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DNTS 1

Developmental toxicity of perfluorinated compounds: A voyage from animal studies to transfected cells

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Abstract

Perfluorinated chemicals (PFAAs) were created for their excellent properties as surfactants, with extensive industrial and consumer applications. However, those properties also resulted in environmental persistence and accumulation in wildlife and humans. These chemicals have been in large-scale production since the 1950s and are detected in the general populations of many countries, increasing the concern regarding the risks to health and the environment. Studies were undertaken at US EPA to assess the developmental toxicity of PFAAs in rats and mice. Developmental toxicity findings included pre- and postnatal lethality, postnatal developmental delay, growth deficits, delayed sexual maturation, and endocrine effects. Cross-foster and restricted periods of exposure studies demonstrated that in utero exposure alone and exposure late in gestation were key to producing the postnatal mortality. Considering the known carcinogenicity of PFOA and that hepatic responses included peroxisome proliferation via activation of peroxisome proliferator activated receptor-alpha (PPARα), the potential for that pathway to be important to the developmental toxicity was examined in our laboratory using PPARα knockout mice. These studies showed that an intact PPARα pathway was required for developmental toxicity of PFOA and PFNA, but other mechanisms were at play for PFOS. With a confirmed receptor pathway implicated in the molecular response, studies were undertaken using cells transfected with reporter constructs of human or mouse PPARα. The *vitro* studies evaluated the relative potency of PFASs and examined the potential additivity of mixtures. Both the studies using animals and the in vitro model advanced our understanding of the developmental toxicity of PFAAs. The animal models revealed species differences (rat vs. mouse), including relevant pharmacokinetic influences, confirmed the role of PPAR, but also showed that some members of the PFAS family can produce developmental toxicity even in absence of the PPAR pathway. The in vitro model of receptor activation was important for comparing larger numbers of PFAA compounds for relative activity and determination of complex and binary mixture behaviors. This research serves as a case study for the importance of including intact animal models and targeted in vitro models to understand the basis for a developmental response and to evaluate risk to human health.

DNTS 2

The gut-brain axis: A primer

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Abstract

The human gut is home to our enteric nervous system, two thirds of our immune system, and at least as many microbes as there are cells in our body. This dynamic metropolis of activity did not evolve merely for nutrient absorption and metabolism! The gut and its microbes respond to cultural habits, stressors, environmental exposures, and pathogens, and in turn relay their status to the central nervous system via hormonal, immune, and neural pathways. The implications of this bidirectional gut-brain axis for nervous system development and function throughout the lifespan are significant. Imbalances in the diversity and species composition of gut microbiota are associated with numerous developmental, psychiatric, and neurodegenerative disorders. Growing efforts to unravel the mechanisms by which the gut-brain axis mediate health and disease have the potential to revolutionize treatment and prevention of many of these disorders.

DNTS 3

Immune and microbiota profiles in children with ASD and co-morbid GI symptoms

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Abstract

Gastrointestinal (GI) symptoms in children with autism spectrum disorders (ASD) have long been reported by parents and in the recent decade studies investigating the link between ASD and GI issues have reported altered microbiome profiles, pro-inflammatory responses and

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impaired intestinal permeability in children with ASD and GI symptoms (ASD^{GI}). However, few studies have compared these findings to ASD children without GI issues (ASD^{NoGI}) or similarly aged typical developing children (TD^{NoGI} and TD^{GI}). Therefore, we sought to determine whether there are biological signatures in terms of immune dysfunction and microbiota composition in ASD^{GI}. For the study, children were enrolled in one of four groups: ASD^{GI}, ASD^{NoGI}, $TD^{GI,}$ or TD^{NoGI}. Peripheral blood mononuclear cells (PBMC) were isolated from the blood, stimulated and assessed for cytokine production; stool samples were analyzed for microbial composition. Following Toll-Like receptor (TLR)-4 stimulation, ASD^{GI} PBMC produced increased levels of mucosa-relevant cytokines: IL-5, IL-15 and IL-17 compared to ASD^{NoGI}. The production of the regulatory cytokine TGFb1 was decreased in ASD^{GI} compared to both ASD^{NoGI} and TD^{NoGI}. Analysis of the microbiome at the family level revealed differences in microbiome composition between ASD and TD children with GI symptoms; furthermore, a predictive metagenome functional content analysis revealed that pathways were differentially represented between ASD and TD subjects, independently of the presence of GI symptoms. Overall our findings suggest that children with ASD who experience GI symptoms have an imbalance in their immune response, possibly influenced by or influencing metagenomic changes, which may contribute to their symptoms and clinical outcome.

DNTS 4

The microbiota-gut-brain axis and neurodevelopment in animal models

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Abstract

There is a growing body of literature recognizing the importance of the intestinal microbiota in the development and function of the central nervous system. Animal studies ranging from those that use germ-free mice (animals that are bred and maintained in the total absence of bacteria), mice that have been treated with antibiotics during various stages in development, pre- and pro-biotic feeding studies, fecal microbial transplant experiments, as well as work that has examined the impact of pathogenic infection have all indicated that the bacterial status of the intestinal lumen has far-reaching and long-term effects on brain neurochemistry, anatomy and behaviour. Within this context, data will be presented from recent animal studies conducted in our laboratory that involve treating mice at various critical developmental windows with low-dose oral penicillin and demonstrate long term, sex-dependent changes to the intestinal microbiome, adult behaviour, brain neurochemistry and gut function. Lastly, I will discuss the hypothesis that specifically targeting the intestinal microbiome will provide promising and novel therapeutic opportunities in the treatment of psychiatric disease.

DNTS 5

The role of the microbiota in disorders of the gut-brain axis

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Abstract

In recent times the influence of the gut microbiota in health and disease has come to the fore. Accumulating data now indicates that central nervous system function and behaviour can be influenced by the gut microbiota. The bi-directional communication between the brain and the gut includes neural, endocrine and immune pathways and understanding the mechanisms underlying such communication especially during early life may be crucial for gaining an understanding of certain diseases. We have used strategies to investigate the brain-gut-microbiota axis including studying the effect of probiotic bacterial strains, which can generate neuroactive metabolites, on behaviour and biochemical responses. We have also assessed the impact of antibiotics in early life and at other time windows. Interruption of the colonization of the neonatal gut with antibiotics or adverse events confers a greater risk of developing disorders of the brain-gut-microbiota axis including psychiatric disease and gastrointestinal disorders. Growing up with a complete absence of microbiota, (germ free) also leads to altered signaling within the axis including increased pain responses, anxiety as well as changes in brain neurotransmitters. Colonization studies in these animals indicate that there is a neurodevelopmental time window where introduction of bacteria normalizes many of the anomalies seen. Also, specific strains of *Lactobacilli* and *Bifidobacteria* have decreased pain sensitivity, depressive and anxiety scores as well as reducing the stress hormone corticosterone when administered to rats with vagotomy preventing some of these beneficial effects indicating that the vagus nerve is essential to the signaling. The emerging concept of the microbiota-brain-gut axis suggests that modulation of the gut microbiota may be an amenable strategy for developing novel therapeutics for disorders of the brain-gut axis ranging from complex central nervous system disorders to functional bowel problems.

DNTS 6

Mitochondrial bioenergetics and gut metabolites: Implications for human disease

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Abstract

Gastrointestinal microbiome-derived metabolites play a significant role in human health and can modulate the nervous system. We are investigating the role of metabolites in cell models for human disease to clarify mechanisms of action and present two examples. (1) The SH-SY5Y neuroblastoma cell line is used to model catecholaminergic neurons in Parkinson's disease (PD). The objectives were to determine the role of the amino acid derivative indole in regulating cell bioenergetics. Indole is a primary metabolite produced by gut bacteria from tryptophan. Differentiated SH-SY5Y cells were treated with indole (0.1–10 mM) for 6, 24, and 48 h; cytotoxicity was observed in a dose and time-dependent fashion. In a mitochondrial stress test (direct injection), both 5 mM and 10 mM indole lowered basal oxygen consumption rates, and 10 mM reduced maximal respiration of SH-SY5Ys. Studies will continue to examine the role of indole on cellular mechanisms of toxicity. (2) The small chain fatty acid butyrate was investigated in a rat model for neurogenic hypertension, a disease that can accompany PD. Oxidative phosphorylation of primary astrocytes derived from spontaneously hypertensive rats did not respond to butyrate as that observed for normotensive animals. Further, butyrate regulated genes associated with oxidative damage and inflammation differently in astrocytes from control and hypertensive animals, suggesting aberrant responses in cells to butyrate. These two examples demonstrate that microbial metabolites modify cell bioenergetics and molecular responses. The microbiome is intertwined with disease etiology, and further understanding is needed to discern therapeutic targets that modify the gut-brain-immune axis to alleviate neurological disease.

DNTS 7

Microbiome-based translational strategy in Parkinson's disease

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Abstract

Host-microbe interactions in the gastrointestinal (GI) tract contributes to individual susceptibility to disease. In Parkinson's disease (PD), GI disturbances are one of the earliest non-motor symptoms. To better understand the cellular-molecular mechanisms underlying early GI dysfunction in PD, we characterized GI dysfunction and intestinal inflammation using the MitoPark transgenic mitochondria-defective mouse model of PD based on specific inactivation of TFAM in dopaminergic neurons. We found that GI dysfunction is one of the earliest non-motor symptoms observed in this model, seen as early as 8 weeks with significantly reduced transit times from 12 weeks onwards. MitoPark mice also exhibited constipation and increased intestinal inflammation. These results suggest that MitoPark mice recapitulate GI dysfunction in PD chronologically and can offer an attractive model for pre-clinically assessing drug efficacy. In terms of translational research, recent research has elucidated gut microbiome interventions for preventing or treating gut disorders, however, microbiome-based therapeutics in PD has never been explored. Herein, we genetically engineered a novel microbiome-based platform for non-pulsatile delivery of L-DOPA as a probiotic alternative to oral tablet-based dopamine replacement therapy that's inherently pulsatile and prone to L-DOPA-induced dyskinesia. Our results demonstrate that orally delivered L-DOPA-producing *E. coli* readily colonize the mouse gut, achieving steady-state plasma L-DOPA levels that correspond to clinically effective plasma levels in PD patients. Our treatment increases L-DOPA and dopamine levels in MitoPark mouse brains and alleviates their locomotor deficits. This talk highlights the GI dysfunction in a transgenic mouse model as well as unique microbiome-based dopaminergic therapeutics for treating PD. (Supported by NIH grants NS100090 and ES027245.)

DNTS 8

Endocrine Disrupting Compounds Change Behavior and Gene Expression Over Multiple Generations

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Abstract

Our lives are flooded with endocrine disrupting chemicals (EDCs). It is well-established that developmental exposure to some EDCs (bisphenol A, vinclozolin, phthalates etc.) have multi- and transgenerational effects on reproductive function, behavior and other aspects of physiology. I will illustrate this with examples from our work, and others, and explore potential mechanisms for these effects. The data are important because, despite efforts to restrict use of EDCs, transgenerational actions persist.

DNTS 9

Organic chemicals in the marine environment and their relevance to human health

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Abstract

More than 3.5 billion people depend on the ocean for their primary source of food, and by the year 2040, that number is anticipated to double to 7 billion. Beyond food, we our health is intimately connected to the ocean in a number of ways, including new medicine, technology, and recreation. However, there is a downside as well on the oceans to our health through the exposure to pathogens, natural toxins and toxicants, and manmade pollutants. In recent decades, climate change has brought with it increased risks of toxic marine blooms and affected the pathways of pollutant inputs into the ocean, affecting modes and extents of exposure through consumption of marine resources or through the atmosphere. This presentation will highlight work at UC San Diego, along with their collaborators, on the origin and transport of toxins, toxicants, and pollutants in the marine environment.

