NTX1

Neurotoxicity of Acute Diesel Exhaust Exposure in Adult Mice

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Increasing evidence suggests that air pollution, in addition to increasing morbidity and mortality caused by respiratory and cardiovascular diseases, may also negatively affect the central nervous system (CNS) and contribute to CNS diseases. Particulate matter (PM), and in particular ultrafine particulate matter (UFPM; <100 nm), is believed to be the most widespread threat and has been heavily implicated in disease. Traffic-related air pollution is a major contributor to global air pollution, and diesel exhaust (DE) is its most important component, as it is a major constituent of ambient PM, particularly of UFPM. Adult mice of both sexes were exposed to DE exhaust (250-300 ug/m^3 for six hours) in the University of Washington exposure facilities. Oxidative stress and neuroinflammation were assessed in several brain regions by measuring lipid peroxidation and a panel of pro-inflammatory cytokines. DE exposure increased oxidative stress and caused neuroinflammation in all brain areas (particularly in the olfactory bulb and the hippocampus), and the effects were more pronounced in male mice, possibly because of lower expression of the antioxidant and anti-inflammatory enzyme paraoxonase 2. Activation of microglia by DE exposure was suggested by increases in Iba1 levels. A primary role played by microglia-related oxidative stress and neuroinflammation was also suggested by in vitro studies with DE particles in neuron-microglia co-cultures. As neuroinflammation may impair adult neurogenesis, the latter was measured in three brain regions (hippocampus, subventricular zone, olfactory bulb). DE exposure impaired neurogenesis in all three areas, particularly in the hippocampus. The effect of DE was more pronounced in male mice. These initial results suggest that even a short-term exposure to DE exhaust negatively impacts the CNS. It also suggests that males may be more at risk for neurotoxicity, possibly because of lower antioxidant abilities.

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NTX2

Microglia as central nervous system sentinels and the detection of air pollution

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Air pollution is being increasingly implicated in central nervous system (CNS) pathology/disease, but the mechanisms for this interaction are poorly understood. Microglia, the resident innate immune cells in the brain, are linked to chronic neuron damage and evidence indicates that microglia detect and respond to diverse forms of air pollution exposures. Diesel exhaust (DE) is a major component of near-road and urban pollution and has received significant attention regarding its human health effects in both ambient and occupational exposure conditions. Adult male rats exposed to DE (2.0, 0.5, and 0 mg PM/m3) for one month by inhalation show elevated markers of neuroinflammation (nitrotyrosine, IL-6, TNFα, and MIP-1α) in the midbrain, cortex, and olfactory bulbs, in addition to altered morphology. The highest levels of neuroinflammation occurred in the midbrain, which also contained the highest level of the IBA-1 microglial marker. Microglia exposed to nanometer-sized DEP (50μg/ml) were activated to produce H2O2 through the MAC1 receptor, indicating that particulate matter is capable of activating microglia through established neurotoxic pathways. Rats exposed to biodiesel exhaust revealed only changes in microglia morphology without any elevation of M1 or M2 factors, demonstrating that the type of microglial activation may be exposure specific. O3 is a prevalent air pollutant recently linked to increased Alzheimer's disease risk and O3 is unable to translocate to the brain parenchyma. However, rats exposed to1 PPM O3 for 4 hours showed persistent changes in microglial morphology. To address whether O3-induced circulating signals may be culpable in the microglial activation, in situ assays testing the pro-inflammatory reactivity of microglia were performed. Serum from O3- exposed rats was shown to augment the microglial pro-inflammatory response (H2O2 and TNFα) to LPS and Aβ-induced neurotoxicity in vitro. Analysis of the O3-induced bioactive serum failed to demonstrate elevation of traditional cytokines and chemokines (TNFα, IL-1β, MIP-1α, CCL1, & CCL2). Taken together, data indicate that air pollution may impact microglia through multiple pathways and point to a "Lung-Brain Axis", where inhaled pollutants may result in circulating signals independent of traditional cytokines that prime microglia to become more sensitive to deleterious stimuli.

NTX3

Prenatal Air Pollution Exposure Effects on Autism Spectrum Disorder and Neurodevelopment

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Autism spectrum disorder (ASD) often has a heterogeneous presentation and broad range of impairment. Prenatal air pollution has been associated with ASD, poorer neurodevelopmental outcomes, and increased cognitive deficits in separate studies. We examined the relationship between prenatal air pollution exposure and ASD risk as well as ASD severity, cognitive ability, and adaptive functioning among children with ASD using data from Childhood Autism Risks from Genetics and the Environment (CHARGE), a population based case-control study of children from California. Separately, we examined the relationship between prenatal air pollution exposure and ASD risk and severity among affected and unaffected sibling pairs from the Autism Genetics Resource Exchange (AGRE). In both studies residential histories were geo-coded and used to develop estimates of average air pollution exposures during pregnancy. Regional air quality measures were assigned based on the EPA's Air Quality Monitoring Network. In CHARGE we examined Mullen Scales of Early Learning (MSEL) composite and subscale scores, Vineland Adaptive Behavior Scales (VABS) composite and subscale scores, and the Autism Diagnostic Observation Schedule (ADOS)-derived Calibrated Severity Score (CSS) as continuous phenotypes. In AGRE, the CSS and Social Responsiveness Scale (SRS) were used as continuous outcomes. Both studies examined risk of ASD using a positive ADOS and Autism Diagnostic Interview-Revised (ADI-R) to determine case status. In CHARGE, increasing NO2 exposure during pregnancy was associated with an 11% (95%CI (-20%, -2%) decrease in the VABS composite and a 26% (95% CI (-34%, -8%)) decrease in composite MSEL scores. Additional deficits were identified among communication and language subdomains with increasing exposure to NO2 and PM2.5. In AGRE, we found that increasing exposure to PM2.5 was associated with increased risk of ASD among siblings. These results highlight the potential for genetic susceptibility to prenatal air pollution exposure and contribute to the growing literature regarding neurotoxic effects of air pollution exposure.

NTX4

Developmental Exposure to Ultrafine Particle Air Pollution Produces Features of the Autism Phenotype

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The brain is increasingly recognized as a target of the inflammatory effects of air pollution. Particularly disturbing are the increasing numbers of epidemiological reports associating air pollution with diagnosis of autism spectrum disorder (ASD), a profound, heterogeneous and intractable neurodevelopmental disorder. Recent studies from our laboratories provide biological plausibility for these epidemiological associations. Mice were exposed to ambient roadside intake ultrafine particle air pollution (UFPs, <100 nm), considered the most toxic component of air pollution, at levels consistent with those in major urban U.S. cities but far below those in more polluted regions such as in China. These represent highly relevant real world exposures. The exposures took place from postnatal days (PND) 4-7 and 10-13, considered equivalent to the human third trimester, that encompasses a period of rapid neuro- and gliogenesis. UFP exposures produced a series of outcomes consistent with the impairments seen in children affected by ASD, with a more severe phenotype occurring in males. These included male-specific persistent disruption of white matter development, including in corpus callosum (CC), the largest white matter tract in brain and critical to inter-hemispheric connectivity, with resulting ventriculomegaly, and persistent microglial activation. Both sexes exhibited increased brain glutamate levels and astrocyte activation, and sex-specific deficits were seen in learning, memory and attention. Although the behaviors examined were not targeted specifically to those used for ASD diagnosis, evidence of repetitive behavior was found in both sexes, and astrocytic activation in amygdala in males would portend aberrant social behaviors. Collectively, these findings suggest that developmental air pollution exposure could represent an environmental risk factor for components of the ASD phenotype, or for other neurodevelopmental disorders known to share these neuropathological features. Further, they carry significant public health implications considering that National Ambient Air Quality Standard regulations exist for particles of sizes ≤10 um and ≤ 2.5 um, (i.e., PM10 and PM2.5), while data for regulating the more toxic UFPs (≤ 100 nm) is still considered equivocal.

NTX5

Epidemiological Studies on Outdoor Air Pollution Exposure and Neuro-psychological Effects: From Cradle to Grave

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Air pollution is a major environmental risk to health. Beside the adverse effects on lung function and the cardiovascular system there is accumulating evidence from epidemiological studies that long-term exposure to outdoor air pollution is associated with adverse effects on the central nervous system (CNS), in particular in children and adults.

Several studies have shown that in the general population these adverse effects include neuropsychological development in children, neurodegeneration in adults. It has been demonstrated that various components of air pollution, such as ultrafine particles, can easily translocate to the CNS where they can activate innate immune responses. Furthermore, systemic inflammation arising from the pulmonary or cardiovascular system can affect CNS health. Despite intense studies on the health effects of ambient air pollution, the underlying molecular mechanisms of susceptibility and disease remain largely elusive.

The aim of this presentation is to provide an overview on the recent epidemiological findings on the association between air pollution exposure and neuropsychological development in children and cognitive impairment in adults.

