croscopy. **Results:** In this study, we first examined the effects of APOE4 genotype on lateral ventricle volume in saline-administered controls. In saline-administered controls, TR-APOE4 mice presented 95% larger ventricle volume compared to TR-APOE3 mice. Additionally, we observed sex differences in lateral ventricle volume amongst saline-administered control TR-APOE4 mice, with males presenting 37% larger ventricles compared to females. Next, we evaluated the effects of PQ on ventricle volume. Compared to saline controls, PQ exposure slightly increased ventricle volume in TR-APOE3 mice, reporting a 13% increase in ventricle volume. However, in TR-APOE4 mice, PQ exposure resulted in a 101% increase in ventricle volume compared to saline controls. When evaluating the sex differences in PQ-exposed TR-APOE4 groups, we found PQ increased ventricle volume by 108% and 88% compared to saline-administered controls in males and females, respectively. Since published studies have shown APOE4 and PQ exposure independently increase α-synuclein deposition and aggregation, we assessed total α-synuclein levels in the substantia nigra via immunofluorescence. In saline-administered TR-APOE4 mice, we observed increased α-synuclein levels compared to TR-APOE3 mice. These saline-administered control groups also showed sex differences on α-synuclein pathology, as TR-APOE4 males presented increased levels compared to TR-APOE4 females. When examining the effects of PQ exposure on α-synuclein pathology, we observed increased α-synuclein levels in PQ-exposed TR-APOE3 and TR-APOE4 groups compared to saline controls. Lastly, we observed sex differences in α-synuclein pathology in PQ-exposed TR-APOE4 groups, as males presented larger increases in α-synuclein levels compared to females. **Conclusions:** This study demonstrates that PQ exposure increases PDD/DLB-related pathology in TR-APOE4 mice. Additionally, in experimental groups treated with PQ, males showed a greater level of PDD/DLB pathology compared to females. Collectively, this suggests that APOE4 genotype and male sex are significant modifying factors for PQ neurotoxicity. *Supported in part by R01ES033892 awarded to JRR.*

PS 4611 Association of Pesticide Exposure with Plasma Aβ-42/40 Ratio: Evidence from Alzheimer's Disease Mouse Models and the Child Health and Development Studies

