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44th Annual Meeting of the Developmental Neurotoxicology Society

Held in conjunction with the 60th Annual Meeting of the Society for Birth Defects Research and Prevention and the 33rd Annual Education Meeting for the Organization of Teratology Information Specialists, Charleston, South Carolina, June 27–July 1, 2020

Meeting Cancelled due to COVID-19 Global Pandemic

DNTS P1
Evaluation of chemical and non-chemical interactions on neurodevelopment of rat offspring

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Abstract

Adverse perinatal conditions experienced by the mother can cause physiological and behavioral abnormalities in children. Although manganese (Mn) is an essential element, exposure above background levels have been linked to neurodevelopmental deficits in humans. To evaluate the neurological impacts of maternal stress presented together with chemical exposure, we exposed pregnant LE rats to Mn through drinking water (0, 2, or 4 mg/ml) from gestational day (GD) 7 to postnatal day (PND) 22. Half of the dams were also exposed to a variable stress paradigm from GD13 to PND9. Somatic development and behavior were evaluated in the offspring. No evidence of overt maternal toxicity was observed, although on GD10 and 21 weight gain was significantly lower in the 4 mg/ml Mn groups. Maternal stress nor Mn exposure affected litter viability, but pup weight was significantly reduced in the 4 mg/ml Mn-exposed group on PND22. Water consumption was decreased in the Mn exposed dams in a dose-dependent manner but was not altered by stress. Stress responses to the maternal manipulations were determined using serum corticosterone (Cort). Baseline Cort levels (GD7) were low and similar in all treatment groups. As expected, Cort levels were elevated in all the Stress groups on GD16 when compared to the Non-Stress groups. A preliminary analysis of Mn tissue concentrations revealed levels elevated similarly in Mn-exposed pups and dams; the addition of stress did not alter the Mn levels. Taken together, these results show that maternal stress did not exacerbate the effects of Mn exposure on these measurements. This abstract does not necessarily reflect EPA policy.

DNTS P2
Adult measures of criminality correlated with reduced regional brain volumes in a cohort with childhood lead exposure

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Abstract

The consequences of childhood lead exposure have been shown to persist into adulthood. Behavioral studies have demonstrated that lead exposure correlates with measures of adult criminality and personality disorders such as psychopathy while neuroimaging studies report abnormalities in the brain, both structurally and functionally. However, little research has connected these relationships. Using data from a longitudinal birth cohort with measured childhood lead exposure, we tested the hypothesis that increased rates of criminality and reduced regional brain volumes, both correlated with increased measures of childhood lead exposure, are associated with each other. We utilized Magnetic Resonance Imaging (MRI) to obtain high resolution T1-weighted anatomical images from one hundred and nineteen participants (mean age 26.8 years, 40% male, 93% African American) from the Cincinnati Lead Study (CLS). Childhood blood lead levels were obtained during CLS visits. Adult criminal records were obtained from public databases following the CLS IRB protocol. Brain volume was estimated using the Computational Anatomy Toolbox in SPM12. Multiple linear regressions were used to investigate the relationship between criminality, childhood lead exposure, and brain volume. Childhood lead exposure was inversely associated with regional brain volume, mainly in the frontal lobe. Adult criminality measures were also inversely associated with regional brain volume in the frontal lobe. A conjunction analysis shows significant overlap between the analyses, primarily in the cingulate. Increased rates of criminality associated with childhood lead exposure may be related to corresponding changes in brain volume.

DNTS P3
Neuronal and neuroimmune dysfunction underlie behavioral and synaptic phenotypes in a mouse model of MEF2C Haploinsufficiency Syndrome

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Abstract

Microdeletions and mutations in the MEF2C gene cause a neurodevelopmental disorder termed MEF2C Haploinsufficiency Syndrome (MCHS). Symptoms of MCHS include core autism symptoms, such as...
deficits in speech and social reciprocity, and repetitive motor behaviors. In addition, individuals with MCHS often present with epilepsy, intellectual disability, sleep disturbances, hypotonia and high pain tolerance. Through mouse models, we aim to uncover the mechanisms underlying MCHS. Here, we show that MCHS-associated MEF2C nonsense mutations cluster in the conserved DNA binding domain, and generation of severe of several of the patient missense mutations produced in a dramatic reduction in MEF2C DNA binding affinity. DNA binding-deficient global Mef2c heterozygous mice (Mef2c-het) display numerous MCHS-related behaviors as well as input-specific reductions in cortical excitatory synaptic transmission. As a regulator of neuronal synapse development, MEF2C hypofunction in neurons is presumed to underlie most MCHS symptoms, but MEF2C is also expressed in neuroimmune cells, specifically microglia. Our ongoing studies suggest critical roles for MEF2C in both neuronal and neuroimmune populations for behavioral and synaptic phenotypes in a genetic mouse model of MCHS.

