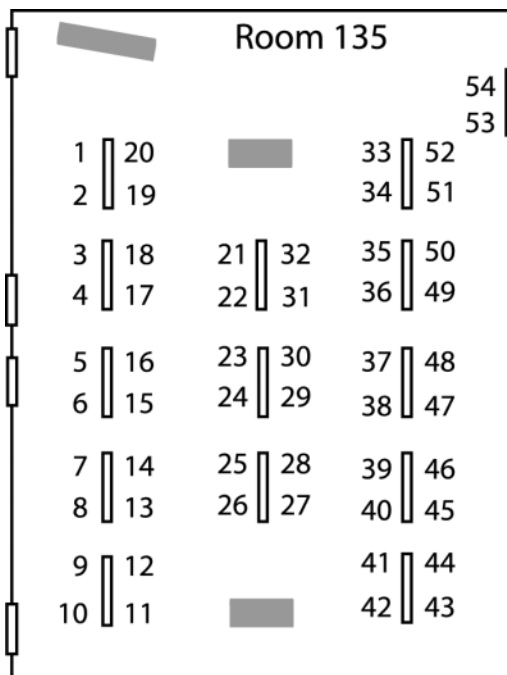


Photo: Erin Grund (poster # 27)

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ABSTRACTS

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WHAT ROLE DOES APOPTOSIS PLAY IN MYOFIBER REMODELING IN NORMAL MATURE EXTRAOCULAR MUSCLES?

Jocelyn A. Rowe (Linda McLoon)

Department of Ophthalmology, University of Minnesota

The extraocular muscles (EOM) are unique when compared to other mammalian skeletal muscles. For example, they continue to express molecules, such as growth factors, that are completely down-regulated in other adult skeletal muscles. Previous studies suggest that the extraocular muscles are continually undergoing a process of myofiber remodeling. If there is indeed addition of new myocytes to existing myofibers in the extraocular muscles, then there must also be a process of elimination of myonuclei and associated cytoplasm within extraocular myofibers. Apoptosis is a process of cell death that occurs normally in the body as a way to keep cell growth in check. The role of apoptosis in EOM remodeling was studied in two ways. First, we stained for the activated Caspase-3 enzyme, an indicator of apoptosis, and counted how many myofibers were undergoing apoptosis in a particular section of muscle. Next, we used a double-labeling procedure to look for nuclear DNA fragmentation associated with apoptosis along with the presence of activated Caspase-3 in serial sections of EOM. This gave us an idea of whether apoptosis occurred only within a segment of the myofiber or if it occurred along the entire fiber. This study has implications in the field of medicine, as there are certain diseases, such as muscular dystrophy, that affect all the skeletal muscles yet leave the EOM untouched. The more that is discovered about the EOM, the greater chance there is to apply this knowledge to other muscle diseases.

02

ESTABLISHING CELL LINES FROM MICE AT DIFFERENT STAGES OF MLL-AF9 LEUKEMIA DEVELOPMENT

Marnie L. Taylor (John H. Kersey)

Cancer Center, University of Minnesota

Over thirty types of leukemias are diagnosed according to the presence of an MLL fusion gene that ensues from a chromosomal translocation. The Kersey lab studies the mechanism by which one fusion gene, MLL-AF9, manifests acute myelogenous leukemia (AML) in infants, a disease that carries with it a poor prognosis. The development of a heterozygous MLL-AF9 transgenic mouse strain has allowed for the direct comparison of mice containing or not containing MLL-AF9. Differences in blood cell maturation can be detected as early as day E13.5, and eventually the MLL-AF9 mouse develops overt leukemia around age five months. While the mouse model is ideal for *in vivo* experiments, cell lines are more practical for molecular studies and testing experimental therapies. The establishment of cell lines possessing the characteristics of MLL-AF9 leukemia was attempted by isolating cells from mice with the fusion gene at different ages (E13.5, post-parturition day 6, 5 weeks, and overt leukemia). Cells isolated from the mice were cultured in a semisolid media containing IL-3, IL-6, GM-CSF, and stem cell factor to promote growth of the heartiest cells. Then the cells were transferred to a liquid media containing the same cytokines, which were subsequently removed except for IL-3. So far, three cell lines exhibiting MLL-AF9 leukemia phenotype (CD11b/Gr-1 positive) have been established from mice with overt leukemia. These cell lines do not require IL-6, GM-CSF, or stem cell factor for growth, but are IL-3 dependent. Cell lines from E13.5, day 6, and 5 weeks have also been established, however none of them exhibit the characteristic MLL-AF9 leukemia phenotype. This finding supports the hypothesis of a multi-step pathogenesis from MLL-AF9 leukemogenesis eventually leading to the development of AML.

03

LEUCINE AMINOPEPTIDASE IN *Caenorhabditis elegans*

Leah E Colvin (Catherine Kirkpatrick)

Department of Genetics, Cell Biology, and Development, University of Minnesota

Leucine aminopeptidases (LAPs) are part of a family of structurally similar metalloenzymes which are expressed in many organisms, including *C. elegans*, *Drosophila*, and humans. LAPs function by hydrolyzing N-terminal peptide bonds adjacent to a N-terminal leucine amino acid or another bulky residue. Catherine Kirkpatrick has found that *Drosophila* LAP CG7340 interacts with a component of the Wnt/Wingless developmental signaling pathway, and is expressed in the developing gut and excretory system. The goal of this research is to determine the function of LAPs in another model organism, *C. elegans*, which has two LAP genes ZK353.6 and W07G4.4. These are structurally similar to the LAPs found in humans and *Drosophila*. The involvement of LAPs in the Wnt pathway of *C. elegans* was studied using in RNA interference (RNAi) techniques. In RNAi techniques, double-stranded RNA from the gene of interest is introduced to the organism either through bacteria expressing it or injection. This can inhibit expression of the gene of interest, inducing a loss of function phenotype. Loss of function in either LAP did not produce embryonic lethality, suggesting that the LAPs are not involved in Wnt signaling. No phenotypes were observed in worms at later developmental stages. Ongoing experiments will investigate the effect of inhibiting both genes simultaneously. The tissue expression patterns of LAPs in *C. elegans* were studied by injecting individuals with vectors encoding GFP chimeric proteins. These proteins from both ZK353.6 and W07G4.4 are expressed in the gut of larvae and adults, suggesting a role for LAPs in digestion, and in different amphid neurons, suggesting an involvement in neuropeptide generation or degradation. W07G4.4 was expressed in the vulva of L4 larvae and adults. Neither gene appears to be expressed in the early embryo, but both are expressed in the gut late in embryonic development.

04

EXPRESSION AND PURIFICATION OF A RAT TESTICULAR PROTEIN

Khaled Dajani, (David W. Hamilton and Kenneth P. Roberts)

Department of Genetics, Cell Biology, and Development & Department of Urologic Surgery, University of Minnesota

Male germ cells are closely associated with Sertoli cells throughout much of their developmental cycle. Without direct interaction with Sertoli cells, germ cells will not develop. The rat testicular protein CRISP-2 (Cysteine **RI**ch **S**ecretory **P**rotein), for which a human homologue exists, has been implicated in adhesion between germ cells and Sertoli cells, and is also thought to be important in sperm function. The objective of this project was to synthesize and purify recombinant rat CRISP-2 (rCRISP-2) to use in further studies of CRISP-2 function. The rat CRISP-2 cDNA was subcloned into the expression vector pMT/V5-His B and the construct transfected into *E.coli* for amplification. Since significant post-translational modification of this protein occurs *in vivo*, a eukaryotic cell line (Schneider-2 insect stem cell; S2) was chosen for expression. The plasmid construct was chemically transfected into the S2 cells, and the cells were induced to express rCRISP-2. The V5 epitope encoded by the construct permitted protein production evaluation via western immunoblotting. Cellular localization of rCRISP-2 was achieved by immunocytochemistry. Recombinant rat CRISP-2 was partially purified by affinity chromatography via the histidine tag sequence, also encoded by the construct. These data show that transfected S2 insect cells secrete rCRISP-2, which can be partially purified by affinity chromatography. Further optimization of the transfection and purification protocols is required to generate the necessary rCRISP-2 for biological function assays, which will be used to test the germ cell-Sertoli cell adhesion hypothesis.

