nation and influx of glial cells into the corpus callosum begins at week 3 and peaks at week 5. However, a decrease in myelin and oligodendrocyte markers, myelin basic protein (MBP) and 2, 3-cyclic nucleotide 3-phosphodiesterase (CNPase), was evident at week one. Increased expression of CD11b and glial acidic fibrillary protein (GFAP), evidence of activated microglia and astrocytes, was also observed at week one. Coincident with these early changes, is an increase in cyclooxygenase (COX)-2 and lipoxygenase (LOX)-15 expression, suggesting that these arachidonic acid metabolism genes are either involved in or respond to the earliest sign of demyelination. Expression of LOX-5 was not significantly changed during the early stages of demyelination but it peaked during week 5, when glial markers and frank demyelination also reached their peak of expression, suggesting that LOX-5 expression is a consequence of the massive influx of inflammatory cells into the area of demyelination. While expression of LOX-12 was not consistently increased during demyelination, increased LOX-12 expression was observed during the remyelination, suggesting a role for this isoform in the recovery process. Our study is the first to demonstrate that multiple enzymes involved in arachidonic acid metabolism are altered in the cuprizone model of demyelination and remyelination. These data may help to develop new therapeutic targets to treat demyelinating diseases, such as multiple sclerosis.

## PS 2156 CORTICOSTERONE ATTENUATES HIPPOCAMPAL NEUROTOXICITY AND REACTIVE GLIOSIS THROUGH REGULATION OF THE BLOOD-BRAIN BARRIER IN C57BL/6J MICE TREATED WITH KAINIC ACID.

S. A. Benkovic, J. P. O'Callaghan and D. B. Miller. *TMBB, CDC-NIOSH, Morgantown, WV.*

High levels of stress or stress hormones have been reported to exacerbate human disorders of several physiological systems. As a model of stress, male mice were implanted with 0, 10, 35 or 100 mg/21d release corticosterone (CORT) pellets (0, 19, 67, and 190 mg/kg/d). After 7d, mice were injected with saline or 25 mg/kg kainic acid (KA), were scored for seizures (Racine scale), and were allowed to recover for 12 or 24h (histology), 7d (ELISA for GFAP), or 1, 3, 6, or 12h (western blot for IgG). Tissue was prepared for histological analysis of neurodegeneration by the cupric-silver stain; astrogliosis by ELISA and immunohistochemistry for GFAP; and microglial activation by Iba-1, CD11c, isolectin, and reactive silver staining. Treatment of mice with CORT caused no neuronal death, and attenuated damage caused by KA. GFAP levels were elevated seven-fold in KA-treated mice. Increasing doses of CORT caused greater decreases in basal GFAP, and CORT pre-treatment attenuated KA-induced protein elevation. GFAP staining revealed hypertrophic astrocytes with thick processes following KA treatment. Astrocytic hypertrophy was attenuated by CORT pre-treatment. Iba-1 and silver staining revealed a population of resting microglia in all brain regions. Basal Iba-1 staining was attenuated by high doses of CORT. Lectin and CD11c stained microglia were observed in regions that displayed KA-induced neurodegeneration and were rarely observed in control or CORT-treated animals. KA treatment caused a breach of the blood-brain barrier (BBB) and resulted in hippocampal levels of IgG that were increased by one hour. IgG levels were maximal at six hours post-treatment, and returned to baseline by 12 hours. CORT pre-treatment attenuated KA-induced BBB opening and IgG influx. Our data indicate CORT does not cause neuronal damage, and attenuates excitotoxic neurodegeneration and glial activation that has BBB disruption as a component of the pathological mechanism.

## PS 2157 CYANIDE-MEDIATED INCREASE IN $CA^{2+}$ AND NITRIC OXIDE IS ASSOCIATED WITH CYTOCHROME OXIDASE INHIBITION.

H. B. Leavesley, S. Mukhopadhyay, L. Zhang, L. Li, X. Zhang, J. L. Borowitz and G. E. Isom. *Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN.*