**DNTS P4**

Using electroencephalography (EEG) in human infants as a measure for environmental epidemiology and developmental neurotoxicology

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**Abstract**

Recent work in environmental epidemiology and developmental neurotoxicology has found that prenatal exposure to phthalates and phenols is linked to negative cognitive, social, and behavioral outcomes in infancy and childhood. However, less is known about how early exposure to these chemicals impacts the brain to produce these outcomes. Here we used electroencephalography (EEG) to measure brain activity in 93, 7–10-month-old infants from a large prospective study in Illinois. Specifically, we measured EEG as infants saw pictures of both novel and repeated (familiar) faces. For each infant, we calculated responses to familiar compared to novel faces for four electro-physiological components associated with perceptual (P290 and P400), attentional (Nc) and memory (PSW) processing. At a group level we found results consistent with past work: infants’ brain responses to perceptual processing were the same for familiar versus novel faces: P290, t(92) = .24, p = .81, P400, t(92) = .34, p = .31, but different for attentional processing: Nc, t(92) = 2.44, p = .017, and memory processing: PSW, t(92) = 2.29, p = .025. These findings validate our measure at the group level and suggest it may be a promising methodology to investigate individual differences. In the future, we will assess associations between infants’ brain responses (investigating perception, attention, and memory) and prenatal exposure to phthalates and phenols. This will allow us to more directly study the effects of chemicals on the brain and specific aspects of cognition. (Supported by ES022848, ES028607, ES007326, OD023272, and USEPA RD83543401.)

**DNTS P5**

The use of vitamin E as a rescue agent in larval zebrafish exposed to benzo[a]pyrene in early development

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**Abstract**

Polycyclic aromatic hydrocarbons (PAHs) are environmental pollutants created by incomplete combustion. Benzo[a]pyrene (BaP), among other PAHs, is known to exert toxicity through oxidative stress which is thought to occur through inhibition of antioxidant scavenging systems. The use of agents that reduce oxidative stress may be a valuable route for ameliorating the adverse effects of PAHs on neural development and behavior. Zebrafish are becoming an advantageous species for assessing potential risks of environmental hazards to the public and have proven their efficacy as a model organism to help identify neurotoxicants and neuroprotectants. This study was conducted to determine if vitamin E supplementation can prevent or reduce neurobehavioral deficits in zebrafish embryos exposed to BaP during early development. Newly hatched zebrafish were assessed on locomotor activity, and light responsivity and habituation, as well as developmental morphogenesis. Zebrafish embryos were exposed to vehicle (DMSO), vitamin E (alpha tocopherol, 0.1 μM–4 μM), and/or BaP (3 μM–10 μM) from 5 to 120 h post-fertilization. This dose range was below the threshold for producing overt dysmorphogenesis or increased survival. BaP (3 μM) was found to cause locomotor hypoactivity in larval fish. Co-exposure of alpha tocopherol (4 μM) led to a restoration of locomotor function, similar to that of the control group. Based on the findings of this study, this model can be expanded to assess the outcome of vitamin E supplementation on other potential environmental neurotoxins, and additionally lead to determination if this rescue persists into adulthood. (Support from the Duke University Superfund Research Center, ES010356.)

**DNTS P6**

Gestational exposure to polybrominated diphenyl ethers and social skills and behavioral problems at age 12 years

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**Abstract**

Polybrominated diphenyl ethers (PBDEs) exposure during early brain development has been associated with cognitive deficits as well as increased hyperactivity, inattention, and aggression. We sought to examine if there is an association with social skills and behavioral problems. In the Health Outcomes and Measures of the Environment (HOME) Study, we examined 239 children with PBDEs measured in maternal serum at 16 weeks gestation. Social skills were evaluated at age 12 years using the child-reported Social Skills Improvement System (SSIS) Rating Scales. Covariates considered for the linear regression models included child sex and race/ethnicity; maternal age, education, marital status, depression, parental relationship frustration; household income; and maternal serum cotinine concentration. Geometric means for PBDE-28, -47, -99, -100, -153 and the sum of all 5 were 1.1, 20.7, 4.8, 4.0, 4.9 and 36.9 ng/g lipid, respectively. Mean SSIS scores were 99.1 (±15.2) for social skills and 96.6 (±12.2) for problem behaviors. Prenatal PBDEs were not significantly associated with social skills scores. There were sex-differential associations between PBDEs and problem behavior scores. No significant associations were observed among females. In males, for each 10 fold increase of PBDE concentrations (-28, -47, -99, -100, and the sum), problem behavior scores increased by 17.0 (95% CI: 9.5, 24.4), 11.0 (4.4, 17.6), 9.6 (2.9, 16.2), 8.4 (1.8, 15.0), and 10.6 (4.0, 17.1), respectively. Prenatal PBDE exposure was associated with an increase in self-reported problem behaviors in males at age 12 years. This adds to the evidence that PBDE exposure may negatively affect child neurodevelopment.

