2659 Paraquat Inhalation, a Translationally Relevant Route of Exposure, Produces Male-Specific Deficits in Locomotor Behavior, Decreased Midbrain Dopamine, and Alterations to Striatal Glutamine and Serotonin

T. Anderson, A. K. Merrill, M. L. Eckard, E. Marvin, K. Conrad, G. Oberdörster, M. Sobolewski, and D. A. Cory-Slechta. *University of Rochester, Rochester, NY.*

Numerous epidemiological studies have reported associations between the broad spectrum herbicide paraquat (PQ) and Parkinson's disease (PD), with findings supported by injection and feeding studies. Despite evidence that inhalation exposure to airborne pesticides, such as PQ, is an occupational and public health concern, the ability of inhaled PQ to reproduce features of PD has not been investigated. The present study was designed to determine if inhalation exposure to PQ would lead to its disposition to the brain, dopaminergic dysregulation, and neurobehavioral outcomes consistent with the trajectory of PD. Adult male and female C57BL/6J mice were exposed to PQ aerosols (130 μg/m^3) in a whole-body inhalation chamber for 4hrs/day, 5 days/week for 4 weeks. Subsets of males were sacrificed during and after exposure and PQ concentrations in various brain regions (olfactory bulb, striatum, midbrain, and cerebellum) were quantified via mass spectrometry. Alterations in motor behavior were examined using spontaneous locomotor activity, rota-rod, and grip strength. Following the conclusion of behavioral assessment 275 days after the end of exposure, mice were sacrificed and neurotransmitters were measured by mass spectrometry. PQ inhalation resulted in significant concentrations in all examined brain regions, with the highest burden observed in the olfactory bulb (5.34 ± 0.72 ng/g tissue), consistent with nasal olfactory translocation. PQ inhalation produced male-specific deficits in locomotor activity and grip strength, but no significant effect on motor coordination on the rota-rod apparatus. Critically, PQ inhalation exposure led to a significant male-specific reduction in midbrain dopamine (25%), even 275 days post-exposure. Further, in the striatum, PQ significantly reduced the dopamine metabolite, homovanillic acid (33%), glutamine (21%), serotonin (33%), and its metabolite 5-HIAA (40%), relative to filtered-air controls. These data highlight the importance of inhalation as route of exposure for neurotoxic pesticides in the airborne state and lend biological plausibility to a causal relation between PQ and PD. *Supported by ES025541, ES00247, ES007026.*

2660 *Aspergillus versicolor* Inhalation Dysregulates Neuroimmune Homeostasis and Augments Alzheimer's Disease-Like Neuropathology

A. L. Dunbar[1], T. B. Ladd[1], J. A. Johnson Jr.[1], C. L. Mumaw[1], H. J. Greve[1], X. Xuei[1], E. Simpson[1], M. A. Barnes[2], B. J. Green[2], T. L. Croston[2], D. H. Beezhold[2], and M. L. Block[1]. *[1]Indiana University School of Medicine, Indianapolis, IN; and [2]NIOSH, Morgantown, WV.*

Increasing evidence associates indoor fungal exposure with deleterious central nervous system (CNS) health, such as cognitive and emotional deficits in children and adults, but the potential impact on CNS disease, particularly Alzheimer's disease (AD), is poorly understood. To characterize how *Aspergillus versicolo*r, a common opportunistic filamentous fungi species associated with damp environments modifies the CNS transcriptional phenotype, 8-week-old female B6C3F1/N mice were exposed to filtered air, heat-inactivated A. versicolor (3 x 10^5 spores), or viable *A. versicolor* (3 x 10^5 spores) via nose-only inhalation twice a week for 4 weeks. Bulk RNA-seq analysis of the midbrain, the brain region determined to have the largest TNFα neuroinflammation response by RT-qPCR, revealed that 4 weeks of viable *A. versicolor* exposure resulted in significant transcriptional enrichment of neuroinflammation, glial cell activation, postsynaptic, and neurotransmission pathways. To discern the effect of *A. versicolor* on neurodegenerative disease processes, 8-week-old male 5xFAD mice were exposed to either filtered air or live *A. versicolor* (3X10^5 spores) twice a week for 13 weeks. Immunohistochemical analysis of AD-like neuropathology in the cortex demonstrated an increase in the number of Thioflavin S+ plaques in the cortex with *A. versicolor* exposure, supporting that inhalation of viable filamentous fungi can augment amyloid plaque pathology in the 5xFAD AD mouse model. Analysis of the circulating factors revealed that serum IL-5 and CXCL10 were elevated in both 5xFAD and control mice, both serum IL-12 and IL-10 decreased in only 5xFAD mice, and HMGB1 was elevated in only 5xFAD mice in response to *A. versicolor* exposure. Together, these findings indicate *A. versicolor* inhalation can dysregulate neuroimmune homeostasis, uniquely modify circulating factors in 5xFAD mice, and augment ongoing AD-like neuropathology, providing much needed insight into how inhaled fungal exposures may affect CNS disease.