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Background and Purpose: Environmental factors are increasingly recognized as significant contributors to Alzheimer's disease and related dementias (ADRD), with epidemiological studies linking pesticide exposure to a decline in cognitive performance. Dichlorodiphenyltrichloroethane (DDT), an organochlorine pesticide used extensively to combat insect-borne diseases in the US until its ban in 1972, persists in trace amounts due to its environmental and biological long half-life, bioaccumulation, and existing dumpsites. Although there has been a steady decline in environmental DDT levels, the persistent metabolite dichlorodiphenyldichloroethylene (DDE) is still ubiquitously detectable and considered non-neurotoxic. This exemplifies the need to investigate legacy exposures as people highly exposed to DDT between the 1940s and 1970s face a significantly increased risk of developing late-onset AD (≥ 65 years). We have previously reported that serum levels of DDE, the long-lived metabolite of DDT, were nearly four times higher in patients with AD compared to healthy individuals. Additionally, we showed that biologically relevant doses of DDT increase amyloid (Aβ) pathology in cell culture, fly, and animal models. However, little is known about the effects of DDE exposure on plasma Aβ-42/40 ratio, a reliable biomarker for prodromal AD, Aβ pathology, and cognitive dysfunction. Here, we sought to assess - i) the impact of subchronic DDE exposure on serum Aβ-42/40 ratio and its association with Aβ pathology, cognition, and neurodegeneration in transgenic 5xFAD mouse model and ii) the effects of human prenatal DDE exposure on plasma Aβ-42/40 ratio in mid-life. **Methods:** 5xFAD mice (6-week-old males), expressing human APP and PSEN1 transgenes with a total of five AD-linked mutations, were exposed to 3 mg/kg *p,p'*-DDE, or corn oil (vehicle control) by oral gavage every 3 days for 3 months. Hippocampal tissue was dissected and sequentially homogenized in N-PER, Guanidine HCl (GuHCl), and formic acid (FA) to extract soluble Aβ, diffuse and dense-core plaques, respectively. Brain and serum Aβ levels were quantified using multiplex MSD assays. Neurodegeneration was assessed by unbiased stereological counting of Nissl$^+$ neurons in the subiculum and measuring serum neurofilament light chain (NfL) levels on the MSD platform. Cognitive impairment was evaluated by measuring spontaneous alternation behavior in a Y-maze. To assess the effects of prenatal *p,p'*-DDE exposure, offspring from the Child Health and Development Studies (CHDS) pregnancy cohort (1960-1963) in Oakland, California, were recruited in 2010 for a follow-up study on health disparities. Participants (average age 50) completed cognitive function assessments and provided interview data and blood samples. Quanterix Neurology 3-Plex A kit was used to measure plasma Aβ-42/40 in mid-life (n=160; 50% males and females). Matched prenatal *p,p'*-DDE, measured in archived maternal pregnancy serum, was available for this pilot sample. Associations were estimated in linear, binomial, and multinomial logistic regression models, adjusted for race and sex. **Results:** In DDE-exposed 5xFAD mice, MSD analysis of hippocampal tissue revealed no significant differences in N-PER soluble (monomeric and oligomeric) Aβ-40 or Aβ-42 levels compared to the control group. Contrastingly, analysis of GuHCl fraction showed significantly higher levels of Aβ-40 (~32 fold) and Aβ-42 (~100 fold), while formic acid soluble Aβ-40 (~3.5 fold) and Aβ-42 (~5 fold) were also higher in DDE-exposed mice suggesting more plaque-associated insoluble Aβ buildup. The serum Aβ-42/40 ratio was significantly reduced by 31% in DDE-exposed mice. Additionally, reduced serum Aβ-42/40 was correlated with elevated hippocampal Aβ-42 in the GuHCl (r = -0.42; p = 0.01) and FA fraction (r = -0.40; p = 0.03). To determine whether DDE promotes neurodegeneration, unbiased stereological counting revealed that DDE significantly reduced ~13% of Nissl+ neurons in the subiculum compared to controls, and this was positively correlated with serum Aβ-42/40 (r = 0.52; p = 0.03). Serum NfL levels were 38% higher in DDE-exposed mice compared to control. Additionally, serum NfL levels negatively correlated with serum Aβ-42/40 ratios (r = -0.399; p = 0.04). Mice exposed to DDE also displayed deficits in working memory and performed significantly worse in the Y-maze, with a 20% decrease in spontaneous alternation compared to control 5xFAD mice. Further, cognitive decline in DDE-exposed 5xFAD mice positively correlated with low serum Aβ-42/40 (r = 0.42; p = 0.02). In the CHDS cohort, higher prenatal DDE was associated with lower Aβ-42/40 in mid-life. In logistic models, the Odds Ratio (OR) for estimating lower Aβ-42/40 was 2.6 (95% CI=1.0-6.9) and 3.2 (95% CI=1.1-9.8) for DDE tertiles 2 and 3, respectively. The test for trend across DDE tertiles was significant (p = 0.04). Higher prenatal DDE was also associated with lower mid-life Wechsler Digit Symbol Substitution Task (DSST) score (β = −0.0221, 95% CI = −0.041 to −0.004; p = 0.02). Associations were robust to multiple alternative modeling approaches. Adjusting for achieved education in mid-life did not alter results. **Conclusions:** Overall, these data indicate that DDE is an active metabolite of DDT and has a long-lasting neurological impact on the amyloid system and cognitive function. In DDE-exposed 5xFAD mice, serum Aβ-42/40 strongly correlates with AD pathology in the brain, suggesting that serum Aβ-42/40 reliably confirms amyloid status, particularly insoluble Aβ-42 and neurodegeneration. In the CHDS cohort, higher prenatal DDE exposure is correlated with lower midlife plasma Aβ-42/40, a potential early marker for ADRD, and lower DSST scores, indicating that prenatal pesticide exposure may have long-lasting effects on cognitive function. Our translational findings in mice and humans provide evidence that prenatal and early-life environmental exposures increase ADRD risk, advancing opportunities for early mid-life interventions to prevent disease progression.