**DNTS P7**

Effects of developmental exposure to manganese and non-chemical environmental factors on tasks of learning and memory, social interaction, motor activity, and anhedonia

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**Abstract**

Manganese (Mn), a toxicant that naturally occurs in the environment, has been shown to produce neurotoxic effects on the developing
young when levels exceed physiological requirements. To evaluate the effects of this chemical in combination with non-chemical factors pregnant Long-Evans rats were treated with 0, 2, or 4 mg/mL Mn in their drinking water from gestational day (GD) 7 to postnatal day (PND) 22. Half of the dams received a variable stress protocol from GD13 to PND9. (Beasley et al. abstract). Motor activity was measured in figure-eight mazes at 3 different ages (PND17, 29, and 79, with each age being a different group of animals). Learning and memory was examined using a Novel Object Recognition (NOR) task (PND34) and the Morris Water Maze (PND62); social interaction (PND48) was tested by looking at time and number of visits to a stranger rat. Anhedonia (PND53), a measure of depression, was assessed by comparing the preference for chocolate milk versus water. There were statistically significant interactions between Mn and stress but the effects differ by sex and endpoint. The only assessment listed above that had no effect of Mn or stress was anhedonia. These data indicate that other factors, extraneous but coexistent with chemical exposures, should be considered in the evaluation of chemical risk. This abstract does not necessarily reflect EPA policy.

DNTS P8
Association of prenatal maternal stress with measures of cognition in 7.5-month-old infants
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Abstract
Studies have shown that prenatal stress can negatively impact neurodevelopment, but little is known about its effect on early cognitive development. We assessed the impact of prenatal stress on cognition in 152 7.5-month-old infants using Cohen’s Perceived Stress Scale (PSS; 10–14 and 34–36 gestational weeks), maternal telomere length (MTL; 16–18 gestational weeks), and a Stressful Life Events (SLE) Scale that ascertained adverse events throughout pregnancy. A visual recognition memory task consisting of nine blocks, each with one familiarization trial (2 identical stimuli) followed by two test trials (one familiar stimulus, one novel), was administered. Five stimulus pairs were human faces and four were geometrical shapes. During familiarization trials, average time looking at stimuli before looking away (a measure of processing speed), and time to reach looking time criterion (a measure of attention) were assessed. During test trials, the proportion of time looking at the novel stimuli (a measure of recognition memory) was assessed. Generalized linear models examined the association of each stress measure with each outcome adjusted for infant age and sex, assessment condition (which of the two stimuli in each set was novel), household income, and maternal age, education, and IQ. Each measure of higher prenatal stress was associated with shorter looking durations [PSS (β = −1.2, 95% CI: −2.1, −0.43); SLE (β = 0.77, 95% CI:0.21,1.33); MTL (β = 1.81, 95% CI:0.18,3.44)] and longer time to criterion [PSS (β = 10.8, 95% CI:4.4,17.2); SLE (β = −14.7, 95% CI: −24.4, −4.9); MTL (β = −23.9, 95% CI: −43.3, −4.5)], suggesting that higher prenatal stress is associated with decreased visual attention in infancy. (Supported by ES007326, ES022848, RD83543401, OD023272, and ES028607.)

DNTS P9
Preschool blood lead level, early substance use, and sexual behavior in ploy-drug exposed adolescents
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Abstract
The objective was to examine the association between early lead exposure assessed at age 4 and adolescent substance use and sexual behavior. Participants were 265 (137 female, 128 male) adolescents, primarily African-American and of low socioeconomic status, prospectively enrolled at birth for a longitudinal study of prenatal cocaine exposure. Blood lead levels (BLL) were measured at 4 years of age (mean = 7.07 µg/dL (SD = 4.12), range 1.3–23.8). At age 15, substance use (alcohol, tobacco, marijuana) was assessed via self-report and biologic assays. Age at first time of sexual intercourse was assessed via self-report, with early sexual behavior defined as sexual intercourse before 15th birthday. Prenatal drug exposure (cocaine, alcohol, tobacco, marijuana), maternal psychological distress (α = .95) via the Brief Symptom Inventory, were assessed at birth. Violence exposure (α = .75), parental monitoring (α = .74), and externalizing behavior, using the Youth Self-Report, were assessed at age 12 and used as covariates. About one-third of the adolescents reported tobacco (31%), alcohol (40%), and marijuana (32%) use; and 36% reported sexual intercourse before 15th birthday. Multiple logistic regression analyses indicated that, after controlling for covariates, higher BLL is related to a greater likelihood of using alcohol (OR = 1.12, 95% CI = 1.04, 1.20, p = .003), tobacco (OR = 1.07, 95% CI = 1.002, 1.15, p = .04), and marijuana (OR = 1.08, 95% CI = 1.001, 1.155, p = .047), and engaging in sexual intercourse before age 15 (OR = 1.12, 95% CI = 1.04, 1.21, p = .004). Elevated BLL in preschool years is a risk factor for adolescent substance use and early sexual behavior.