NTX6

Inhaled Ultrafine Particles Increase Inflammatory Markers in Rodent Brains and May Contribute to Neurodegeneration

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Exposure to particulate matter (PM), a component of urban air pollution, may cause adverse effects in the brain. Since the main route of exposure to particulate matter is through inhalation, there is a potential for compounds to directly enter the brain and alter normal cellular function. Several studies show that in rodent models, inhaled particles enhance inflammatory markers in a region-specific manner. Enhancement in neuroinflammatory markers has been observed in neurodegenerative disorders, such as Alzheimer's disease (AD), and PM-induced potentiation of these events may accelerate the disease process. In a pilot study, we demonstrate that exposure to ultrafine particles, derived from heavily polluted area in California, upregulates inflammatory and disease markers in a transgenic animal model of AD. Using normal human brain cells, we further demonstrate that neuronal cells may be specifically vulnerable. These data validate the concept that the brain is a target for the adverse effects of ambient ultrafine particles and support the need for more thorough future investigations.

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NTX7

Inhaled Ultrafine Particulate Matter and Neurodegeneration; On the Biological Plausibility of Mechanisms

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The aetiology of neurodegenerative disorders such as Alzheimer's and Parkinson's disease is complex and multifactorial. Environmental factors, including air pollution, reportedly play a major role. However, knowledge about which factors and their exact role in degenerative disorders is fairly limited. Air pollution is an increasing problem on a global scale. It comprises a complex mixture of (semi-) volatiles, gaseous compounds and particulate matter (PM) originating from diverse sources. Part of the inhalable fraction of PM is ultra-fine particulate matter (UFPM; aerodynamic diameter <0.1µm). UFPM is able to enter the alveolar part of the respiratory system and, depending on its physicochemical properties, can translocate to the systemic circulation and extra-pulmonary organs including the brain. Also, transport of UFPM from the nasal cavity to the brain via the olfactory nerve has been demonstrated. In the lung and the cardiovascular system UFPM can evoke oxidative stress and inflammatory processes and exposure is related to (lung) fibrosis and atherosclerosis. *In vitro* as well as *in vivo* studies demonstrate that inhalable UFPM is also able to induce a wide array of effects in the brain that may link exposure to the pathophysiology of neurodegenerative diseases. However, the components responsible for these effects and underlying mechanisms remain to be elucidated.

NTX8

The aerotoxic syndrome: Is there a new low-level neurotoxic syndrome in the air?

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Airmonitoring studies in aircrafts and biomonitoring among passengers showed that exposures to tri-cresylphosphates (TCPs), including the neurotoxin tri-ortho-cresylphosphate (ToCP), during single and repeated flights are very low. However, by using the phosphorylation of butyrylchonilneesterase (BChE), a highly sensitive method for the detection of possible exposures to ToCP and its metabolite cresyl saligenin phosphate (CBDP), such low-level exposures could be confirmed in some jet airplane passengers. Regardless of the possible exposure to toxic chemicals in aircraft cabins there is a sufficient number of affected people that complain about symptoms related to impaired nervous system functioning (e.g. loss of memory). However, the modes of action of the individual TCP isomers are unknown and the effects of exposures to TCP mixture has not been subjected to systematic investigations. TCP isomers are not the only pollutants in cabin air and a more comprehensive analysis of the various health hazards is still lacking.
The AS is not unique with regard to environmentally caused health effects. The possible health effects of low-level exposure to chemicals in indoor environments have often been studied in the context of the sick-building syndrome (SBS) or more general idiopathic environmental intolerance (IEI). Even thought multi-organ effects, including the respiratory tract, characterize these syndromes, the obvious parallelism might help to shed light on the etiology of AS. Airborne chemicals are usually sensed by the chemosensory systems (i.e. olfaction and trigeminal chemoreception) and have also been described as triggers for subsequent illness. Inter-individual differences (e.g. negative affect) are known modulators of health complaints due to low-level chemical exposures. The impact of these factors is often stronger than the concentration of the chemical that causes the perception. Moreover, unspecific health complains like headaches, irritability and gastrointestinal symptoms in response to odors are also more likely to be elicited in certain individuals. Besides that, we recently showed that acute stress reduces the detection threshold for foul-smelling chemicals. Such results need to be included into future studies investigating the AS in humans.

NTX9

Can ozone-initiated chemistry explain symptoms among air crewmembers?

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Epidemiological studies of air crewmembers show a similar pattern of symptoms as encountered in offices within the umbrella of the so-called "Sick-building Syndrome" symptoms: eye related symptoms, e.g. dry or tired eyes; and CNS related symptoms, like headache and tunnel vision. However, VOCs generally have thresholds for sensory irritation that are one to three orders of magnitude higher than cabin air concentrations, except for formaldehyde. Thus, the reactive chemistry, i.e. ozone-initiated reactions with terpenes and surface deposited reactants, e.g. squalene, producing a host of oxygenated reaction products and ultrafine particles, was suggested as causative. Thus, two hypotheses have been proposed to encounter the sensory related symptoms: reaction products from ozone-initiated chemistry and low relative humidity. This review will briefly report about recent measurements in air cabins of VOCs and ozone-initiated reaction products, and assess their potential health effects. Air cabin measurements are risk assessed by the health index for sensory irritation of target cabin air pollutants; health effects are assessed in context of low relative humidity and reduced cabin pressure. Major VOCs were toluene, xylenes, limonene, and TCPs; ozone-initiated reaction products formaldehyde, acrolein, 6-MHO, 4-OPA, formic and acetic acid, C6-C10 aldehydes, and ozone. Except for ozone, the air cabin pollutant concentrations are comparable with data from residences and offices. The health index of maximum reported mean cabin air concentrations of the target pollutants for sensory irritation was less than 0.2, and dominated by formaldehyde. The contribution of major cabin air target pollutants, that included formaldehyde, could not explain eye irritation symptoms, even when normal addition was assumed. Several of the pollutants, in particular the aldehydes, have low odor thresholds; thus, they are likely to influence perceived air quality. Elevated ozone events and temporary events like deicing may alter the air quality. Occupational, thermal climate, and personal risk factors should be assessed for ocular symptoms. The reported cabin air concentrations and the presence of ultrafine particles are not considered causative. Rather, elevation of the relative humidity improves eye tear film quality and reduce the eye symptoms. Reported mean cabin air concentrations appear too low to be associated with CNS effects.

NTX10

Towards a Clinical Diagnosis of the Aerotoxic Syndrome, Possible Methods and Challenges

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In the last years, aviation companies in the Netherlands were increasingly confronted with employees reporting symptoms they claimed to be attributed to the neurotoxic effects of Tricresylfosfate (TCP) in contaminated bleed air due to motor oil leaks. The repeated national media attention raised awareness of the problems to a wider public. After an investigation of a news report television program “Zembla”, the Dutch Inspectorate of Environment and Transport invited concerned employees to report themselves to offer clinical and etiological diagnostics and care.

The Netherlands Center for Occupational Diseases was requested to evaluate the nature and severity of the symptoms of those employees and to assess whether the symptom cluster could possibly be regarded as a new occupational disease. In contrast to what was expected only a few employees reported themselves for investigation.

In absence of a golden standard for the diagnosis of the Aerotoxic syndrome, we choose a multidisciplinary approach, in analogy to the method used in the “Solvent Team project” for the diagnosis of Chronic Solvent induced Encephalopathy. The assessment included an interview by an occupational physician to assess the occupational history and the development of symptoms during employment, routine laboratory blood tests, an estimation of the extent of the exposure by flight hours and the number of ‘fume events’, and a neuropsychological assessment to objectify signs of neurotoxicity. If needed a neurologist would be consulted and neuroimaging could be incorporated in the diagnostic evaluation.

The Inspectorate of Environment and Transport have received our report and they have sent it to the Dutch Secretary of State of Infrastructure and Transport. For now the results are not yet open to the public but will be when the INA conference is held. In the presentation results of the clinical assessment will be discussed in the light of the stepwise assessment procedure of an occupational disease and literature on ATS, together with the implications for further research.

NTX11

Neurotoxic Hazard Characterization and Risk Assessment of Different TriCresyl Phosphate (TCP) Isomers

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TriCresyl Phosphates (TCPs) are widely used as plasticizers, flame retardants, and as additives in lubricants and hydraulic fluids to prevent wear and tear of aircraft engines. The resulting possible exposure to TCPs in cabin air and the suggested association with the so-called aerotoxic syndrome has led to concerns among aircrew. TCP neurotoxicity is mainly attributed to *ortho*-isomers including Tri-*ortho*-Cresyl Phosphate (T*o*CP), which inhibits acetylcholine esterase (AChE) and neuropathy target esterase (NTE), potentially resulting in cholinergic complaints and organophosphate-induced delayed neuropathy (OPIDN). The neurotoxic potential of *ortho*-isomers led to a strong reduction of the commercial use of T*o*CP and it is now absent from most commercial aircraft engine oils. Consequently, recent exposure studies indicate that T*o*CP levels in cabin air are very low. Our recent risk assessment therefore indicates it is unlikely that exposure to T*o*CP is responsible for the reported health complaints. Other causes for these health complaints must thus be considered, including exposure to non-*ortho* TCP isomers.

