3035 Neuroinflammation and Gulf War Illness: The Role of Microglia in the Response to In-Theater Toxicant Exposure

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Gulf War Illness (GWI) is a multi-symptom disorder with similarities to the features of sickness behavior. Previously, we have demonstrated that, like sickness behavior, GWI is associated with underlying neuroinflammation. In particular, toxic exposures experienced by soldiers during the Gulf War such as pesticides and nerve agents, as well as physiological stress, have led to a chronic, primed neuroinflammatory state that results in an exacerbated response to subsequent inflammatory challenges. The significant elaboration of inflammatory cytokines related to the neuroinflammatory priming observed in our GWI mouse model indicates that this illness may be the result of long-term alterations in the brain's resident immune cells, namely microglia. Here, we have investigated the potential role of microglia in GWI using the CX3CR1 -/- mouse strain and minocycline, an anti-inflammatory drug with effects on microglia. Adult male C57BL/6J or CX3CR1 -/- mice were exposed to our GWI model consisting of corticosterone (CORT) in the drinking water at levels associated with high physiological stress for 7 days followed by exposure to the nerve agent surrogate, diisopropyl fluorophosphate (DFP), on day 8 and a subsequent immune challenge with lipopolysaccharide (LPS) on day 10. C57BL/6J mice that were given minocycline received a single dose 30 minutes prior to LPS. To test whether minocycline or CX3CR1 -/- disrupted LPS-induced inflammation, an additional cohort of animals were exposed to LPS exposure on day 8 in place of DFP. Interestingly, neither CX3CR1 -/- nor minocycline-treated mice exhibited major differences in cytokine mRNA expression following CORT+LPS exposure compared to controls. However, both CX3CR1 -/- and minocycline treatment removed the contribution of DFP to the GWI phenotype, reducing cytokine mRNA expression to levels comparable to CORT+LPS treatment. The recovery of the GWI phenotype to CORT+LPS levels is clinically significant, because this condition mimics a "healthy sick" state in which stress may potentiate inflammatory conditions. These results suggest that microglia play a crucial role in the development/maintenance of GWI particularly in response to DFP and that drugs with modulatory effects on microglia show promise for the treatment of veterans suffering with GWI.

3036 Mitochondrial Bioenergetics following Ozone Exposure in Sedentary Versus Active Lifestyle of Female Long-Evans Rats

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Mitochondrial bioenergetics play a key role in the mechanisms of neurodegenerative disorders and chemical induced neurotoxicity. However, mitochondrial bioenergetic parameters have not been systematically evaluated within multiple brain regions in sedentary versus active lifestyle following environmental pollutant exposures. In the present study, we measured complex I, complex II and complex IV enzymes in the frontal cortex (FC), cerebellum (CER), hypothalamus (HYP), and hippocampus (HIP) in female Long-Evans rats (N = 5 per group) that were sedentary or active with continuous access to running wheels starting at postnatal day (PND) 22 until the age of PND 100 and subjected to Ozone (O_3) exposure (0, 0.25, 0.5 or 1.0 ppm O_3 for 5 h/day for two consecutive days). Immediately following O_3 exposure, rats were sacrificed, brain regions were dissected on ice, quick frozen on dry-ice, and stored at -80°C until analysis. Complex 1 enzyme activity (NADH dehydrogenase, EC 1.6.5.3) in CER and FC was not significantly altered by O_3 exposure; however, there was an increase of activity in active animals following 0.5 ppm O_3 ($p<0.05$) exposure. A similar effect was observed in HIP, but it was not significant ($p=0.0561$). Complex II enzyme activity (succinate dehydrogenase, EC 1.3.5.1) was increased in the FC of active animals but was not significant ($p=0.05627$). The HIP showed a significant reduction in Complex II activity at 0.25 ppm ($p=0.0041$) in active versus sedentary animals. Complex IV enzyme activity (cytochrome c oxidase, EC 1.9.3.1) was reduced at 1 ppm in the FC in the running wheel group but was not significant ($p=0.062$). These results suggest that brain mitochondrial bioenergetics were altered in actively trained rats and O_3 exposure had effects only on active rats but not sedentary suggesting an interaction of lifestyle and O_3 effects on brain mitochondrial bioenergetics parameters. *This abstract does not necessarily reflect US EPA policy.*