05

QUANTITATIVE ANALYSIS AND DESCRIPTION OF BEHAVIORS DURING CHIMPANZEE (*Pan troglodytes*) BORDER PATROLS

Miranda J. Oliver (Anne Pusey)

Department of Ecology, Evolution and Behavior & The Jane Goodall Institute's Center for Primate Studies, University of Minnesota

Chimpanzees (*Pan troglodytes*) engage in aggressive interactions with neighboring communities in which male alliances occasionally attack or kill neighboring individuals. Male chimpanzees form border patrol parties that monitor border areas of their home range that overlap with neighboring communities (Goodall 1986, Wilson 2001, Watts and Mitani 2001, Manson and Wrangham 1991). Behavior during border patrols differs from behavior during non-patrol times. Behaviors during patrols include unusual silence; cautious travel; intense listening and watching of neighboring communities; tense behavior; sniffing and bipedal standing (Mitani 2000, Mitani and Watts 2001, Goodall 1986, Goodall 1979, Nishida 1979). Although border patrols have been identified in several communities, little quantitative data is published regarding behavior during border patrols. In addition, little quantitative data has been gathered about border patrols occurring in the Kasakela community of Gombe National Park. This paper describes and quantifies border patrols and intergroup interactions in the Kasakela community of Gombe National Park and examines whether or not border patrol parties suppress vocalizations during patrols. The data for this study was taken from the 1978 field notes recorded at Gombe National Park. During 1978 there were 15 patrols, seven of which were identified as patrols by the observer and eight in which patrol behaviors occurred. Males patrolled more frequently than females, however, females were not completely absent from all patrol parties. The patrol parties patrolled the southern part of their range 13 times out of 15 patrols. These findings are consistent with the history of the Kasakela community in the few years prior to 1978. Patrol behaviors were exhibited outside of the home range as well as inside the home range close to the border. The patrol parties did not completely suppress pant-hoot vocalizations during patrols; however, the frequency of pant-hoots on patrol days may be less than that on normal days.

06

USE OF SCANNING ELECTRON MICROSCOPY FOR IDENTIFYING JUVENILE MUSSELS

Whitney Taylor, (Mark Hove), Pat Cliff, Tessa Diedrich, Matt Haas, Marissa McGill, Carrie Nelson, and Anne Kapuscinski

Department of Fisheries, Wildlife, and Conservation Biology, University of Minnesota

Freshwater mussels are important members of a healthy ecosystem, yet nearly two-thirds of Minnesota's species are state or federally listed. As larvae (glochidia) most mussel species must attach to a specific fish species to facilitate metamorphosis into a juvenile. Effective conservation of mussels frequently requires knowledge of mussel-host relationships. We collected freshwater drum, walleye, smallmouth bass, shorthead redhorse, white bass, long and shortnose gar, northern pike, emerald and mimic shiners, and lake sturgeon naturally infested with glochidia from the St. Croix River during 2000-2001. Fish were held in individual aquaria and aquaria were siphoned to recover juvenile mussels. Juveniles were sorted using life history information and identified with scanning electron micrographs. Most juveniles were in the subfamily Ambleminae or Lampsilinae. Images are being analyzed to improve our identifications. Additionally, we are developing a species identification key to upper Mississippi River mussel glochidia using characters visible with scanning electron microscopy. We appreciate the administrative and financial support provided by the St. Croix National Scenic Riverway and the NRPP-Threatened and Endangered Species Fund, U.S. Geological Service, Macalester College, and the University of Minnesota's Undergraduate Research Opportunities Program.

07

EVOLUTION OF COOPERATION

Kristine C. Paul (Ahmed Naumaan)

Department of Computer Science, University of Minnesota

Understanding how cooperation emerged in populations has been the subject of many theories, including those based on kin selection and reciprocity. These theories can be investigated through the use of computer simulations. A model for the evolution of cooperation in the absence of reciprocity has also been proposed in the literature. The published model uses a population of creatures with a certain identifying characteristic. When two creatures from the population have values for this characteristic that are close to each other, one of the creatures *always* has to help the other. The creature that has been helped receives some benefit, which increases its chances for reproductive success. The authors show that under these circumstances cooperative behavior becomes established within the population. I assert that a more realistic case is one where each of the creatures has a “choice” of whether to help the other creature or not. I have designed a software-based experiment to investigate whether cooperative behavior (i.e. a tendency to provide benefit to other creatures) will emerge and stabilize within the population under these more stringent circumstances created by the availability of choices. In my experiment the creatures’ characteristics and behaviors are encoded in a “gene.” Genetic algorithms are employed to evolve the genetic string to produce new creatures that exhibit changed behavioral patterns. Experiments employing different combinations of the creatures’ characteristics are currently underway.

08

PARTURITION IN WILD CHIMPANZEES

Sara E. Bebus (Anne Pusey)

Department of Ecology, Evolution, and Behavior, University of Minnesota

Parturition behavior in wild chimpanzees (*Pan troglodytes schweinfurthii*) has been observed very rarely, and parturition by captive individuals occurs in highly artificial conditions and may be aberrant. This study examines the course of three chimpanzee births that were witnessed in Gombe National Park, Tanzania. The births occurred between August 1992 and January 1999. All pregnancies appeared to be full-term and, all infants were born healthy. One female was primiparous and the other two multiparous. The lengths of labor ranged from approximately 2 hours to more than 7.5 hours. During the labors, the females held their vaginal areas and often sniffed or licked their fingers. All females gave birth in day nests (one on the ground, two in trees). Each female delivered in a different position, one was standing tripodally, one squatting, and the other lying on her side. The primiparous female gave birth at a feeding station and was joined by three females and a young male. The other two females were with their older offspring. All females and the other individuals present at the births showed interest in the placenta and birth fluids. Birth fluids were licked off of fingers and leaves, while the placenta was eaten in two of the three cases by the female. Older offspring of two of the females begged for the placenta, but it was not shared in either case. Chimpanzees will eat meat whenever they have the opportunity, and the placenta and fluids provided a similar desirable food. Interest in the newborn was shown only by the youngest individuals present. Two of the females experienced aggression from other chimpanzees after the births. The aggression was in an attempt to take the newborn in one case and the placenta in the other. Further observations are needed to paint a more complete picture of chimpanzee parturition in their natural habitat.

09

EXPRESSION, PURIFICATION, AND CHARACTERIZATION OF THE PROTEIN PhoN-Se

Brian J. Tienor (Alex J. Lange)

Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota

Diabetes is a disease that affects a relatively large percentage of the U.S. population, and the disease rates are increasing. Diabetes is characterized by dysregulated glucose metabolism in the liver and typically requires life-long treatment. An enzyme involved in hepatic glucose metabolism is glucose-6-phosphatase. Dysregulation of this enzyme causes overproduction of glucose, which leads to high blood glucose levels, or diabetes. We are looking for proteins that model glucose-6-phosphatase in order to study its function. Specifically, we think an acid phosphatase found in *Salmonella enterica* (PhoN-Se) would serve as a good model. If the active site of PhoN-Se is similar to that of glucose-6-phosphatase, and it displays similar phosphatase activity, it may one day be possible to localize an engineered protein in the liver cells of diabetes patients where it can take over for the dysfunctional glucose-6-phosphatase. We are interested in expressing the protein, purifying it, and finally characterizing its phosphatase activity and determining its three-dimensional structure. For expression we are using a plasmid vector (pBAD/gIIIa) in *Escherichia coli* that codes for a recombinant version of the protein and directs it to the periplasmic space following induction. Osmotic shock is used as an initial purification step to release the protein from the periplasmic space. After further purification, we found the optimum conditions for phosphatase activity and ran assays to determine the purity of the protein solution. We plan on using x-ray crystallography to determine the protein structure and study the active site. With this information, we will be more able to determine if PhoN-Se would be a viable substitute for glucose-6-phosphatase.

10

DEVELOPMENT OF A SPECTROPHOTOMETRIC ASSAY FOR NUCLEOSIDE PHOSPHORAMIDASES

Janelle Johnson (Carston R. Wagner)

Department of Medicinal Chemistry, University of Minnesota

3'-Azido-3'-deoxythymidine (AZT) is a nucleoside analog reverse transcriptase inhibitor which is used as an antiviral agent. AZT is one of several nucleoside analogs whose biological activity requires intracellular metabolism to a 5'-nucleoside triphosphate by kinase-mediated phosphorylation. One mechanism of AZT resistance is the down regulation of thymidine kinase, which decreases the efficacy of AZT. An approach for solving this problem is to deliver the AZT monophosphate as a neutral prodrug into the cell where it is subsequently converted to AZT monophosphate. AZT phosphoramidates represent one example of this approach, which have been shown to exhibit activity in PBMC and CEM cells. Conversion of the phosphoramidate to AZT monophosphate, which is believed to be the metabolic pathway, has been difficult to detect in the past, due to the lack of a real time assay. My project was to develop an assay that can be monitored spectrophotometrically. A coupled assay was investigated in which the decrease in absorbance, caused by conversion of NADH to NAD⁺, can be correlated with the release of AZT monophosphate. Experiments were performed to determine necessary concentrations of reactants for sufficient activity of uridine monophosphate, thymidine monophosphate and AZT monophosphate. Initially enzymatic experiments were carried out with NMP Kinase showing specificity for UMP but poor turn over rates for TMP. A new *E. coli* enzyme Thymidylate Kinase (TMPK) was obtained and found to be much more specific for TMP resulting in good turn over rates. TMPK was then investigated in experiments with AZT-MP. Results indicate that the enzyme has less substrate specificity for AZT-MP allowing for a more flexible assay. This assay will be used in the future in analyzing structure activity relationships on the enzymatic activity of phosphoramidases.