DNTS P10
Manganese consumption and perinatal stress cause persistent, sex-dependent, and complex changes on attentional function in rats
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Abstract
The developmental effects of chemicals that co-occur in vulnerable populations with elevated physical and psychological stress are of increasing concern to risk assessors. In order to assess causality of these factors we developed a rodent model of co-occurring perinatal manipulations and conducted a series of behavioral assessments in male and female offspring. Manganese (Mn), a potential neurodevelopmental toxicant, was delivered in drinking water (0, 2, or 4 mg/mL Mn) of pregnant rats from gestational day (GD) 7 to postnatal day (PND) 22. A variable perinatal stress paradigm (PS) was applied to half of the animals from GD13 to PND9. Acoustic startle response (ASR) and choice reaction time (CRT) were evaluated in adults. The ASR and its habituation were unaffected by stress or Mn, whereas prepulse inhibition of the ASR yielded a significant interaction of Mn, Stress, and prepulse noise; however, contrasts were not significant. Mn reduced cued but not uncued CRT accuracy in males, and PS alone reduced accuracy in 0 mg/ml Mn-males compared to non-stress males. PS increased female movement time (MT) but not decision time on both CRT tasks. A significant interaction of PS and Mn occurred on cued MT at the 4 mg/ml dose in both sexes. These data demonstrate the ability of both PS and Mn to impair attentional function in adult animals. Furthermore, the data show evidence of PS modifying the effect of Mn on movement in both sexes. This abstract does not reflect EPA policy.

DNTS P11
Is coal keeping children off the honor roll? Coal flyash exposure and school performance in children aged 6–14 years
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Fly ash is a coal-combustion byproduct composed of particles with diameters less than ten micrometers (PM10) that may contain neurotoxic metals such as aluminum, lead, manganese, and arsenic. Little research exists examining the effects of fly ash exposure on neurobehavioral outcomes in children, but metal components have been independently associated with adverse neurobehavioral effects. To assess school competency associations related to fly ash, we analyzed data from 189 children aged 6 to 14 years living near coal ash storage sites using personal modular impactors, lift tape samples, the School Competency scale of the Child Behavioral Checklist (CBCL), and parent report of school grades. Statistical methods used to analyze the data included multinomial and binomial logistic regression and the likelihood ratio test. Fifty (26.5%) participants had low School Competency t-score values on the CBCL and 147 (77.8%) participants had fly ash in their homes. The odds ratio for low school competency and in-home presence of any fly ash was 3.4 ($p = 0.02$, 95% CI = 1.23–9.53), adjusting for age and sex. A separate analysis of parent-reported grades and fly ash exposure for 206 children revealed that a lack of fly ash in the home predicted better school performance. Odds ratios of 0.19 ($p = 0.04$, 95% CI = 0.04–0.93) for “excellent” grades and 0.22 ($p = 0.07$, 95% CI = 0.04–1.13) for “average” grades, with “poor” as the reference and adjusting for sex, were determined. These findings suggest that school competency impairments and lower parent-reported grades for children living near coal ash storage sites may be related to fly ash exposure.

**DNTS P12**

5-State Alliance for Child Development Research (5-STAR)

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**Abstract**

The 5-State Alliance for Child Development Research (5-STAR), a consortium of five universities (Case Western Reserve University (CWRU), University of California, San Diego (UCSD), University of New Mexico (UNM), Oklahoma State University (OSU), and Emory University, works collaboratively to 1) design the protocol of a large, multisite study to examine children’s brain, cognitive, behavioral, social, and emotional development prenatally through ages 9–10 and 2) determine the impact of pre/postnatal opioid exposure on development. Methods: 5-STAR working groups will 1) plan children’s long-term follow-up; 2) pilot protocols for maternal/child assessments, including novel technologies; and 3) identify methods for ethical recruitment. Sites will: conduct focus groups to ascertain subject recruitment and retention preferences; collect information on maternal drug use and infant birth outcomes; conduct neuroimaging and multiple developmental assessments on children 0–2 years of age; and pilot an experimental crib monitor of sleep patterns and heart rate variability. Each site contributes uniquely: CWRU will pilot: MR fingerprinting (MRF), a novel technology to reduce MRI scanning time; UNM: MEG-EEG and infant self-regulation data; Emory: assessment of infant cardiac orienting response and non-nutritive sucking; OSU: will administer the PICCOLO language assessment; and UCSD: will inform sampling drug exposures from their breast milk repository. Descriptive statistics will summarize maternal/infant characteristics and child outcome across sites. Findings will elucidate the elements of a successful longitudinal study conducted prenatally through early childhood to assess prenatal opioid exposures and environmental influences on children’s brain/behavioral development.

**DNTS P13**

Associations of prenatal phthalate exposure and neurobehavioral outcomes in 7.5-month-old infants