Since the knowledge about the neurotoxic potency of these non-*ortho* TCP isomers is rather sparse, we initiated an *in vitro* screening to compare the effects of different TCP isomers on viability and spontaneous electrical activity of primary rat cortical neurons. Our initial results using neutral red and CFDA-AM assays demonstrate that neuronal viability is not considerably affected following 24h or 48h exposure to different TCP isomers up to 10 µM. While some mild cytotoxic effects are observed at 100 µM, all isomers induce a profound increase in an alamar blue assay at 10-100 µM, indicative for mitochondrial hyperactivity. Next, acute effects of TCP isomers on spontaneous activity of rat cortical neurons were investigated at non-cytotoxic concentrations using a multi-electrode array (MEA) approach. None of the TCP isomers show a profound effect on the mean spike rate (MSR) at concentrations up to 1 µM. At 10 µM, T*p*CP and T*m*CP decrease MSR by ~20%, whereas T*o*CP increases MSR by ~30%. Despite these seemingly opposite effects, the lowest-observed-effect-concentrations (LOECs) are comparable. We may therefore need to consider the potential neurotoxicity of non-*ortho* TCP isomers for risk assessment purposes.

NTX12

Tri-ortho-cresylphosphate and TCP isomers – neurotoxic effects in addition to OPIDN?

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Exposures to tri-cresylphosphates cause various types of neurotoxicity. One isomer, tri-ortho-cresylphosphate (ToCP), is a well-studied organophosphate (OP) which is known to cause delayed neuropathy (OPIDN) by inhibiting neuropathy target esterase (NTE). TCP isomers, including ToCP and its metabolite cresyl salignin phosphate (CBDP), have been discussed as causative agents of the aerotoxic syndrome (AS) based on their use as additives in jet gear oil. Symptoms of AS encompass headache and problems in coordination. These symptoms are more likely to be based on impaired central nervous processes and can hardly be explained by OPIDN. Thus, alternative modes of action of the different TCPs have to be investigated. Weused a battery of in vitro approaches to identity such neurotoxic effects of TCPs and CBDP on function and structure of mouse cortical neurons.
Cell viability, neurite morphology as well as the responsiveness of the neurons to glutamate stimulation were assessed after different treatment conditions. After 24 h treatment with TCPs, neurite structures were altered in a concentration-dependent manner. By using fluorescence-based life-cell calcium imaging we could show that low concentrations of ToCP (nanomolar range) not affecting cell viability or neurite structures, impair glutamate sensitivity. The effects of TCP isomers after similar treatment on glutamate sensitivity were obviously weaker. In contrast higher concentrations (1 to 10 µM) reduced the glutamate responsiveness. In the OPIDN literature CBDP is described as more toxic than ToCP and of greater relevance for AS. Our results show that CBDP cause no significant effect on glutamate sensitivity after 24 h pretreatment. Moreover, ToCP in contrast to the other TCPs and CBDP acts specifically as a concentration-dependent blocker for glutamate receptors. To investigate the underlying mechanisms of reduced glutamate sensitivity and to identify the affected receptor subtypes we used qRT-PCR and gene-array technique.
Our results show that ToCP affects the functionality and morphology of cortical neurons at lower concentrations than the other TCPs. This effect is specific for glutamate receptors and is not observable for CBDP. Thus, the ban of ToCP from TCP mixtures in lubricants is an important step to reduce the exposures to neurotoxic compounds in aircrafts.

NTX13

In vitro neurochemical screening assays to predict adverse outcomes of a set of potentially neurotoxic chemicals in fish, birds, and mammals

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The overall hypothesis of this study is that several toxicants will emerge and interact with, and disrupt the function of neurotransmitter receptors and enzymes that mediate vertebrate reproduction and behavior. As current animal studies are expensive, time-consuming, and overlook many at-risk organisms, development of a new, high-throughput screening method is required. Cell-free methods are now a component of the U.S. EPA's ToxCast program, and development of such bioassays for predicting adverse outcomes is particularly attractive in ecological risk assessment owing to the lack of screening methods that span several taxa and a limited ability to conduct whole-animal bioassays. Thanks to these methods, chemicals can be prioritized according to their toxicity for more in-depth animal dose-response studies. Here, we conducted a series of in vitro screening assays assessing 6 neurotransmitters receptors and 3 enzymes associated with essential behavior and reproduction via the glutamatergic, GABAergic, dopaminergic, serotonergic, cholinergic, and other neurochemical pathways. Such neurochemical receptors and enzymes were isolated from different organisms (n=20) of multiple taxa, including freshwater and marine fish, birds, terrestrial and marine mammals, and biomedical species including human. The isolated neurochemical receptors and enzymes were dosed in vitro with a diverse set of 80 potentially neurotoxic chemicals, such as metals, rare-earth elements, pesticides, personal care products, flame-retardants, and others. Initial results indicate that high-quality data was collected across multiple organisms not easily studied in the lab, or in the field, with consistent ‘hits' found across species (such as tin and DDE). We generated important data on several toxicant classes (including real-world mixtures) that are of ecological relevance. This work resulted in the establishment of a mid-throughput screening assay that can be used to predict neurochemical effects across multiple ecologically relevant species (birds, fish and mammals). In this presentation, we will also discuss how to identify hits from the preliminary results and prioritize chemicals for further investigations.

NTX14

Lead-induced disruption of brain barriers and its mechanisms

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Lead (Pb) is a widespread environmental pollutant that is known to cause a wide range of neurotoxic effects, especially in children. The knowledge about Pb-induced cognitive deficits has increased in the recent years; however, the distribution and transport of Pb in brain were less studied. In our study using SD rats with gestational Pb exposure, the Pb levels in difference brain regions in the pups was determined. Autometallography showed that Pb accumulated in the hippocampus, cortex, and piriform lobe. The results from X-Ray Fluorescence (XRF) confirmed that Pb mainly accumulated in hippocampus and frontal cortex. Interestingly, Pb exposure also resulted in altered levels of some essential elements, such as Zn, Fe and K, in the brain. Since the homeostasis of elements in brain is regulated by brain barriers, the impact of Pb exposure on blood-brain barrier (BBB) and the blood-cerebrospinal fluid barrier (BCB) were investigated. The results demonstrated that Pb exposure resulted in disruption of both BBB and BCB, accompanying with decreased levels of tight junction proteins, such as occludin and ZO-1. In the in vitro BBB model using rat brain microvascular endothelial RBE4 cells, Pb exposure was found to significantly increase the level of Src phosphorylation. The Pb-induced down-regulation of occludin, but not ZO-1, was rescued by of Src inhibitor, dasanitib. By using siRNA knockdown, glucose-regulated protein 78 (GRP78) was found to be an upstream molecule regulating Src phosphorylation and then affected occludin level. In the in vitro BCB model using rat choroidal epithelial Z310 cells, Pb exposure was also found to activate Src and specifically regulated occludin level. Although Pb treatment did not affect GRP78 protein level in Z310 cells, Pb was found to have high binding affinity to GRP78 and then decreased its function in the folding of proteins. In addition, we found that a gap junction protein, Connexin-43, plays an important role in the Pb uptake and release process in Z310 cells. Together, Pb enters brain parenchyma through brain barriers and accumulates mainly in the hippocampus and cortex. The activation of Src signaling and up-regulation of GRP78 contribute to the Pb exposure-induced disruption of brain barriers.

NTX15

-NMDA R/+VDR pharmacological phenotype as a novel therapeutic target in relieving motor-cognitive impairments in Parkinsonism

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A common mechanism in the cause and progression of drug/chemotoxin induced Parkinsonism involves calcium signalling in oxidative stress, autophagy, cytoskeletal instability and excitotoxicity. This study sets to target calcium controlling receptors, specifically activation of Vitamin D3 receptor (VDR) and inhibition of N-Methyl-D-Aspartate Receptor (NMDAR) in the motor cortex of mice model of drug induced Parkinsonism. Also we demonstrated how these interventions improved neural activity, cytoskeleton, glia/neuron count and motor-cognitive functions in vivo.
Adult mice were separated into six groups of n=5 animals each. 5mg/Kg body weight of haloperidol was administered intraperitoneally for 7 days to block dopaminergic D2 receptors and induce degeneration in the motor cortex, following which an intervention of VDR agonist (VDRA), and (or) NMDAR inhibitor was administered for 7days. Control animals received normal saline while a separate group of control animals received the combined intervention of VDRA agonist and NMDAR inhibitor without prior haloperidol treatment. Motor and cognitive functions were tested at the end of the treatment and intervention periods, and neural activity in the motor cortex was recorded in vivo using unilateral wire electrodes. We also employed immunohistochemistry to demonstrate neuron, glia, neurofilament and proliferation in the motor cortex after haloperidol treatment and the intervention. We observed a decline in motor function and memory index in the haloperidol treatment group when compared with the control. Similarly, there was a decline in neural activity in the motor cortex (a reduced depolarization peak frequency). Cell loss (neuron and glia) and depletion of neurofilament were observed in the motor cortex of this group. However, Vitamin D3 intervention facilitated an improvement in motor-cognitive function, neural activity, glia/neuron survival and neurofilament expression. NMDAR inhibition and the combined intervention improved motor-cognitive functions but less than that observed in VDRA intervention. Our findings suggest that calcium mediated toxicity is primary to the cause and progression of Parkinsonism and targeting receptors that primarily modulate calcium reduces the morphological and behavioural deficits in drug induced Parkinsonism. VDR activation was more effective than NMDAR inhibition and a combined intervention.
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NTX16