PS 4612 Impact of stress hormone on brain damage and inflammatory responses resulting from methamphetamine exposure in rats

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Background and Purpose: Over the last several years, illicit drug use has increased in the U.S. including drugs like methamphetamine. As a dopaminergic neurotoxicant, methamphetamine exposure poses risk of injury to dopaminergic neurons, brain inflammation, and mortality. While illicit drug use constitutes an obvious health risk to the user, public safety personnel including law enforcement, emergency medical service, and corrections officers, are at risk for unintentional exposure to these drugs when interacting with contaminated materials or individuals under the influence. While unintentional exposure is likely to be at a lower drug concentration, a health risk may exist particularly when considering other work environment conditions. Work-related stress is a major concern for public safety personnel due to the high risk of exposure to repeated high-stress incidents, as well as work organizational stress factors. In previous studies, subchronic exposure to stress hormone was shown to exacerbate the neuroinflammatory and neural injury responses to several neurotoxicants in rodent models. The aim of the current study was to identify low doses of methamphetamine that did not produce neuroinflammation or neural damage on their own and evaluate the ability for prior stress hormone exposure to modulate these responses in a rat model of exposure. **Methods:** Adult male Sprague-Dawley rats were given corticosterone (200 mg/L in 0.6% ethanol) in their drinking water for 7 days followed by a single subcutaneous injection of methamphetamine (5, 7.5, or 10 mg/kg). Body temperature was measured at 1 and 2 hours post-methamphetamine exposure. Markers for neuroinflammation and dopaminergic neuron damage were assessed in the striatum, hippocampus, and cortex. Inflammatory cytokine mRNA was measured in the brain by qRT-PCR. Glial fibrillary acidic protein (GFAP), a marker of astrogliosis, and tyrosine hydroxylase (TH), a marker for dopaminergic neurons, proteins were measured by ELISA. **Results:** Exposure to corticosterone significantly modulated the body temperature response to methamphetamine exposure; all doses tested showed a significant temperature elevation compared to corticosterone alone while only the 10 mg/kg, s.c. methamphetamine dose resulted in significant hyperthermia on its own. Neuroinflammatory changes were observed as early as 12 hrs post- methamphetamine. In the striatum, which is the site of dopaminergic nerve terminals from the substantia nigra and methamphetamine -induced damage, the 10 mg/kg, s.c. dose of methamphetamine significantly increased several cytokines regardless of corticosterone exposure status. However, the 7.5 mg/kg, s.c. dose significantly increased inflammatory cytokine mRNA expression only with prior corticosterone exposure. Interestingly, while methamphetamine typically targets the dopaminergic nerve terminals of the striatum, prior exposure to stress hormone expanded the neuroinflammatory effects of methamphetamine to the hippocampus and cortex. **Conclusions:** Stress is a common workplace environmental factor and the nature of public safety work constitutes a high work-related stress environment due to exposure to traumatic events, as well as work organizational factors. Here, prior stress hormone exposure, a surrogate for stressors, was demonstrated to mod-

ulate the response to low dose methamphetamine exposure increasing its negative effects on the brain. These data also highlight the correlation between methamphetamine-induced hyperthermia and detrimental effects on the brain, with prior stress increasing the risk of hyperthermia with lower dosages of methamphetamine. Overall, these findings suggest that the chronic stress experienced by public safety personnel may pose a greater risk for adverse neurological health outcomes resulting from unintentional exposure to methamphetamine. Considering the association between dopaminergic neuron loss and long-term neurological disease, such as Parkinson's Disease, further work is needed to assess the potential long-term consequences of these combined exposures.

