4569 Expression of Copper Chaperone for Superoxide Dismutase (CCS) in the Blood-CSF Barrier and Impact of *In Vivo* Lead Exposure in Mice

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Copper chaperone for superoxide dismutase (CCS), also named superoxide dismutase 4 (-SOD4), is a copper (Cu) chaperone protein with a primary function to deliver Cu to SOD1 for cellular defense against oxidative damage. CCS also participates in regulation of cellular Cu levels by its influence on the expression of Cu binding proteins, e.g., MT-I, MT-II, ATOX1, COX17, and ATP7A. CCS has been found to be expressed in high amounts in the choroid plexus, a brain tissue that constitutes a barrier between the blood and cerebrospinal fluid (CSF) in brain ventricles and is known to play a vital role in maintaining Cu homeostasis in the central milieu. Previous data from this lab has established that exposure to toxic metal lead (Pb) causes substantial accumulation of Pb in the choroid plexus. This study was designed to test the hypothesis that Pb accumulation in choroid plexus following chronic exposure interfered with CCS expression in two major cell types of choroid plexus (i.e., choroidal epithelia and endothelia), which may contribute to altered Cu brain homeostasis. CD-1 mice received oral gavage at the dose of 13.5 mg Pb/kg body weight as Pb acetate (saline for control mice), once daily for 28 days. Atomic absorption spectroscopy (AAS) analysis revealed that blood lead levels (BLLs) were 0.1 ± 0.2 (SD) µg/dL and 35.0 ± 8.0 µg/dL in control and Pb-exposed animals, respectively (n=4, p=0.01). Immunohistochemistry was performed with rabbit anti-SOD4 antibody and, rat anti-CD31 antibody, followed by fluorophore-conjugated secondary antibody to visualize the impact of Pb exposure on CCS expression in brain tissues by confocal microscopic imaging analysis. The data showed that Pb groups had a significantly increased CCS expression in the choroidal epithelial cells by 92.9% ($p<0.01$), but with no changes in the choroidal endothelial cells, in comparison to controls. Interestingly, CCS was found to express abundantly in the subventricular zone (SVZ) at levels equivalent to that in the choroid plexus. Pb exposure appeared to increase CCS expression in SVZ by 20.2%, although this increase did not meet statistical significance ($p<0.05$). These results prove the presence of CCS in both choroidal epithelial and endothelial cells in the blood-CSF barrier in the choroid plexus. Exposure to environmental Pb causes an increased expression of CCS specifically in the epithelial cells that are the structural basis of the blood-CSF barrier, suggesting an activated defense against Pb toxicity and possible interference with Cu regulation by the blood-CSF barrier. Further research to explore the mechanisms whereby Pb altered the CCS with resulting Cu dyshomeostasis in the CSF is in progress. *Supported by NIH/NIEHS R01ES027078.*

4570 Variable Activity of Lead (Pb) on Inflammatory Cytokine Gene Expression in SIM-A9 Murine Microglia (MC) Stimulated with Endotoxin

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Despite regulatory restrictions, Pb continues to pose a risk to human health, particularly the nervous system (NS). Pb has been shown to induce oxidative stress, impair neurotransmitter signaling, and cause the death of the different neuronal populations. Given its environmental ubiquity, Pb poisoning accounted for 50% of the deaths due to exposure to chemicals in 2019 and 30% of idiopathic cognitive dysfunction, according to the WHO. While MC play a protective role in the CNS, they have also attracted significant attention as effectors of neuroinflammation and neurodegeneration (ND). Yet few studies have addressed the effects of Pb on MC. The present study used murine SIM-A9 MC to determine the effects of Pb acetate (1 µM - 1 mM) on MC viability at 24 hours. At these concentrations, Pb did not affect viability using the MTT assay, nor did these concentrations alter NO production, as indicated by nitrite analysis of cell supernatant using the Griess method. However, while 100 µM Pb alone did not induce changes in gene expression, determined by RT-qPCR, for IL-1β and IL-6, it enhanced LPS (100 ng/mL)-induced MC activation, as evidenced by the significantly elevated expression of these markers ($p<0.001$). Interestingly, for COX-2, TNF-α and iNOS gene expression was negligible in the presence of Pb (100 µM) alone, but that concentration significantly ($p<0.05$) reduced gene expression in the presence of LPS (100 ng/mL) compared to MC treated with LPS (100 ng/mL) only. These latter results are at odds with reports of enhanced expression in murine MC primary cultures. However, those studies relied on immunohistochemistry and not qPCR. These preliminary studies indicate that Pb may exacerbate activity of primed MC in the presence of ongoing injury, given that Pb is known to directly induced neuronal damage, and warrants further investigation, particularly as to its inflammatory role.