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11

CILIARY MICROTUBULES IN MAMMALIAN DEVELOPMENT

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Cilia and flagella are present throughout protist and animal phyla. Cilia and flagella are constructed from an evolutionarily conserved, 9-fold arrangement of doublet and triplet microtubules, known as the axoneme. In particular, the A-microtubule (from a doublet microtubule complex) contains a specialized set of protofilaments called the "ribbon." Ribbon proteins such as rib43a are specifically present in cilia and centrioles from organisms such as sea urchins and *Chlamydomonas reinhardtii*, to mice and humans. Cilia are required in retinal photoreceptors and olfactory receptors; they are also critical for normal left-right axis determination in embryonic development, and later for motility in respiratory and reproductive tracts. In this project, the gene *RIB43a* for a ciliary microtubule-associated protein, rib43a, from *Chlamydomonas* has been shown to have high homology to a gene in the mouse, *Mus musculus*. A tentative Mm-RIB43a cDNA has been cloned from an adult mouse expression library. Its nucleotide sequence and the predicted amino acid sequence of the protein are being determined. Mm-RIB43a probes are being used to study its expression in fetal and adult mouse tissues by northern blot, *in situ* hybridization and immunofluorescence microscopy techniques.

12

CHARACTERIZATION OF *BLD1*, A GENE REQUIRED FOR FLAGELLAR ASSEMBLY IN *CHLAMYDOMONAS*

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Chlamydomonas reinhardtii is a unicellular green alga widely used as a model system for cytoskeleton and photosynthesis research. *Chlamydomonas* cells use two anterior flagella to swim in liquid media, and mutations in flagellar proteins result in defective cell motility. Cells affected by one such mutation, known as *bld1*, are unable to assemble flagella. We have previously shown that the gene affected by the *bld1* mutation encodes a protein that shares significant sequence homology with proteins in nematodes, fruit flies, mice, and humans. Nematodes harboring a mutation in the homologous *osm6* gene cannot assemble sensory cilia, which are structurally similar to *Chlamydomonas* flagella. Therefore, it is likely that the *BLD1* gene is involved in a conserved cellular mechanism responsible for the assembly of cilia and flagella in many different types of organisms. Cilia and flagella play wide-ranging roles in human physiology, including sperm motility, movement of fluids in the lungs and female reproductive system, and regulation of embryonic development. A better understanding of the function of *BLD1* will help to elucidate the importance of proper assembly of cilia and flagella to diverse organisms and biological processes. We will report on the further characterization of *BLD1*, including the localization of the gene product within the cell.

13

SEQUENCE REQUIREMENTS FOR TRYPANOSOME RNA EDITING

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Some species within the trypanosome family of protozoa are human parasites that are causative agents of several diseases, including African Sleeping Sickness and Chagas Disease. The mitochondria of these organisms exhibit a unique type of RNA editing; several mRNAs do not encode a functional open reading frame until uridine insertions or deletions occur at specific sites after transcription. The editing is directed by guide-RNA (gRNA) that binds to a mRNA immediately adjacent to the editing sites and contains a sequence complementary to the edited mRNA that directs the editing machinery to insert or delete the appropriate number of uridines. The lab previously demonstrated that the cytochrome b mRNA has U-insertions added in a gRNA-independent manner at specific sites of the mRNA which had been identified and shown to be critical for the guide-RNA independent reaction. This project attempts to identify the critical features of the AU region necessary for the gRNA-independent U-insertions and to determine if other RNAs of the trypanosome mitochondrial genome have analogous sequence elements. Selection amplification was used to analyze the possible permutations of the AU element that support editing. After randomizing the AU sequence those RNAs that are efficient substrates for the editing reaction were selected from the random population. Because the starting random population of RNA was so large, the small sub-population that was edited needed to be amplified by RT-PCR and T7 transcription for additional enrichment. After four cycles of enrichment, the RNA pool was edited more efficiently than the wild type A-U sequence. This population was cloned into a vector for sequencing and an analysis of the generated phylogeny is providing insights into the RNA structural requirements for this intriguing form of RNA processing.

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23S RIBOSOMAL MUTATION SUFFICIENT FOR ERYTHROMYCIN RESISTANCE IN

Bordetella pertussis

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Infections due to *Bordetella pertussis*, the causative agent of pertussis (whooping cough), have steadily been on the rise since 1980. The infection is treated with erythromycin, but recently erythromycin resistant isolates of *B. pertussis* have been reported. In these isolates, resistance has been associated with an A to G transition mutation in the 23S rRNA gene. To confirm that this mutation is responsible for erythromycin resistance in *B. pertussis*, a 1.5 kb PCR product containing the mutation was introduced into an erythromycin-susceptible strain of *B. pertussis* through homologous recombination. Transconjugants were screened by PCR-RFLP and for resistance to erythromycin. Four of the eight transconjugants tested had the mutation in one or more copies of its 23S rRNA genes as revealed by PCR-RFLP. Strains containing the mutation were erythromycin-resistant when tested with an erythromycin disk while the strains without the mutation remain susceptible to the antibiotic. The results showed that the mutation in the 23S rRNA gene was sufficient to confer erythromycin resistance. Knowing the mutation is responsible for resistance provides a means of screening for resistance through PCR method I in lieu of the slower culture method.

15

GENETIC STUDY OF A RED LIGHT SIGNALING MUTANT IN ARABIDOPSIS

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The mechanisms by which eukaryotic cells perceive and transduce external signals to regulate their development are of central interest in biology. The response of plants to red and far-red light signals, mediated by phytochromes, is an excellent system to study such a signaling process. For years, *Arabidopsis* genetic screening has been a powerful tool to isolate the components involved in the signaling cascade. However, few red light mutants and red/far-red light mutants have been identified. The majority of the red light or red/far-red light mutants have only a single allele isolated, indicating that the genetic screen under red light condition has not been saturated. We have isolated an EMS mutant, *rli1* for red light insensitive 1, with a long hypocotyl phenotype specifically under red light. The *rli1* mutant was demonstrated to be recessive and to be non-allelic with the *phyB-9* mutant by genetic crosses. In order to clone the corresponding gene in the newly identified mutant, we have crossed the mutant to *Landsberg er. ecotype* to generate a mapping population. Future research efforts will be directed to the mapping and cloning of the corresponding gene by chromosome walking and the characterization of its involvement in red light signaling.

16

INTERACTION OF THE SCA1 GENE PRODUCT WITH CELLULAR MICROTUBULES AND DYNEIN FOR TRANSPORT TO THE NUCLEUS

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Spinocerebellar ataxia type 1 (SCA1) is a rare autosomal-dominant neurodegenerative disorder. The mutation responsible for the disease is an unstable expansion of a CAG repeat in the SCA 1 locus which produces an abnormally long glutamine tract in the ataxin-1 protein product. Previous research has shown that nuclear localization of mutant ataxin-1 is required for the onset and progression of the neurodegeneration characterizing the disorder. The cellular network of microtubules is suspected to play a role in trafficking the ataxin-1 via dynein motors towards the nucleus. Current experiments using Chinese Hamster Ovary (CHO) cells expressing ataxin-1 are under way to help identify possible interactions between ataxin-1 and the microtubules. Immunoprecipitation and immunofluorescence techniques are being utilized to see if the ataxin-1 protein will either co-precipitate or co-localize with microtubular components. Thus far, the ataxin-1 protein has not co-precipitated with dynein; however this may be due to the amount of antibodies used in the immunoprecipitation. The immunofluorescence of the CHO cells with anti-ataxin and anti-tubulin did not provide any conclusive evidence regarding the potential interactions between ataxin-1 and the microtubules. Experiments are being performed as well to determine if the nuclear localization of the ataxin-1 protein is affected by disruption of the microtubule network with colchicine treatment. To observe the effects of colchicine treatment in real-time, a stable cell line expressing a GFP-ataxin-1 fusion protein will be used. This cell line is expected to provide a strong tool for studying the potential trafficking of ataxin-1 within the cytoplasm.