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**Abstract**

Phthalates are ubiquitous endocrine-disrupting chemicals. Research indicates that gestational exposure alters neurodevelopment. In a prospective birth cohort study, five first morning urine samples collected across pregnancy were pooled and the following exposures assessed: Σdi-(2-ethylhexyl) phthalate metabolites (ΣDEHP), monoethyl phthalate (MEP), Σdiisononyl phthalate metabolites (ΣDINP), Σdibutyl phthalate metabolites (ΣDBP), Σanti-androgenic phthalate metabolites, and Σall phthalate metabolites (ΣPhthalates). Communication, problem solving and personal-social domains were assessed via the Ages and Stages Questionnaire (ASQ-III), a standardized parent-reported age-adapted screening tool. We obtained ASQ scores for 157 7.5-month-olds. Generalized linear regression examined associations of prenatal phthalate exposure with ASQ scores (assuming a Poisson distribution) after adjusting for maternal age, infant age at assessment, gestational age, sex, income, and maternal education. A ΣDINP × sex interaction revealed that compared with girls, ΣDINP exposure was associated with higher communication scores in boys ($β = −1.292; 95\%CI: −2.509, −0.074; p = 0.038$). This was maintained upon stratifying by sex (boys:$β = 1.111; 95\% CI: 0.020,2.203; p = 0.046$; girls:$β = −0.094; 95\% CI: −0.687,0.499; p = 0.756$). Higher MEP concentrations were associated with lower problem solving scores ($β = −0.252; 95\% CI: −0.493, −0.011; p = 0.041$), and higher ΣDEHP concentrations were associated with lower personal-social scores ($β = −1.329; 95\% CI: −2.175, −0.483; p = 0.002$), while ΣPhthalates were positively associated with personal-social scores ($β = 0.059; 95\% CI: 0.0004, 0.118; p = 0.052$). A ΣDINP × sex interaction demonstrated that increasing ΣDINP was associated with higher personal-social scores in boys compared with girls ($β = −1.179; 95\% CI: −2.290, −0.069; p = 0.037$). This was maintained upon stratifying by sex (boys:$β = 1.145; 95\% CI: 0.064,2.226; p = 0.038$; girls:$β = −0.014; 95\% CI: −0.362,0.335; p = 0.938$). Findings suggest that prenatal phthalate exposure influences neurobehavioral outcomes, some in a sex-specific manner. (Supported by ES007326, ES022848, RD83543401, and OD023272.)

**DNTS P14**

Regulation of mood by dopamine D1 receptors on cerebral cortical interneurons

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**Abstract**

Depression is a multifactorial brain disorder caused by a variety of alterations in neural structure and function. We have used the Cre/loxP system to selectively delete dopamine D1 receptors developmentally from GABAergic neurons from the Nkx2.1 lineage within the medial ganglionic eminence (GABA-D1-cKO), which include a subset of crucial cerebral cortical interneurons within the frontal cortex. Cell-type specific loss of D1 receptor expression was validated by in situ hybridization. Neurobehavioural analyses of GABA-D1-cKO mice revealed normal patterns of locomotor activity, anxiety, and spatial and nonspatial memory. However, GABA-D1-cKO mice exhibit decreased immobility in the forced swim test and decreased latency to consume a palatable...
liquid in a novel stressful environment; these results are both indicative of an antidepressant-like effect. GABA-D1-cKO mice also show reduced basal plasma corticosterone levels, altered TrkB levels, and down-regulation of the neurexin Cnnap4 in cerebral cortical interneurons. Conditional deletion of dopamine D1 receptors from telencephalic glutamatergic neurons (using Emx1-Cre as a deleter) does not induce these phenotypes. These data suggest a new mechanism by which the development of cerebral cortical interneurons may contribute to mood regulation and identify a new potential mechanism to exploit in the treatment and prevention of this prevalent brain disorder.

DNTS P15
Perinatal exposure to sevoflurane based general anesthesia in the absence of hypoxia causes neurodegeneration but has little effect on behavior later in life
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Abstract
Concerns have arisen about general anesthesia use in pediatric populations. An overwhelming body of preclinical evidence indicates that early life exposure to prolonged general anesthesia can cause neurodegeneration and impact cognition. This agrees with the limited clinical literature on extended exposures. Standard rodent models of early life anesthesia-related neurotoxicity, however, have come under criticism. Most rodent studies do not continuously monitor for hypoxia which may occur in those standard models. Here, we present data on the neurodegenerative and later behavioral effects of prolonged sevoflurane exposure in post-natal day (PND) 7 rats without hypoxia. PND 7 Sprague-Dawley rats were exposed to sevoflurane (2.5%) or the carrier gas (75% oxygen/25% nitrogen) for 6 h and then returned to their dam. During exposure, blood oxygen saturation levels ($S_{O2}$) of anesthetized animals were continuously monitored. Animals were euthanized (2–72 h post-exposure) for tissue collection or maintained for later adult behavioral analysis. Animals whose $S_{O2}$ level fell below 90% were excluded from the study. A comprehensive histological analysis of the forebrain showed an increased incidence of cell death in the indusium griseum, portions of the hippocampus, nucleus accumbens, caudate/putamen, and elsewhere. Moreover, a different profile of neurodegeneration was evident depending on the post-exposure interval. In contrast, few effects of anesthesia were seen on a battery of behavioral measures in adulthood. While hypoxia may exaggerate the effects of anesthesia, these data indicate that prolonged anesthesia in the absence of hypoxia elevates markers of neuronal death in young rats. (Supported by NCTR Protocol E7601.)