DNTS 10

Research in the time of hurricanes: Puerto Rico 2017

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Abstract

In September 2017 two hurricanes, Irma and Maria, hit Puerto Rico (PR) and the Caribbean within two weeks. These category 4 storms cause massive destruction in Puerto Rico and many Caribbean island and a large death toll during and following the event. All utilities were severely impacted, including electricity, water, communication and transportation. Many lost their home, and this created the largest disaster response ever faced by the US Federal Emergency Management Administration in its history. In Puerto Rico, research centers, some industries, and academic centers that were resilient or mostly resilient during the disaster became locales for community support. The PROTECT and CRECE Centers examine the role of environmental exposures during pregnancy on the high rate of preterm births in PR and the impact of prenatal exposures on children's neurodevelopment. Located at the exit point of Hurricane Maria in northern PR, the area sustained major damage, our field office lost electricity and operated on emergency generators for several weeks. Our team was directly impacted by the hurricane, but no one was hurt. Two staff lost their home, and six reported no water service and having fetching water from local rivers or wells. Loss of wireless communication made contact with study participants very difficult. The local community health centers became the key point of contact with study participants. Within two weeks most had been contacted, but some moved to the US mainland and did not return. We quickly learned about the major unmet needs: water filters for safe drinking water, solar chargers, small size diapers for premature babies, toiletries, and many others. A coalition of academic centers in the US mainland and PR was formed very quickly and shipped many of the articles needed. Our field office became the distribution center for our area for direct relief to local communities and community health centers. Large water filtration systems were installed at schools and health centers. Thanks to the major support, PROTECT and CRECE were able to resume operations of recruitment and follow up of participants within a month and has become more resilient for future events.

DNTS 11

Domoic acid developmental neurotoxicity: New insights from human and animal studies

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Abstract

An example of ocean-borne risks to pregnant women and their children is found in neurotoxins generated from harmful algal blooms. In particular, Domoic Acid (DomA) produced by pseudo-niche has been identified as the cause of Amnestic shellfish poisoning. A number of studies, mostly in rodents, have addressed the issue of DomA toxicity and neurotoxicity upon perinatal exposure. Overall, DomA does not appear to act as a classic teratogen, however, both pre- and post-natal exposure to DomA have been found to cause hippocampal damage, seizure disorders, and persistent behavioral abnormalities. Most importantly, such effects are seen at doses lower than those required to cause neurotoxicity in adult rodents. Pre-natal oral exposure to DomA was associated with subtle but long-lasting behavioral impairments in the locomotor and cognitive domains. Overall, these studies found behavioral, electrophysiological and morphological changes in mice exposed in utero even to a single, low dose of DomA, indicating that this toxin passes the placental barrier to affect the developing brain. Our pharmacokinetic studies show that after oral exposure, DomA can accumulate in fetal tissue in utero, thus reinforcing the importance of determining the neurodevelopmental potential for this toxin. Recent reports in primates confirm these observations from oral exposures and suggest even lower levels of exposure result in altered neurodevelopment. Limited assessments in human populations suggest the need for additional qualitative and quantitative epidemiological assessments. Endpoints of interest across species include tremor, learning and memory, and possibly autism – like neurodevelopmental impacts.

DNTS 12

What can fish tell us about developmental neurotoxic risks of the sea?

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Abstract

Zebrafish provide an important complementary model for neurobehavioral teratology. Zebrafish are vertebrates with a complexly organized brain and sophisticated behavioral function. Their clear chorion provides an unrestricted view of the organizational processes of brain development. Cellular reporter systems elucidate processes of proliferation, differentiation, migration and circuit formation in brain development. A variety of molecular tools enable the study of the genetic bases of neurodevelopment and its perturbation. Behavioral function can be readily characterized at larval, juvenile, adult and aged life stages. We and other researchers have developed and validated sensitive and reliable tests of zebrafish sensorimotor response, emotional function, cognition and social affiliation, which can be assessed with computerized video tracking analysis. Zebrafish are quite economical to use, so a much greater number of compounds can be evaluated for neurobehavioral teratological effects for the same cost compared with rodent models. Zebrafish can serve a useful complementary role in the spectrum of neurotoxicity models from in vitro cell-based assays through invertebrate models such as *C. elegans* and drosophila at more elementary levels to mice, rats, monkeys and humans at more complex levels of analysis. We have successfully used zebrafish to characterize the neurobehavioral teratology of pesticides, metals, flame retardants, polycyclic aromatic hydrocarbons, drugs of abuse, therapeutic drugs and nanoplastic particles. The use of a battery of behavioral tests to assess short and long-term neurobehavioral effects provides characterization of the specificity of the neurobehavioral effects in the dose range below that that causes increased lethality or dysmorphogenesis. The mechanisms of the specific behavioral impairments can then be tracked back to converge with the incipient effects of the exposures on neurodevelopment. (Supported by ES010356.)

DNTS 13

From neural tube defects to environmental contaminants

Hrubec T

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Abstract

My initial research focused on development of the immune system. This reversed 180° into studying the role of the immune system in directing development. That transitioned into teratology when I began to investigate role of the maternal immune function in causing and preventing neural tube defects (NTDs). When my controls developed NTDs, I had to figure what exposure was causing the birth defect, and my research focus switched environmental causes of birth defects. My current research focuses on the toxicity of quaternary ammonium compounds (QACs). This class of chemical is common in household, commercial and industrial products. Approximately 80% of individuals have detectable concentrations in their blood. Long considered safe, my research was the first to demonstrate that QAC exposure in mice to either females or males resulted in NTDs. QAC exposure also resulted reproductive toxicity in both males and females. Some of the reproductive effects carried over into the next generation. Additional work has demonstrated QAC induced immunotoxicity as well. Again, some of the effects were seen in subsequent generations. Other research labs have independently found that QACs inhibit cholesterol synthesis, inhibit mitochondrial function, and are implicated in inflammatory bowel disease. Collectively, these studies indicate that QACs are not safe, and could be contributing to many disease processes we see today.

DNTS 14

Environmental influences on child health outcomes (ECHO): A national research program

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Abstract

Environmental Influences on Child Health Outcomes (ECHO) is an NIH-Office of the Director nationwide program of interconnected observational and intervention research whose mission is to enhance the health of children for generations to come. In its observational research, ECHO leverages nearly 70 longitudinal cohorts of mothers and children into what will become a national research resource during the next five years: a single ECHO-wide Cohort comprising ~50,000 children and

their families. Its overall goals are to examine how a broad array of early environmental factors interact with biology and genetics to influence outcomes of pregnancy, common childhood conditions such as obesity, asthma, and autism spectrum disorders, and positive attributes of child health. The vision for ECHO cohorts is to identify and quantify modifiable factors—especially in early developmental periods—that lead to worse or better health outcomes among a diverse sample of our nation's children as they grow and develop through the life course. By doing so, the cohorts will develop evidence that informs policies, practices, and programs. ECHO encourages researchers to leverage this national resource and bring innovative ideas to studying early exposures on childhood diseases, conditions, health, and well-being. In addition to the pediatrics cohorts, there is an intervention arm to prevent or treat pediatric conditions that is the purview of ECHO's IDeA States Pediatric Clinical Trials Network (ISPCTN), a partnership with *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) and National Institute of General Medical Sciences. The priorities of ISPCTN are to create evidence to influence pediatric practice, programs, and policies through providing access to state-of-the-art clinical trials among children from rural and underserved backgrounds. An ECHO pediatric cohort grantee will provide the perspective of a site and how ECHO is being leveraged to promote site-specific science in the effect of prenatal exposures on the autonomic nervous system, as well as help build research infrastructure and resources in South Dakota.

DNTS 15

Choline and brain development: An overview

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Abstract

Choline is an essential nutrient required for multiple cellular functions, such as the formation of the neurotransmitter acetylcholine, synthesis of phosphatidylcholine and sphingomyelin which are important components of membranes, and production of betaine—a metabolite involved in the generation of methyl groups necessary for DNA, RNA, and protein methylation. The importance of adequate choline supply for brain development has been well established. If choline availability is increased above adequate during a critical period of embryonic development in rodents, the offspring perform better on memory-related tasks, such as Morris Water maze and radial arm maze, throughout life. In people, higher maternal intake of choline during pregnancy is associated with better information processing speed and better performance on memory tests in the children of these mothers. Insufficient supply of choline during development increases the risk for neural tube closure defects in people and leads to aberrant genesis of the cerebral cortex and hippocampus in rodent models. Under low choline conditions in fetal rodent brain, fewer neural progenitor cells are formed, and though enough are available to form early born layers of cortex and hippocampus, there are deficient numbers to form the later born layers. Some of the molecular mechanisms whereby choline affects brain development have been recently described. In the developing cerebral cortex and hippocampus, choline acts to modulate neural progenitor cell proliferation and differentiation by regulating the synthesis of the epidermal growth factor receptor (EGFR)—an essential component of a signaling pathway involved in cortical neurogenesis. Choline availability controls EGFR synthesis by suppressing expression of a specific miR, miR-129-5p, which is upregulated under low choline conditions. This miR binds to the 3′ untranslated region (UTR) of *Egfr* and inhibits its protein synthesis. We suggest that choline administration enhances neural progenitor cell self-renewal with resulting increased neural progenitor cell reserves, thereby creating a developing brain that is more resilient to the effects of toxic exposures that normally affect brain development.

DNTS 16

Maternal choline supplementation: A therapy for Down Syndrome with population-wide cognitive benefits

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Abstract

Down syndrome (DS) is estimated to affect ~400,000 people in the USA, with ~5000 infants born each year with the disorder. DS is caused by the triplication of human chromosome 21 and is the primary genetic cause of intellectual disability. Although DS can be diagnosed prenatally, there are no effective treatments to lessen the intellectual disability or to prevent the early onset of Alzheimer's disease, now known to occur in most individuals with DS. A potential therapeutic strategy emerging from our studies of a trisomic mouse model of DS is to supplement the maternal diet with additional choline, an essential nutrient with many critical roles in fetal neurodevelopment. Our studies demonstrate that maternal choline supplementation (MCS) markedly improves spatial cognition and attentional function in the trisomic offspring, and also protects basal forebrain cholinergic neurons and adult hippocampal neurogenesis. It is currently unknown whether similar benefits would be derived in human DS pregnancies, although anecdotal data from women who have increased choline intake due to our rodent studies are encouraging. The convergence of several factors makes it challenging to conduct a clinical trial of MCS in human DS pregnancies: 1) the majority of DS births are not diagnosed prenatally; 2) prenatal intervention is likely to be critical for MCS benefits to be observed; and 3) most early DS diagnoses end in abortion. Thus, the only way for MCS to widely offer protection to DS individuals would be for all women to increase choline consumption during pregnancy—an intervention likely to benefit typically developing children as well. Currently ~90% of pregnant women consume substantially less than the recommended amount of choline, and choline is absent from most prenatal multivitamins. Rodent studies and a small controlled human intervention study conducted in our lab indicate that choline supplementation during pregnancy is entirely safe and would substantially improve attention, memory and problem-solving for all children. Although confirmation by a larger study is needed, these findings suggest that the addition of choline to standard prenatal vitamin regimens would be beneficial to all children and would also provide an early effective intervention for individuals with DS.

DNTS 17

Can choline ameliorate the neurodevelopmental effects of prenatal exposure to alcohol?

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Abstract

Despite the fact that complete avoidance of alcohol throughout pregnancy has long been the official recommendation by the US Surgeon General and in most other countries, a substantial proportion of pregnancies continue to be exposed to alcohol, particularly in the early weeks of gestation prior to pregnancy recognition. Thus, a need to identify interventions that could help ameliorate the

neurodevelopmental effects of prenatal alcohol is pressing. Animal data have provided compelling evidence that prenatal and postnatal choline levels and choline supplementation can attenuate the adverse effects of prenatal alcohol. This work has shown clear differences in the outcomes affected depending on the timing of the supplement intervention. Findings in human studies regarding the same intervention have been less consistent. Two prenatal intervention trials have been completed, using two different supplementation doses in different populations. The trial using the higher dose demonstrated significant improvement on selected infant performance measures in treated infants compared to infants with similar prenatal alcohol exposure but no choline supplementation. Two postnatal intervention trials have been completed in children ranging from preschool through school age who were prenatally exposed to varying levels of alcohol. Neither showed significant benefit in improving performance post-baseline; however, there was some evidence that the very youngest age group was more sensitive to choline. An ongoing trial in that younger age group is awaiting results. Given that alcohol-exposed pregnancies are likely to continue to occur, choline still holds promise in a field where few interventions have been proven to help ameliorate alcohol-related neurodevelopmental deficits.