Deficits in neural responses to manganese exposure in Huntington's Disease models

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The essential micronutrient manganese is enriched in brain, especially in the basal ganglia. We sought to identify neuronal responsive markers to neurologically relevant manganese levels, as previous data suggested that alterations in striatal manganese handling occur in Huntington's disease (HD) models. We report that p53 phosphorylation at serine 15 is the most responsive cell signaling event to manganese exposure (of 18 tested) in human neuroprogenitors and a mouse striatal cell line. Manganese-dependent activation of p53 was severely diminished in HD cells. Analysis of ATM activation and inhibition support a role for ATM in the activation of p53 by manganese and that a Mn-specific defect in this process occurs in HD. We hypothesized that the modulation of Mn neuronal biology in HD is associated with compensatory metabolic responses. We used an untargeted metabolomics approach by performing ultraperformance liquid chromatography-ion mobility-mass spectrometry (UPLC-IM-MS) on control and HD immortalized mouse striatal neurons to identify metabolic disruptions under three Mn exposure conditions, low (vehicle), moderate (non-cytotoxic) and high (cytotoxic). Our analysis revealed lower metabolite levels of pantothenic acid, and glutathione (GSH) in HD striatal cells relative to control cells. HD striatal cells also exhibited lower abundance and impaired induction of isobutyryl carnitine in response to increasing Mn exposure. In addition, we observed induction of metabolites in the p53 regulated pentose shunt pathway in HD striatal cells after high Mn exposure. These findings provide evidence of an interaction between the HD genotype and biologically relevant levels of Mn. Ongoing studies aimed at dissecting the molecular underpinnings of the alterations in neuronal Mn handling in HD will also be presented.

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NTX17

Introduction to Session on the Neurotoxicity of Brominated Flame Retardants and the Quest for Safer Alternatives

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Some brominated flame retardants (BFRs), such as polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD), are progressively being phased out due to their neurotoxic properties. As a result, there is growing interest in finding alternative halogen-free flame retardants (HFFRs), such as organophosphate- and metal-based flame retardants (OPFRs and MBFRs). However, to date, the neurotoxicologic properties of HFFRs are understudied, hampering proper human risk assessment. This session examines current research on the neurotoxicity of BFRs being phased-out by industry, and on some of the alternative flame retardants that are taking their place. A brief overview of the different classes of flame retardants, including halogenated, organophosphorus, nitrogen-based, and inorganic classes, and their use and chemical structures, will be given to orient attendees to the different flame retardants that will be discussed during the session.

NTX18

Cognitive and Motivational Impacts of Developmental PBDE Exposure in Rats

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Polybrominated diphenyl eithers (PBDEs) are synthetic flame retardants that were used heavily in the manufacturing of polymer products from the late 1970s through the early 2000s. Their use has been curtailed recently because of their bioaccumulative and potentially toxic properties. However, PBDEs persist in the environment and continue to be detected in biota. In rodent models, perinatal exposure to PBDEs in food, dust, and air disrupts endocrine and neurobehavioral functioning, and some of these effects persist throughout adulthood. Neurobehavioral effects include hyperactivity, mild learning deficits, mild attentional deficits, and alterations in behavioral responses to challenges with cholinergic drugs. However, recent studies in our laboratory have also revealed effects on motivation and hedonic tone: pups exposed to the commercial PBDE mixture DE-71 voluntarily consume more ethanol and sucrose than do their non-exposed counterparts, yet they demonstrate reduced motivation to engage in cognitively demanding tasks unless large rewards are offered. The motivational effects of PBDE exposure are stronger than the cognitive effects, and in fact they may be more fundamental to the neurobehavioral profile of exposed individuals.

This research was supported by Colorado College.

NTX19

Neurobehavioral Function and Low-level Exposure to Brominated Flame Retardants in Adolescents: A Cross-sectional Study

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Animal and in vitro studies demonstrated a neurotoxic potential of brominated flame retardants. The aim of this study was to investigate the associations between biomarkers of internal exposure to brominated flame retardants [serum levels of polybrominated diphenyl ether (PBDE) congeners 47, 99, 100, 153, 209, hexabromocyclododecane (HBCD), and tetrabromobisphenol A (TBBPA)] and cognitive performance. We assessed the neurobehavioral function using the Neurobehavioral Evaluation System (NES-3) battery of tests and analyzed blood samples of high school students. Cross-sectional data on 515 adolescents (13.6-17 years of age) was available for the analysis. A two-fold increase of the sum of serum PBDE's was associated with a decrease of the number of taps with the preferred-hand in the Finger Tapping test by 5.31 (95% CI: 0.56 to 10.05, p=0.029). Serum levels above the level of quantification were associated with an average decrease of FT3 level by 0.18 pg/mL (95% CI: 0.03 to 0.34, p=0.020) for PBDE-99 and by 0.15 pg/mL (95% CI: 0.004 to 0.29, p=0.045) for PBDE-100, compared with concentrations below the level of quantification. PBDE-47 level above the level of quantification was associated with an average increase of TSH levels by 10.1% (95% CI: 0.8% to 20.2%, p=0.033), compared with concentrations below the level of quantification. HBCD and TBBPA did not show consistent associations with performance in the neurobehavioral tests. To conclude, consistently with experimental animal data, PBDE exposure was associated with changes in the motor function and the serum levels of the thyroid hormones.

NTX20

Neurotoxicity assessment of 15 brominated- and halogen-free flame retardants

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Following the phase out of polybrominated diphenyl ethers (PBDEs), Organophosphate FRs (OPFRs), and in particular tris (1,3-dichloropropyl) phosphate (TDCPP), became the primary FRs applied to foam padding used in upholstered furniture and baby products. Like PBDEs, OPFRs are additive and can more readily leach out into the surrounding environment. OPFRs have been detected in the environment at concentrations similar to or exceeding that of PBDEs. Although OPFRs have been in use for several decades, until recently relatively little was known regarding their potential for adverse human and environmental health consequences. However, based on their structural similarity to OP pesticides, they may have analogous mechanisms of toxicity. The main objective of this research project was to evaluate the toxicity of four structurally similar OPFRs (TDCPP; tris (2,3-dibromopropyl) phosphate, (TDBPP); tris (1-chloropropyl) phosphate (TCPP) and tris (2-chloroethyl) phosphate (TCEP)) in comparison to chlorpyrifos (CPF), a well-studied OP pesticide. A combination of in vitro and in vivo models was used to elucidate potential mechanisms and functional consequences of exposure in developing organisms. We used PC12 cells to evaluate the effects of four structurally similar OPFRs (TDCPP, TDBPP, TCEP, or TCPP) and CPF on neurodevelopment. In general, TDCPP elicited similar or greater effects when compared to an equimolar concentration of CPF. All OPFRs tested produced similar decrements in cell number and altered phenotypic differentiation. Next, zebrafish (Danio rerio) were used to evaluate the effects of the same suite of chemicals larval swimming activity following a developmental exposure (0-5 days post fertilization (dpf)). All test chemicals affected larval swimming behavior on 6 dpf at concentrations that were not overtly toxic. These results will be put in context with recent work by other research groups that can further elucidate the mechanisms of toxicity of these chemicals.

NTX21

A comparison of the in vitro and ex vivo neurotoxicity of brominated and halogen-free flame retardants: Prioritization in search for safe(r) alternatives

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Many brominated flame retardants (BFRs) are infamous due to their persistence and abundance in human and environmental samples. A vast amount of in vivo and in vitro data indicates the potential neurotoxicity of BFRs, thereby underlining the need for replacement by halogen-free flame retardants (HFFRs). However, the neurotoxic potential of many HFFRs is currently unknown.
We therefore investigated the in vitro neurotoxicity of 12 HFFRs and 3 BFRs using PC12 cells. Our data demonstrate that the majority of FRs induced negligible cytotoxicity, except zinc hydroxystannate (ZHS) and zinc stannate (ZS) which decrease cell viability already in the submicromolar range. Additional single-cell fluorescent Ca2+-imaging demonstrated that at (sub)micromolar concentrations tetrabromobisphenol-A (TBBPA) as well as aluminium trihydroxide (ATH), ZHS and ZS increased the basal intracellular calcium concentration ([Ca2+]i). Importantly, many FRs, including TBBPA, triphenylphosphate (TPP), ZHS and ZS are potent inhibitors of voltage-gated calcium channels. In a separate set of experiments, we demonstrate using Xenopus oocytes expressing nicotinic acetylcholine receptors (nACh-R) that some FRs, including TBBPA, TPP and aluminium diethylphosphinate (Alpi), act as nACh-R antagonists.
Based on the combined in vitro data we could identify suitable (e.g. Alpi) and less suitable (e.g. ZS) candidates for replacement of BFRs. To substantiate this notion, we investigated synaptic plasticity in mouse hippocampus ex vivo following a single neonatal exposure to TBBPA, Alpi or ZS. Surprisingly, none of these flame retardants significantly affected long-term potentiation or the expression of postsynaptic proteins. Subsequent analysis of brain, liver and muscle indicated only significant levels of TBBPA in liver, but FRs were not detectable in brain, suggestive for low bioavailability and/or rapid elimination/metabolism. Although data on (in vivo) (neuro)toxicity following prolonged (developmental) exposure is yet lacking, our combined in vitro and ex vivo findings already indicate that several HFFRs may be suitable alternatives for BFRs.
This work was supported by a grant from the European Union (ENFIRO; grant agreement FP7-ENV-2008-1-226563).