DNTS 27
Comparison of single fraction whole brain conventional or FLASH proton irradiation in neonatal Sprague-Dawley rats: Cognitive and behavioral effects
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Abstract
Proton radiation reduces off-target damage compared with X-rays but few studies have examined neurocognitive effects after developmental exposure. We compared whole brain conventional vs. FLASH proton irradiation using a comprehensive assessment of behavior and cognitive function in rats. In a split-litter design (19 litters), male/female pairs from each litter were exposed on postnatal day (P)11 under isoflurane anesthesia to physical doses of 0, 5, or 8 Gy single fraction whole brain proton uniform field plateau irradiation at 250 MeV. As adults (P64) rats were tested for locomotor activity, acoustic and tactile startle (ASR, TSR) with or without prepulses, novel object recognition (NOR), striatal dependent egocentric learning (Cincinnati water maze (CWM-V)), prefrontal dependent working memory (radial water maze (RWM)), hippocampal dependent spatial learning (Morris water maze (MWM)), amygdala dependent conditioned freezing, and the mirror image CWM-W. With the exception of 5 Gy FLASH, center ambulation was decreased in irradiated groups. ASR and TSR were reduced in the 8 Gy FLASH group compared with controls; there were no differences on PPI. There were no effects on NOR, MWM, or conditioned freezing. Irradiated groups with the exception of 5 Gy FLASH had longer escape latencies in the CWM-V, RWM, and CWM-W compared with controls. The results show striatal and PFC involvement in proton irradiation induced cognitive deficits at 5 and 8 Gy conventional and at 8 Gy FLASH proton exposure, but protective effects of FLASH at 5 Gy proton exposure. (Supported by a grant from Varian Medical Systems.)
affinity AHR and functioning CYP1A2 (AhrbCyp1a2+/+)

carbon receptor pathway increases susceptibility to BaP exposure during a mouse model to determine if genetic differences in the aryl hydrocarbon receptor pathway increase susceptibility to BaP exposure during early brain development. We compared wild type mice with a high affinity AHR and functioning CYP1A2 (AhrbCyp1a2+/+) mice with Cyp1a2(−/−) knockout mice having either the high affinity Ahrβ or poor affinity Ahrβ alleles. Dams were exposed to 10 mg/kg/day BaP from gestational day 10 (GD10) through weaning at postnatal day 25 (P25). A battery of cognitive and motor function tests were performed when the mice reached young adulthood (P60). We found a significant gene x treatment interaction in Novel Object Recognition with AhrbCyp1a2(−/−) mice showing impairments. We found a significant gene x treatment interaction in the Pole Climb test with wild type AhrbCyp1a2(−/+) having significantly longer latencies to turn before descending a 50 cm pole while AhrbCyp1a2(−/−) mice were significantly faster than corn oil-treated controls. There was a trend for significance (P = 0.1) for the total descent time. In Rotarod, mice were tested for 5 days with the rod accelerating from 0 to 20 rpm. There was a significant sex x treatment interaction on Day 4 with BaP-treated females having shorter latencies to fall. Together, these results indicate that both genes play a role in risks associated with prenatal exposure to BaP.

DNTS 31
Paternal aspartame exposure produces working memory deficit and anxiety-like phenotype in the offspring
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Abstract
The use of low-calorie sweeteners, such as aspartame, as weight loss tools is highly prevalent. In the US, approximately 25% of children and 40% of adults report using low-calorie sweeteners. We exposed adult (6-8-week-old) male C57BL/6 mice to aspartame in drinking water (0.03% or 0.015%; equivalent to 100% and 50%, respectively, of FDA’s acceptable daily intake). Following 12 weeks of exposure, we analyzed anxiety-like behavior (elevated zero maze and open field test), spatial working memory (Y-maze), and depression-like behavior (tail suspension test). Aspartame produced significant effects on anxiety-like behavior and spatial working memory (one-way ANOVA; p < 0.05), but not on depression-like behavior (p > 0.05). Post hoc multiple comparisons test revealed that mice exposed to both the concentrations had significant anxiety-like behavior and working memory deficit (p < 0.05). Next, we bred the 0.015% aspartame-exposed and control mice with drug-naïve females to produce the F1 generation. Paternal aspartame exposure produced significant effects on anxiety-like behavior and spatial working memory in the F1 mice (two-way ANOVA; p < 0.05). Post hoc multiple comparisons test showed that male and female F1 mice derived from the aspartame-exposed male mice showed significant anxiety-like behavior, while only the male F1 had a significant spatial working memory deficit (p < 0.05). In summary, aspartame exposure at levels as low as 50% of the FDA recommended human daily intake may carry the risk of adverse behavioral phenotypes not only in individuals directly exposed to the aspartame, but also in their descendants.

DNTS 32
Paternal cannabis extract exposure (containing 4 mg/kg/day of THC, sc) for 28 days caused significantly increased risk-taking behavior on the elevated plus maze in male offspring. This was also seen in the offspring of males exposed to the same duration and THC content in cannabis extract. Importantly, stopping cannabis exposure for 56 days before mating showed this impairment too. In the radial maze, female offspring of the cannabis-exposed fathers showed a choice accuracy impairment. This effect was attenuated in the cannabis abstinence offspring. These studies show that paternal preconception drug use can produce deleterious effects in the behavioral function of their offspring. Paternal preconception exposure to other exogenous chemicals like environmental toxicants may also pose risks to offspring neurobehavioral development. (Supported by the Templeton Foundation (60564, 60957), USEPA (RD83543701), and NIEHS (ES022831).)