DNTS 18

Perinatal choline supplementation improves social behavior in rodent models of Autism

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Abstract

Autism is a neurodevelopmental disorder with multiple genetic and environmental risk factors. Choline is a fundamental nutrient for brain development and high choline intake during prenatal and/or early postnatal periods is neuroprotective. We examined the effects of perinatal choline supplementation on three rodent models of autism: 1) prenatal exposure to the inhibitor of the histone deacetylase, valproic acid (VPA), in rats; 2) BTBR T + Itpr3tf /J (BTBR) mouse model; and 3) contactin associated protein-like 2 (Cntnap2) mutant mouse model. We evaluated social behavior in these animals as well as assessed anxiety and repetitive behaviors. Prenatal exposure of rats to VPA causes multiple anatomical and behavioral abnormalities similar to those seen in autistic patients. Perinatal choline supplementation dramatically improved VPA-associated behavioral deficits in social interaction in males in both adolescence and adulthood using the three-chamber test for social interaction. Prenatal VPA exposure reduced mRNA and protein levels of serotonin 5-HT1A and 5-HT2A receptors in the hippocampus of male rats, whereas choline supplementation prevented this reduction. The protein levels of the serotonin transporter 5-HTT/SLC6A4 were also decreased in the hippocampus of VPA-exposed males but were rescued by choline supplementation. BTBR mice are an inbred strain that exhibit many of the behavioral anomalies observed in patients with autism, including deficits in play and social approach and excessive self-grooming behavior. Cntnap2 mice lack functional CNTNAP2 protein and show stereotypic motor movements, behavioral inflexibility and impaired communication and social behavior. In both mouse models, choline supplementation significantly decreased digging behavior in a marble burying test, increased the number of open arm entries and time spent in open arms in the elevated plus maze, but had no effect on locomotion as determined in the open field test. Choline supplementation also improved impairments in social interaction in the three-chamber test in these mice. Overall, these results suggest that high choline intake during early development can prevent or dramatically reduce deficits in social behavior and anxiety in rodent models of autism, revealing a novel strategy via maternal and infant nutrition for the treatment/prevention of autism spectrum disorders.

DNTS 19

How Targeted Choline Supplementation Reverses the Negative Neurocognitive and Genomic Impacts of Early-Life Iron Deficiency

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Abstract

Iron is an essential nutrient for brain development involved in neuronal energy metabolism, neurotransmitter synthesis, myelination and regulation of synaptic plasticity genes. Late gestational/early postnatal iron deficiency particularly targets the developing hippocampus, the brain area that mediates recognition and spatial memory. Newborns with brain iron deficiency have compromised recognition memory at birth and neurocognitive deficits that persist into adolescence in spite of iron repletion. Animal models reveal a hypometabolic state in hippocampal pyramidal neurons, characterized by lower neuronal oxygen consumption rates and mitochondrial dysmotility. These lead to reduced dendritic arborization, altered expression of synaptic plasticity genes and epigenetic modifications to chromatin, largely through iron-dependent histone demethylase activity (JARIDs). Rodents that were iron deficient only during gestation and early postnatal life have impaired recognition memory behavior throughout their lifespan in spite of iron repletion in the newborn period, suggesting an early-life programming effect. Choline is a nutrient that improves hippocampal structure and function in normal and pathological conditions (e.g., Fetal Alcohol syndrome rat, Down's mouse, Rett's mouse) when provided during one of two critical periods in the rodent; mid-gestation and during the period of rapid postnatal hippocampal development. Provision of choline in either critical time period to a rat model of gestational dietary iron deficiency reversed hippocampal structural deficits, reversed JARID-induced repression of specific synaptic plasticity genes (e.g., BDNF) and genome-wide networks, improved mitochondrial oxygen consumption and partially rescued recognition memory deficits in the adult animal. The mechanism(s) by which choline reversed the impacts of early life iron deficiency remain unclear. However, choline appears to modify iron-deficiency induced epigenetic changes potentially through its role as a methyl donor and it appears to improve neuronal mitochondrial efficiency. Since many populations around the world have difficulty maintaining iron sufficiency during pregnancy and early childhood, choline supplementation may be an adjunct strategy to protect the brain from a life-time of structural and functional damage induced by early life iron deficiency.

DNTS 20

Monkeys, memories, and Merle

Buffalo E

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Abstract

In this talk, I will share memories of Merle Paule as my mentor at the National Center for Toxicological Research in the early 1990's. Merle inspired my career in science and instilled a deep appreciation for the value of the monkey model in understanding cognition. Here, I will discuss recent work from my laboratory that has examined neural activity in the hippocampus and adjacent entorhinal cortex in monkeys

performing behavioral tasks including free-viewing of complex natural scenes and memory tasks in a virtual environment. I will also discuss new research involving chronic, large-scale recordings throughout the primate brain and other areas of opportunity for future research to further our understanding of mechanisms in the hippocampal formation that support memory formation.

DNTS 21

Regions of the basal ganglia and primary olfactory system are most sensitive to the initial and more prolonged increases in neurodegeneration seen in 7 d old rat pups after 6 h of sevoflurane anesthesia

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Abstract

There were two initial objectives of this study that evaluated the neurotoxic effects at 2, 24, and 72 h after a 6 h bout of sevoflurane-induced anesthesia (SIA), carried out under fully oxygenated conditions in PND 7 rats. The first was to determine the brain regions where the most intense neurodegeneration (above basal levels) occurred at the three-time points after SIA and the second was to quantify the regional neurodegeneration at those same time points. This effort aided in determining whether there were any regions in which a "secondary" wave of neurodegeneration was present at 24 h or 72 h and whether specific neuronal pathways or networks are more susceptible. FJc and TUNL labeling were initially used to detect regions with high levels of neurodegeneration in 25 μm formalin fixed forebrain sections. Subsequently, FJc was used to detect the total number of degenerating neurons per region. This value was "normalized" using the total area (in mm^2) of the region within the brain section being evaluated. The most intense SIA-related neurodegeneration was found in specific regions of the primary olfactory system including hippocampal rudiments (IG), the posterior dorsomedial hippocampus (CA1), the stria terminalis and the shell of the nucleus accumbens. Significant increases in neuro-degeneration also occurred in the basal ganglia (caudate putamen, globus pallidus and associated thalamic regions) and motor cortex. Sevoflurane-induced neurodegeneration was minimal or not detected in the ventral tegmentum, most of the hypothalamus, and limbic regions of the frontal cortex. In most of the regions where neurodegeneration was increased 2 h post sevoflurane, the levels returned to near control apoptotic levels by 24 h. Also, there were no regions where there was a significant increase in neurodegeneration at either 24 or 72 h in the absence of an increase at 2 h. Thus, there was no evidence of a "secondary wave" of neurodegeneration in most of the regions affected by SIA. However, in IG, CA1, stria terminalis, globus pallidus and substantia nigra there was evidence of either a prolonged period of neurodegeneration, lasting 24 h or more, or a secondary degenerative event occurring after the 2 h time point. The specific regional increases in neurodegeneration due to SIA would imply that behavioral deficits that might result from such exposure could involve impairment of olfaction, learning/motivation, and/or locomotor capabilities.

DNTS 22

From the lab to the clinic and back again: The relevance of behavioral endpoints in nonhuman subjects to clinical outcomes in humans

Chelonis J

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Abstract

For over 30 years Dr. Merle Paule has led a collaborative research effort to demonstrate the validity of a battery of behavioral measures that has been extensively used at the National Center for Toxicological Research to determine the pharmacological underpinnings of cognition and perform toxicological assessments in nonhuman primates. We have taken a three-pronged approach to demonstrate the clinical relevance of these tasks in humans, and that comparable data can be generated across species. First, we have directly compared performance of adult monkeys and children on these tasks. Our data reveal that for most tasks and endpoints, younger children perform worse than monkeys, while older children perform better, with the age of cross over being task and endpoint dependent. Secondly, we have explored how the presence of various psychological disorders, the administration of drugs, and exposure to various toxins affects performance on these tasks in humans. Performance on these tests is influenced by levels of depression and anxiety and is sensitive to medication status in children with Attention Deficit/Hyperactivity Disorder. Lastly, we are conducting research to determine the relationship between the endpoints generated from this task and those generated by measures that are typically used to assess clinically relevant endpoints in children. Although there is still much work to be done regarding the validation of these behavioral tests, the approach we have taken can serve as a model for how laboratory tests can be validated for assessing and predicting clinical outcomes. (Supported by NCTR protocol E07355).

DNTS 23

Celebrating with Dr. Merle Paule

Slikker W

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Abstract

We have gathered to celebrate the tremendous research accomplishments of Dr. Merle Paule. Dr. Paule received his B.S. in Biochemistry and his Ph.D. in Pharmacology and Toxicology at the University of California at Davis. In 1983 he began work at the FDA's NCTR, where he was the Director, Division of Neurotoxicology from 2005 to 2018. In 2000, he attained certification as one of FDA's Senior Biomedical Research Scientists. Dr. Paule has played a major role in developing an automated system for monitoring multiple complex brain functions in nonhuman primates, children, and rodents. Utilization of similar behavioral tasks across species serves to facilitate the interspecies extrapolation of data and, thus, the risk assessment process. He also oversaw the development of NCTR's bio-imaging capability including two PET/CT and two MRI instruments used to visualize toxicological insults in animals. Dr. Paule has supervised 15 undergraduate interns, served on 12 doctoral dissertation committees and trained 16 Postdoctoral fellows. Merle is past President of the Behavioral Toxicology Society, the Neurobehavioral Teratology Society and the Neurotoxicology Specialty Section of the Society of Toxicology and Associate Editor for *NeuroToxicology* and *Neurotoxicology and Teratology*. He has published over 225 research articles and 30 book chapters and held Adjunct Professorships at the University of Arkansas for Medical Sciences in the Departments of Pharmacology and Toxicology and in Pediatrics. In 2017, he was selected as an Arkansas Scholar by the Arkansas Research Alliance. Dr. Paule is a Fellow in the Academy of Toxicological Sciences and in the International Behavioral Neuroscience Society.

DNTS 24

Domoic acid exposure in pregnancy produces behavioral neurotoxicities in mothers and offspring

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Abstract

Domoic acid (DA) is a potent neurotoxin, produced by marine algae and found in seafood worldwide. In recent years, DA algal blooms have been increasing in frequency and severity, and there has subsequently been a growing interest in health effects related to low-level, chronic exposure in sensitive populations, especially developing infants and children. To assess the neurodevelopmental effects of prenatal exposure to DA, 32 *Macaca fascicularis* were orally exposed to 0.00, 0.075, or 0.150 mg DA/kg/day throughout pregnancy. These doses were chosen to bracket the current estimated tolerable daily intake (0.075–0.1 mg/kg). We observed a subtle behavioral neurotoxicity in the mothers; increased intention tremors when performing a reaching task ($p = 0.02$). Tremors were significantly correlated with decreased structural integrity in white matter, as shown with MRI ($p = 0.048$). Like the dams, a subset of prenatally exposed infants demonstrated tremors when performing a reaching task during early postnatal development. To better understand the neuropathological underpinnings, follow-up at 1 year was conducted with sedated EEGs and MRIs in a subset of offspring. These results, however, did not reveal any exposure related changes in power or coherence on EEGs or in brain structural changes as measured with MRI. Overall, we found that low-level, chronic exposure to this common neurotoxin at levels near the current regulatory limit result in behavioral neurotoxicities in pregnant macaques and their infants, but the underlying neuropathological changes are presently unknown.

DNTS 25

Prenatal exposure to PCBs in Cyp1a2 knock-out mice interferes with F1 fertility, impairs long-term potentiation, reduces acoustic startle, and impairs conditioned freezing contextual memory with minimal transgenerational effects

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Abstract

Polychlorinated biphenyls (PCBs) are toxic environmental pollutants. Humans are exposed to PCBs mixtures via contaminated food or water. PCB exposure causes adverse effects in adults and after exposure in utero. PCB toxicity depends on the congener mixture and *CYP1A2* gene activity. For coplanar PCBs, toxicity depends on ligand affinity for the aryl hydrocarbon receptor (AHR). Previously, we found that perinatal exposure of mice to a 3-coplanar/4-noncoplanar PCB mixture induced deficits in novel object recognition and trial failures in the Morris water maze in *Cyp1a2*$^{-/-}$::*Ahr*^{b1} C57BL6/J mice compared with wildtype mice (*Ahr*^{b1} = high AHR affinity).Here we exposed gravid *Cyp1a2*$^{-/-}$::*Ahr*^{b1} mice to a PCB mixture on embryonic day 10.5 by gavage and examined the F1 and F3 offspring (not F2 s). PCB-exposed F1 mice exhibited increased open-field central time, reduced acoustic startle, greater conditioned contextual freezing, and reduced CA1 hippocampal long-term potentiation with no change in spatial learning or memory. F1 mice also had inhibited growth, decreased heart rate and cardiac output, and impaired fertility. F3 mice showed few effects. Gene expression changes were primarily in F1 PCB males compared with wildtype males. There were minimal RNA and DNA methylation changes in hippocampus from F1 to F3 with no clear relevance to the functional effects. F0 PCB exposure during a period of rapid DNA de/re-methylation in a susceptible genotype produced clear F1 effects with little evidence of trans-generational effects in the F3 generation. While PCBs show clear developmental neurotoxicity, their effects do not persist across generations for effects assessed herein.