17

THERMAL WITHDRAWAL RESPONSE OF *C. elegans* AS AN INDICATOR OF NOCICEPTION

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We studied somatosensory processing in *C. elegans* using acute exposure to thermal stimuli. A typical thermal withdrawal response consists of the worm stopping its forward progression, moving backwards and changing its direction. To analyze this thermal avoidance behavior we developed a heating filament that gives reproducible stimulation across a range of temperatures. For each worm strain tested, we calculated a population response percentage by dividing the number of worms that responded by the total populations. The upper limit of thermal stimuli for all strains is 36°C, at which point the percentage of the population responding to stimuli approaches 100. Testing worms at 24, 27, 33°C uncovered significant variations between mutant worm and wild type responses. We tested the thermal avoidance behavior of the wild type worms as well as a variety of worms that have mutations in genes implicated in decreased sensitivity to thermal stimuli¹. Of these strains several mutations in the *eat-4* gene were tested. The *eat-4* gene encodes a protein homologue to the mammalian sodium-dependent inorganic phosphate co-transporter I, which is localized in the thermosensory M3 neuron, and has been implicated in glutamatergic neurotransmission. The results show that thermal responsiveness varies with stimulus intensity. In addition, thermal responsiveness varied significantly among different mutations of the same gene, as determined by population response percentage. Studying withdrawal responses using multiple stimuli produces a graded response that can be used to characterize additional mutations.

¹Wittenburg, Nicole, and Baumeister, Ralf thermal Avoidance in *Caenorhabditis elegans*: An approach to the study of nociception (1999) *Proc. Natl. Acad. Sci USA* 96, 10477-10482.

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REAL-TIME PCR SYBR GREEN GENOTYPE ASSAY FOR A MURINE KNOCK-OUT MODEL OF MUCOPOLYSACCHARIDOSIS TYPE I

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Current studies of gene therapy in a mouse model of mucopolysaccharidosis type I (Hurler syndrome and variants) are dependent upon efficient and reliable identification of heterozygotes and affected homozygotes for this autosomal recessive defect. Such genotyping must be accomplished in a large colony and, ideally, could be done in newborn pups. Toward this objective, we explored an assay exploiting SYBR Green (a dye that intercalates into the minor groove of double stranded DNA) and the PE-Biosystems Model 7700 instrument for quantitative PCR. Two sets of primers were designed with Primer Express 1.5 software. For the wild type (WT) allele, primers were developed in exon 6 of the *IDUA* gene, one on each side of the *BstEII* restriction enzyme site (where the PGK-Neo resistance cassette was placed to create the gene knock-out). For the knock-out (KO) allele, primers were developed in the Neo resistance cassette created from the plasmid pKO Select Neo (Stratagene). In comparison to TaqMan probe methods or enzyme assays, this real-time PCR SYBR Green approach is more rapid, facilitates higher throughput, and is more economical. Importantly, this method should be applicable to extremely small specimens from newborn pups. Of broader significance, this approach can be applied to the vast variety of knock-out models that are being generated by DNA insertion. Supported by NIH PO1-HD32652.

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DISRUPTING A NOVEL MOLECULAR PATHWAY MAY CONTRIBUTE TO CANCER DEVELOPMENT

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Low levels of the tumor suppressor p27 are a prognostic indicator for more aggressive human cancers. The mechanistic contribution of p27, however, has not been elucidated. A yeast two-hybrid screen revealed a novel binding interaction between p27 and GRB2, an adaptor protein involved in Ras activation. Ras is a proto-oncogene that activates multiple pathways leading to proliferation, and is inappropriately activated in 30% of all human cancers. We hypothesize that p27 may negatively regulate Ras activation by binding GRB2. We have confirmed that GRB2 and p27 bind using proteins purified from *E. coli* cells. The binding of endogenous proteins in fibroblasts is induced by stimulating quiescent cells with mitogenic signals, and requires transport of p27 from the nucleus to the cytoplasm. Transport occurs following phosphorylation of p27 by MAP Kinase, a protein kinase activated by Ras signal transduction. These data establish an additional role for p27 as a member of a negative feedback loop preventing inappropriate activation of Ras, and may help us explain its role as a tumor suppressor in cancer development.

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A FUNCTIONAL ANALYSIS OF ALTERNATIVE SPLICING OF THE CYTOPLASMIC DYNEIN INTERMEDIATE CHAIN IN DROSOPHILA

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Cytoplasmic dynein is a multi-subunit complex composed of heavy, intermediate, light intermediate, and light chain polypeptides. It is known to be employed in axonal trafficking of vesicles, mitochondria and golgi vesicle movements, mitotic spindle assembly, and chromosome movement. How dynein associates with diverse cargoes is not well understood. The dynein intermediate chain (DIC) subunit has been identified as important for binding of cargo to the dynein complex. The dynein intermediate chain gene in *Drosophila melanogaster* and *Rattus norvegicus* expresses multiple alternatively spliced transcripts or isoforms. These isoforms potentially encode dynein intermediate chain protein variants that contribute to specialized dynein functions. The expression of several DIC isoforms in *Drosophila* has been demonstrated to be constitutive; expression of other isoforms has been shown to be tissue specific. The question of whether these isoforms actually encode functionally distinct DIC proteins is unresolved. Through the use of transgenic flies that express a single constitutively expressed DIC isoform, we are attempting to answer this question. We created a transgene from a single isoform cDNA (cdic2a) fused to the endogenous promoter of the genomic DIC gene. This was assembled through multiple sub-clones using an *E. coli* plasmid vector. The transgene was microinjected into early stage *Drosophila* embryos. An eye color marker was used to determine progeny flies that had the transgene inserted into their genome. We have recovered and established several independent *Drosophila* lines containing the DIC cDNA transgene. Utilizing these flies, we will be able to test whether the single isoform encodes sufficient function to rescue DIC lethal mutations. We will accomplish this by crossing the transgenic lines to fly lines with 3 different, recessive lethal mutations in the endogenous DIC gene to test for viability or the presence of tissue specific phenotypes

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HUA AND TRISTETRAPROLIN COMPETE FOR BINDING TO A SUBSET OF T LYMPHOCYTE AU-RICH ELEMENT SEQUENCES

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Many growth regulatory genes, such as proto-oncogenes and cytokine genes, that are expressed following T lymphocyte activation contain AU-rich elements (AREs) in their 3'-untranslated regions (3' UTRs). AREs regulate mRNA degradation through their interaction with ARE-binding proteins within the cytoplasm, however the biochemical mechanisms underlying ARE-mediated mRNA degradation are poorly understood. Our work aims to characterize the binding interaction between ARE sequences and two ARE-binding proteins, HuA and tristetraprolin (TTP), both of which are expressed in activated T lymphocytes. Cytoplasmic HuA expression is induced rapidly, within one hour after T lymphocyte activation, whereas TTP expression occurs three to six hours after activation. In other systems, overexpression of HuA has led to specific stabilization of ARE-containing transcripts while overexpression of TTP has led to specific degradation. We have used a HeLa cell transfection system, in which TTP was produced from a full-length cDNA expression construct and HuA was constitutively expressed, to assess the relative affinities and binding specificities of HuA and TTP for a variety of T lymphocyte ARE sequences. Binding by HuA and TTP to ARE sequences was assessed using an RNA-protein UV cross-linking assay. HuA recognized specific AU-rich sequences found in c-jun or c-myc mRNA that were poorly recognized by tristetraprolin. In contrast, tristetraprolin recognized an AU-rich sequence in IL-2 mRNA that was poorly recognized by HuA. HuA and tristetraprolin, however, competed with each other for binding to certain ARE sequences, including sequences from c-fos, interleukin-3, tumor necrosis factor-alpha, and granulocyte-macrophage colony stimulating factor mRNA. These findings support the hypothesis that the RNA-binding specificities and relative abundances of HuA and TTP may contribute to the differential regulation of ARE-containing transcripts within the cell. This would provide a mechanism by which genes could be transiently expressed and then turned off following cellular activation.

22

INVESTIGATING GERMINATION PERFORMANCE OF STANDER AND ITS PROGENY

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The malting barley variety Stander was introduced with high expectations, but germination and quality problems began to appear, and malters now reject the variety. My objectives were to test whether there is a statistically significant difference in the germination rates of the varieties Robust (the industry standard) and Stander, in conditions similar to malting and the field, and whether there is a correlation between the germination rates of Stander and the age of the seed. Additionally, I attempted to observe whether Stander's germination shortcomings have been passed on to its progeny in the Minnesota barley germplasm and whether there is a relationship between the germination of Stander progeny and the amount of Stander germplasm it contains (i.e., do Stander half-sibs have lower germination rates than Stander quarter-sibs). Stander and the other major Minnesota barley varieties (Robust, Morex, MnBrite, and Lacey) from the years 1997, 1998, 1999, and 2000 were germinated in germination paper at 20°C and 10°C. The M-lines produced in crosses with Stander were also germinated in germination paper at 20°C with Stander and Robust as checks. The comparison of Stander and Robust seed grown 1997, 1998, 1999, and 2000 showed that at $\alpha = 0.05$, there is a difference between the mean germination at both 20°C and 10°C, while the relationship between seed age and germination was not significant. I was not able to observe whether Stander's germination shortcomings had been spread through its progeny or if the amount of Stander's genetic material in a line affected the percent germination.