3037 Relevance of Gender in Tobacco Smoking-Dependent Dysregulation of the Iron Exporter Slc40a1

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Cerebral iron homeostasis is crucial to maintain optimal CNS functionality. Iron is a vital cofactor involved in the process of energy production, thus alteration of its transmembrane efflux can impact cell and mitochondrial viability. Specifically, the excess of iron resulting from unwarranted intracellular accumulation can reacts with oxygen to form cytotoxic free radicals leading to cellular damage. Previously, the functional role of Slc40a1 (solute carrier family member 1 or ferroportin 1), a transmembrane iron exporter, has been well characterized in enterocytes, hepatocytes and reticuloendothelial system. Activity of this transporter is linked to Nrf2-ARE pathway to regulate cellular ion metabolism and redox balance. Our previous findings demonstrated that pharmacological activation of Nrf2 by Sulforaphane produced a dose-dependent upregulation of the mitochondrial Slc40a1 (both gene and protein) in various *in vitro* models of mouse and human blood-brain barrier endothelium. We have also shown that, tobacco smoking progressively downregulate Nrf2 in total brain homogenates following 4 weeks of exposure in mice. Based on these premises we tested the hypothesis, chronic tobacco smoke exposure to mice for 4 weeks can impact Slc40a1 mediated iron exporter function at the neurovascular unit. Both male and female mice were divided into two groups, chronically exposed (via direct inhalation of side-stream smoke) either to tobacco smoke mixed with oxygenated air or oxygenated air alone following modified International Organization for Standardization/ Federal Trade Commission (ISO/FTC) standard smoking protocol (4 sec puff duration, 56 sec delay, 35 ml puff volume, 1 puff/min). Following 4 weeks of exposure (2 cigarettes/hr, 6 times/day -7 days/week) mice were sacrificed and brain were collected. Western blot analysis of whole brain homogenate interestingly reveals the unaltered levels of Slc40a1 in male mouse while significant downregulation in female mice. Although needs further validation by downstream relevant assays, this gender difference in Slc40a1 expression and possibly iron shuttling is indeed a novel finding suggesting that tobacco smoke could differentially impact the cerebrovascular system (and possibly CNS functionality) based on gender.

3038 Is BMAA an Agent of ALS? A Preliminary Study in Zebrafish

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Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurodegenerative disease with a prevalence of about 3.9 out of 100,000 people. Individuals with ALS are usually diagnosed between the ages of 40 and 70, and have an average life expectancy of three years post-diagnosis. The disease is characterized by motor neuron degeneration, leading to muscle atrophy, paralysis, organ systems failure, and ultimately death. Ten percent of ALS cases are familial, with the remaining 90% classified as sporadic. While many gene mutations have been associated with ALS, it is likely that a combination of genetic and environmental factors contributes to the disease. β-methylamino-L-alanine (BMAA) is a non-protein amino acid produced by cyanobacteria, which has been implicated in several neurodegenerative diseases, including ALS. It has been postulated that exposure to BMAA could lead to formation of protein aggregates, oxidative stress, or sequestration of glutamate receptors, mechanisms that have been implicated in the etiology of ALS. However, no causal relationship between BMAA exposure and the onset of ALS has been proven. We are leveraging a transgenic zebrafish line carrying the *sod1* G93R mutation to explore the possible connection between chronic and acute exposure to BMAA and ALS-like phenotypes. Wild type (WT) and *sod1* mutant embryos were exposed to BMAA from six hours post-fertilization to seven days post fertilization (dpf). BMAA exposure in WT fish was associated with reduced density of neuromuscular junctions (NMJ), as determined by immunohistochemistry, and hyperactive-like behavior. By contrast BMAA did not elicit a significant behavioral change in the *sod1* mutants, leading us to speculate that increases in superoxide radicals in *sod1* mutants might ameliorate BMAA neurotoxic properties. Overall, our preliminary results suggest that, although some effects of neurotoxin exposure were seen, these short-term developmental exposures are not sufficient to make definitive conclusions about any possible role of BMAA in the etiology of ALS. Long-term exposures are underway to more closely assess the onset and severity of ALS-like symptoms in zebrafish exposed to BMAA and to examine motoneuron viability and function over time, and how such potential compromises might reveal mechanisms involved in neuromuscular degeneration in ALS when analyzed by behavioral, histological and proteomic assays.