DNTS 26

Chronic Steady Low-Dose Prenatal Diazinon Exposure of Rats Causes Locomotor Hyperactivity, Increased Risk-taking Behavior and Cognitive Impairment

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Abstract

Diazinon is a widely-used organophosphate (OP) pesticide. It can with pulsatile exposure during neonatal development cause long-term neurobehavioral impairment in rats. This experiment evaluated in Sprague-Dawley rats the persisting behavioral effects of chronic zero order kinetic low-level infusion of 0, 0.5 and 1.0 mg/kg/day of diazinon throughout pregnancy by osmotic minipumps ($N = 13–15$ litters per condition). One male and one female from each litter was assessed with a battery of behavioral tests that continued from four weeks of age into adulthood. Litter was used as the unit of variance for the analysis of variance test of significance, with sex as a within litter factor. Diazinon dose was the between-subjects factor and time within session or sessions were repeated measures. Chronic steady diazinon exposure from pre-mating until the postnatal period caused a significant ($p < 0.05$) increase in percent of time spent on the open arms of the elevated plus maze, an index of an increase in risk taking behavior. Prenatal diazinon exposure also caused during adolescence a significant ($p < 0.05$) degree of hyperactivity in the Figure-8 apparatus during the early part of the hour-long test session. This effect had dissipated by the time the rats reached adulthood. Diazinon exposure also caused a significant impairment in novel object recognition, a test of cognitive function. The 1 mg/kg diazinon dose ($p < 0.05$) showed significantly less preference for the novel vs. familiar object than controls during the first five minutes of the novel object recognition test. These behavioral tests and companion neurochemical assessment will be used to guide future tests of drug treatment to alleviate the persistent behavioral impairment due to prenatal exposure to diazinon. (Supported by ES010356.)

DNTS 27

State-of-the art statistical solutions for litter effects: Litter as a random effect in generalized linear regression models

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Abstract

Cross-species comparisons of biological systems and mechanisms are a foundation of teratological research, and comparisons of toxicant

effects in rodent and human offspring are an essential component of developmental neurotoxicological research. Differences in reproductive biological characteristics however can confound cross-species comparisons. For developmental neurotoxicology, one issue has concerned managing issues associated with multiparous pregnancies. Since at least the 1970's, researchers have noted two main issues that arise when all pups from all litters are included in statistical analyses, broadly referred to as "litter effects." These include excessively large sample size producing statistically significant *p*-values for non-meaningful effect sizes; and "intralitter likeness," which violates the critical statistical assumption of observation independence. A historical review of the literature revealed that the long-preferred approach was to model litter as a random effect in regression models. This solution addressed both types of litter effects. Limitations in computing capacity and software development prior to 2000 however appear to have led researchers and guidance authorities to endorse the alternative method of using a litter-based design. Current statistical software combined with remarkable laptop computing capability have the potential to transform how this methodological issue is addressed going forward, while perhaps adding a new capacity to begin using intralitter likeness as a meaningful additional outcome for enhanced understanding of interactions between genetics and contaminant exposure. The workshop will consider relevant details from the historical literature, disadvantages of the litter-based design, and details regarding application of the long-preferred statistical solution that models litter as a random effect in regression analyses. The laptop accessibility of highly sophisticated and efficient statistical software now allows all neurotoxicologists to use the preferred statistical solution to the problem of intralitter likeness.

DNTS 28

Federal agency activities to identify and implement alternatives

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Abstract

The recently published US Strategic Roadmap for Modernizing Safety Testing discusses new processes for developing, and establishing scientific confidence in, alternative testing strategies that are more relevant to human health. A cross-agency ICCVAM working group has been formed focusing on developmental and reproductive toxicity and identifying opportunities for use of in vitro alternatives. The workgroup scope and charge include developing a scoping document of regulatory needs and decision contexts, creating a catalog of existing technologies, and mapping new and emerging technologies to known mechanisms of developmental toxicity and relevant adverse outcome pathways. Data from the National Toxicology Program has been used to identify environmental chemicals with developmental toxicities in multiple species, ranging from overt malformations to subtle effects, that could serve as reference chemicals for evaluation of new approaches. Results from testing alternative systems, such as a human stem cell metabolomics platform, against sets of reference chemicals derived from the NTP and from recent ICH guidance, will also be discussed.

DNTS 29

In Vitro to In Vivo extrapolation using maternal-fetal models

Lumen A

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Abstract

Understanding how in vitro assays can inform risk assessment for developmental toxicity requires translation of observed activity concentrations into predicted exposures, specifically with respect to the developing fetus. Reverse dosimetry models are being developed and applied to data from stem cell platforms to yield exposure-based hazard predictions in the human fetus. The micromolar concentrations at which the targeted biomarker reaches a teratogenic potential are translated using a "human pregnancy physiologically based pharmacokinetic (PBPK) model" to infer predicted margins of exposure. The model incorporates maternal-fetal physiology and transport at differing stages of pregnancy to calculate maternal and fetal concentrations based on human exposure scenarios. Applications using the known human developmental toxicant, retinoic acid, and a series of analogues with varying teratogenic potential which have all been tested in the stem cell platform, will be discussed.

DNTS 30

International progress on alternative approaches for developmental neurotoxicity: Evaluation of assay batteries and development of IATAs and AOPs

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Abstract

Developmental neurotoxicity (DNT) is an important adverse outcome for which <150 compounds have been evaluated in Guideline DNT studies, and for which New Alternative Methods (NAMs) need to be established and evaluated. In the past decade, a battery of high-throughput NAMs to assess DNT hazard has been developed, but not yet fully evaluated. The Organization for Economic Cooperation and Development (OECD) and other international regulatory partners are working to evaluate this battery through several activities. First, it has worked to establish reference chemical lists for DNT and test these chemicals in the battery. Second, it is supporting the development of Integrated Approaches to Testing and Assessment (IATAs) to provide examples of how data from this battery can be used to inform decisions regarding potentially developmentally neurotoxic compounds. Finally, work is ongoing to elucidate adverse outcome pathways (AOPs) that for DNT will map in vitro assays to key molecular and cellular events. To evaluate the battery of ~12 in vitro assays to screen and prioritize compounds for DNT hazard, a set of compounds for which there is evidence of DNT in mammals in the literature, have been tested in guideline DNT studies, or are putative negative compounds has been developed and is being tested. Determining the specificity and sensitivity of each assay individually and collectively with a set of positive and negative controls will increase the confidence in the battery as unknown compounds are tested. Data from this chemical set will be used to develop IATAs to provide examples of how the data can be applied to regulatory decision-making. Finally, because the individual assays evaluate processes that are critical to nervous system development, (e.g. proliferation, differentiation, synaptogenesis, etc.), they comprise Key Events in Adverse Outcome Pathways (AOPs) for developmental neurotoxicity. Further development of DNT AOPs will provide scientific context for the observed in vitro effects and increase confidence in the outcomes. Collectively, these international efforts will develop NAMs for generation of DNT hazard information on greater numbers of chemicals and establish guidance for the use of that data in environmental decision-making. This abstract does not reflect US EPA policy.

DNTS 31

Integrated testing strategies for developmental neurotoxicity testing

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Abstract

The need for reliable and efficient screening tools to identify, prioritize, and evaluate chemicals for their potential to induce developmental neurotoxicity (DNT) is well recognized. This talk will highlight advancements in a paradigm shift from using the traditional in vivo guideline DNT studies to using an integrated approach of New Alternative Methods (NAMs) that can be combined with advancements in in vivo DNT Guideline studies. The National Toxicology Program recently hosted a workshop that was attended by global experts from academia, industry, Government and Regulatory agencies to discuss how different assays may be combined into a "battery" of medium- and high-throughput cell-based models, and alternative animal systems to prioritize compounds for further in vivo testing and/or to complement current regulatory DNT guideline studies. NTP created a free, publicly available interactive web-application known as Developmental NeuroToxicity Data Integration and Visualization Enabling Resource (DNT-DIVER) to analyze, compare, and visualize multiple DNT screening assays. This talk will provide guidance on how DNT DIVER can support the use of NAMs for DNT screening. It also highlights some key considerations needed while putting together a battery of assays to include: 1) identifying assays critical to the battery; 2) developing a common data analysis approach to compare disparate assays across the battery; 3) identifying knowledge gaps and methods to improve them; 4) application of a battery approach in regulatory decision making; and 5) relating outcomes from these assays with human exposure. Finally, we shed light on how this approach may be combined with guideline studies to improve DNT Testing.

DNTS 32

Association between prenatal bisphenol exposure and problematic behaviors at age 2

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Abstract

Effects of prenatal BPA exposure on child behavior are mixed, including reports of increased aggression, emotional reactivity, and other problematic behaviors in girls, and increased externalizing behaviors in boys but decreased problematic behaviors in girls. We identified no studies on prenatal bisphenol S (BPS) exposure and child behavior. In a prospective cohort study, 5 maternal prenatal urine samples collected across pregnancy were pooled and analyzed for BPA and BPS concentrations. We assessed child behavior at 2 years using the Child Behavior Checklist (CBCL). We used generalized linear regression, adjusting for child age, sex, gestational age at birth, sex by phenol interaction, household income, maternal parity, marital status, age, education, and race. Mean maternal age was 29.7 years ($N = 68$). The majority were White (88%), had an annual household income ≥$50,000 (66%), and at least a college degree (81%). Median concentrations were 1.3 ng/mL (range 0.4–7.2) for BPA and 0.3 ng/mL (range 0.1–3.5) for BPS. Sex modified the effect of BPS on internalizing behaviors. Girls in the highest exposure tertile (≥75%) had increased internalizing behaviors compared to those in the lowest tertile (< 25%), whereas boys in the highest tertile did not differ from those less exposed ($\beta = 14.14$, 95% CI 0.01–28.27; $p = 0.003$). Similarly, girls in the highest BPS tertile exhibited significantly more sleep problems than those in the lowest tertile, whereas boys in the highest tertile did not differ from those in the lowest ($\beta = 1.39$, 95% CI 0.27–2.51; $p = 0.015$). BPA exposure was not consistently associated with CBCL outcomes. (Supported by ES022848, OD023272, and RD83543401.)

DNTS 33

Characterization of performance on an automated visual recognition memory task in 7.5-month-old infants

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Abstract

Infant looking behaviors measured during visual assessment paradigms may be more reliable predictors of long-term cognitive outcomes than standard measures such as the Bayley Scales of Infant Development typically used in epidemiology. In this study, 318 7.5-month-old infants from a prospective birth cohort were assessed using a visual recognition memory (VRM) paradigm. Infrared eye tracking was used to record looking time as infants were shown pairs of identical faces or shapes followed by two trials in which the familiar stimulus was paired with a novel one that appeared on the right in one trial and on the left in the other. Infants were assessed in one of four conditions. In conditions A and B, stimulus set 1 were the familiar stimuli and set 2 were novel; in conditions C and D, set 2 were familiar and set 1 novel. The novel stimuli were presented on the right first in conditions A and C and on the left first in conditions B and D. Infants showed a preference for the novel stimuli similar to that in published in prior studies, a significant right side preference which has not been reported before, and a strong preference for eyes versus the rest of the face (p-values < 0.0001). Novelty preference was significantly higher when set 1 stimuli were novel (p-value < 0.0001), suggesting a preference among infants for set 1 stimuli compared to set 2 stimuli. These findings show that side and stimulus preferences should be considered in infant VRM assessment. (Supported by ES022848, ES028607, OD023272, and USEPA RD83543401.)