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THE ROLE OF X-CHROMOSOME INACTIVATION IN THE MANIFESTATIONS OF X-LINKED DISEASES

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Chromosome delegation that determines one's sex (XX in females and XY in males) gives a double dose of X-chromosome characteristics to females. To compensate for this, X-chromosome inactivation occurs. This can occur by transcriptionally silencing one X-chromosome in females. Theoretically half of the X-chromosomes from the mother and half from the father should be inactivated. However, skewing can occur for various reasons, including random and nonrandom X-chromosome inactivation. For example, Alport syndrome, an X-linked disease causing kidney failure, can be variably expressed in the kidney due to skewing. This project began looking at the role of X-chromosome inactivation in the manifestations of this X-linked disease in a mouse model, which is under development. By searching biotechnology databases, variable chromosome sequence differences, polymorphisms, were found on the X-chromosome. After isolating and verifying a sequence, an assay was developed that tested for polymorphisms between two mice strains. Information collected from the assay, could then be applied to the hypothesis. Hypothetically, if X-chromosome inactivation is skewed, then varying severities of X-linked diseases may be explained.

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CHLAMYDOMONAS & THE LF2 GENE: HOW THIS GENE MEASURES UP IN CONTROLLING FLAGELLA LENGTH

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The unicellular green alga, *Chlamydomonas reinhardtii* has two flagella, a cup-shaped chloroplast that encloses the nucleus, and an eyespot for sensing light. The Lf2 gene plays a key role in regulating the length of the flagella, because the Lf2 mutants have flagella two to three times the normal length. My research involved the sub-cloning of the Lf2 gene in preparation for sequencing. To prepare a sample for sequencing, I used DNA from a *Chlamydomonas* cDNA library and performed a Polymerase Chain Reaction (PCR) to amplify the DNA. Next, I inserted the DNA fragment via ligation into a plasmid-cloning vector, P-Blue Script (PBS). I mixed the bacteria and the PBS-Lf2 vector and via electroporation induced transformation of the bacteria. I cultured the mixture on nutrient agar plates containing Ampicillin to select for the transformed bacteria—only the bacteria containing the PBS-Lf2 vector survived on the plate. I then picked individual bacterial colonies, isolated, and purified their DNA. My attempt to prepare the Lf2 gene for sequencing was unsuccessful because the bacteria would not retain the PBS-Lf2 vector. I performed gel electrophoresis with the expectation that a 2.4 Kilobase (Kb) band from the Lf2 insert would result, but a band at either 2 or 3 Kb was consistently obtained on the agarose gel. Since the bacteria would not remain transformed, it was uncertain whether the PBS-Lf2 plasmid was obtained from the bacteria during the lysis procedure. Toothpick lysates were then performed to obtain DNA from numerous, mixed bacterial cultures. This step attempted to determine whether any of the different bacterial colonies would retain the Lf2 insert, but was also unsuccessful. Since it was not conclusive that the PBS-Lf2 vector was isolated and purified, the sequencing step for the Lf2 gene was not performed.

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THE GLUTAMATE RECEPTOR EAAC1 IS HIGHLY CONCENTRATED IN HIPPOCAMPAL CELLS WHEN COMPARED TO STRIATAL CELLS.

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Glutamate, the most common excitatory neurotransmitter in the brain can, under certain conditions prolong its stay in the synaptic cleft. Prolonged periods lead to uncharacteristically large amounts of glutamate in the extracellular space. Receptors on the post-synaptic cells develop a greater affinity for glutamate binding. The increasingly bound glutamate ultimately leads to excitotoxicity, which can be detrimental to the entire cell. Glutamate transporter knockout studies in astrocytic processes have demonstrated that astrocytes are mostly responsible for the rapid uptake of the bound glutamate in glutamatergic synapses (Rothstein et al. 1996). Furthermore, striatum and hippocampal slices have shown that astrocytes contain GLAST transporters, while post-synaptic dendrites contain EAAC1 transporters (Rothstein et al. 1994). Previous data have suggested that cultures of striatum are more vulnerable to glutamate toxicity than hippocampal cultures (Brustovetsky and Dubinsky, unpublished data). Huntington's Disease involves degeneration of neurons in the caudate and putamen, (parts of the striatum) which eventually leads to increased excitation in the motor cortex. Comparative studies in hippocampus and striatum cultures were examined to compare concentrations of EAAC1 and GLAST glutamate transporters. We show here by way of immunocytochemistry that hippocampal culture (thirteen days after plating on a glial feeder layer) indicated a stronger concentration of EAAC1 than the striatal cells (plated on the same day with glial feeder layer).

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AN ALTERED ACETOHYDROXYACID SYNTHASE SITE THAT CONFERS TOLERANCE TO IMAZAMOX HERBICIDE IS LOCATED ON CHROMOSOME 6D IN COMMON WHEAT (*Triticum aestivum*)

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There are currently no selective herbicides that will kill jointed goatgrass (*Aegilops cylindrica* Host.) in wheat (*Triticum aestivum* L.). The common wheat cultivar 'Fidel' had previously been mutated and selected for resistance to imidazolinones. Imidazolinones are a class of herbicides that inhibit acetohydroxyacid synthase (AHAS) and are effective against a broad spectrum of monocot and dicot weedy species, including jointed goatgrass. Previous research using durum (*T. turgidum* L. var. durum Desf.) crosses with the AHAS resistant Fidel (IMI-Fidel) has indicated that the mutated AHAS gene was located on chromosome 6D. This study was done to confirm the location of the imidazolinone-resistant AHAS gene on chromosome 6D in a common wheat genetic background. Two populations of F₂-derived F₃ families developed from common wheat cultivars 'Cashup' and 'Madsen' each crossed with IMI-Fidel were treated with imazamox in a spray cabinet and evaluated for herbicide resistance in the greenhouse. Microsatellite markers from chromosome 6D were used to screen the populations. The marker *Xgwm55* was determined to have a linkage distance of 3.6 cM from the AHAS gene in the Cashup/IMI-Fidel population, and the marker *Xgdm127* had linkage distances of 18.4 cM and 24.8 cM from the AHAS gene in the Cashup/IMI-Fidel and Madsen/IMI-Fidel populations, respectively. A screening of group 6 nulli-tetrasomics, ditelosomics, and deletion lines confirmed the location of the marker *Xgdm127*, and therefore the linked AHAS gene, to be on chromosome 6D.

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QUANTITATION OF GLOMERULAR CELLS

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Approximately 17 million Americans have diabetes, and between ten and twenty-one percent of diabetics have nephropathy, comprising around 0.89% of the total population.¹ To understand effects of diabetes on kidneys, the glomerulus must be studied. Three cell types are involved in filtration across glomerular capillary membranes: endothelial, epithelial, and mesangial. As diabetic nephropathy progresses, the secretion of mesangial cells, mesangial matrix, increases in volume.^{2,3} This increased mesangial matrix may decrease function of the glomerulus by decreasing the surface of capillary walls available for filtration. To accurately examine if this increased mesangial matrix is due to proliferation of mesangial cells, glomerular cells must be quantitated using an unbiased, objective technique. The research objective was therefore to study diabetic nephropathy through its effects on glomerular cell number. The first phase of research was to establish which of two techniques, the Physical Disector or Weibel-Gomez, would be used. Particle size, number, and shape assumptions of the Weibel-Gomez method added bias and thus resulted in decreased accuracy. The Physical Disector method does not make the same assumptions, more accurately quantitating the density of glomerular cells, and therefore was used in the following stages of research. The second phase involved counting the number of endothelial, mesangial, and epithelial cells in biopsies from diabetic patients and non-diabetic controls, and comparing the results to determine if the number of cells changes due to diabetes. This involved serial sectioning kidney tissue into section pairs, 2 μm apart, at 20 μm intervals. Sections were stained, digital images captured, images viewed in Adobe Photoshop, and nuclei of glomerular cells were counted using the Physical Disector. Currently, several biopsies have been counted but several more must be completed before analysis of the data. These comparisons will hopefully provide insight into renal cellular reactions due to diabetes.