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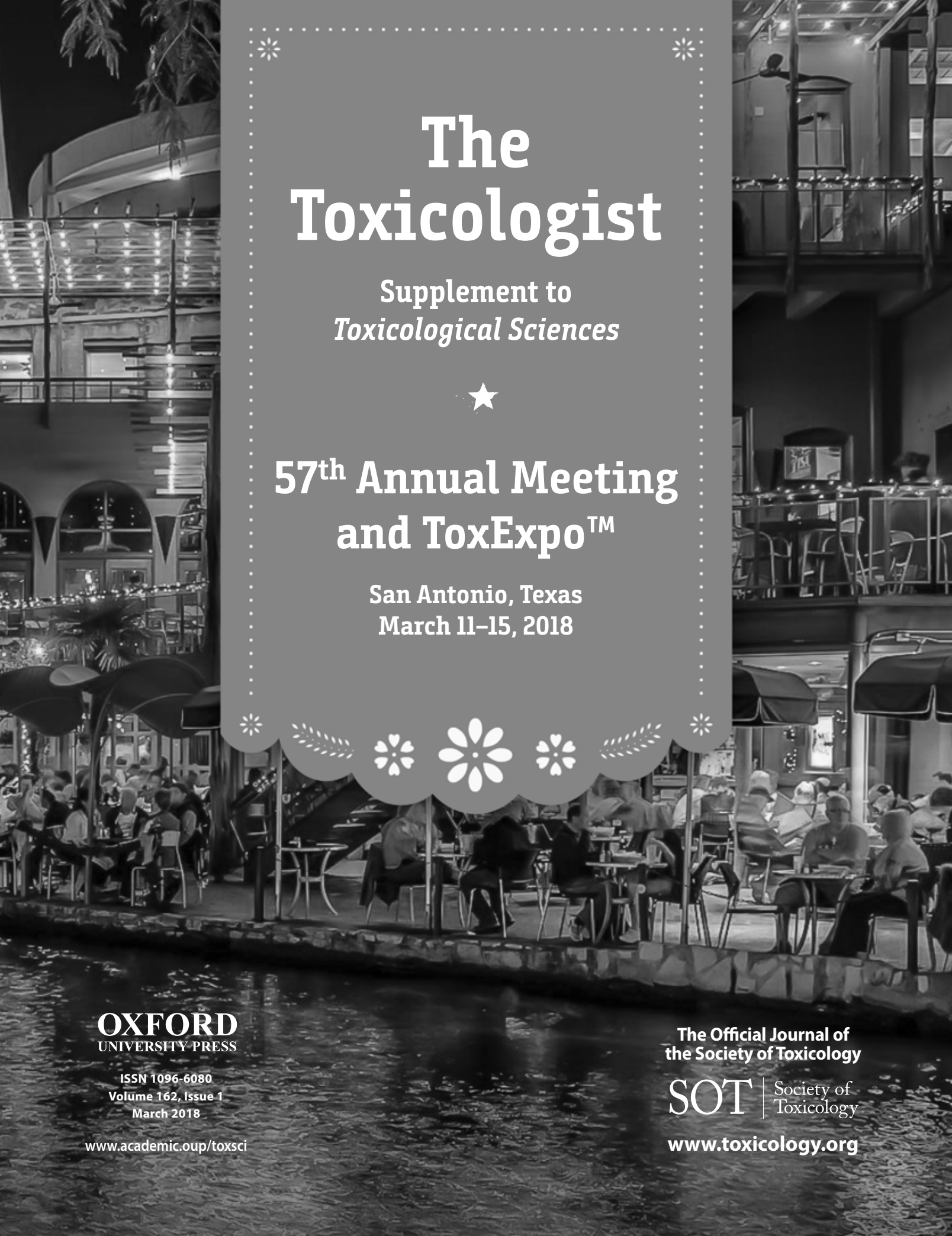

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