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## FROM THE DIRECTOR

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There have been a number of CERH-sponsored activities during the last few months, and these include several hands-on workshops developed by the Facility Cores. Larry Dangott and the Protein Technologies Facility Core held a four-day workshop on two-dimensional electrophoresis/proteomics. Scientists from laboratories of several CERH investigators attended this intensive workshop and received scholarships to cover costs. K.C. Donnelly (Analytical Core) coordinated a  $^{32}\text{P}$ -post-labeling workshop at the Institute for Biosciences and Technology where this service for the Analytical Core is carried by Dr. Zhou Guo-Dong. The Genomics & Bioinformatics and Transgenic Cores have also been holding workshops, which have been attended by the CERH faculty and their associates. The Image Analysis Core has initiated a new type of training for the techniques available in this facility. Bob Burghardt has been meeting with research groups of one or more CERH faculty and has been providing individualized training for these groups. Please contact Bob if you want to participate in these sessions.

The Scientific Advisory Group has been meeting every month to discuss CERH business, and currently we are preparing for the External Advisory Board meeting on December 9. The meeting will be held in the CERH conference room (#423) in the Veterinary Medical Research Building and all CERH co-investigators and associates are invited to attend. (8:30am – 1:00pm). The search for a new director is continuing, and I hope that within the next four to six weeks we will be successful in filling this position.

December 10 is another important date for the CERH. The CERH and the Center for Biological Clocks will be hosting our annual Scientific Symposium on December 10 from 8:30am – 3:45pm in the Clayton Williams Alumni Center. Four outstanding scientists will be presenting their research, and the afternoon session from 2:15 will feature a poster presentation from CERH faculty and their colleagues. For more information on this event, log on to CERH.TAMU.EDU. Please keep this date open, and your attendance and participation by your research group will be appreciated.

Sincerely,

Steve Safe

**Be sure to cite center support (P30-ES09106) on relevant publications.**

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## PILOT PROJECTS

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One of the major missions of the CERH is to fund pilot projects that will lead to NIH funding of research related to environmental health issues. Last year (2002), the CERH funded six projects for a total of \$150,000. Twenty-three applications for pilot projects were received for 2003 and five were funded. In this issue, we focus on progress of a previously funded pilot project under the direction of Dr. Louise Abbott.

Methylmercury (MeHg) is a potent environmental neurotoxicant, which causes degeneration of neurons including cerebellar granule cells. Recent studies using cultured cerebellar granule cells exposed to MeHg *in vitro* indicate that calcium ion homeostasis is altered, leading to increased intracellular calcium concentrations and eventual cell death. However, gaps still exist in our knowledge concerning the specific mechanisms leading to neuronal cell death following acute *in vivo* MeHg exposure. Altered intracellular calcium ion homeostasis can have profound effects on mitochondrial function and/or survival and damage to mitochondria in general results in decreased ATP production and release of cytotoxic/cell death signals. Because MeHg appears to alter calcium ion homeostasis we also are studying a spontaneously occurring mutation in mice called the "leaner" mutation, which also alters intracellular calcium ion homeostasis. Homozygous leaner (*tg1a/tg1a*) mice carry an autosomal recessive mutation in the carboxy-terminus of the pore forming  $\alpha_{1A}$  subunit of P/Q-type voltage-gated calcium ion channels. These channels are highly expressed by cerebellar granule cells. Leaner cerebellar granule cells begin to die via apoptosis on postnatal days (P) 10-12, with peak cell death at P20.