NTX22

Prenatal cocaine, alcohol, and tobacco effects on adolescent attention/inhibition

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Objective: Prenatal cocaine exposure (PCE) can affect cognitive development through its actions on neurotransmitter systems affecting fetal brain development. We investigated attention and response inhibition in 348 15-year-old children (177 PCE) enrolled in a prospective longitudinal study of prenatal drug exposure since birth. The cohort was primarily urban, African-American, of low socioeconomic status. All subjects were given the Integrated Visual and Auditory Continuous Performance Test (IVA/CPT) which discriminates between auditory vs. visual distractibility. Effects of PCE were assessed with multiple regression analyses controlling for multiple confounders.

Methods: PCE predicted lower visual response control, (β = -.13, p<.04), consistency (β = -.13, p<.06), and stamina (β = -.17, p<.02). Prenatal alcohol exposure predicted lower visual (β = -.14, p<.02), and auditory speed (β= -.13, p<.03), but better visual response control, (β = .15, p<.01), and visual stamina (β = .14, p<.04). Current caregiver tobacco exposure was related to lower Full Scale (FS) response control (β = -.12, p< .03), and lower visual response control (β = -.12, p<.03), prudence (β = -.12, p<.03), and consistency (β = -.14, p<.01).

Results: Adolescent's concurrent drug use had additional effects on attentional measures. Tobacco use was associated with lower FS (β = -.11, p<.04) and auditory response control (β =-.16, p<.006), and lower auditory prudence (β = -.12, p<.03). Alcohol use predicted lower FS attention (β = -.09, p<.08), visual attention (β = -.10, p<.04), and vigilance (β = -.13, p<.01), while marijuana use predicted poorer visual (β = -.12, p<.03), and auditory focus (β = -.10, p<.06). Boys in general performed more poorly and higher IQ overall predicted better performance. When race was a significant factor, African-American children performed better.

Conclusions: Prenatal cocaine exposure has persistent negative effects on visual attention and response inhibition in mid adolescence. Adolescent concurrent use of alcohol, tobacco, and marijuana has additional detrimental effects on attention.
Prenatal drug exposures and concurrent substance use contribute to adolescent attentional performance. Secondhand smoke through caregiver tobacco use also has negative effects on visual attention.

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NTX23

Effects of prenatal cocaine exposure and externalizing behavior on adolescent substance use (15-17 years)

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Introduction: Previous findings from this prospective longitudinal birth cohort study indicate that prenatal cocaine exposure (PCE) increased odds of tobacco, marijuana, alcohol and any drug use at age 15 and externalizing behaviors at 12 years. This study evaluated the effects of PCE on substance use at 15 and 17 years and examined whether externalizing behaviors mediate PCE effects on adolescent substance use.

Method: 365 adolescent subjects (186 PCE; 179 NCE), at ages 15 (n=358) and 17 (n=349), supplied drug use information using the Youth Risk Behavior Surveillance System. The cohort was primarily urban, African-American, and low socioeconomic status. The effects of PCE were assessed using General Estimating Equation (GEE) analyses controlling for significant confounding demographic factors, violence exposure and preschool lead level. Youth Self-Report externalizing behavior assessed at 12 years was evaluated as a mediator of PCE effects.

Results: PCE predicted higher odds of tobacco, marijuana and any drug use (p's <.008). Teens with PCE were on average 2 times more likely to have used tobacco (OR=2.18; CI 1.25-3.82; P<.006), marijuana (OR=1.82; CI 1.16-2.85; P<.009) or any drug (OR=1.84; CI 1.18-2.88; P<.008) in the past 30 days than NCE teens controlling for covariates. Violence exposure was also a significant independent predictor of tobacco (p<.05), marijuana (p<.0006) and any drug use (p < .0003). A more positive home environment was associated with lower odds of marijuana (p<.03) and any drug use (p<.008). Externalizing behavior at age 12 mediated PCE effects on tobacco and any drug use but not marijuana use. Each of the substance use types reported in the past 30 days increased between 15 and 17 years, with no differences in the rate of increase between PCE and NCE groups.

Conclusion: Prenatal cocaine exposure has persistent negative effects on teen use of tobacco, marijuana and any drug use, with externalizing symptoms at age 12 partially mediating the effects of cocaine on tobacco and any drug use. Exposure to violence continues to have negative effects on teen substance use independent of PCE. These data suggest that specialized drug use prevention measures for PCE children are necessary.

NTX24

Neonatal (+)-methamphetamine exposure impairs egocentric, allocentric, and working memory in rats

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Neonatal treatment of rats with (+)-methamphetamine (MA) on P6-15 or P11-20 induces egocentric learning deficits in the Cincinnati water maze (CWM) and allocentric learning deficits in the Morris water maze (MWM; Vorhees et al., 2009). The mechanism for these effects is unknown. We previously showed that reducing MA-induced corticosterone release does not prevent these effects. In adult rats, MA-induced dopamine depletion can be blocked by pretreatment with the spin-trapping agent, N-tert-butyl-α-phenylnitrone (PBN), implicating reactive oxygen species (ROS) in this neurotoxicity (Cappon et al., 1996). Here we tested whether PBN could attenuate the effects of developmental MA. Using a split-litter design, male/female pairs within each litter were treated with 10 mg/kg x 4/day MA at 2 h intervals on P6-15 or saline (Sal) with or without PBN (40 mg/kg, 30 min prior to MA or Sal) forming 4 groups: Sal-Sal, PBN-Sal, Sal-MA, PBN-MA. Progeny were tested in the CWM, MWM, and radial-arm water maze (RWM) as adolescents (males) or adults (females). MA-exposed rats were impaired learning the CWM, MWM, and RWM at both test ages. There was no main effect or interaction with PBN. In the RWM, MA-treated rats showed working and reference memory impairments in adolescent males and reference memory-only impairments in adult females. In the MWM, MA-treated rats showed impaired acquisition but no changes on probe trials and no differences in swim speed. In the CWM, MA-treated rats showed increased errors at both ages with no changes in swim speed in a straight channel prior to maze testing. The data show that neonatal MA (1) induces multiple types of cognitive deficit, (2) each type of deficit emerged early and persisted, (3) cognitive deficits did not recover as the rats matured to adulthood, (4) deficits were not accompanied by performance impairments, and (5) deficits were not attenuated by PBN; the latter suggesting that neonatal MA-induced cognitive deficits are not mediated by ROS. (Supported by T32 ES007051)

NTX25

Loss of dopamine D2 receptors increases parvalbumin-positive interneurons in the anterior cingulate cortex

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Alterations to dopamine (DA) homeostasis during development can have long-term consequences and have been implicated in the manifestation of neuropsychiatric disorders such as depression and schizophrenia later in life. Similarly, aberrant expression of GABAergic markers is common in postmortem brains of people suffering from such disorders. Furthermore, during development, DA D2 receptors (D2R) are found to be necessary for the appropriate migration and subsequent expression of GABAergic interneuron markers within the frontal cortex, a region implicit in the neuropathology of neuropsychiatric disorders. However, it is unclear if developmental aberrations to these interneurons caused by disrupted D2R activity normalize later in life, nor is it clear which GABAergic marker subsets are altered by D2R activity. Using the D2R knockout mouse, we investigated which interneuronal subpopulation was altered following loss of the D2R. Furthermore, we determined if this loss of D2R would alter long-term behavior associated with human psychiatric disorders. We found an increase in GAD67+ cells within the anterior cingulate cortex of adult D2R knockout mice relative to wild-type controls. No such difference in GABAergic neurons were found in the striatum and somatosensory cortex between the genotypes. Similarly, parvalbumin+ (PV+) cells, one such subset of GABAergic interneurons, were also elevated (~20%) in the ACC of the knockout mouse; however, there was no difference in expression levels of other GABAergic markers (i.e., calretinin or somatostatin) in any of the brain regions examined. PV cell density was also elevated at postnatal day (P)14, a period in which PV expression rapidly increases, in the knockout mouse, indicating that these abnormalities are present throughout development and not just confined to adulthood. The effects of D2 receptors on PV levels may be direct, as approximately 24% of PV+ cells within the anterior cingulate cortex also co-express the D2R, visualized using the D2eGFP BAC transgenic mouse. Finally, we found that D2R knockout mice were significantly less immobile in the tail suspension test compared to control mice, indicating that disruptions of the D2R can contribute to depressive-like behaviors. These data indicate that developmental alterations to D2R can have long-lasting effects on interneuron number and functional circuits.