DNTS 34

Association of prenatal maternal stress with visual recognition memory in 7.5-month-old infants

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Abstract

Recent evidence has shown that prenatal maternal stress can negatively impact neurodevelopment, but little is known about its effect on early cognitive development. We assessed the impact of prenatal stress on recognition memory of 7.5-month-olds in a subsample of 188 infants from an ongoing prospective study. Maternal stress was assessed at 10–14 and 34–36 gestational weeks using the Perceived Stress Scale. The median was used to classify women: those scoring below the

median at both times (low stress), above the median at one of the two times (medium stress), and above the median both times (high stress). Infants' looking time was recorded via infrared eye tracking. The infants were shown identical black-and-white photographs of human faces displayed side-by-side, then the familiar face was paired with a novel face. Infants were tested in one of four conditions. The conditions varied by which face was familiar and by which side the novel face appeared on first. A generalized linear model was used to examine the association of novelty preference (proportion of looking time spent looking to novel stimulus) with prenatal stress, after adjusting for infant age, sex, assessment condition, household income, maternal education, maternal IQ, and a sex-by-stress interaction. The model revealed a significant sex-by-stress interaction (p-value = 0.033). In girls, higher prenatal stress was associated with higher novelty preference; whereas, in boys higher stress was associated with lower novelty preference. However, post hoc analysis revealed there was not a significant effect of stress on recognition memory in either sex. (Supported by ES007326, ES022848, RD83543401, and OD023272.)

DNTS 35

Co-exposure to tobacco and cannabis: Effects on stress reactivity at early school age

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Abstract

Tobacco and cannabis are two of the most commonly used substances among pregnant women, with high rates of co-use of both substances. However, the literature on the effects of co-use on child outcomes is small. We examined the role of tobacco and co-exposure to tobacco and cannabis on stress reactivity in early school age by measuring child cortisol responses to two laboratory stressors. We also examined child sex, maternal postnatal mood (depression/stress), and parenting behavior in early childhood as moderators. The sample consisted of 238 children (67 tobacco only; PTE; 83 co-exposed to tobacco and cannabis, PTCE; and 88 non-exposed, all with low alcohol exposure and no other drug exposure) with cortisol data at kindergarten age, and their mothers recruited in the first trimester of pregnancy. The sample was diverse with mostly low-income mothers with relatively low education. Prenatal substance exposure was measured using a combination of maternal self-reports in each trimester, maternal salivary nicotine and cannabis metabolites in each trimester, and infant meconium assayed for tobacco and cannabis metabolites. Child salivary cortisol was collected at 4-time points in response to a laboratory stressor at kindergarten age. Results from multilevel models indicated significant individual differences in cortisol changes over time. There was a significant interaction of tobacco exposure and maternal mood on intercept, significant interaction of co-exposure and parenting (harshness, warmth) on linear slope, and a significant interaction of co-exposure and parenting on quadratic slope that indicated a complex interplay of exposure and parenting on stress reactivity.

DNTS 36

Thyroid hormone disruption in the fetal and neonatal rat: Predictive hormone measures and bioindicators of hormone action in the developing cortex

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Abstract

Adverse neurodevelopmental consequences remain a primary concern when evaluating the effects of thyroid disrupting chemicals. However, OECD and US EPA primarily detect antithyroid activity in vivo by deficiencies in serum thyroid hormones (THs). Neither brain TH concentrations or TH-mediated signaling are evaluated, as it is not understood how brain hormone homeostasis is controlled during development. To address these knowledge gaps and to enhance chemical testing, dose response experiments were performed in pregnant rats using the goitrogen propylthiouracil (PTU) (dose range 0.1–10 ppm). THs were quantified in the sera and brains of offspring on gestational day 20 (GD20) and postnatal day 14 (PN14), two developmental stages included in OECD and EPA regulatory studies. Using the dose response data, the quantitative relationships between THs in the serum and brain were determined. Next, targeted gene expression analyses were performed in the fetal and neonatal cortex to determine if changes in hormone action are linked to changes in brain TH concentrations. Results show a significant reduction of T4/T3 in the serum and brain of the GD20 fetus in response to low doses of PTU; interestingly, very few genes were significantly different at any dose tested. In the PN14 pup significant reductions of T4/T3 in the serum and brain were also detected; however, twelve transcriptional targets were identified in the neonatal cortex that correlated well with reduced brain THs. These results show that serum T4 is generally a good predictor of brain THs,and offer several target genes that could serve as readouts of T4/T3 dysfunction within the PN14 cortex. We are now testing the utility of these identified molecular bioindicators under conditions of TH insufficiency induced by the environmental contaminants perchlorate, triclosan, and perfluorohexanesulfonic acid.

DNTS 37

The developmental and toxicological role of an evolutionarily conserved AHR-dependent long non-coding RNA in zebrafish

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Abstract

Structurally diverse groups of chemicals, including polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and dioxins interact with and activate the aryl hydrocarbon receptor (AHR) leading to adverse health effects. There is mounting evidence that AHR ligand structure can differentially modulate AHR activity, producing disparate biological effects. Despite decades of research, the precise molecular events downstream of AHR activation necessary for developmental toxicity remain elusive. In the zebrafish model we profiled hundreds of AHR ligands revealing a multitude of in vivo outcomes. For example, following exposure to a number of ligands, AHR dependent repression of the transcription factor *sox9b* caused several developmental effects, including craniofacial cartilage malformations; however, the mechanism of *sox9b* repression remains unknown. Since AHR activation generally increases gene transcription, repression of *sox9b* was difficult to explain. We identified a long non-coding RNA, *slincR*, induced during embryonic development by multiple AHR ligands. We determined how *slincR* expression was affected in response to sixteen different environmentally relevant PAHS. We demonstrated that *slincR* induction absolutely required AHR and that *slincR* was necessary for the

repression of *sox9b* expression. We investigated the mechanism of *slincR* repression of *sox9b* using Capture Hybridization Analysis of RNA Targets (CHART) to determine if *slincR* bound to the *sox9b* locus. We also used a *sox9b* morpholino to determine if *sox9b* expression regulated *slincR* expression. To understand *slincR*'s role in the TCDD-induced toxicity pathway, we performed RNA sequencing (RNA-seq) and Gene Ontology (GO) enrichment analysis on 48 h post fertilization (hpf) control and *slincR* morphants exposed to 0.1% DMSO or 1 ng/mL TCDD. To investigate the phenotypic impact of *slincR* expression on TCDD-induced jaw malformations, we measured the cartilage of 72 hpf control and *slincR* morphants treated with 0.1% DMSO or 1 ng/mL TCDD. At 48 hpf, we performed a hemorrhaging screen on TCDD-exposed morphants. Finally, we mined multiple RNA-seq datasets to identify potential mouse and human orthologs of *slincR*. The results established that slincR bound to the *sox9b* 5′ untranslated region to repress transcription, regulated cartilage development, had a causal role in the TCDD-induced hemorrhaging phenotype, and was upregulated by multiple environmentally relevant PAHs. These findings have important implications for understanding the ligand-specific mechanisms of AHR-mediated toxicity.

DNTS 38

Developmental neurotoxicity of perfluoroalkyl substances

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Abstract

Perfluoroalkyl substances (PFAS), chemicals used to make products stain and stick resistant (among other uses), have been linked to health effects in adults and adverse birth outcomes. A growing body of literature also addresses health effects in children exposed to PFAS. This presentation will discuss neurodevelopmental outcomes associated with PFAS exposure in children and from experimental studies in zebrafish. We performed a systematic review of the literature related to PFAS exposure in association with children's health outcomes including neurodevelopment and attention. For inclusion in the systematic review, studies must have included a measured concentration of a PFAS in human biological media (i.e., blood, serum, or plasma) and a health effect in a child. Studies that examined childhood developmental milestones or neurodevelopment reported primarily null results, though some observed positive associations. There were multiple studies of attention, impulsivity and inhibition, which provided mixed results with multiple studies reporting positive associations and some studies reporting null or inverse associations. A study of congenital cerebral palsy reported a higher risk in boys with higher maternal PFAS levels. An examination of autism in a case-control study found no association with certain maternal plasma PFAS (e.g. PFOA, PFNA, PFHpS or PFDA) but PFOS and PFHxS had elevated odds ratios, although PFOS confidence intervals were wide. The epidemiologic literature is largely comprised of studies on legacy PFAS chemicals (e.g. PFOA, PFOS). Very little is known about the newer chemistry perfluorinated or polyfluorinated compounds, especially their potential to induce developmental neurotoxicity. To help fill this data gap, EPA is evaluating the developmental neurotoxicity of legacy PFAS as well as newer replacement chemistries including polyfluorinated ethers in zebrafish using automated behavior assays. This abstract does not r*epresent the positions or policies of the US EPA*.

DNTS 39

Your teenager's brain on pot: what do we have to look forward to?

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Abstract

With increasing legalization of marijuana, the public hears little about the toxic effects of the drug. Marijuana can interfere with experience-dependent plasticity, the foundation of cortical neural circuitry development during adolescence which is largely completed by adulthood. During adolescence, delta-9-tetrahydrocannabinol (THC) administration alters brain-behavior relationships within circuits subserving affect, reward and cognition. These alterations exhibit period-specific and sex-specific vulnerabilities. We will review the three major domains of behavior: cognition, mood (anxiety, depression and psychosis) and reward as they are altered by cannabis consumption during adolescence in human and preclinical models. Emphasis will be placed on marijuana toxicities determined in relevant animal models in comparison with available human data in this relatively understudied population. Also, early stress alters the endocannabinoid system differently in males and females establishing a sex-dependent tone within the system which in turn alters stress and drug responses throughout life. THC administration during the prepubescent period produces significantly greater effects on affective behavior than later exposure and since males and females undergo puberty at different ages, the age of maximal effect is different in males and females. Also, studies have demonstrated that adolescent exposure to THC produces permanent effects on reward circuits by increasing the consumption of drugs of abuse in adulthood. The dose-response nature of the relationship between marijuana and neurotoxicity will be highlighted throughout to emphasize the message that consuming less marijuana, less frequently and beginning at an older age will reduce the overall impact of smoking marijuana during adolescence on adult brain function.

DNTS 40

Serotonin transporter (SERT) binding potentials in brain of juvenile monkeys one year after discontinuation of a two-year treatment with fluoxetine

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Abstract

The potential long-term effects of childhood fluoxetine therapy on brain serotonin systems were studied in the rhesus monkey (*Macaca mulatta*). Juvenile male rhesus were treated orally with fluoxetine (2 mg/kg) or vehicle daily for two years. Each treatment group was assigned an equal number of subjects with low and high transcription polymorphisms of the monoamine oxidase A (MAOA) gene. One year after discontinuation of treatment, positron emission tomography (PET) scans were conducted ($N = 8$ treated, 8 control) using [^{11}C]DASB to quantify serotonin transporter (SERT) in 16 cortical and subcortical regions. Fluoxetine treated monkeys with the MAOA low transcription-polymorphism had significantly *lower* [^{11}C]DASB binding potentials than controls. This finding was seen throughout the brain but was strongest in prefrontal and cingulate cortices. The MAOA*fluoxetine interaction was enhanced by binding potentials that were non-significantly *higher* in monkeys with the high transcription polymorphism. Residual post-treatment effects of juvenile fluoxetine treatment on brain SERT that depend on MAOA genotype may be important to consider when treating children with fluoxetine. (Supported by HD06586 and OD011107).

DNTS 41

Impacts of dim light at night and phthalate exposure on circadian locomotor and feeding behaviors and hypothalamic gene expression in male and female mice

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Abstract

The circadian system synchronizes physiology and behavior with the environment. This timekeeping system is regulated by exogenous and endogenous cues, such as light and hormones, respectively. Exposure to other environmental factors, including dim light at night and phthalates, modifies brain physiology. Phthalates, including di-(2-ethylhexyl) phthalate (DEHP), are ubiquitous endocrine disruptors capable of modulating behavior. However, the effects of dim light at night (dLAN) and phthalate exposure on circadian behavior have remained largely unexplored. We tested the hypothesis that dLAN and DEHP disrupt rhythmic locomotor activity and feeding behaviors. Adult CD-1 male and female intact mice were individually housed with running wheels for locomotor behavior analysis. Mice (8/sex/group) were treated for 30 days with one of the following conditions: 12 h:12 h light:dark (Control), 12 h:12 h light:dLAN with 5 lx light during the dark (dLAN), 12 h:12 h light:dark with 50 μg/kg/day DEHP (DEHP), or 12 h:12 h light:dLAN with 5 lx light during the dark and 50 μg/kg/day DEHP (dLAN-DEHP). DEHP was administered orally by gently pipetting into the cheek. For feeding behavior, light phase and dark phase food consumption was measured every 12 h. DEHP males increased the percent of food consumed in the dark cycle, and both DEHP and dLAN-DEHP females reduced nocturnal feed consumption. DEHP males had reduced total activity, while females in all three treatments had increased diurnal behavior. We are currently analyzing target genes in several hypothalamic regions to understand the impact of our treatments on the brain. Together, these data provide some of the first evidence that dLAN and DEHP impact circadian behaviors.