4571 Aberration of Corticothalamic Brain Regions in Rats Exposed to Welding Fumes

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The potential for developing Parkinson's disease (PD)-like neurological dysfunction following occupational exposure to welding fumes (WF) is an emerging concern. Manganese (Mn) in welding consumables is suspected to cause the neurological deficits seen in welders. Indeed, we have shown that Mn-containing WF causes dopaminergic neurotoxicity in rats by provoking neuroinflammation, and reducing PD-related (PARK) proteins in the striatum and midbrain, areas typically affected in PD. Recent studies show that chronic exposure to low doses of Mn causes fine motor and cognitive impairment. Functional magnetic resonance imaging studies of welders reveals Mn accumulation and altered metabolites in cortical and thalamic regions, which correlated with reduced performance in fine motor, working memory, and executive function tasks. Such subclinical motor and non-motor dysfunction often precede clinical motor symptoms linked to dopaminergic neurodegeneration. These findings suggest that the neurological underpinnings in PD, manganism, and WF-mediated PD-like manifestation encompasses much more than degeneration of the nigrostriatal dopaminergic pathway and involves brain areas associated with sensorimotor, fine motor, and cognitive tasks, such as the cortex, thalamus, and cerebellum. Here, we examined the effects of WF on the frontal cortex (FCT, including pre-frontal cortex), parietal cortex (PCT, including motor cortex), and thalamus (THL, including subthalamic nucleus) of rats to determine if it instigates neurochemical and synaptic changes that are predictive of sensorimotor and cognitive impairment. Sprague-Dawley rats (male; 3 months old) were exposed by whole-body inhalation to fumes (4 - 6 mg/m^3; 3 hours/day × 4 days/week × 5 weeks; for a total of 20 days) generated by gas metal arc-stainless steel (GMA-SS / WF), humanely euthanized at 1, 7, 28 or 112 days post-exposure, and brain areas collected. WF upregulated (1.4 to 1.7-fold) the mRNA transcripts for interferon-gamma (*Ifng*), inducible nitric oxide synthase *(Nos2*), matrix metalloproteinase 9 (*Mmp9*), dopamine D2 receptor (*Drd2*), and the solute carrier family 18 member A2 (*Slc18a2 / Vmat2*) in FCT after 1 d, suggestive of neuroinflammation and altered monoamine neurotransmitter signaling. Small decrements (10 - 20 %) in norepinephrine (NE) and serotonin (5-HT) were detected in the FCT at 7, 28, and 112 days. Reduced (22 - 30 %) NE, 5-HT, and dopamine (DA) was also seen in THL at 28 days, and the levels remained persistently lower at 112 days. Synaptophysin 1 protein increased (43 %), while ubiquitin C-terminal hydrolase L1 (UCHL1 / PARK5) protein levels decreased (44 %) in the FCT at 28 days. The FCT, primarily the prefrontal cortex, is known to coordinate and regulate cognitive tasks, including working memory, decision-making, attention, and learning. NE, DA, and 5-HT principally modulate this brain region, and dysregulation or imbalance in their levels affect neuronal circuits involved in cognitive processing. In conjunction, increased DRD2 expression is linked to poor cognitive performance. Together, our findings support the notion that a complex interplay of overlapping neural circuits, primarily involving nigrostriatal, cortical, thalamic, and cerebellar tracts are critical for eliciting key motor and non-motor symptoms in PD, and perhaps manganism, as well as welding-mediated PD-like manifestation. As dysregulation of corticothalamic region, linked to subclinical and non-motor symptoms, often precedes clinical motor signs, it may provide early insight into the neurodegenerative process. More research is necessary to identify biomarker signatures linked to sensorimotor and cognitive impairment that can aid early detection, intervention, and prevention of motor dysfunction associated with welding and Mn exposure.

4572 Cadmium Exposure Alters the Gut Microbiome and Microbial Metabolites before the Onset of Learning and Memory Deficits in Mice

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Cadmium (Cd) is a heavy metal that has been recognized as one of the most toxic environmental pollutants. Increasing evidence suggests that Cd is a neurotoxicant, however, the underlying mechanisms remain poorly understood. The gut-brain axis is a communication pathway between the gut microbiome and the central nervous system. Studies in the literature have found that alterations of the gut microbiome are implicated in various neurological diseases and manipulating the gut microbiome can affect neurological functions in animals. Because we previously showed that the gut microbiome is a target of Cd toxicity, we hypothesize that the gut-brain axis may mechanistically contribute to Cd-induced impairment of learning and memory. As a first attempt to test our hypothesis, we tested if Cd induces gut dysbiosis preceding the onset of cognitive deficits, and how distinct neuroactive microbial metabolites mechanistically contribute to Cd-mediated cytotoxicity. We exposed 8-week-old C57BL/6 male mice to 3 mg/L Cd through drinking water for 9 weeks. The Novel Object Location test, which probes for hippocampus-dependent spatial memory deficits, was performed weekly during the exposure period to detect the onset of cognitive deficits. Fresh fecal pellets were collected weekly to examine the alterations of the microbiome over the time course. We found Cd exposure caused hippocampus-dependent learning and memory impairments starting at 4 weeks into exposure, without affecting activity or anxiety in mice. Hippocampus-specific RNA-sequencing at the terminal time point showed that Cd reduced the

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