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SUBSTANCE P AND CALCITONIN GENE RELATED PEPTIDE IMMUNOREACTIVE EPIDERMAL NERVE FIBERS IN A MURINE MODEL OF CANCER PAIN

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Although pain is a significant consequence associated with cancer, the mechanisms by which tumors interact with nerves to produce pain are unknown. We have shown previously that a tumor in the hindpaw of mice causes degeneration of epidermal nerve fibers (ENFs), suggesting that tumors produce a neuropathic condition. A number of different types of ENFs could potentially be affected when tumor tissue invades adjacent structures. One thing that characterizes different types of fibers is the type of neurotransmitters they contain and release. In order to better understand cancer pain, we used a murine model of fibrosarcoma to investigate the changes that occur during tumor progression with ENFs containing the neuropeptides Substance P (Sub P) and calcitonin gene-related peptide (CGRP), which are contained in pain afferent fibers. To determine this, skin biopsies were taken from mice that were hyperalgesic to mechanical stimulation. Then tumor ENFs were immunostained for either SubP or CGRP. This was done at days 10, 14, and 18 after tumor cell injection so that changes in the numbers and proportions of Sub P and CGRP immunoreactive ENFs could be observed. Specimens were imaged with confocal and fluorescence microscopy to analyze the tissue based on the number of ENFs per epidermal length. The results indicate that the number of Sub P and CGRP immunoreactive ENFs initially increase, peak around tumor day 14, and decrease thereafter. However, these changes were not significant, which suggests that Sub P and CGRP immunoreactive fibers, which presumably transmit painful information, are conserved during tumor growth.

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FUNCTIONAL ELECTRICAL STIMULATION

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Functional electrical stimulation, stimulation of the muscles via electrical pulses, is changing many aspects of the medical world. Not only is functional electrical stimulation (FES) being used to train animals and help children learn to read silently, it is also being utilized to build underdeveloped muscles and enhance rehabilitation techniques. Even more exciting, the utilization of FES has allowed some paraplegic people to walk 15 consecutive steps before they become fatigued. My experiment will offer further insight into the possibilities of electrical stimulation. This study will test the hypothesis that electrical stimulation of muscle using doublet activation (two closely spaced stimulation pulses with a recurrent relaxation period in between pulse sets) will result in the ability to track a closed-loop isometric force trajectory longer than when activating with singlets (single stimulation pulses separated by relaxation periods). A closed-loop isometric force trajectory means that the subject's quadriceps will be stimulated until it reaches a target force that is a fixed percentage of the maximum voluntary contraction. The quadriceps muscle will be stimulated while the knee is fixed in a 90° position. Testing will be stopped when the subject fails to attain the target force three consecutive times. If this research shows that the quadriceps muscle fatigues more slowly when using doublet stimulation, a stronger case for the importance of utilizing doublet stimulation in restoration of muscle function will be provided. Currently, the functional electrical stimulation (FES) chair in which the subjects will sit is being built, the stimulation apparatus is under construction and the protocol for the experiment is under review.

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THE TRANSLATION INITIATION APPARATUS AS A TARGET FOR ANTIFIBROTIC THERAPY

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Idiopathic pulmonary fibrosis (IPF) is a relentless disease of the lung resulting in death by suffocation. In IPF, lung fibroblasts proliferate leading to occlusion of alveolar airspaces. Therapies to limit fibroblast accumulation or promote regression have been ineffective. Fibroblasts isolated from IPF lung tissue have elevated levels of the mRNA cap binding protein, translation initiation factor 4E (eIF4E), a component of the protein synthesis apparatus that we have previously shown results in cellular resistance to apoptosis. eIF4E, along with eIF4G and eIF4A, is part of the trimolecular translation initiation complex eIF4F. This complex associates with the 40S subunit of the ribosome via eIF3 to initiate cap-dependent translation. The translational repressor protein 4E-BP1, sharing the eIF4E-binding domain with eIF4G, competitively inhibits association of eIF4E with eIF4G. Based on this, we hypothesize that transfer of 4E-BP1 into IPF fibroblasts will sensitize them to apoptosis. When fibroblasts isolated from IPF lung tissue were transduced in vitro with genes encoding 4E-BP1wt and 4E-BP1 T70A (a more active hypophosphorylated mutant) using retroviral vectors, gene transfer efficiency and expression of the ectopic gene were excellent. Unfortunately, these primary fibroblast cultures became senescent. To overcome this problem, we turned to a well-established murine trachea allograft model of airway fibroproliferation. Tracheas of donor mice are subcutaneously implanted into the backs of recipient mice. Tracheas become completely occluded with proliferating fibroblasts 21 days after transplantation. High titer retroviruses coding for empty vector, 4E-BP1wt, or 4E-BP1 T70A in cis with a GFP reporter are injected into the lumen of the tracheas. Histological examination is carried out to verify reporter gene expression. Our immediate goal is to develop a method that will ensure therapeutic gene expression in the target fibroblasts in the tracheas. Once this method is refined, we will be positioned to test the efficacy of translational repressors as antifibrotic agents alone and in combination with proapoptotic pharmaceuticals.

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PROTECTIVE EFFECT OF VEGF DURING RENAL ISCHEMIA

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Vascular endothelial growth factor (VEGF) a proangiogenic protein inhibits the tubular fibrosis, peritubular capillary loss and reduced function that occurs during renal failure (D-H Kang et al, J. Am. Soc. Nephrol 12:1448-1457, 2001). In renovascular hypertension, stenosis of a single renal artery leads to gradual atrophy of the affected kidney. We hypothesized that during onset of renal ischemia, growth factors, ie, VEGF and bFGF, exert a protective effect as described above. In 14 anesthetized rats a small cortical sample was removed, and in one group (Ischemic, n=8) a Goldblatt clip placed on the left renal artery while the other group (Sham n=6) underwent sham operation. After 2.5-3 hrs., the rats were sacrificed and cortical tissue removed from both left and right kidneys. VEGF and bFGF mRNAs in the tissue samples were analyzed by RT-PCR. In the Ischemic group, VEGF message was increased above control in both left and right kidneys, whereas, there were no changes in the Sham group. In contrast, bFGF was unchanged in both the Ischemic and Sham groups. These results suggest that in the early stage of renovascular hypertension, VEGF is elaborated in the kidney subjected to reduced blood flow. VEGF may serve to temporarily inhibit renal atrophy and preserve renal function.

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HIGH FREQUENCY CHEST COMPRESSION THERAPY FOR CYSTIC FIBROSIS PATIENTS

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Cystic fibrosis is a genetically inherited disease affecting 30,000 children and adults in the United States. One of the complications affecting CF patients is a thick, sticky mucus buildup in their lungs, which must be cleared away in order to keep the airway clear as well as prevent infection. Historically this was done by manually compressing the chest, using cupped hands on the chest and back. About a decade ago a new therapy device was invented at the University of Minnesota, a machine that clears mucus away much more efficiently by using high frequency pulses of air in a vest worn by the patient to compress the chest. This project tested the third generation of the machine, which makes use of a different pulse pattern than the first two. The pattern of air compression in the vest resembles a triangle wave, with the vest quickly filling with air and reaching a peak, then quickly deflating. This quick inflation and deflation is believed to create a strong shear force that physically knocks mucus from the patient's lungs. Comparisons of new and old machines were made by collecting sputum samples from patients, and it was found that in all cases the new machine resulted in better lung clearance. In addition, the machine was made to be much more portable, adding to the ease of use for the patient.

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MAREK'S DISEASE VIRAL TITERS IN PRIMARY AND IMMORTAL CHICKEN CELL LINES

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The virus producing capabilities of two newly discovered spontaneous immortal cell lines of chicken origin were determined. Cultures of these cell lines (SC1 and SC2), along with several control cell lines including a primary chicken fibroblast cells (CEF^o) and one previously developed immortal chicken cell line (DF1), were infected with the Marek's disease virus. Viral titers were then determined by counting the number of viral plaques per plate of each culture, first by light microscopy, and then by an immunofluorescent antibody technique for confirmation. The results of the experiment showed that, while viral titers for the SC2 cell line were significantly lower than that of the controls, the titers for the spontaneous immortal SC1 cell line were comparable to that of the primary fibroblasts and greater than those of the DF1 cell line. These results suggest that, under similar growth conditions, the SC1 cell line would be a potential candidate for commercial Marek's disease viral production, while the SC2 cell line would not.

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HIV INFECTION OF EPITHELIAL CELLS: A ROLE IN TRANSMISSION

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HIV is a retrovirus that infects cells of the immune system, specifically CD4 positive T lymphocytes and macrophages. Eventually, people who are infected with HIV will develop AIDS (Acquired Immunodeficiency Syndrome) and succumb to secondary bacterial and fungal infections. Since the main mode of virus transmission occurs across epithelial layers, and considering that the exact mechanism is still unknown, our research has focused on HIV transmission in various human organ cultures and primary cell cultures containing epithelial cells. We analyzed how seminal fluid disrupts and/or infects stratified squamous epithelium (palatine tonsil and ectocervix), reticulated epithelium (tonsillar crypts), and single cell columnar epithelium (endocervix). By simulating the physiological and biological conditions present during a natural transmission, we have found that HIV virions and seminal cells exhibit a low frequency of transmission across intact epithelial layers. HIV infected lymphocytes and seminal cells bind to, and actively interact with intact epithelial surfaces, remaining tightly bound after many washings. By staining for cytokeratin we can distinguish between cryptal epithelium and stratified epithelium to visualize exactly where infection is occurring and which cell types are more susceptible to infection. Immunohistochemistry, along with fluorescent staining, allows visualization of HIV infected cells. Due to the fact that some epithelial surfaces have a mucus layer protecting the tissue from the immediate environment, the majority of seminal cells do not penetrate into the endocervical layer. Currently, we are examining how the disruption of various epithelial layers can have an effect on the frequency of transmission.