DNTS 42

Deltamethrin exposure to Sprague-Dawley rats from postnatal days 3–20 alters dopaminergic and glutamatergic activity and long-term potentiation when tested as adults

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Abstract

Pyrethroids are a prevalent class of synthetic insecticides that act through voltage gated sodium channels to prolong channel opening leading to depolarization. Pyrethroids are used in many settings where children are present, such as schools and parks, as well as for treating head lice. Epidemiological studies find that developmental exposure to pyrethroids is associated with neurological and behavioral abnormalities. The effects of Type II pyrethroids, such as deltamethrin (DLM), on development have received relatively little attention. We previously showed that Sprague-Dawley rats exposed to DLM from postnatal day (P)3–20 had deficits in egocentric and allocentric learning and memory as adults. We also observed increased hippocampal long term-potentiation (LTP) at P28–35 in P3–20 DLM treated rats. Here we exposed Sprague-Dawley rats to 0 or 1.0 mg/kg/day DLM by gavage from P3–20. LTP was assessed at P60–90 in brain slices in CA1 and dentate gyrus (DG). Males and females treated with DLM had decreased LTP in both the CA1 and DG of the hippocampus compared with controls. Amphetamine-stimulated dopamine release was assessed via microdialysis. DLM-treated rats exhibited decreased dopamine release in the nucleus accumbens. Western blots revealed that DLM-treated rats did not differ in dopamine receptor D1 (DRD1) protein in the nucleus accumbens or hippocampus, but exhibit increased levels in the striatum. No differences were observed for DRD2 levels or the NMDA receptor subunit NR1 in examined regions. However, NMDA receptor subunit NR2A protein was increased in the hippocampus and NR2B protein was decreased in the striatum. The data indicate that rats developmentally exposed to DLM have altered CA1 and DG LTP, decreased extracellular n. accumbens dopamine release, increased DRD1 and decreased NR2B protein expression in striatum, and increased NR2A expression in the hippocampus. Experiments are ongoing for the effects of developmental DLM exposure on LTP and protein expression in both male and female rats. (Supported by T32 ES007051).

DNTS P1

Effects of domoic acid exposure on maternal reproduction and infant birth characteristics in a preclinical primate model

Burbacher T, Grant K, Petroff R, Shum S, Crouthamel B, Stanley C, McKain N, Jing J, Isoherranen N

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Abstract

Domoic Acid (DA) is a naturally-occurring excitotoxin produced by marine algae. Because algal blooms that produce DA are becoming more widespread and very little is known about the dangers of chronic, low-dose exposure, we initiated a preclinical study focused on the reproductive and developmental effects of DA in a nonhuman primate model. To this end, 32 adult female *Macaca fascicularis* monkeys were orally exposed to 0, 0.075 or 0.15 mg/kg/day DA on a daily basis, prior to and during pregnancy. Females were bred to non-exposed males and infants were evaluated at birth. Results from this study provided no evidence of changes in DA plasma concentrations with chronic exposure. DA exposure was not associated with reproductive toxicity or adverse changes in the physical characteristics of newborns. However, in an unanticipated finding, our clinical observations battery revealed the presence of subtle neurological effects in the form of intentional tremors in the exposed adult females. While females in both dose groups displayed increased tremoring, the effect was dose-dependent and observed at a higher frequency in females exposed to 0.15 mg/kg/day. These results demonstrate that chronic, low-level exposure to DA is associated with injury to the adult CNS and suggest that current regulatory guidelines designed to protect human health may not be adequate for high-frequency shellfish consumers.

DNTS P2

Effects of in utero domoic acid exposure on neonatal behavior and infant memory in a preclinical primate model

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Abstract

Domoic Acid (DA) is a naturally-occurring neurotoxin that is increasingly documented in ocean waters around the globe. In both humans and marine animals, prenatal DA exposure occurs through the

maternal consumption of contaminated shellfish and finfish. To better understand the public health risks associated with fetal exposure, we initiated a longitudinal, preclinical study focused on the reproductive and developmental effects of chronic, low-dose oral DA exposure during pregnancy. To this end, 32 adult female *Macaca fascicularis* monkeys were orally dosed with 0, 0.075 or 0.15 mg/kg/day DA on a daily basis throughout breeding and pregnancy. The doses included the proposed human Tolerable Daily Intake (TDI) (0.075 mg/kg/day) for DA. Adult females were bred to nonexposed males. To evaluate development during early infancy, offspring were administered a Neonatal Assessment modeled after the human Brazelton exam and a series of Visual Recognition Memory problems. Results indicated that prenatal DA exposure did not impact early survival reflexes or responsivity to the environment. Findings from the recognition memory assessment, given between 1 and 2 months of age, showed that exposed infants demonstrated robust novelty scores when test problems were relatively easy to solve. However, when more difficult recognition problems were introduced, infants in the 0.15 mg/kg DA group failed to show differential visual attention to novel test stimuli. The disruption of memory in these infants demonstrates that chronic, fetal exposure to DA, at levels near the human TDI, may impact developing cognitive processes.

DNTS P3

Exposure to manganese, lead, and barren cage stress during development has adverse long-term effects on neurocognitive, behavioral, and monoamine outcomes in adult Sprague-Dawley rats

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Abstract

Developmental stress can cause dysregulation of the hypothalamic-pituitary-adrenal axis. Stressors such as low socioeconomic status (SES) may induce adverse long-term neurodevelopmental effects. Children in lower SES households experience more stress and are more likely to be exposed to environmental neurotoxins like lead (Pb) and manganese (Mn). Developmental co-exposure to stress, Pb, and Mn may increase the risk of abnormal CNS function. Within each litter of Sprague-Dawley rats, one male/female pair were assigned as follows: 0 (vehicle), 10 mg/kg Pb, 100 mg/kg Mn, or 10 mg/kg Pb + 100 mg/kg Mn (Pb—Mn). Treatment was from postnatal day (P) 4–28 with half the litters reared in cages with standard bedding and half without bedding (Barren). Offspring were tested as adults. Mn and Pb—Mn groups had decreased anxiety, reduced acoustic startle, transient hypoactivity, increased activity following (+)-methamphetamine, egocentric learning and memory deficits in Cincinnati water maze (CWM), and deficits in latent inhibition. Pb increased anxiety and reduced open-field activity. Barren-reared rats had decreased anxiety, CWM deficits, increased startle, and transient hyperactivity. The Mn, Pb—Mn, Pb, and Barren-reared groups also had impaired Morris water maze performance. Pb altered neostriatal 5-HT and norepinephrine, Mn increased hippocampal 5-HT in males, Mn + Barren-rearing increased neostriatal 5-HT, and Barren-rearing decreased neostriatal dopamine in males. The most pronounced effects were in the Mn and Pb-Mn-exposed groups, suggesting that Mn exposure drove most of the detrimental effects on behavior and neurotransmitters. Few interactions between Mn and Pb with barren cage rearing stress were found. (Supported by ES015689 and ES007051.)

DNTS P4

Ultrasonic vocalizations in rat pups to examine the impact of neurodevelopmental stressors: Preliminary results from perinatal stress and manganese

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Abstract

Rodents emit ultrasonic vocalizations (USVs) for intra-species communication. The ability to measure these emissions has become a valuable tool in the assessment of neurobehavioral development, communication and affect, but has not been widely applied in a toxicological context. Due to the influence of the external environment on USV emissions and the complexity of USV analyses, we first examined the ontogeny of these emissions in Long-Evans rat offspring during 3 min of maternal separation. Number of calls, duration, mean peak frequency, and bandwidth were compared between repeated (n = 7–8/sex/day) and cross-sectional (n = 14/sex/day) recordings in male and female pups across postnatal days (PDs) 6 to 21. USV emissions peaked around PD 8 and fell to near 0 by PD 21. Both repeated and cross-sectional studies followed similar patterns with few differences between USV characteristics. Examination of call characteristics led us to differentiate calls into 2 classes based on peak frequency and duration. These calls became most evident beginning on PD 13. We have begun evaluating these calls as part of a battery of assessments to evaluate a rodent model of co-occurring neonatal stressors. Manganese (0, 2 or 4 mg/ml), an established neurodevelopmental toxicant, was delivered in drinking water to pregnant dams from gestational day (GD) 7 to PD 22. A variable stress paradigm was applied to half of these dams from GD 13 to PD 9 and USVs examined on PD 13. Preliminary findings of the this noninvasive USV testing paradigm as a metric of neurodevelopmental disturbance will be presented. Abstract does not necessarily reflect EPA policy.

DNTS P5

Adult offspring performance in trace fear conditioning after concurrent developmental exposure to variable stress and manganese

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Abstract

This study is focused on the interaction of developmental manganese (Mn) exposure with stress on learning changes in the adult offspring of Long Evans (LE) rats. Pregnant LE rats were exposed to an unpredictable series of stressful events, which had previously been shown to increase maternal corticosterone levels. These stressors were presented daily from gestational day (GD) 13 through postnatal day (PND) 9. Groups of stressed dams and control dams were exposed to 0, 2, or 4 mg/mL of Mn in drinking water from GD 13 through PND 22. Starting at PND 97, male and female offspring were trained with a trace fear conditioning (TFC) protocol, whereby rats were exposed to a compound cue (light and tone) followed by 30 s (trace period) and a mild foot shock (1 mA, 0.5 s). Five paired training sessions occurred on the training day. The following day, context and cue learning was assessed by measuring motor activity. Preliminary data suggests that all treatment groups displayed learning during context testing. No significant treatment effects were noted for stress, Mn, or concurrent

exposure to both when compared to control offspring performance. Ongoing research with two additional cohorts will increase sample size and examine transcriptional changes in the hippocampus and amygdala of adult rats after learning the TFC task. This abstract does not necessarily reflect EPA policy.

DNTS P6

Using evidence mapping to refine the evaluation of neurological endpoints in a systematic review of polychlorinated biphenyls (PCBs)

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Abstract

Systematic reviews of animal toxicology literature often encompass a wide range of health outcomes associated with a chemical exposure. These reviews are challenging to conduct when the chemical under evaluation is well-studied, resulting in a large database of studies for consideration. Here, we describe the application of evidence mapping as an organizational principle for evaluating a large and complex database using animal toxicology studies on the neurological effects of polychlorinated biphenyls (PCBs) as an example. >138 studies evaluating PCB neurotoxicity in animal models were identified through a literature search of scientific databases (PubMed, Web of Science, Toxline). Key scientific information (experimental design and health outcome information) from these studies was then extracted into literature inventory tables. The endpoints evaluated in each study were categorized by outcome type to facilitate further evaluation (e.g. cognitive behavioral tests, activity level/motor function, brain size, structure, and histology, etc.). To identify the health outcomes most relevant to support human health risk assessment, major considerations include the severity/biological significance of the outcomes identified, the number and types of studies that evaluated each outcome, and the sensitivity of each outcome to the chemical exposure. *The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.*

DNTS P7

Examination of the combined effects of iodine deficiency and developmental perchlorate exposure on fear learning in rats

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Abstract

Severe iodine deficiency (ID) has long been associated with hypothyroidism and neurological impairments in humans. The environmental contaminant perchlorate also reduces thyroid hormone (TH) production by interfering with iodine uptake into the thyroid gland. Our previous studies independently examined the impact of either dietary ID or drinking water exposure to perchlorate. Although these treatments both reduced serum THs and impaired hippocampal synaptic transmission, no deficits were observed in a hippocampal-dependent task of contextual fear learning. In the present study we hypothesized that an ID diet in conjunction with perchlorate exposure may exacerbate TH insufficiency and result in fear learning deficits. Female rats were maintained on either an iodine sufficient (IS) or ID diet for a minimum of four weeks before breeding. A subset of each group was administered ammonium perchlorate via the drinking water (0 or 300 ppm) beginning on gestational day 6 until weaning on postnatal day (PN) 21 creating four treatment conditions. Offspring were euthanized during the neonatal period to assess serum THs. Trace fear conditioning was later examined in adult male offspring. Results revealed that ID reduced serum T4 by 35% in naive female rats prior to breeding. On PN2, serum T4 in pups born to ID dams decreased by 20% compared to IS dams and by 57% if dams were both ID and treated with perchlorate. Behavioral results of adult male offspring show that combined ID and perchlorate exposure during development did not result in a significant learning deficit. Evaluation of serum and brain THs are underway and will provide additional context for interpretation of these preliminary findings. These data suggest that more robust and sensitive behavioral assays may be required to adequately assess the consequences of early thyroid disruption on brain function. *Does not reflect EPA policy.*