2661 Paraquat Primes the Microglial NLRP3 Inflammasome via the Voltage-Gated Proton Channel Hv1

C. Kim[1], M. Neal[1], A. Boyle[2], L. Wu[3], and J. R. Richardson[1]. *[1]Florida International University, Miami, FL; [2]Kent State University, Kent, OH; and [3]Mayo Clinic, Rochester, MN.*

Paraquat (PQ) is a widely used herbicide and can increase the risk of developing Parkinson's disease (PD) by ~2.5 times. PQ treatment of mice induces selective nigrostriatal degeneration, aggregation of α-synuclein, and increased neuroinflammation. Recently, NOD-like receptor protein 3 (NLRP3) inflammasome was increased in the brain of PD patients, indicating a potential role in PD. Hvcn1/Hv1 is a voltage-gated proton channel selectively expressed in microglia and other immune cells. It has been shown to be required for NADPH-oxidase (NOX)-dependent production of reactive oxygen species (ROS) under pathological conditions. The purpose of this study was to determine the potential for PQ to prime/activate the NLRP3 inflammasome and the potential for Hv1 to regulate this process. Direct PQ treatment induced *Hvcn1* mRNA levels 2-3 fold in primary microglia (PMG) and mRNA expression of *Nlrp3* in C57 PMG. PMG isolated from global Hv1 knockout (Hv1 KO) mice displayed significantly reduced production of ROS and mRNA levels of *Nlrp3* and *Il1b* following PQ treatment. PQ treatment of wild-type (WT) PMG increased expression levels of the NLRP3 inflammasome-related proteins including NLRP3, ASC, cleaved caspase-1, and cleaved IL-1β, which were abolished in PMG from Hv1 knockout mice (KO). The ability of PQ to prime the NLRP3 inflammasome was confirmed by increased protein levels of NLRP3, ASC, cleaved caspase-1, and cleaved IL-1β following a second PQ challenge or lipopolysaccharide priming. These effects were attenuated or abolished in Hv1 KO PMG. Similar effects were observed for Il1β measurements in conditioned media. Following a single injection of 10 mg/Kg PQ to C57BL/6J mice, mRNA levels of *Hvcn1* and IL-1β were increased by 6-fold and 2-fold in the striatum, respectively. As an indicator of NLRP3 activation, ASC protein levels were increased by 6-fold in the striatum and 5-fold in substantia nigra. These effects were abolished in Hv1 KO mouse brain. Collectively, these data demonstrate that direct PQ treatment can both prime and activate the microglial NLRP3 inflammasome and that voltage-gated proton channel Hv1 plays a key role in this process. *Supported in part by R01ES021800 and The Michael J Fox Foundation.*

2662 Brain TSPO Levels Are Associated with Sex-Dependent Cognitive Function Deficits in the 5XFAD Animal Model of Alzheimer's Disease

J. L. McGlothan Dziedzic, D. Albores-Garcia, D. R. Brooks, and T. R. Guilarte. *Florida International University Robert Stempel College of Public Health & Social Work, Miami, FL.*

Alzheimer's disease (AD) is an irreversible neurodegenerative disease with memory loss and dementia. Neuroinflammation is thought to play an important role in AD pathogenesis and appears to be an early event in the progression of the disease. Here, we used the 5XFAD transgenic mouse model which expresses five of the major human familial mutations associated with AD. These mutations cause this mouse model to develop AD pathogenesis rapidly, with advanced disease observed by 7-10 months of age. We assessed cognitive function using the Barnes Maze in male and female wildtype (WT) and 5XFAD mice at 3 months (3M) and 7 months (7M) of age. We found no significant differences in performance at 3M between WT and 5XFAD male ($F_{1,15}$=0.583, p=0.457) and female ($F_{1,15}$=0.438, p=0.518) mice. At 7M, we observed no significant differences in learning performance between WT and 5XFAD male mice ($F_{1,15}$=1.646, p=0.219). However, there was a highly significant difference in the performance of female mice by genotype ($F_{1,15}$=18.170, p=0.001). That is, we observed a marked impairment in the performance of 5XFAD female mice in the Barnes maze relative to WT. Translocator Protein 18 kDa (TSPO) is a validated biomarker of neuroinflammation that has been used in preclinical animal models of human neurodegenerative disease and in a variety of neurodegenerative conditions including AD. To assess the level of neuroinflammation in the brain of 7M WT and 5XFAD mice, we performed quantitative autoradiography using the TSPO-specific radioligand [^{3}H]-DPA-713. Brain regions analyzed included the cerebral cortex, striatum, septal nuclei, amygdala, hippocampus, thalamus, and hypothalamus. Using an unpaired t-test adjusted for multiple comparisons (p=0.0065), regional analysis of WT and 5XFAD male and female brains at 7M indicated no significant changes in the brain of 5XFAD male mice relative to WT. However, significant increases in TSPO levels were found in the cerebral cortex (p=0.001) and thalamus (p=0.001) of female 7M 5XFAD mice relative to WT. The increase in TSPO levels in the 7M 5XFAD female brain relative to WT was inversely associated with the marked deficits in performance of the 7M 5XFAD female mice

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