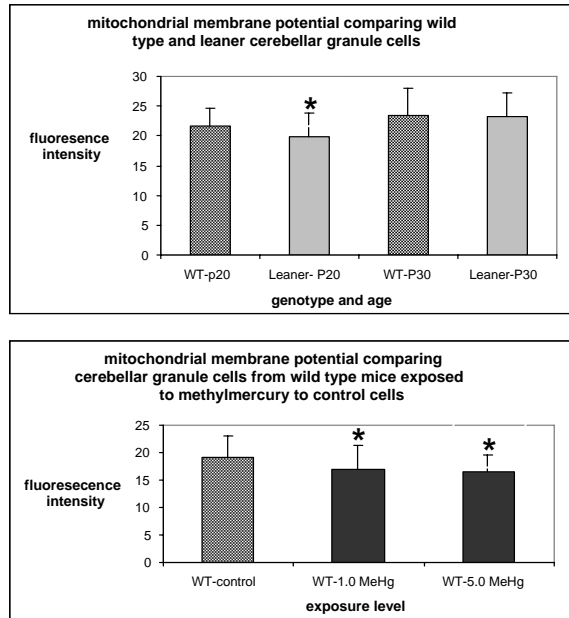
First, we examined mitochondrial function by comparing mitochondrial membrane potentials of cerebellar granule cells from mice that were "wild type" or controls, (C57BL/6J mice, not exposed to mercury) or wild type mice exposed to 1.0 mg/kg and 5.0 mg/kg MeHg, via their food. We also examined homozygous leaner mice (also on the C57BL/6J background) at two different ages, P20 and P30. We used both male and female mice. All mice were anesthetized using isoflurane, decapitated and the brains removed. Cerebellar granule cells were acutely isolated using dissociation medium containing protease, and plated onto poly-D-lysine coated coverslips. Cell viability is routinely greater than 90% using this protocol. Granule cells were loaded with tetramethylrhodamine-methyl ester (TMRM), which is a lipophilic cation that accumulates in mitochondria in proportion to the membrane potential. With sufficient accumulation, the dye exhibits a red shift in absorption and fluorescence emission spectra. Fluorescent images of granule cells from all groups were acquired and analyzed.

At P20, the mitochondrial membrane potential in leaner granule cells was significantly lower than in wild type cells (Figure 1A). However, at P30 no difference was observed between leaner and wild type granule cells. We speculate that changes in intracellular homeostasis alter leaner granule cell mitochondrial integrity, leading to activation of neuronal cell death signaling pathways. At P30, death of leaner cerebellar granule cells has decreased significantly and surviving leaner cells exhibit no differences in mitochondrial membrane potential from age-matched wild type granule cells.

The mitochondrial membrane potentials observed in granule cells from wild type mice (P45-50) treated with either 1.0 mg and 5.0 mg MeHg were significantly lower than from wild type granule cells not exposed to MeHg (Figure 1B). This suggests that *in vivo* MeHg exposure diminishes mitochondrial function in cerebellar granule cells, which ultimately could lead to death and/or dysfunction of these neurons. Since MeHg toxicity

may function through altering intracellular calcium levels in neurons, and since intracellular calcium levels of leaner mouse granule cells are already altered at P20 we plan to expose leaner mice to MeHg. We hypothesize that MeHg will have an even greater affect on the mitochondrial membrane potential of leaner cerebellar granule cells when compared to MeHg treated wild type mice and non-treated leaner mice. We are completing these experiments now. If this hypothesis proves to be true, then differences in neuronal calcium homeostasis capabilities could provide one explanation for different susceptibilities to MeHg observed in the human population.

Two toxicology graduate students, Dr. Sairam Bellum and Kerry Thuett, are carrying out this research under the direction of Drs. Louise Abbott and Ron Tjalkens. Dr. Tjalkens is a collaborator on this project and we greatly appreciate his expertise and assistance. This work was supported by NIEHS (CERH) support to LCA (P30-EF09106).



**Figure 1.** Graphs showing the relative fluorescence intensity of mitochondria acutely isolated from cerebellar granule cells and labeled with TMRM. The top graph shows fluorescence measurements obtained from leaner (*tg<sup>la</sup>/tg<sup>la</sup>*) and wild type mice at two different ages (P20 and P30). The fluorescence intensity is significantly lower in leaner granule cells at P20 when compared to age-matched wild type mice (\*,  $p < 0.05$ ). The bottom graph shows results obtained from exposing wild type mice to 1.0 mg/kg and 5.0 mg/kg MeHg. Both exposures resulted in a significantly lower mitochondrial membrane potential when compared to control mice (\*,  $p < 0.05$ ). A two-way ANOVA was used to account for both treatment (or genotype) and gender, followed by post hoc analysis. No effects of gender were observed. Error bars indicate standard deviation. 120-130 granule cells were examined for each group and cells were obtained from a minimum of 4 mice per group, two males and two females.