Cyanide is a potent neurotoxicant that inhibits mitochondrial (mt) respiration at the level of cytochrome c oxidase (CcOX). Acute cyanide exposure causes dopaminergic cell death in the *substantia nigra*, which may lead to a delayed-onset Parkinson-like syndrome. Our studies have shown that nitric oxide (NO), produced by nitric oxide synthase (NOS), plays a role in cyanide inhibition of CcOX and the subsequent death of dopaminergic cells. Cyanide (5 μM) increased generation of mt NO ($NO_{mt}$) in mesencephalic cell culture. The rise in $NO_{mt}$ could originate from cytosolic NOS or mt (mtNOS). In this study, we examined the mechanism by which cyanide increases $NO_{mt}$ in N27 rat dopaminergic cells. Since $Ca^{2+}$-bound calmodulin ($Ca^{2+}$-CAM) is an activator of NOS and cyanide increases cytosolic free $Ca^{2+}$ ($Ca^{2+}_{cyt}$), we compared cyanide-induced changes in $Ca^{2+}_{mt}$ to $NO_{mt}$. Cyanide-mediated changes in $Ca^{2+}_{cyt}$ and $Ca^{2+}_{mt}$ were monitored in whole cells using the fluorescent probe Fluo-4 AM. It was noted that changes in $Ca^{2+}_{mt}$ did not correlate with changes in $NO_{mt}$. Within the 1-10 μM KCN range, $Ca^{2+}_{mt}$ peaked at 8 μM KCN. In contrast, $NO_{mt}$ increased at 3 μM KCN, as determined by DAF-FM fluorescence. To further examine the mechanism of $NO_{mt}$ elevation, ruthenium red was employed to block $Ca^{2+}_{mt}$ uptake. Ruthenium red treatment enhanced $NO_{mt}$ by 50%, suggesting that elevated $Ca^{2+}_{mt}$ did not increase $NO_{mt}$. Examination of $Ca^{2+}_{cyt}$ showed a direct correlation between cyanide-mediated changes in $NO_{mt}$ and $Ca^{2+}_{cyt}$. Thus, it appears that cyanide increases $NO_{mt}$ primarily through activation of cytosolic NOS, not mtNOS. It is concluded that the cyanide-induced rise in $Ca^{2+}_{cyt}$ stimulates to the cytosolic production of NO, which then enters mt to interact with CcOX, thereby enhancing cyanide inhibition of oxidative phosphoryation. (Supported in part by NIH Grant ES04140.)

## PS 2158 INDUCIBLE EXPRESSION OF BNIP3 IN CLONAL MESENCEPHALIC CELLS LEADS TO NECROPTOTIC DEATH.

S. Mukhopadhyay, L. Li, L. Zhang, H. B. Leavesley, J. L. Borowitz and G. E. Isom. *Purdue University, West Lafayette, IN.*

BCL2/adenovirus E1B 19 kDa interacting protein 3(BNIP3) is a member of the BH3-only subfamily of pro-apoptotic Bcl-2 proteins and is associated with hypoxia-mediated cell death. To study involvement of BNIP3 in KCN-mediated cell death, a tetracycline (tet) inducible stable cell line was constructed. The cell line was prepared by stable transfection of the TetR plasmid in immortalized dopaminergic neuronal cells (N27), to obtain N27-TetR clones. The clones were stably transfected with expression plasmids containing BNIP3 gene downstream of cytomegalovirus promoter carrying tetO sequences. In the cells BNIP3 expression increased in a dose-dependent and time-dependent manner following tet treatment. Both Propidium Iodide (PI) and trypan blue staining showed that minimal necrotic death occurred within 24 hrs of Tet-on (0.5μg/ml). However cell viability decreased within 24hrs of tet (0.5μg/ml) treatment as detected by the MTT assay. Upon Hoescht 33258 staining, apoptotic death was observed based on nuclear morphological changes. Nuclear fragmentation, typical of apoptosis, was not observed but chromatin condensation was arrested at stage I in which nuclei exhibit a wrinkled pattern of peripheral chromatin condensation. DNA laddering was detected. At higher concentrations of tet(>0.5μg/ml) the DNA fragmentation was reduced and cell death shifted to necrosis as indicated by a smear on 1.5% agarose gel. Prolonged tet treatment for >48 hrs or treatment with cyanide (400μM) shifted the mode of cell death to necrosis as confirmed by trypan blue and PI staining. Cell viability decreased following KCN treatment (400μM) as measured by trypan blue cell counting. Neither the apoptosis nor necrotic death was associated with caspase 3 activity. It was concluded that following tet induced expression of BNIP3, a continuum of caspase-independent cell death from apoptosis to necroptosis to necrosis could be produced depending on the dose and duration of exposure to tetracycline and cyanide. (Supported by NIH grant ES04140)