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IDENTIFYING MOLECULAR SENSORS OF LUNG EPITHELIAL CELL HOMEOSTASIS, ADAPTATION, INJURY & APOPTOSIS AFTER EXPOSURE TO HAZARDOUS AIR POLLUTANT PARTICLES

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Hazardous air pollutant (HAP) particles travel into lungs and cause a variety of respiratory problems in addition to increased morbidity and mortality. We hypothesize that the biological response to this type of environmental stress begins at the cellular level and should follow a characteristic pathway; that of homeostasis, adaptation, injury, and death. To better understand the mechanism behind this stress response, we have quantified cellular toxicity of HAP particles and identified molecular sensors that will predict the physiological or pathological outcomes mentioned above after exposure to HAP particles. Cytotoxicity of HAP particles on lung epithelial cells was measured using the lactate dehydrogenase (LDH) assay, which measures cell damage. Of the six HAP particles tested, vanadium oxide (V_2O_5) was the most toxic at low concentrations, thus making it a strong efficient stimulus. For characterizing homeostasis and injury, we chose heat shock protein (HSP) and nuclear factor- $\kappa\beta$ (NF- $\kappa\beta$), respectively, for activation testing. HSP and NF- $\kappa\beta$ promoter elements were each linked to luciferase reporter genes and these DNA constructs were transiently transfected into MP48 rat lung epithelial cells. Following transfection, the cells were exposed to various concentrations of V_2O_5 , and activation of HSP and NF- $\kappa\beta$ sensors was measured by the levels of luciferase produced. HSP was found to be an inadequate predictor of homeostasis upon HAP particulate stress as low levels of activation were consistently obtained. However, NF- $\kappa\beta$ was identified as a sensor of cellular injury with 3- to 4-fold increases in activation over the control at a V_2O_5 concentration of 25 $\mu\text{g}/\text{cm}^2$. This information could be used to further establish the dose, composition, and size of HAP particles that define the threshold between cell adaptation and injury, generating guidelines for EPA regulation of airborne pollutant particles. Additional sensors, such as for adaptation and apoptosis, are currently being investigated using a faster method called microarray processing.

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THE REWARDING AND LOCOMOTOR-STIMULATING EFFECTS OF COCAINE ARE INFLUENCED BY G-PROTEIN GATED INWARDLY RECTIFYING K^+ (GIRK) CHANNELS

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Although G-protein gated inwardly rectifying K^+ channels (GIRK 1-4) have been identified at a molecular level, the phenotypic effects of these channels are not fully understood. GIRK 2 and GIRK 3 subunits are widely expressed in regions of the brain that are associated with the mechanisms of drug addiction such as the nucleus accumbens (NAc), ventral tegmental area (VTA), and the periaqueductal gray (PAG). However, no pharmacological ligands currently exist that modulate these channels. Thus, by studying GIRK 2 and GIRK 3 single knockout mice and GIRK 2/GIRK 3 double knockout mice, the role these channels play in the rewarding and locomotor stimulatory effects of cocaine may be ascertained. *Methods:* A genotype-blind study was conducted in which subjects were put on a fixed-ratio 1 schedule of cocaine self administration (1 lever press response per infusion) for 3-hour access periods in order to test the rewarding effects of cocaine. Doses of cocaine (0.125, 0.25, 0.5, 1.0, 2.0 mg/kg/infusion) were non-systematically varied in order to generate a dose response curve, or behavioral profile of the subjects. To test the effects of cocaine on locomotor behavior, subjects were habituated with saline injections to a plexiglas chamber for 2 days in order to observe baseline activity levels. Following this habituation period, mice were injected for 5 days with cocaine (15 mg/kg), and movement was observed. *Results:* Dose response curves indicated that GIRK 2/GIRK 3 double knockout mice and wild type mice self-administered more cocaine when compared to both GIRK 2 and GIRK 3 single knockout mice. Locomotor behavior results demonstrated that baseline activity did not differ among the four genotypes. Following cocaine injections, all genotypes exhibited a significant increase in locomotor activity. *Conclusion:* Cocaine's effect on reward and locomotor behavior is dependent upon the GIRK subunits expressed and the combinations of those subunits within the areas of the brain implicated in drug addiction.

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A META-ANALYSIS OF OUTCOMES FOLLOWING FEMORAL NECK FRACTURES IN THE ELDERLY

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One of the major consequences of an increase in life expectancy has been a marked increase in fractures of the femoral neck in the elderly population. However, there is currently a lack of consensus in the orthopedic community as to which type of surgical treatment has the best results. *METHODS*: Meta-analysis, a technique for the combination of data from multiple sources, was applied to analyze the four main techniques for treating femoral neck fractures: internal fixation (both cannulated screws and a dynamic hip screw), unipolar hemiarthroplasty, bipolar hemiarthroplasty, and total hip arthroplasty. Using a *MEDLINE* search, all available literature from 1995 through 2002 on fractures of the femoral neck were obtained and analyzed, and included or excluded based on the predetermined criteria. The data from the eligible articles was combined to determine statistical trends in three main outcomes: mortality, functioning, and revision rates. *RESULTS*: Although definite results have not yet been obtained, some general trends have been noted. Techniques of internal fixation, although slightly less invasive, usually result in the highest rate of re-operation. Both types of hemiarthroplasty (unipolar and bipolar) are major procedures and generally become less suitable as age increases, as a consequence of degenerative joint disease. Total Hip Arthroplasty has shown good results, with a lower rate of revision and an increase in functional results. *CONCLUSIONS*: Research is still being conducted, so a formal conclusion cannot be made.

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CYSTIC FIBROSIS SWEAT TEST

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Cystic Fibrosis (CF) is an inherited disease, which causes the exocrine glands in the body to function abnormally. The sweat test is the current method for the diagnosis of CF. Pilocarpine is delivered by iontophoresis to the sweat glands to stimulate sweating, which is collected on a gauze pad so its chloride content can be measured. This current method has problems. For accuracy, it must be conducted by trained technicians. It takes a long time, is expensive, and uses obsolete equipment. I am attempting to find a faster, easier, more reliable and cost-effective way of delivering pilocarpine to the sweat glands. The ultimate goal is to have a self-stimulating sweat test patch that will measure the amount of sweat and its chloride content. I have looked into four methods: microneedles, electroporation, ultrasound, and DMSO. While microneedles can increase skin permeability up to six orders of magnitude, these patches require more refinement before they can be marketable. Electroporation is the use of electrical current to create transient pores in the plasma membrane through which macromolecules can enter a cell. However, electroporation is costly, has a high pain potential, and is not ready for clinical use. We are also investigating sonophoresis, the use of ultrasound to enhance transdermal drug delivery by producing temporary defects in the stratum corneum. The most promising method is the use of dimethyl sulfoxide (DMSO), which, as absorbed, can carry non-ionized molecules with it across the skin. We are conducting two experiments. One measures the amount of sweat produced over time for different dilutions of pilocarpine in 50% DMSO solution. The other tests how the partial removal of the uppermost layer of the stratum corneum affects transdermal drug delivery. Success in this project could point the way to better and faster treatments for skin and systemic diseases as well as make possible the diagnosis of CF as early as the second day of life.

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B-MODE AND DOPPLER ULTRASONOGRAPHIC GASTROINTESTINAL MOTILITY PATTERNS IN THE HORSE

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Colic is a combination of symptoms that indicate abdominal pain in the horse and represents a significant cause of morbidity and mortality. The abnormal gastrointestinal conditions that cause colic include gas distension, impaction, displacements, infections, and vascular lesions. This study was designed to evaluate gastrointestinal motility patterns in clinically healthy horses using B-mode and Doppler sonography to quantify base line levels and provide information to identify abnormal function in the digestive tract. B-mode sonography uses sound waves to compose a two-dimensional picture of the internal anatomy and was utilized to measure parameters such as wall thicknesses of the stomach, cecum, small and large intestine, number and diameter of loops in the small intestine, and large colon distension. Doppler sonography was studied in isolated regions to measure the frequency and intensity of the waves to determine the type of motility in the small intestine. Preliminary data indicate the most active area is the large intestine, followed by the slightly less motile cecum. Stomach activity is usually minimal. Activity in the small intestine is more difficult to quantify, because in many trials it was not seen. Decreased motility patterns were observed when the horse was taken off feed for more than eight hours or sedated. Data collection and analysis is ongoing.