DNTS 34
Developmental MeHg exposure alters neuroprogenitor differentiation in a human-induced pluripotent stem cell model
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Abstract
In vivo and historic human methylmercury (MeHg) exposures have suggested that both dopaminergic and glutamatergic neurons are targeted by MeHg. Despite this, little is known about which neuronal lineages and stages of development are most sensitive to MeHg exposure or the mechanisms of differential sensitivities. Therefore, using the human-induced pluripotent stem cell (hiPSC) model, we aimed to
understand which neuronal lineages and stages of development are most sensitive to MeHg. hiPSCs were differentiated down the cortical glutamatergic and floor plate dopaminergic lineages, and exposed to 0, 0.1, or 1 μM MeHg. Exposures occurred five days over the course of development to the neuroprogenitor (NP) stage (D4 to 10 of differentiation) and/or five days over the course of development of early post-mitotic neurons (D14 to 20 of differentiation). Differentiation potential was determined by examining expression of lineage-markers with qRT-PCR and immunocytochemistry at D11 and D21. In either the cortical or floor plate lineages, MeHg did not significantly affect expression of NP markers at D11 with qRT-PCR or immunocytochemistry. However, with qRT-PCR at D21, MeHg exposure increased the expression of FOXL1 (p = 0.038), a telencephalic NP marker, and a correspondingly decreased the expression of TBR1 (p = 0.027), an early cortical glutamatergic neuron marker. Additionally, the percentage of cells expressing Map2 was decreased in MeHg-exposed cortical neurons at D21 (p = 0.01). Preliminary data examining uptake of MeHg suggests differences in transport or storage of MeHg between the different developmental stages may play a role in the sensitivity of differentiating NPs to MeHg. (Supported by NIEHS, R01ES007331.)

**DNTS 35**

Essential amino acids are crucial for manganese induced increase of S6 but not AKT Phosphorylation.

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**Abstract**

Manganese (Mn) is crucial for optimal brain functioning as kinases of the insulin/IGF signaling cascade are Mn-dependent. We seek to identify direct molecular targets in the insulin signaling pathway that are altered at non-cytotoxic Mn exposures. Mn and IGF synergistically augment AKT, but not S6 kinases; therefore, we hypothesized that essential amino acids (EAA) play a role in Mn dependent activation of S6. We serum deprived murine striatal cell lines then exposed to Mn (100 μM), and/or IGF (1 nM) and IR/IGFR inhibitor (BMS-536924 at 10 nM) in both presence and absence of EAA for 3 h. AKT phosphorylation increased at 200 μM Mn but not at 100 μM Mn. Mn mediated increases in AKT were seen in both the presence and absence of EAA which was inhibited by BMS. Mn mediated S6 phosphorylation increased exclusively in the presence of EAA which was mitigated by BMS. IGF alone did not alter S6. Mn potentiates the effect of IGF on S6 only in the presence of EAA, in a Mn-dependent manner through IGF signaling pathway. This suggests a potential parallel pathway through which EAA is facilitating the Mn-dependent phosphorylation of S6. We are exploring the effects of Mn on insulin/IGF signaling using a similar exposure paradigm in developing cortical, floorplate and medium spiny neurons in control and Huntington’s disease lines. This study will improve our knowledge on the effects Mn has on insulin signaling pathway, which is crucial in context Mn neurotoxicity, neurodevelopment and neurodegeneration. (Supported by R01ES010563.)

**DNTS 36**

Thyroid hormone action controls cell signaling in the developing ventricular epithelium

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**Abstract**

Maternal thyroid hormone (TH) insufficiency is associated with neurodevelopmental disorders in children, although the underlying mechanisms are often unclear. Previously, we characterized that transient developmental hypothyroidism alters cell adhesion and migration in the neonatal rat brain. These abnormalities were largely localized to the ventricular epithelium, a progenitor cell niche, and later resulted in birth defect formation. Here we employed laser capture microdissection and RNA-Sequencing (RNA-Seq) to further evaluate how TH action controls this cell population. Pregnant rats were treated with propylthiouracil (PTU, 0.0003%) through the drinking water to induce TH insufficiency from gestational day 6 until postnatal day 14 (PN14). This exposure significantly reduced thyroxine (T4) and triiodothyronine (T3) in the sera of dams and pups during the postnatal period. Both T4 and T3 were also significantly reduced in the telencephalon of exposed neonates relative to controls. Next, frozen sections were collected from pup brains on PN2, the ventricular epithelium microdissected, and total RNA sequenced using Illumina HiSeq. We identified 271 differentially expressed genes in the ventricular epithelium of PTU-exposed animals (Q-values < 0.05). This included downregulation of Hairless (Hr) and Calcium Calmodulin Kinase IV (Camk4), consistent with our previous work. Intriguingly, we identified a 245-fold upregulation of an unannotated gene (Q < 0.001); bioinformatic analyses suggest that this is a paralog within the Alpha Mannosidase Class 1 family. This work supports the hypothesis that TH signaling controls vital processes in the ventricular epithelium and has identified novel pathways that hormone action may control. This work does not reflect US EPA policy.