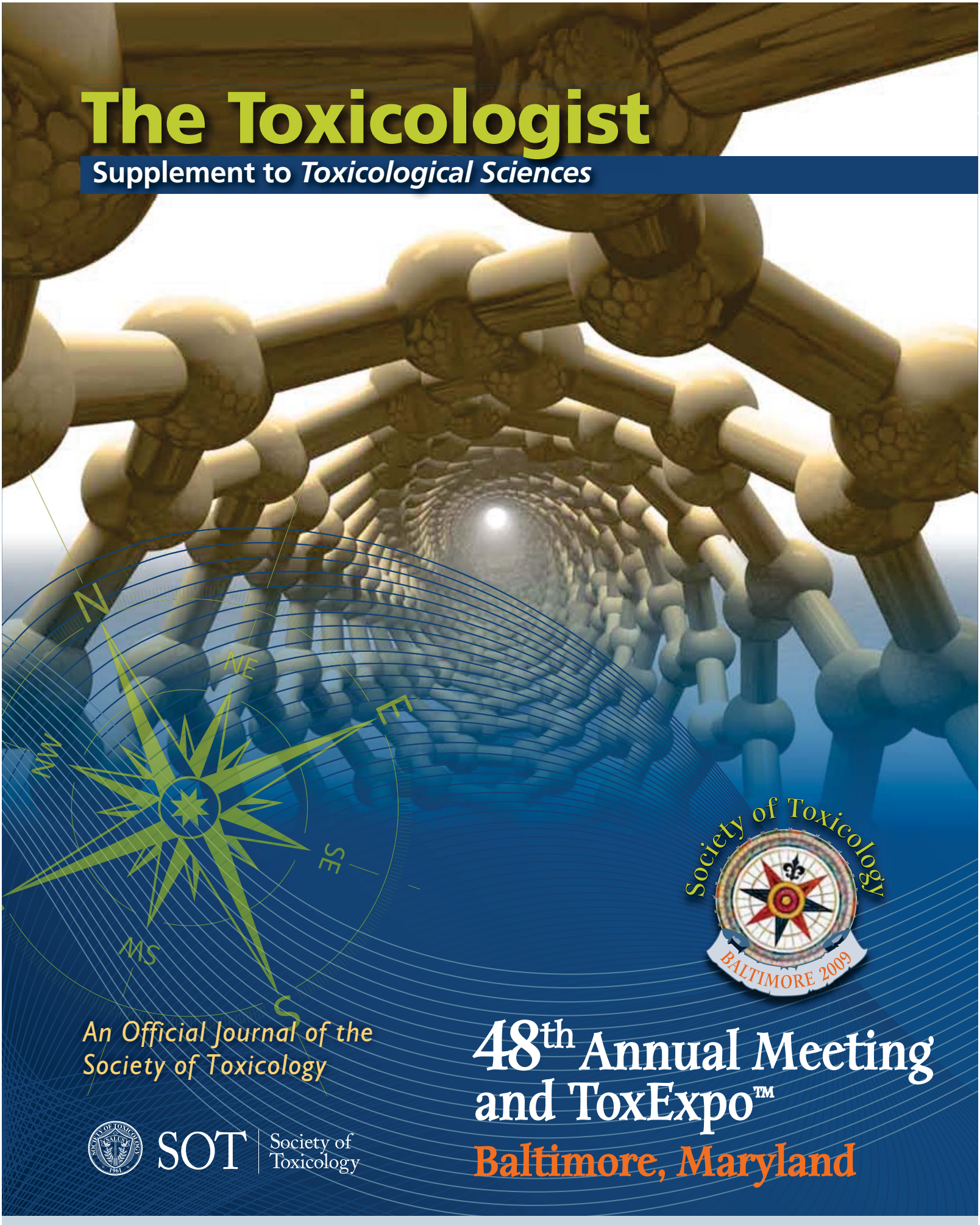
## PS 2159 EARLY LETHAL EPILEPSY IN MICE WITH COMBINED GLUTAMATE-CYSTEINE LIGASE MODIFIER SUBUNIT AND L-GULONOLACTONE OXIDASE DEFICIENCY.