4613 Acute neurotoxic effects of antioxidant by-products in rat cortical neurons

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Background and Purpose: Owing to their affordability and versatility, plastics have become integral to modern life. Consequently, plastics are extensively used in modern day products such as clothing, car tires, food packaging and drug packaging, automotive components, electronics, and many more. To enhance the durability and longevity of plastics additives are used, including antioxidants, which protect them from degradation. Without such additives, plastics degrade more rapidly under prolonged exposure to stress factors, such as heat, UV-light, and mechanical wear. Antioxidants in plastics prevent oxidation by sacrificing themselves to neutralize free radicals, resulting in the formation of Antioxidant By-Products (ABPs). Given the widespread applications of plastics, humans are exposed to plastic leachates, including ABPs, although the potential health impacts, especially neurotoxic effects remain largely unexplored. The aim of this study was to investigate the acute neurotoxic effects of ABPs on rat cortical neurons. **Methods:** Effects on cell viability experiments were determined using an Alamar blue assay following exposure of rat cortical cells for 21 days, 48 hrs and 30 minutes to ABP 1-10 at concentrations of 0.01, 0.1, 1, 10 and 100 µM. Additional experiments were performed using 48-well microelectrode array (MEA) plates to record changes in spontaneous neuronal activity evoked by acute exposure to ABPs. Prior to cell seeding, the MEA plates were coated with a 0.1% PEI solution in borate buffer to enhance cell adhesion. Cells were then seeded at a density of 1 × 10^5 cells per well. After 11 days, baseline neuronal activity was recorded for 30 minutes using the Maestro Pro™ system. Following baseline recording, cells were exposed to ABPs at concentrations of 0.01, 0.1, 1, 10, and 100 µM, with neuronal activity monitored for an additional 30 minutes. Data acquisition was controlled by Axion's Integrated Studio (AxIS version 2.6), with raw data files sampled concurrently across channels at a gain of 1200x and a sampling rate of 12.5 kHz per channel, using a band-pass filter. Raw data were pre-processed to generate spike files. Spike detection was performed with AxIS's spike detector, and the results were further analyzed using the Neural Metrics Tool (v3.1.7, Axion BioSystems) and custom Excel macros. ABPs 1, 2, 3, 4, 6, 7, 9, and 10 were purchased commercially, while ABPs 5 and 8 were synthesized and provided by LyondellBasell, as they are not commercially available. **Results:** ABPs 1, 2, 3, 5, 7, 8, 9, and 10 did not induce cytotoxic effects after 21 days of exposure. However, significant reductions in cell viability were observed at 100 µM for ABPs 4 and 6 under the same conditions. Following a shorter 48-hour exposure, no cytotoxic effects were noted for ABP 6, while ABP 4 exhibited a significant decrease in cell viability at 100 µM. Finally, no cytotoxic effects were observed for ABP 4 at any tested concentration after a 30-minute exposure. With respect to changes in neuronal activity, a significant reduction in the numbers of recorded spikes was observed at 100 µM for ABP 1, 2, 3, 4, 8 and 9. ABP 5, 6, 7 and 10 on the other hand did not appear to affect neurological activity. The reduction in the number of spikes was often associated with a reduced number of bursts and network bursts. Effects on synchrony were not detected, unless the number of spikes was close to zero, in which case synchrony was also reduced. For ABP 4, the number of spikes was also observed at 10 µM. This reduction also resulted in a reduced number of bursts and network bursts, although synchrony was not affected. **Conclusions:** To conclude, acute exposure of ABP at concentrations of 10 to 100 µM resulted in reduced spontaneous activity. Reduced number of spikes, bursts, network bursts appear to be the most significantly impacted parameters. These novel neurotoxicological findings suggest that ABP exposure at extremely high levels may affect human brain health.

4614 Botanical extracts and active constituents dose-dependently modulate neuronal activity in rodent primary cortical cultures measured using microelectrode array (MEA) recordings