**DNTS 37**

Impulsivity, working memory, and dopamine dynamics in Lphn3 knockout rats

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**Abstract**

Lphn3 gene variants predispose individuals to ADHD, predict ADHD severity and treatment response, and increase the likelihood of substance use disorder (SUD). We examined impulsivity, working memory, and dopamine (DA) dynamics in Lphn3+/− (knockout, KO) and Lphn3−/− (wildtype, WT) rats. Impulsive action was measured via a differential reinforcement of low rates (DRL) task, impulsive choice via a delay discounting (DD) task, working memory via a delayed spatial alternation (DSAT) task, and DA dynamics using in vivo fixed potential amperometry (FPA). For DRL, a single lever was presented, and rats had to wait 15 s between presses to earn a food reinforcer. For DD, a press on one lever resulted in immediate delivery of one food pellet, while a press on the other delivered three food pellets after a 0, 4, 8, 12, or 16 s delay. DA incorporated delays of 0, 5, 10, and 20 s between trials and required lever alternation between trials to earn a reinforcer. FPA measured stimulated DA release in medial prefrontal cortex (mPFC) and nucleus accumbens core (NAC). KOs had a lower ratio of reinforced:non-reinforced responses during DRL and a lower percent correct during DSAT indicative of impulsive action and working memory deficits, respectively. Surprisingly, the KOs exhibited less impulsive choice on the DD task. FPA data analysis revealed lower DA release in KOs in NAC, with no genotype difference in mPFC. These, results indicate Lphn3 deletion impairs executive function and alters DA release in a way that may contribute to the behavioral and pharmacological profile of ADHD and SUD. (Supported by The University of Memphis Faculty Research Grant Fund.)

**DNTS 38**

Prenatal cocaine exposure and other substance exposure: Pathways
to individual differences in stress reactivity in adolescence.

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Abstract

Prenatal cocaine (PCE) and other substance exposure (alcohol-PAE, tobacco - PTE) are often markers of continued adversities throughout childhood. We examined associations between substance exposure (cocaine, alcohol, tobacco) and individual differences in stress reactivity (both cortisol and stress ratings) in adolescence, sex differences, and if these associations were mediated via parenting from infancy to school age. The sample was 216 families recruited at birth (116 PCE, 100 control; 49% boys). Substance exposure was ascertained at delivery through self-reports, mother and infant urine toxicology, and maternal hair. Adolescent cortisol was assayed from saliva samples at four time points and adolescent stress ratings were collected at five time points before and after the Trier Social Stress Test. Unconditional Latent Growth Curve (LGC) models indicated that a quadratic model (increase from baseline to post-stressor and decrease 20 min after) fit the data well and significant variability in intercept and slopes of both measures. Conditional models revealed that PCE was associated with lower baseline cortisol among boys but not girls. The final conditional model indicated a direct association between PTE and greater increase in cortisol and stress ratings from baseline (pre-stressor) to post-stressor and lower baseline and greater recovery in adolescent stress ratings; a direct association between PAE and higher stress ratings at baseline; and an indirect association between PTE/PCE and greater cortisol recovery via maternal harshness. Results highlight continued role of prenatal exposure on adolescent stress reactivity, the importance of recovery via maternal harshness. Results highlight continued role of prenatal exposure on adolescent stress reactivity, the importance of recovery via maternal harshness. Results highlight continued role of prenatal exposure on adolescent stress reactivity, the importance of recovery via maternal harshness.

DNTS 40

Greater exposure to acetaminophen during the first trimester is associated with poorer language development in boys, but not girls, at 2 years of age.

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Abstract

Acetaminophen is an analgesic women are told they can take during pregnancy. However, recent studies suggest that acetaminophen use during pregnancy may impact language development. In a prospective cohort study, women reported number of acetaminophen doses during the first trimester. When children were 26–30 months of age, mothers completed the MacArthur-Bates Communicative Development Inventory (MB-CDI), a standardized parental report measure of language development, specifically productive vocabulary, mean length of utterance, and language complexity. Associations of acetaminophen use with MB-CDI outcomes were assessed using generalized linear models adjusted for maternal education, child sex, and child age at assessment for 211 children, 119 girls and 92 boys. Most mothers were White, had a college education, and an annual household income of $60,000 or greater. Most women took little acetaminophen during the first trimester (median 1 ± 1.04;95%CI 0–256 doses). Mean length of utterance was not associated with first trimester acetaminophen use. The trends observed for the dose by sex interactions for both vocabulary (β = 4.641, 95%CI −0.813–10.096, p = 0.095) and complexity (β = 0.370, 95%CI −0.027–0.768, p = 0.068) suggested there may be sex-specific differences in these two outcomes. When stratified by sex, greater acetaminophen use was associated with decreased language complexity in boys, but not girls (boys: β = −0.356, 95%CI −0.651–0.061, p = 0.019; girls: β = −0.015, 95%CI −0.184–0.153, p = 0.856). Similar yet non-significant trends were observed for vocabulary (boys: β = −3.839, 95%CI −8.809–1.131, p = 0.128; girls: β = 0.002, 95%CI −1.822–1.827, p = 0.997). These findings suggest that higher exposure to acetaminophen during the first trimester of pregnancy may have an adverse impact on early language development in boys. (Supported by ES022848, ES028607, OD023272, and RD83543401.)