## Symposium

# Environmental Adaptation and the Circadian Response

**Wednesday, December 10, 2003**  
**Clayton Williams Alumni Center,**  
**Texas A&M University**

- 8:00 Registration  
8:30 Opening Remarks  
Steve Safe, Director CERH  
8:45 **Joe Takahashi, Ph.D.**  
*Howard Hughes Medical Institute*  
*Northwestern University*  
*Title: Circadian Clocks and Gene Expression In Mammals*  
9:45 **Steve McKnight, Ph.D.**  
*UT Southwestern Medical Center*  
*Title: Reciprocal Coupling of Circadian Rhythm And Metabolism*  
10:45 Break  
11:00 **Vincent Cassone, Ph.D.**  
*Texas A&M University*  
*Title: Interactions of Metabolic and*  
*Transcriptional Oscillations in Vertebrate Circadian Clocks*  
12:00 Lunch  
1:15 **Chris Bradfield, Ph.D.**  
*University of Wisconsin*  
*Title: The Ah Receptor: Adaptive, Toxic and Developmental Pathways*  
2:15 Post-doctoral/Graduate Student Poster Presentations  
3:45 Poster Presentation Awards/Adjourn



Sponsored by The Center for Environmental and Rural Health and The Center for Biological Clocks

**Registration is free, but spaces are limited, so reserve your spot now at CERH.TAMU.EDU. If you have any questions, call 458-0562.**

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## CERH NEWS & NOTES

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Joanne Lupton has recently received a grant from NASA to examine “Gene Expression in Radiation-Enhanced Colon Cancer”. She was also appointed by HHS Secretary Tommy G. Thompson and Agriculture Secretary Ann M. Veneman to serve on the Dietary Guidelines Advisory Committee. This committee is responsible for utilizing the current scientific and medical information to review the Dietary Guidelines for Americans report, which is published every 5 years.



Farida Sohrabji, Assistant Professor in the Department of Human Anatomy and Neurobiology, received the Excellence in Research Award for Junior Faculty in the College of Medicine. Dr. Sohrabji’s research focus is Alzheimer’s Disease and the effects of estrogen on the aging process. The National Institutes of Health has continuously funded Dr. Sohrabji’s research since 1997, and she recently has received an additional grant from the Alzheimer’s Association. She has served at the national level on grant review committees, and some of her numerous publications have received more than 200 citations.



The Superfund Basic Research Program Project annual meeting was held at Dartmouth College, Hanover, New Hampshire, November 9-12, 2003, and several faculty, staff and students attended. Awards were given for biological and non-biological research presentations and these were both won by Texas A&M Superfund graduate students Mindy Wiles and Denise Hill. Congratulations to both students for their excellent presentations.



The School of Rural Public Health received a Notice of Grant Award for the Project “Issues in Human Health Risk Assessment: Novel Mechanistic Approaches in Human Health Risk Assessment”. The investigators on this grant include K.C. Donnelly (SRPH), R.H. Finnell (IBT), S.H. Safe (TAMU-CVM) and G.M. Shaw (California Birth Defects Monitoring Program). The total project award for three years is \$888,929 from the USEPA. The objective of the project is to improve exposure models for use in human health risk assessment. The study will measure concentrations of environmental chemicals in a refugee population living in contaminated areas of Azerbaijan.

**Be sure to check the weekly email announcements from the Director for current information on seminars, workshops and other events of interest to CERH Investigators. Send items to be included in upcoming newsletters to [parrish@medicine.tamu.edu](mailto:parrish@medicine.tamu.edu)**