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Background and Purpose: The use of botanical extracts for medicinal, cosmetic, and dietary purposes is continuously increasing globally. Botanical extracts are naturally complex as growing conditions, extraction processes, and/or changes to the finished product can all potentially influence phytochemical composition even within samples from the same species. While the public often thinks these botanical products are safe as they have a natural origin, their use is associated with a wide range of unintended adverse effects, including neurotoxicity. Since botanical extracts are often used intentionally, ensuring the safety of these products is a priority. However, toxicity testing of botanicals is challenging because of their chemical complexity and variability. Moreover, due to the presence of many constituents botanicals extracts may affect multiple modes of action. Consequently, costly, lengthy and ethically-debated animal toxicology testing is often inadequate. Therefore, there is increasing interest in using new approach methodologies (NAMs), such as microelectrode array (MEA) recordings of neuronal activity, to evaluate the neurotoxic/active potential of botanical extracts. The aim of the current study was to use MEA recordings to derive full dose-response curves of 13 neuroactive botanical extracts to determine their potencies and activity profiles. Moreover, we aimed at revealing the contribution of specific constituents to the activity phenotypes evoked by the neuroactive botanical extracts. **Methods:** Primary cultures of rat cortical neurons were grown on 48-well microelectrode array (MEA) plates at a density of 1 x 10^5 cells/well. Cells were cultured in 5% CO_2/95% air atmosphere at 37°C until use after 15 days in culture. MEA plates were used to record changes in spontaneous neuronal network activity in vitro following acute (30 min) exposure to 13 botanical extracts and 8 active constituents. All botanical samples were tested at a dose of 50 µg/mL down to the no-observed effect level. The botanical test samples were provided as dry extracts by the Botanical Safety Consortium (https://botanicalsafetyconsortium.org/; Mitchell et al., 2022), except for aconite, oleander, and tripterygium, which were provided as a stock solution of the extract (100, 88, and 55 mg/mL, respectively) in dimethyl sulfoxide (DMSO). Details on the sourcing, chemical analysis, and botanical details can be found at NIEHS's CEBS website (https://cebs-ext.niehs.nih.gov/cebs/paper/15717). Botanicals were selected based on existing literature with respect to toxicity or safety, from human (adverse event reporting and clinical trials), animal, or mechanistic studies, and because of their demonstrated neuroactive potential (van Kleef et al., 2024). **Results:** Our results demonstrate that exposure to extracts from milk thistle, usnea, green tea, aristolochia, ephedra, and trypterygium resulted in a dose-dependent, mild inhibition of neuronal activity, albeit with distinct neuronal activity phenotypes. Extracts of yohimbe, kratom, and kava potently inhibited neuronal activity, with cessation of activity at 50 µg/mL. Despite differences in the maximum degree of inhibition, all these inhibitory extracts show No Observed Effect Levels (NOELs) of 1-5 µg/mL. Exposure to blue cohosh, aconite, goldenseal and oleander results in specific activity phenotypes characterized by hyperexcitation and/or intensification of (network) burst activity. While the different extracts exhibit a relatively narrow effective dose range, the extracts from aconite, goldenseal and oleander are most potent with NOELs of 0.25 - 0.5 µg/mL. To reveal if the specific neuronal activity phenotypes are due to individual active constituents, we also tested the effects of aconitine, berberine, dihydrokavain, epigallocatechin, mitragynine, oleandrin, silybin B and yohimbine. Exposure to the active constituents resulted in comparable activity phenotypes compared to the corresponding botanical extracts, albeit that the extracts generally exhibited a higher potency to modulate neuronal activity. **Conclusions:** Diverse botanical extracts have a clear neuroactive potential. The distinct neuronal activity phenotypes suggest that the botanical extracts have diverse modes of action, resulting either in inhibitory patterns or excitatory patterns with increased and/or intensified (network) bursting. While the active constituents largely recapitulate the phenotype of the botanical extract, the higher potency of the extract indicates that there are additional constituents present in the extract. Unfortunately, however, attempts to relate these in vitro findings to human exposure levels and possible health effects are currently hampered by the scarcity of in vivo and human exposure data.

4615 *MT1-G* Activation for Identifying Chemical Chelators Causing Cytotoxicity of Human Dopaminergic Neurons

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Background and Purpose: Human neurotoxicity is a top concern among drug safety and environmental risk factors to human health. Identification of environmental chemicals that cause neurotoxicity is essential to investigating their relevance to human neurodegenerative diseases. A great challenge for *in vitro* neurotoxicity testing is to establish an experimental model for *in vivo* neurotoxicity with sufficient sensitivity and throughput to search chemical libraries in quantitative high throughput screening (qHTS). To meet this challenge, we built a foundation for qHTS by establishing: (1) LUHMES-derived dopaminergic neurons are highly susceptible to known neurotoxicants causing apoptosis and/or ferroptosis (Tong et al, J Appl Toxicol 37:167, 2017; Tong et al, Neurotox Res, 40:1526, 2022); (2) seven toxicants

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