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SYNTHESIS AND CHARACTERIZATION OF PROTEIN ADDUCTS FORMED FROM EXPOSURE TO FURAN

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Protein adducts are formed from the covalent interaction of proteins with *cis*-2-butene-1,4-dial, the microsomal metabolite of the hepatocarcinogenic compound, furan. Furan is an important industrial solvent. It is also found in smog, cigarette smoke, and in many beverages and foods. Furan is both hepatotoxic and hepatocarcinogenic in rats and mice. However furan is classified as a nongenotoxic compound because it does not induce a DNA repair response in rodents. Furan is activated by cytochrome P450 to a reactive intermediate that alkylates proteins and DNA to elicit a toxic response. This toxic response, through an unknown mechanism, stimulates liver cell proliferation and can then lead to liver tumor formation. Furan is metabolized to *cis*-2-butene-1,4-dial in a reaction catalyzed by cytochrome P450. This metabolite is thought to be the metabolite responsible for the toxic and carcinogenic properties of furan. To determine the potential structures of the metabolites and protein adducts formed as a result of furan exposure, *in vitro* experiments were performed to first determine the amino acid adducts. The reactive metabolite *cis*-2-butene-1,4-dial was reacted with various nucleophilic amino acids such as N-acetyl-cysteine, N-acetyl-Lysine, glutamate, and glutathione, in sodium phosphate buffer at room temperature. HPLC, NMR, and electrospray LC-MS/MS analysis of the reaction mixtures show that *cis*-2-butene-1,4-dial readily interacts with nucleophiles. It forms thiol-amine pyrrole cross-links which indicate that *cis*-2-butene-1,4-dial has the potential to form intra and inter protein cross-links. Currently these results and additional future studies of the interaction of *cis*-2-butene-1,4-dial with other nucleophilic amino acids will be used as standards in identifying *in vivo* protein adducts formed from the exposure to furan.

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ECHOCARDIOGRAPHIC, ANGIOGRAPHIC AND ALGORITHMIC PREOPERATIVE PREDICTION OF AORTIC VALVE ANNULUS SIZE FOR PREOSTHETIC VALVE PLACEMENT

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Prior to implanting a valve within the heart, it is important to know the size of the seating place, or implantation site, which is known as an annulus. The purpose of this research was to investigate various ways of predicting annulus sizes within the heart, specifically focused on the aortic valve. The research was performed in the Experimental Surgical Services (ESS) laboratory. Gross predictive factors were studied utilizing historical ESS data. Three different diagnostic tools: angiographic, transthoracic echocardiographic and transesophageal echocardiographic approaches, were studied through literature research and interviews with specialists. In addition, a mathematical algorithm for predicting annulus size was found in the literature, and it was subsequently examined via historical data and experimental research. Gross predictive factors such as age and mass did not provide appropriate predictions of annulus size. Angiography and both types of echocardiography were found to be accurate tools; and degree of invasiveness was then considered. The experiment which was designed and performed, in addition to supplemental historical ESS data, shed doubt on the relevancy of the published algorithm. Huge standard deviations from the algorithmically predicted sizes were found – these correspond to my research revealing published literature that yields inaccurate values. All of these findings/techniques combined provide a more accurate visualization of the inside of the heart, and will aid in the future implantation of prosthetics, including heart valves.

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TRIPLE IMMUNOHISTOFLUORESCENT LABELING PROVIDES INSIGHT INTO CELLULAR PROLIFERATION AND DIFFERENTIATION IN REGENERATING RAT ADRENAL GLANDS

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The adrenal cortex, which produces essential hormones in response to stress, has the uncommon ability to regenerate both morphologically and functionally following injury. A greater understanding of the steps that occur in regeneration will allow us to develop therapies to reduce injury and increase the rate of recovery in trauma patients or patients suffering from septicemia or extensive surface burns. To better understand the steps of differentiation and proliferation in the regenerative process, a triple labeling technique for identifying cellular phenotype and proliferating cells was developed. The phenotype of the cells of the zona glomerulosa and zona fasciculata/reticularis, producing the hormones aldosterone and corticosterone respectively, were labeled via the specific enzymes they produce; immunohistofluorescent labeling of cytochrome P450 aldosterone synthase enzyme marked the cells of the zona glomerulosa, and P450 11 β hydroxylase enzyme marked the cells of the zona fasciculata/reticularis. Immunohistofluorescent labeling of Ki67 protein was used to mark proliferating cells. Proper controls were used to ensure no nonspecific labeling occurred. Images of the fluorescently marked cells were collected and overlapped to create a single image of a regenerating gland identifying the phenotype of proliferating cells. This diagnostic tool was then used to examine regeneration in various injury models including ischemia-reperfusion injury and adrenal enucleation. Data from two days postsurgery indicates that as expected, proliferation increases in the injury models. Currently, experiments are underway to use triple immunohistofluorescent labeling to accurately enumerate proliferating cells of each phenotype across the various injury models.

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ARTIFICIAL LIFE SIMULATIONS

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Computers have often been used to simulate real world events. In the field of artificial life, computers are used to simulate evolution of creatures. Usually the experimenter specifies a fitness function to select creatures for reproduction. The fitness function expresses the experimenter's criterion for promoting certain characteristics among the creatures. System parameters are adjusted by the experimenter using trial and error to yield a stable system state. Lack of inherent system stability calls into question the validity of conclusions drawn from experiments conducted using such a system. The goal of our project is to design a simulated environment which does not require any intervention by the experimenter (other than the initial design of the system) to achieve stability. We will present results that show how a typical, simplistic system design involving relatively homogeneous creature populations leads to systems that are not stable with respect to requiring continuous intervention by the experimenter, by creating a design that promotes diversity in creature types and behavioral patterns. Finally, we will provide some guidelines for designing inherently stable artificial life systems. Such systems can be used to yield meaningful results to questions about the evolution of various creature characteristics.

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IN SITU DIELS-ALDER REACTIONS OF MALEIMIDES WITH CYCLIC KETONES AND NITROGEN HETEROCYCLES TO GIVE TETRAHYDROCARBAZOLES

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The synthesis of tetrahydrocarbazoles has been approached by an acid-catalyzed condensation of indole with cyclic ketones to make a vinylindole followed by an *in situ* trapping of the intermediates using a Diels-Alder cycloaddition with a maleimide. The cycloaddition is followed by an isomerization of the resulting double bond into the indole nucleus to give a tetrahydrocarbazole. The major stereochemistry of the newly formed tetrahydro ring of the carbazole product has the hydrogens all-*cis*. The compounds made in this study may have anti-tumor potential and will be offered to the NIH for testing.

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EFFECTS OF ETHANOL AVAILABILITY ON DEMAND FOR SMOKED COCAINE BASE IN RHESUS MONKEYS

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Cocaine abusers frequently use cocaine and ethanol concurrently. Studies have shown that alcohol acts as a complement to cocaine, whereas a decrease in one would lead to a decrease in the other. It has also been shown that as the price for cocaine increases, consumption of cocaine decreases. However, it is unknown whether ethanol, at a fixed price, can be substituted for cocaine when cocaine is unattainable to the abusers because of an increase in price. In order to determine if ethanol can substitute for cocaine, ethanol was alternatively available at a fixed ratio of 8 to monkeys who were previously trained to receive cocaine inhalations by means of lever pressing. The amount of ethanol consumed was measured in relation to cocaine consumption at different prices. Each price of cocaine (64, 128, 256, 512, 1024, and 2048 lever presses) was tested for each monkey to determine if ethanol consumption would increase as the price for cocaine base increased and subsequent consumption of cocaine decreased. The data for this study are currently incomplete and no clear trends have presented themselves up to this point.

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DEVELOPMENT OF A MOUSE COCHLEA DATABASE

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Both the National Science Foundation and the National Institutes of Health have reported that future scientific information will arise not only by laboratory experimentation but also by discovery based upon information contained in community-accessible databases. The purpose of this research is to develop a collection of databases, called the Mouse Cochlea Database (MCD) that will contain anatomical images and complete bibliographic information of the mouse cochlea. The MCD will consist of four databases that will provide a comprehensive collection of information on the anatomy of the mouse cochlea. The four, Web-accessible databases are: bibliographic, image, cytochleogram, and 3D reconstructions. The bibliographic database presently contains 1142 citations, spanning a period of 1965-2001. These citations were obtained from PubMed, book chapters, and meeting abstracts. These records are searchable online and also can be downloaded as a single file. In addition, a concordance program was developed using visual basic to extract frequency information from the database. This program parses each of the bibliographic database's records and retrieves the frequencies of individual words. To date, mouse strain, discipline of study, and author productivity have been obtained using this program. Currently, a prototype image database is being developed and will contain anatomical data from normal cochleas using light microscopy and immunohistochemical methods for the purpose of compiling the molecular anatomy of the mouse cochlea. The MCD has received funding from the NIDCD for its development; however, much work remains to be accomplished to make it a useful community resource.