NTX26

Use of non-mammalian animal models in neurotoxicology testing in the National Toxicology Program

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There is limited information on the potential health effects of organophosphorus flame retardants (OPFRs) used as brominated flame retardants (BFRs) replacements. The effects of 8 OPFRs: triphenyl phosphate (TPHP), isopropylated phenyl phosphate (IPP), ethylhexyl diphenyl phosphate (EHDP), butylated phenyl diphenyl phosphate (BPDP), trimethyl phenyl phosphate phosphate (TMPP), isodecyl diphenyl phosphate (IDDP), tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) and trichloroethyl phosphate (TCEP) were compared with 2 BFRs tetrabromobisphenol A (TBBPA) and brominated diphenyl ether (BDE 47) using alternative model systems [e.g, cell systems, C. elegans, zebrafish (Danio rerio)]. In mouse embryonic stem cells, none of the compounds (0.03-100 µM) affected the expression of goosecoid (cellular differentiation maker) in the absence of cytotoxicity. Neural proliferation and neurite outgrowth were more sensitive in human stem-cell derived neuroprogenitor cells and neurons than in rat primary neuronal cultures. Proliferation was affected in 5/10 FRs with BPDP and IPP being the most toxic [point of departure (POD)< 10 µM]. In the human neurite outgrowth assay 7/10 compounds were toxic, of which 5 had PODs <10 µM, and were cytotoxic (BDE-47, TOCP, BPDP EHDP, IDDP). In rat cultures, IPP and BPDP were the only two compounds that impaired neurite ourgrowth in the absence of cytotoxicity. In primary cultures of rat cortical neurons, four OPFRs (BPDP, IPP, EHDP, TMPP) selectively decreased spontaneous network firing rate in the absence of cytotoxicity at < 10 µM. Embryonic and larval development were assessed in zebrafish and C. elegans. While BDE-47 decrease. C. elegans development at < 10 µM, this assay appeared to be preferentially targeted by the aromatic phosphates with 5/7 compounds(TPHP, BPDP, IPP, EDHP, IDDP) with PODs <10 µM; in addition, this assay was also the most sensitive for TBBPA and TDCIPP (POD ~ 10 µM). In the developing zebrafish (0.4 µM - 120 µM), TPHP, IPP, BPDP, and TDCIPP were as toxic as TBBPA (POD < 10 µM), while BDE-47 TMPP and EHDP were slightly less toxic (30µM>POD > 10 µM), and TCEP and IDDP produced no toxicity. For some endpoints, the OPFRs showed comparable (or greater) toxicity to the BFRs suggesting that OPFRs should be further examined for hazard characterization.

NTX27

The RAS/PI3K pathway involved in the damage on long-term potentiation of acute aluminum treatment

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The RAS/PI3K signal transduction pathway appears to be involved in the mechanism of AMPA receptor trafficking and Long-term potentiation (LTP). Our previous study showed that acute aluminum treatment obviously suppressed the hippocampal LTP of rats in vivo, and it may be relate to the decreased trafficking of AMPA receptor subunits. Here we explored the RAS activity of rat hippocampus after acute aluminum exposure and the antagonism of RAS activator EGF on hippocampal LTP suppressed by aluminum and on the PKB activity and the phosphorylation of GluR1 S831和S845. First, acute aluminum treatment, by intracerebroventricular injection (i.c.v.) with different dose of aluminum-maltolate complex (Al(mal)3), produced a dose-dependent decrease of RAS activity in rat hippocampus. Second, the early suppression of hippocampal LTP by aluminum could be antagonized by the RAS activator EGF. Finally, the same changes with LTP were showed in the PKB activity and the phosphorylation of GluR1 S831和S845 after EGF treatment. It was concluded from the results that RAS→PI3K/PKB→GluR1 S831和S845 signal transduction pathway may be involved in the damage on hippocampal LTP by aluminum in rats. However, the mechanisms underlying this observation need further investigation.

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NTX28

Lysosomal dysfunction caused by the environmental neurotoxicant manganese increases exosome-mediated cell-to-cell transfer of α-synuclein by a prion-like mechanism

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Many age-related neurodegenerative disorders share a common neurotoxic mechanism involving the aggregation and deposition of misfolded proteins. In Parkinson's disease (PD), the accumulation of misfolded and aggregated α-synuclein (αSyn) is considered a key pathophysiological feature. Generally, these misfolded proteins can be degraded by autophagy via the lysosomal pathway. However, the lysosomal degradation pathway is impaired in the diseased state, leading to a significant accumulation of autophagic vesicles in the neuronal body. We recently reported that an interaction between the divalent metal manganese (Mn) and αSyn promotes protein misfolding. In the present study, we further explored the molecular mechanisms underlying cell-to-cell transfer of Mn-induced αSyn aggregates. After establishing dopaminergic neuronal cells stably expressing wild-type human αSyn, we treated them with a non-toxic dose of Mn (300 μM) for up to 24 h. In Western blot analysis comparing αSyn-expressing cells to vector alone cells, Mn increased expression of the autophagosomal markers LC3-II and Beclin-1, whereas it decreased expression of the lysosomal marker LAMP2, suggesting that Mn impairs the autophagic/lysosomal degradation pathway. Interestingly, Mn also induced the release of αSyn into the extracellular media in a time-dependent manner. Electron microscopic examination of extracellular media readily detected the membranous nano-sized vesicles with characteristic hallmarks of exosomes. Nanosight particle analysis further showed that Mn exposure markedly increased the number of released exosomes. Slot blot analysis with anti-oligomer antibody revealed that exosomes contained misfolded proteins, and ELISA studies further confirmed that the released exosomes were indeed packaged with αSyn. High resolution image analysis revealed that exosomes released into the extracellular milieu entered into adjacent cells, thereby propagating protein aggregates. Collectively, our data show that Mn-induced autophagic/lysosomal dysfunction results in the accumulation and secretion of αSyn-containing autophagosomes in the form of exosomes, which contributes to a prion-like propagation of misfolded neurotoxic αSyn aggregates. (NIH grants ES19267, ES10586, and NS088206)

NTX29

Can zebrafish be used to identify developmentally neurotoxic chemicals?

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The U.S. Environmental Protection Agency is evaluating methods to screen and prioritize large numbers of chemicals for developmental neurotoxicity. We are exploring behavioral methods using zebrafish by designing a behavioral testing paradigm capable of assessing the effects of sublethal and sub-teratogenic concentrations of developmental neurotoxicants. The behavioral paradigm simultaneously tests individual 6 day old zebrafish under both light and dark conditions in a 96-well plate using a video tracking system. By controlling the duration and intensity of light, we are able to assess changes in locomotion during light-dark transitions, and adaptation to both light and dark during approximately 1-2 hours of testing. The testing format allows evaluation of large numbers of larvae, chemicals and chemical concentrations. Using this paradigm we have tested a training set of chemicals that are either known to or generally considered positive or negative controls for producing developmental neurotoxicity in mammals. We have found that many developmentally neurotoxic compounds perturb behavior at sub-teratogenic doses, while many developmentally non-neurotoxic compounds do not perturb behavior. Exposure to developmental neurotoxicants may alter the overall level of activity in light and dark conditions and/or the pattern of activity. Therefore, results from the training set indicate that careful behavioral evaluation of zebrafish larvae may be able to identify some mammalian developmental neurotoxicants. This abstract may not necessarily reflect official Agency policy.

Research supported by the U.S. Environmental Protection Agency

NTX30

Persisting Impacts of Organophosphate and Neonicotinoid Pesticides on Neurobehavioral Function in Zebrafish.

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Zebrafish provide excellent models of neurodevelopment with continuous visual access available in an anatomically and temporally intact system and many available molecular tools with which to monitor and manipulate developmental processes. Importantly, behavioral tests have been developed to evaluate cognitive, emotional and sensorimotor function in zebrafish so that the persisting functional effects of developmental neurotoxic exposure. Basic processes of neurodevelopment have been studied in zebrafish embryos as have applied studies of neurotoxicology. In recent years development of behavioral tests to assess a broad range of functions from sensorimotor processes and stress response to cognition and social behavior have been developed in a variety of labs. We have found that embryonic exposure to the organophosphate (OP) pesticide chlorpyrifos caused persistent impairment in learning. More recently we have shown that embryonic exposure of zebrafish to the prototypic neonicotinoid pesticide imidacloprid causes persistent behavioral effects on …

Zebrafish are an outstanding complementary model of developmental neurotoxicity. They are economic and provide both molecular and morphological tools for mechanistic analysis and the ability to determine neurotoxic consequences on behavioral function.