DNTS P8

Extrathyroidal sites of chemical action and neurodevelopmental outcome - An examination using triclosan and perfluorohexane sulfonate

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Abstract

Developmental neurotoxicity is a primary concern for thyroid disrupting chemicals (TDCs). Environmental TDCs are often detected by changes in serum thyroid hormone (TH). This study investigated the neurotoxicological effects of the antimicrobial agent, triclosan, and a perfluoroalkyl chemical, perfluorohexane sulfonate (PFHxS). These agents reduce serum TH by increasing liver catabolism or disrupting serum TH binding. Pregnant rats were dosed by gavage (triclosan: 300 mg/kg/day, PFHxS: 50 mg/kg/day, Control: vehicle) from gestational day (GD) 6 to postnatal day (PN) 21. No changes in litter size, body or liver weights in dams or pups were seen. Expression of liver metabolism genes was increased in dams and pups. Both chemicals reduced serum T4 in the dam (GD20, PN21) and pup (PN0, PN2, PN6, PN14) to varying degrees. Thyroid stimulating hormone (TSH) was unchanged. Reductions in brain T4 were observed on PN0 in PFHxS-exposed pups, whereas triclosan reduced brain T4 and T3 on PN0 and PN2. Despite continued reductions in serum TH, brain TH recovered by PN6. Expression of TH-related genes was not altered in PN14 cortex and there was no evidence of a TH-dependent phenotype (heterotopia). Behavioral tests of fear conditioning and prepulse inhibition were unchanged in adult offspring. These data suggest that TDCs with 'extrathyroidal sites of actions' may differ from model chemicals like PTU in their ability to impact brain TH and neurodevelopment. In the neonate, serum TH decrements may not necessarily translate to brain deficits in TH, but this may not be the case for the fetus/newborn. Additional studies of the age-dependent quantitative relationship between THs in the serum and brain as well as sensitive metrics of neurotoxicity are needed to evaluate the risk of these and other TDCs with similar mode (s) of action. *Does not reflect EPA policy.*

DNTS P9

Brain thyroid hormone measurements in developing rats

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Abstract

Thyroid hormones (THs) are essential for the growth and development of the brain. Due to technical limitations, measuring TH in developing brain tissue is not common, largely due to a lack of methods to isolate THs from a complex tissue matrix, and instrumentation capable of quantifying low concentrations of THs. Recently, a sensitive method to assess THs in rat brain (detection limits of 50 pg/mL for T3 and T4) using liquid-liquid extraction with liquid chromatography/mass spectrometry (LC/MS/MS) was developed at US EPA. We present here a preliminary evaluation of TH measures in control rats using this methodology. Our goals were four-fold: 1) determine if brain TH of neonatal rats could be measured with this analytical method; 2) identify age-controlled ranges in brain concentrations of TH; 3) examine within study biological variability across ages; 4) document study-to-study variability. Brain samples from 3 to 4 studies were analyzed within each age group (n = 3–5 samples/age/study). All samples were well above the detection limits of the instrument and a clear ontogenetic progression in brain TH concentrations was evident. Averaged across studies, brain T4 increased from 0.422, 1.20, 2.11, to 2.86 ng/g from postnatal days (PN) 0, 2, 6 and 14, respectively. Average T3 concentrations were generally higher than that obtained for T4 and also increased with age from 0.740, 1.31, 2.48, to 5.74 ng/g from PN0 to PN14. Coefficients of variation (CV) of brain T4 and T3 within individual studies were commonly below 10% at any given age. The CV between studies, however was substantially higher for both analytes, exceeding 50% in some cases. We anticipate that inclusion of additional studies, continued improvements in tissue harvest/extraction procedures, and assessment using the same analytical instrument will reduce variation between studies. In the 3 most recent studies where these factors were controlled, lower CVs of 20% and 5.9% for T4 and T3 were obtained for the same historical control (HC) sample run over 7 analytical runs. Further work to identify and control factors to limit variability is needed, but low within study CVs and stable HC indicate reliable estimates of brain TH are attainable.

DNTS P10

Association between prenatal exposure to environmental phenols and attention to the eyes in 7.5-month-old infants

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Abstract

In previous research, children later diagnosed with autism spectrum disorder (ASD) showed a decreased time looking at eyes within the first six months of life when compared to typically-developing children. Exposure to bisphenol-A (BPA) has been associated with ASD, but it is unknown whether its replacement chemicals, bisphenol-F (BPF) and bisphenol-S (BPS), have similar effects. In a prospective cohort study, five maternal urine samples collected across pregnancy were pooled and analyzed for BPA, BPF and BPS. Attention to the eyes was assessed in 7.5-month-old infants using eye-tracking technology. Infants viewed an animated video of a teddy bear in five trials. We used generalized linear regression models adjusted for infant sex, age at assessment, maternal education, and household income to assess looking time at the eyes in the last two trials. 206 infants were included in the analysis. Most of their mothers were White, had at least a college education, and had an annual household income greater than $60,000. Median concentrations were 0.50 ± 0.26 ng/mL for BPS; 0.30 ± 0.21 ng/mL for BPF; and 1.00 ± 0.12 ng/mL for BPA. Looking at the eyes during the last two trials was not significantly associated with BPS or BPF exposure, but higher prenatal BPA exposure was associated with more time spent looking at the bear's eyes in the last two trials (β = 488.45, 95% CI 71.25–905.65, p = 0.02). Future analyses will examine infants' looking time at the bear's eyes in the earlier trials to see if there is a different pattern of results. Supported by ES022848, OD023272, and RD83543401.

DNTS P11

Coal fly ash, aluminum, and short-term memory among children 6–14 years old

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Abstract

Fly ash is a coal-combustion residual composed of particles with diameters less than ten micrometers (PM_{10}) that may contain neurotoxic metals, including aluminum. Little research exists examining the effects of fly ash exposure on neurobehavioral outcomes in children, however Al has been associated with memory problems in previous studies. To assess memory effects related to fly ash and aluminum exposure, we collected data from 230 children aged 6 to 14 years living near coal ash storage sites using personal modular impactors, lift tape samples, nail samples, an immediate object memory test, and questionnaires. Statistical methods used to analyze the data included linear and binomial logistic regression and the likelihood ratio test. Thirty-eight (16.5%) participants had abnormal immediate recall t-score values on the Differential Ability Scales while 153 (77.7%) participants had fly ash in their homes. The odds ratio for abnormal immediate recall and presence of fly ash on impactor filters was 3.9 (p = 0.01, 95% CI = 1.4–10.8), when adjusting for age. Immediate recall raw score values were reduced by 0.03 points per one unit (ppm) increase in aluminum in nail samples (p = 0.05), when adjusting for age and sex. Immediate recall raw score values were reduced by 23.2 points per one unit (microgram per cubic meter) increase in aluminum on impactor filters (p = 0.07), when adjusting for age. These findings suggest that immediate memory impairments in children living near coal ash storage sites may be related to fly ash and aluminum exposure.

DNTS P12

Maternal pre-pregnancy body mass index and behavioral problems in offspring

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Abstract

Background: In the United States, the obesity prevalence is 36.5% among women aged 20 to 39. Pre-pregnancy obesity has been identified as a risk factor for gestational diabetes mellitus, pregnancy induced hypertension, large for gestational age (LGA), preterm birth, macrosomia, and caesarean delivery as well as structural anomalies in the

offspring, including both heart and neural tube defects. In addition, recent literature suggests that higher pre-pregnancy BMI may be associated with adverse neurodevelopmental outcomes in the offspring. Methods: Eligible participants were children aged 5–12 whose mothers previously participated in a case–control study of risk factors for hemifacial macrosomia (HFM) and were born between 1996 and 2002. Measures of internalizing and externalizing behavior problems were obtained from mothers using the Child Behavior Checklist (CBCL) and teachers using the Teacher Report Form (TRF). Pre-pregnancy BMI (kg/m^2) was categorized as: underweight ($\leq$ 18.5), normal (18.5–24.9), overweight (25–29.9) and obese ($\geq$ 30). Using normal BMI as the reference, we calculated adjusted mean differences in t-scores for behavioral outcomes using linear regression. Results: Of the 1106 subjects eligible for follow-up, 704 (64%) participated in the study. The present analysis is restricted to singleton control children (n = 560). The proportion of women in the normal, overweight, and obese groups were 63%, 21%, and 12%, respectively. Although differences were slight, externalizing behavior scores were worse based on mother's report when overweight and internalizing behavior and somatic complaint scores were worse based on teacher's report of children of obese women. Conclusions: Evidence of pre-pregnancy BMI as a risk factor for childhood behavior problems is weak in this study due to the lack of consistency in results according to reporter, slight differences, and instability of differences of this study suggest higher pre-pregnancy BMI is associated with adverse behavior in the offspring.

DNTS P13

Prenatal cocaine exposure and the transition from teen drug use to problematic drug use (17–21 years)

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Abstract

Introduction**:** Although previous studies indicate associations between prenatal cocaine exposure (PCE) and teen substance use, little is known whether PCE is related to transitions to substance abuse during emerging adulthood. Methods**:** Participants were 302 birth cohort subjects (149 PCE and 153 non cocaine exposed (NCE)), primarily African American, of low socioeconomic status. Alcohol, tobacco and marijuana use were assessed at ages 17 using biologic assays and self-report coded as 0 (no use) or 1 (use before 17). At age 21, substance abuse symptom counts coded as 0 (0–1) or 1 ($\geq$ 2) were measured using Substance Abuse Module. Four groups were formed: no teen substance use (TSU) with no problematic use at 21 (PU), TSU with no PU, no TSU with PU, and TSU with PU. Results: No associations were observed between PCE and transitions to PU in tobacco and marijuana. Four alcohol use groups were: no TSU, no PU at 21 (69.9%); TSU, no PU (15.2%); no TSU, PU (8.9%); and TSU, PU, (6%). Multinomial regression analyses indicated that PCE and being male were interactively associated with the TSU, PU group for alcohol (p = 0.02), compared to the no TSU, no PU group. Sexual victimization and childhood maltreatment were also independently associated with the pattern of the transition. Conclusion**:** Additional research is needed to replicate findings PCE males being at higher risk for transitioning from teen alcohol use to problematic use at 21, given the relatively small sample size.

DNTS P14

Prenatal cocaine exposure predicts adult behavior problems

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Abstract

Previous studies have revealed associations with prenatal cocaine exposure (PCE) and offspring behavior such as early substance use and externalizing behavior problems. The current analyses are from a longitudinal study of PCE in which women were recruited early in pregnancy and interviewed at multiple postpartum phases about their substance use and other characteristics. Offspring were assessed at delivery and at multiple phases through adulthood with age-appropriate assessments. Prenatal cocaine use was moderate; most women decreased or discontinued use after the first trimester. During the first and third trimesters, 40% and 11% used cocaine, respectively. This cohort represents the most common pattern of PCE in non-treatment samples. At the most recent follow-up, the adult offspring were, on average, 27.2 years old, had 13.4 years of education, 82% were employed, 56% were African-American, and 55% were female. Offspring who were prenatally exposed to cocaine during the first trimester were significantly more likely to use marijuana and other illicit drugs, to have more arrests and higher scores on the Adult Self-Report rule breaking scale, and to have poorer scores on the Iowa Gambling Task. These analyses controlled for other prenatal substance exposure and current demographic and socioeconomic factors. These findings are consistent with those from previous phases and are an indication that there are detrimental effects of PCE that persist across developmental stages and into adulthood. (Supported by DA008916).