Y. Chen[1], E. Johansson[1], C. P. Curran[1], X. Liu[2], K. D. Holland-Bouley[2], S. C. Danzer[2], H. G. Shertzer[1], D. W. Nebert[1] and T. P. Dalton[1]. *[1]Department of Environmental Health, University of Cincinnati, Cincinnati, OH and [2]Department of Neurology, Cincinnati Children's Hospital, Cincinnati, OH.*

Reduced glutathione (**GSH**) and ascorbic acid (**AA**) are two major antioxidants in maintaining redox homeostasis and protecting against oxidative damage to the central nervous system (**CNS**). GSH and AA can interact as a redox couple, such that depletion of either one of them can be compensated for by the continued presence or compensatory rise of the other. In addition, both antioxidants have been suggested to act as neuromodulators in the glutaminergic system. We have generated the *Gclm(-/-)* knockout mouse line by disrupting the gene encoding the modifier subunit of the rate-limiting enzyme in GSH biosynthesis. *Gclm(-/-)* mice exhibit 15-40% of normal tissue GSH levels with a compensatory increase in ascorbate and reveal no overt phenotype. The *Gulo(-/-)* mouse, having ablation of the *L*-gulono-γ-lactone oxidase gene, cannot synthesize AA and, like humans, depends on dietary ascorbate for survival. In the current study, we generated *Gclm(-/-)Gulo(-/-)* double-knockout mice to test the hypothesis that deficiency in both GSH and AA will render these mice highly susceptible to endogenous oxidative damage. Unexpectedly, we observed that the concomitant loss of both *Gclm* and *Gulo* genes in mice causes growth retardation and lethal spontaneous epilepsy between the 2nd and the 3rd

# The Toxicologist

Supplement to *Toxicological Sciences*




*An Official Journal of the Society of Toxicology*

SOT | Society of Toxicology

## 48th Annual Meeting and ToxExpo™

## Baltimore, Maryland

www.toxsci.oxfordjournals.org

OXFORD UNIVERSITY PRESS

ISSN 1096-6080
Volume 108, Number 1, March 2009


Photos courtesy of Salt Lake Convention and Visitors Bureau. Photographed by Richard Cheski, Steve Greenwood, and Eric Schramm.

# Deadline for Proposals for SOT 2010 Annual Meeting Sessions: April 30, 2009

## WHY SUBMIT A PROPOSAL?

1. To present new developments in toxicology.
2. To provide attendees an opportunity to learn about state-of-the-art technology and how it applies to toxicological research.
3. To provide attendees an opportunity to learn about the emerging fields and how they apply to toxicology.

## SESSION TYPES

**Continuing Education**—Emphasis on quality presentations of generally accepted, state-of-the-art knowledge in toxicology

*Note: CE Courses will be held on Sunday.*

**Symposia**—"Cutting-edge" science; new areas, concepts, or data

**Workshops**—State-of-the-art knowledge in toxicology

**Roundtables**—Controversial subjects

**Historical Highlights**—Review of a historical body of science that has impacted toxicology

**Informational Sessions**—Scientific planning or membership development

**Education-Career Development Sessions**—Sessions that provide the tools and resources to toxicologists that will enhance their professional and scientific development

## 2010 Thematic Approach

The Scientific Program Committee will continue the thematic approach for the 2010 Annual Meeting. All proposal submissions will be reviewed for their relevance under the following themes—***Cell Signaling, Gene-Environment Interactions, Metabolic Disease, Mitochondrial Basis of Disease, Toxicity Testing in the 21st Century,*** and ***Translational Toxicology*** for the 2010 meeting. Please note that while we are actively soliciting proposals for the themes listed above, all proposal submissions will be reviewed under the current criteria for their timeliness and relevance to the field of toxicology.

Please refer to the SOT 2009 *Program,* Scientific Program Overview on the fold-out cover for a list of 2009 sessions highlighted under the thematic approach.

**You can now submit your proposal on-line at www.toxicology.org**

# Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the continuing education, symposia, workshop, roundtable, platform, and poster discussion sessions of the 48th Annual Meeting of the Society of Toxicology, held at the Baltimore Convention Center, March 15–19, 2009.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 469.

The issue also contains a Key Word Index (by subject or chemical) of all the presentations, beginning on page 487.

The abstracts are reproduced as accepted by the Scientific Program Committee of the Society of Toxicology and appear in numerical sequence.

Copies of *The Toxicologist* are available at $45 each plus $5 postage and handling (U.S. funds) from:

Society of Toxicology
1821 Michael Faraday Drive, Suite 300
Reston, VA 20190

www.toxicology.org