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NTX31

Detection and validation of molecular biomarkers for neurotoxicity in fish embryos

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Many pollutants entering the aquatic environment are of neuroactive potency and cause considerable hazard to aquatic organisms and consumers. However, neurotoxic effects are of subordinate relevance within the current legislative frameworks regulating chemicals and their environmental release. Developmental neurotoxicity is of particular concern because the developing nervous system is susceptible to injury caused by neurotoxic substances. Current regulatory tests for developmental neurotoxicity (DNT) are based on expensive, complex and ethically disputed mammalian experiments, which are also unsuitable for large scale screening applications.
In our studies we use the zebrafish embryo assay as a screening tool for DNT, combining morphometric parameters with molecular and cellular markers. We evaluated the severity of neurotoxic damage to individual primary and secondary motor neurons in 48 hpf (hours post fertilization) zebrafish embryos by classifying the motor neuron defects after whole-mount immuno-staining, using a transgenic GFP-expressing zebrafish line. Glial fibrillary acidic protein (GFAP) is a marker of radial glial cells, which are progenitors of neuronal precursors, astrocytes and oligodendrocytes. We have used this approach to investigate the neurotoxicity of thiocyclam, disulfiram, cartap, genistein, metals and reference compounds. We were able to link neuronal defects to characteristic morphological phenotypes and behavioral deficits affecting the escape response at 72 hpf. A comparison of the effective concentration values (EC50) for neurotoxicity and for teratogenicity led to an index, which provides an estimate of the DNT hazard potential of a chemical. Neurotoxicity index values of < 1.0 were determined for the positive controls ethanol and nicotine and the model compound cartap, values clearly > 1.0 for 3,4-DCA and triclosan. For metals, we are investigating mechanisms of DNT by transcriptome analysis and have already identified potential gene markers like nkx2.2a or stat3. Furthermore, behavioral assays are important in our DNT studies since endpoints like the photomotor response (PMR) or other light-dark transition responses enable sensitive and reliable read-outs for high-throughput screening applications for the detection of DNT or the identification of neuroactive or neuroprotective drug candidates.

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NTX32

Neurogenetics of toluene in Drosophila

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A toxicity pathway is a conceptual series of mechanistic links between effects of a chemical on molecular targets and adverse outcomes in the whole organism. This study applied the Drosophila Genetic Reference Panel (DGRP) to determine whether genetic correlates of toluene-induced sedation in fruit flies would point to CNS processes associated with narcosis. We exposed 123 lines of DGRP flies to toluene vapor and mapped genetic variation across the lines to the phenotypic variation in the sedation response. Single flies were placed in 5 mm diameter glass tubes and exposed to 750 ppm of toluene vapor (n = 24-50 / line); paired controls received air. Motor activity was recorded at 10-minute intervals for 1 h before, 4 h during, and 15 h after exposure. Toluene reduced activity during exposure in 104 of the 123 lines; the magnitude of this acute effect differed quantitatively across lines (range: -324 to +89 counts). We carried out a genome-wide association analysis for sensitivity to toluene using the DGRP web portal (http://dgrp2.gnets.ncsu.edu/).We tested 1,891,456 DNA variants and found 82 polymorphisms located in or near 66 candidate genes that were associated with phenotypic variation for sensitivity to toluene at P < 5 x10-5. These genes are enriched for Gene Ontology terms associated with cell surface receptor linked signal transduction, neuron differentiation and olfactory learning. The five most-populated clusters of human orthologs of these genes reflected biological process involved in cellular development (22 genes), nervous system development and function (14 genes), cellular assembly and organization (13 genes), neurological disease (10 genes), and cellular compromise (6 genes). The lack of correspondence between the Drosophila genes identified here and known molecular targets for toluene-induced narcosis in mammals may be due to (a) insufficient annotation of the functions of these Drosophila genes; (b) different molecular targets for toluene in Drosophila and mammals; (c) imperfect mapping of these genes to human orthologs; and (d) incompleteness of the canonical glutamate and GABA pathways. This abstract does not reflect U.S. EPA policy.

NTX33

Molecular Neurotoxicology Insights from C. elegans

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The extraordinary conservation of both genetic elements and differentiation processes between mammals and non-mammalians has been revealed during the last two decades. *C. elegans* genome, biosynthetic and metabolic pathways are highly conserved with mammals, yet the small size (adults are ~1 mm long), ease of maintenance, speedy generation time (3 days), and large brood size (>300 progeny per hermaphrodite) allow for a nearly limitless supply of worms for cellular, molecular, and genetic analyses. The transparency of the worm and the ease of making reporter gene fusions facilitate visualization of neuronal morphology and protein expression patterns within the living nervous system. Moreover, the availability of a complete three-dimensional map of the 302-cell nervous system allows for the identification of most synapses between neurons. The self-fertilizing hermaphrodite permits quick and easy homozygosity of mutations; males can be used for mating to generate lines with multiple mutations. The completed sequence of the genome, the ability to perform whole animal PCR ("single worm PCR"), and the existence of a high-density polymorphism map of a related strain of the wt *C. elegans* allows for quick and easy mapping of mutations within practically any gene. Gene knockdowns can be generated with RNAi techniques and loss-of-function mutants can be produced by site-directed mutagenesis. This presentation will discuss validated *C. elegans* disease models, emphasizing conserved pathways between the nematode and mammalian systems focusing on its utility as a model for neurodegenerative studies.

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NTX34

The Objective Measurement of Drug and Environmental Influences on Brain Function

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There are two types of cognitive processes: cold cognitive processes, such as working memory and planning, and hot cognitive processes, such as risky decision-making. In healthy people, these cold and hot processes are well-balanced. An example of cold planning and decision-making might be formulating a business plan, where you decide in a non-emotional way how much of the product you plan to produce, what it will cost and who you plan to sell it to. Hot cognitive decision making might be when an investor has to 'emotionally read' business people pitching their business plans. The investor has a lot to gain financially if they make the correct decision and choose the best plan, but has a lot to lose if they do not. Often, these hot emotional decisions are time-limited and have to be made relatively rapidly.

In this lecture, I will discuss the objective measurement of forms of cold and hot cognition using CANTAB and EMOTICOM, including attention, learning and planning I will also discuss the assessment of environmental influences, such as drugs, on cognition. Finally, I will give examples of the importance of considering neurodevelopment and pharmacogenomics when determining the impact of drugs on the brain.

NTX35

Developmental Causes and Consequences of Drug Abuse

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Brain formation and function relies on the complex interplay of a variety of genetic and environmental factors through protracted periods of gestational and postnatal development. Abnormalities in neurodevelopmental programming contribute to developmental delays and multiple neurological and psychiatric disorders, often with symptom onset much later than the actual induction of pathology. This talk will review several genetic and pharmacological models of monoamine modulation during pre- and post-natal development, each of which produces long-lasting changes in brain function and behavioral responsiveness. Clinical studies and significance will be integrated with mechanistic preclinical studies to define our current knowledge base and identify gaps for future investigation.

NTX36

Reexamining the Developmental Neurotoxicity Study and Risk Assessment

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The rodent Developmental Neurotoxicity (DNT) study paradigm has evolved over time with the most recent test guidelines updated in 2007 with the introduction of Organization for Economic Co-operation and Development (OECD) guideline 426. Over the past four years, a joint USEPA-PMRA intergovernmental group has been working to create an internal guidance document for regulatory reviewers in both countries. As a NAFTA-inspired multi-governmental initiative that involved consultation with both governmental and nongovernmental stakeholders, this initiative was undertaken to provide better context to key parameters necessary for the review of a DNT study, not only for the individual behavioural tests, but for their integration into the weight of evidence for the entire study and for the ultimate assessment of hazard and risk. Over the past year, efforts have focused on providing the content for the internal guidance document. A breakdown of the regulatory importance of the major themes and the key outputs is provided in order to inform external stakeholders of key elements identified in this internal evaluation guidance document.

NTX37

Evaluating Data Variability for Neurobehavioral Measures: How Much is Too Much?

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Neurobehavioral testing is a foundational component of studies designed to assess developmental toxicity and developmental neurotoxicity with pharmaceuticals and chemicals, including pesticides. Computer-automated tests of motor activity and the acoustic startle response are common methods of such studies. Variability of neurobehavioral endpoints is often relatively high, especially at early stages of development, which undermines reviewer confidence in study results and has impacted the interpretation and application of additional safety factors for infants and children. Thus, it is important to control environmental factors that contribute to variability and to understand what is normal or excessive for different endpoints. This presentation will set the stage for the workshop, by using new data comparisons of neurobehavioral endpoints from different types of guideline studies to illustrate how variability in control animals is increased, due to study design requirements, relative to what is achievable under optimal conditions. This presentation will illustrate how to examine variability in the context of particular stages of development, including how to differentiate between excessive variability and highly-consistent results that are inherently more variable.