DNTS P15

Phonological processing outcomes in prenatally cocaine exposed late adolescents

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Abstract

Objective: Research from longitudinal cohort studies of children prenatally exposed to cocaine (PCE) suggest deficits in phonological processing—the cognitive ability to decode, manipulate and recall speech sounds critical for reading and writing—when compared to non-cocaine exposed children (NCE). This study examines if the phonological impairments identified in early adolescence persist into late adolescence. Methods: Adolescents (N = 336; 169 NCE, 167 PCE; 183 female, 153 male; mean age = 17.25 (SD = 0.48)) of primarily African American heritage and of low socioeconomic status were enrolled prospectively at birth. The Comprehensive Test of Phonological Processing (CTOPP) was used to assess phonological processing. Multiple regression analysis was conducted on the composite and subtest scores while controlling for the covariates such as other prenatal substance exposure, child IQ, early life home environment and caregiver characteristics. Results: PCE was significantly associated with lower phonological awareness total scores (b = $-$3.66, t(261) = $-$2.43, p = 0.016; 95% CI = -6.63, $-$0.69) and lower Elision subtest scores (b = $-$0.98, t(268) = $-$3.08, p = 0.002; 95% CI = -1.61, $-$0.35) compared to NCE while controlling for relevant covariates. PCE status did not have a statistically significant association with any of the other subtests or composite scores. Home environment, child IQ, birth head circumference, caregiver marijuana use and psychological distress were significantly associated with phonological awareness and Elision score. Conclusion: These findings partially replicate and expand on prior research of younger PCE exposed children and suggest that PCE's effect on phonological processing continues throughout adolescence.

DNTS P16

Association of fatty acid ethyl esters in meconium and behavior during childhood

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Abstract

Objective: To examine the association between amount of fatty acid ethyl esters (FAEEs) and behavioral outcomes in children prenatally exposed to alcohol and drugs. Methods: A secondary analysis was conducted of a prospective cohort of cocaine, polydrug exposed children, primarily urban, African-American, low socioeconomic status, recruited at birth into a longitudinal study. FAEEs were quantified with gas chromatography via a flame ionization detector. Meconium was analyzed for 216 newborns, with 194 assessed with the Child Behavioral Checklist (CBCL) at ages 4, 6, 9, 10, 11, and 12. Longitudinal mixed model analyses assessed the relationship of quantity of FAEEs to outcomes, controlling for maternal psychological distress. Results: Higher concentrations of FAEEs (ethyl myristate, ethyl palmitate, ethyl oleate, and ethyl linoleate) were associated with greater aggressive behavior at 2 of the 6 ages. A marginal ($p < 0.10$) FAEE by age interaction effect was found. When levels of FAEEs were dichotomized at the median, negative effects were noted on aggressive behaviors at ages 10 and 12. The proportion of children with T scores ≥65 below the median concentration of ethyl linoleate was 0.095 vs 0.197 above the median, and 0.093 vs 0.202 for ethyl palmitate (p's < 0.05). Conclusions: Higher concentrations of FAEEs in meconium are potential markers for children at risk for aggressive behaviors related to the effects of prenatal alcohol exposure.

DNTS P17

Prenatal Substance Exposure and Trajectories of internalizing problems across childhood: The maternal lifestyle study

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Abstract

Objective: The present study aimed to: 1) identify developmental trajectories of internalizing problems; and 2) examine whether trajectories differ by prenatal substance exposure (PSE). Methods: Participants were 1257 mother-infant/child dyads, primarily African-American and of low socioeconomic status, prospectively enrolled in a longitudinal, multisite study of prenatal cocaine or opiate exposure at birth from 1993 to 1995. Internalizing problems were assessed with the Child Behavior Checklist at ages 3, 5, 7, 9, 11, and 13 (91% retention). Results: Five internalizing behavior trajectory groups were identified using latent class growth modeling: 1) no-risk group (26%) constantly reporting below the mean *T*-score; 2) normative-decreasing group (37%) with *T*-scores below the mean at age 13; 3) increasing group (12%) with *T*-scores reaching the borderline range (60) at late childhood; 4) high-decreasing group (22%) with *T*-scores hovering in borderline range; and 5) very high-chronic group (3.4%) with *T*-scores above the clinical cut-point (67) throughout the entire assessments. Multinomial regression analyses, controlling for maternal psychological distress, indicated that, compared to the no-risk group, prenatal nicotine exposure was associated with increased odds of being in the normative-decreasing (OR = 1.36 (95% CI = 1.01–1.83)) and in the high-decreasing (OR = 2.03 (1.42–2.90)) groups. Prenatal opioid exposure (OR = 3.78 (1.36–10.48)) and maternal abuse (OR = 3.22 (1.40–7.42)) were associated with increased odds of being in the very high-chronic group. Boys were more likely to be in the high-decreasing (OR = 1.54 (1.09–2.19)) and the very high-chronic groups (OR = 2.37 (1.08–5.22)). Conclusions: Prenatal exposure to nicotine and opioid differentiated developmental trajectories of internalizing behavior.

DNTS P18

Frontal brain volume asymmetry: A neurobiological marker for prenatal tobacco exposure in young adults

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Abstract

Prenatal tobacco exposure is associated with an increased risk for attention problems and a diagnosis of ADHD. Attention circuits in the brain rely on the function and efficiency of frontal lobe brain regions. This study analyzed the structural changes found in the brains of young adults with ($N = 17$) and without ($N = 25$) prenatal tobacco exposure (PTE) while controlling for current smoking and other demographic and environmental factors. Forty-two participants were assessed from the Maternal Health Practices and Child Development Project, a longitudinal study of the effects of prenatal drug exposure on developmental outcomes. Magnetic resonance images were processed using the automated labeling pathway technique. Volume and asymmetry were calculated using voxel count for all brain regions (L-R/L + R). The PTE group showed significant ($p < 0.05$) increases in asymmetry in frontal brain regions that were associated with reduced volume in the frontal lobes, overall, and reductions in the left hemisphere, specifically. No differences were found in the parietal, temporal, or occipital lobes suggesting that PTE may interfere with the normal development of the frontal lobes. PTE was associated with self-reported attention deficit symptoms experienced during childhood as measured by the Wender Utah Rating Scale. PTE was also associated with attention deficits in adulthood as measured by the Adult ADHD Self-Report Scale. These results suggest that prenatal tobacco exposure is related to structural brain differences in the frontal lobe as well as attention difficulties that persist into young adulthood.

DNTS P19

Chronic paternal THC in rats causes long-lasting behavioral disruption in the offspring

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Abstract

The potential health risks of cannabis are of growing concern, including effects on reproduction and development. Extensive research has investigated risks associated with maternal exposure to this psychoactive compound, yet little has been done regarding paternal exposure to THC prior to conception and its impacts on the development of offspring. As offspring inherit a portion of their DNA methylation pattern from paternal sperm and we have previously found that paternal THC exposure in rats causes changes in this methylation, chemical exposures of the father have the potential to lead to abnormal genetic expression and

neurobehavioral development in the offspring. The current study investigated the intergeneration effects of chronic THC exposure of young adult male rats (0, 2, or 4 mg/kg/day SC for 28 days) prior to mating with drug naïve female rats. Although the clinical health of the offspring was not significantly affected by paternal exposure, preliminary data from these offspring show significant alterations in memory and locomotor activity when compared to controls. Non-significant trends are also apparent when rats were tested in a signal detection attention task. Specifically, during adolescence there was significant locomotor hyperactivity in the offspring of fathers exposed to 2 mg/kg/day of THC prior to mating. This pattern of hyperactivity did not persist into adulthood. Rather, offspring of fathers exposed to 4 mg/kg/day showed significant hypoactivity early in the test session, when tested as adults. During the novel-object recognition task, the controls maintained their relative preference for the novel object across the duration of the ten-min session while the rats whose fathers received THC (2 mg/kg/day) showed a significantly greater drop-off in interest in the novel object during the second half of the session. Our preliminary data also shows that offspring in the exposure groups are trending toward attentional impairment during a signal detection attention task, but more subjects will need to be added to provide sufficient statistical power to test for this possible effect. This study shows that premating chronic paternal THC exposure can cause detrimental behavioral effects, including abnormal locomotor activity, attentional impairment, and reduced memory and motivation. Future studies should investigate the underlying mechanisms driving these aberrant developmental outcomes and seek to identify possible behavioral or pharmacological treatments.

DNTS P20

Prolonged methamphetamine exposure during a critical period in neonatal Sprague-Dawley rats does not exacerbate egocentric and allocentric learning but increases reference memory impairment

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Abstract

Methamphetamine (MA) abuse is a worldwide problem. Associated with MA abuse are children who were exposed to MA in utero and have cognitive deficits. Rat models show developmental MA affects cognitive processes when MA is administered for 5–10 days during a critical period of postnatal development, a period of brain development associated with the last half of human pregnancy. Therefore, this study examined whether extending MA administration (>10 days) beyond the identified critical period would exacerbate the allocentric and egocentric learning and memory deficits observed in rats as adults. Sprague-Dawley female and male offspring (split-litter design) were administered saline (SAL) or MA (10 mg/kg) four times daily from postnatal day (P)6–20 to create four groups: (1) SAL from P6–20, (2) MA from P6–15 and SAL P16–20 (MA6–15), (3) SAL from P6–10 and MA from P11–20 (MA11–20), or (4) MA from P6–20 (MA6–20; the extended exposure group). Egocentric (Cincinnati water maze), allocentric (Morris water maze), and conditioned freezing testing began on P60. The MA6–15 and MA6–20 groups had egocentric deficits and all MA-treated groups had allocentric deficits compared with SAL controls. No differences in latent inhibition of conditioned freezing were found for any MA group compared with the SAL group. Overall, the MA6–15 and MA6–20 groups had similar deficits in learning and memory that were larger than in the MA11–20 group. Sex differences were seen on learning in both mazes, but no interactions with MA were found. The data indicate that extending the exposure period of MA to span overlapping days of two previously established sensitive periods (P6–15 and P11–20) did not increase the cognitive deficits compared with P6–15 exposure.

DNTS P21

Executive function in the LPHN3$^{-\backslash-}$ rat: Relevance to attention-deficit/hyperactivity disorder

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Abstract

Children diagnosed with attention-deficit hyperactivity disorder (ADHD) often suffer from executive function deficits including problems with inhibitory control. The orphan receptor gene Latrophilin-3 (*Lphn3*) has been identified as conveying a high risk for ADHD. *Lphn3*$^{-/-}$ (knockout) rats were generated at Cincinnati Children's Genome Editing Core and shipped along with wildtype (*Lphn3*$^{+\backslash+}$) rats on approximately postnatal day (PND) 45. Male and female rats were food restricted to 90% of their free-feeding weight beginning on PND 60. On PND 70 they began daily operant testing to examine impulsive action via several differential reinforcement of low rates of responding (DRL) tasks where rats were required to wait 5, 10, or 15 s between lever presses in order to earn a reinforcer. Analysis of the DRL data revealed a lower ratio of reinforced:non-reinforced responses in the *Lphn3*$^{-/-}$ rats. This result occurred due to a higher proportion of short inter-response times (IRTs) and lower proportion of long IRTs in the knockouts. Overall, these results suggest inhibitory control (particularly impulsive action) is impaired in *Lphn3*$^{-/-}$ rats, thereby providing supporting evidence for the validity of these knockouts as an animal model of ADHD.

DNTS P22

Altered dopaminergic markers and striatal dopamine release dynamics in hyperactive *Lphn3* KO rats

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Abstract

Attention deficit hyperactivity disorder (ADHD) is a heterogeneous neurodevelopmental disorder affecting ~5% of children in the United States. Genetic, imaging, and pharmacological data suggest impairment of dopaminergic neurotransmission in these children. Latrophilin-3 (LPHN3) is a brain specific G-protein coupled receptor that has been associated with increased risk of ADHD. Rats with a constitutive knockout (KO) of *Lphn3* are hyperactive and under-respond to amphetamine; they are also, hyperreactive to startle stimuli, and less anxious (females) in an elevated zero-maze. Western blots were used to examine key neurotransmitter and receptor markers. Extracellular dopamine transients were measured using fast-scan cyclic voltammetry (FSCV) using implanted carbon-fiber electrodes in brain slice preparations. Increased expression was seen in striatal tyrosine hydroxylase and the dopamine transporter, whereas decreased expression of dopamine receptor 1 and DARRP-32 were seen in *Lphn3* KO rats. FSCV showed increases in dopamine content per transient and an increase in transient frequency in KO rats. The *Lphn3* KO rat is a new model for understanding what LPHN3 does in the brain and how it might be connected to ADHD. (Supported by the L.I.F.E. Foundation).