NTX38

New Insights into Analysis of Highly Variable Data: Motor Activity As a Case Study

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Motor activity (MA) testing is a requirement of both EPA and OCED DNT guidelines as well as the new OECD extended one-generation reproductive toxicity test guideline. Thus, MA will continue to play an important role in children's health risk assessment. The test guidelines are relatively nonspecific about test procedures and data reporting, and high variability in MA data directly impacts interpretation of both study conduct reliability as well as decisions on whether there are effects on the developing nervous system. Similarly, shortcomings in the reporting of MA data from testing laboratories also contribute to uncertainty about test results. This presentation will discuss the type of data that needs to be reported so that regulators can assess the quality of activity data including "normal" activity patterns for the strain, species and equipment. This presentation will provide guidance on expected MA variability under required testing conditions based on recent retrospective analysis of control data from DNT studies submitted to Health Canada. The impact of software and equipment settings on activity of control animals at different developmental stages will be presented. The use of different covariates that can address MA variability will be compared.

NTX39

Hypothesis-Driven Testing and Analysis: Auditory Startle As a Case Study

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DNT studies conducted for regulatory purposes incorporate multiple endpoints that vary in the specific parameters measured as well as the metrics used to evaluate results. Regulatory guidelines provide flexibility in study design, placing responsibility on the individual investigators to precisely define the hypothesis being tested and design appropriate analyses. Available equipment can record multiple parameters, not all of which convey biologically meaningful information. Thus it is useful to focus the study design on detection and analysis of those effects whose biological significance is meaningful in the risk assessment context. Although general guidance has been provided, practical recommendations on implementation of such approaches have been limited, and differences in guideline specifications suggest different biological hypotheses that could be tested within the general guidance provided. For example, the new OECD extended 1-generation study includes a test of auditory startle habituation at PND 24 in 1 male or 1 female/litter. OECD DNT guidelines specify some test of sensory and motor function at two ages in 1 male AND 1 female/litter (i.e. a test of habituation is not required). Analysis of auditory startle data could include treatment, sex, trial block, and day as potential factors in a hierarchical model; analyses of these data could include multiple main effects as well as a many levels of interactions, for multiple measured parameters. Using auditory startle as an example, types of parameters typically measured, ways in which measured parameters may be summarized or combined, and the utility of various types of information in evaluating biological effects of treatment will be explored. Discussion will focus on identifying parameters that have biological relevance for use in risk assessment, and how that and other practical considerations (e.g. type of device) might impact evaluation and graphical and tabular presentation of data. This is an abstract of a proposed presentation and does not reflect the policy of the US Environmental Protection Agency.

NTX40

Standardization of SOPs to Evaluations: Impacts on Regulatory Decisions Using Learning and Memory As Case Studies

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In an era of global trade and regulatory cooperation, consistent and scientifically based interpretation of developmental neurotoxicity (DNT) studies is essential, particularly for nonstandard assays and variable endpoints. Because there is flexibility in the selection of test method(s), standardization can be especially challenging for learning and memory tests required by US EPA and OECD DNT test guidelines (chemicals and pesticides) and recommended in ICH prenatal/postnatal guidelines (pharmaceuticals). No one cognitive test is clearly superior, yet detection of treatment effects may depend on this choice. An understanding of the purpose behind the tests and expected outcomes is critical, and attention to elements of experimental design, conduct, and reporting can improve data collection by the investigator as well as accuracy and consistency of interpretation by regulatory evaluators. This understanding also directs which information must be clearly described in study reports. Standard operating procedures (SOPs) may contain important experimental features, but if not clearly reflected in thorough report submissions there may be questions and misunderstandings by evaluators which could impact risk assessments. A practical example of learning and memory tests will be presented to provide insights into important experimental variables, reporting methods, and approaches for data assessments. Cognitive functions most often tested in DNT guidelines studies include associative, positional, sequential, and spatial learning and memory in weanling and adult animals. These complex behaviors tap different brain areas and develop at different rates. The relative involvement of different neural systems depends on the specific task; however, there is considerable overlap across brain areas during the process of acquisition (learning) and consolidation of memory. Evaluation should include integration of treatment data including performance assessments (motor, sensory), control data (concurrent, historical, positive), and additional measures of neuro- or other toxicity. Doing so can empower consistent and defensible risk evaluations. These considerations provide a stronger scientific basis for standard evaluation approaches for review and interpretation of DNT studies. *This is an abstract of a proposed presentation and does not reflect US EPA policy.*

NTX41

Weight of Evidence (WOE) and Benchmark Dose (BMD) Analysis: Brain Morphometry and Startle Behavior As Examples

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The new OECD 443 extended 1-generation study and also OECD 426 and EPA OPPTS 870.6300 developmental neurotoxicity (DNT) studies have been conducted more frequently for pesticides than other chemicals. Guidelines for these studies include neuropathology and brain morphometry as test article effects on these endpoints may be considered more “severe” than effects in dams. Concerns about increased qualitative (i.e., greater severity) or quantitative sensitivity in offspring have informed DNT regulatory decisions based on the Pest Control Products Act (PCPA) in Canada or the Food Quality Protection Act (FQPA) in the United States, and in Europe may lead to classification as a developmental toxicant. The aforementioned guidelines focus on evaluating offspring, with limited analysis of adults. Therefore, conclusions about relative sensitivity of offspring vs. adults must compare the WOE from other adult toxicity studies with any changes in DNT parameters. Improved selection of brain morphometry measures that take into account brain cytoarchitecture coupled with the systematic collection of maternal toxicity data strengthens the scientific basis for WOE assessments. Although direct effects on the nervous system cannot completely be ruled out, decreases in morphometric measurements and brain weight of juveniles in conjunction with decreased dam and/or juvenile body weights can be interpreted as evidence that the brain alterations are related to a more general effect on growth rather than a specific neurotoxic effect. Absolute morphometric values can be compared with relative values based on brain weight or brain volume in evaluating whether or not alterations might be related to a more general effect on growth. Appropriate selection of a BMR for DNT endpoints also impacts interpretation of relative sensitivity. Statistical and toxicological evidence support setting BMR s for behavior endpoints higher than the default 5% level used by European Food Safety Authority (EFSA). These guiding principles for evaluating WOE and selecting BMR can improve risk assessment decisions and BMD analysis for DNT data.

NTX42

A retrospective of studies on toxic induced loss of color vision and contract sensitivity: What have we learned?

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In the 1970's Cristina Raitta, an ophthalmologist at Helsinki University showed acquired color vision loss among workers exposed to n-hexane and carbon disulphide. Electrophysiological studies confirmed the damage to retino-cortical pathways in these workers. Since that time, acquired color vision loss has been associated with exposure to several occupational and environmental toxics, as well as with prenatal exposure. In the 1990's, near visual contrast sensitivity was shown to be sensitive to mixed solvent exposures among microelectronics workers and has since been used in a number of studies to demonstrate neurotoxicity. Color vision and near visual contrast sensitivity assessments are easily performed under field conditions, including with populations with little or no formal education. Still, most studies of neurotoxic effects of occupational and environmental exposure do not assess visual deficits although many of the neurobehavioral tests have an important visual component. Poor performance on these tests may arise from alterations to the retina and/or retino-cortical pathways. This presentation will: 1) provide an overview on retinal/visual system structure, function and assessment techniques, 2) review the human data on organic solvents and metals that produce altered color vision, visual fields and spatial contrast sensitivity, and 3) examine the association between these deficits and performance on neurobehavioral tests, using data from our studies on several toxic agents.

NTX43

Gestational lead exposure in humans and experimental animals: Novel functional and morphological phenotype and late-onset retinal degeneration

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Offspring of occupationally lead-exposed workers have relatively increased blood lead levels. Previous studies show that low-level gestational lead exposure in humans produces a novel and selective rod vision-mediated increase in the electroretinograms (ERGs) of 7-10 year old children: termed rod supernormality. In the prospective epidemiological study, rod supernormality was associated with lead exposure only during the first trimester in humans: the period of retinal cellular proliferation and beginning of neuronal differentiation. In mice, gestational lead exposure increased the number and prolonged mouse retinal progenitor cell proliferation, delayed the onset of rod photoreceptor and bipolar cells differentiation (late-born neurons), selectively increased the number of these late-born neurons, and delayed the functional development of these neurons. Alterations in cell cycle entry and exit as well as kinetics underlie these proliferative changes, whereas alterations in selective proneural genes/proteins underlie the increased neuronal differentiation. During aging, the age-related apoptosis of rods and bipolar cells was accelerated and increased in retinas of gestationally lead-exposed mice relative to age-matched controls. These latter results were associated with increased para-inflammation implicating lead exposure as a risk factor in retinal degeneration consistent with the association of retinal lead exposure and increased age-related macular degeneration. Moreover, these late-onset findings suggest that children with gestational lead exposure should be monitored for age-related visual dysfunction and retinal degeneration.

NTX44

Impact of mercury vapor toxicity on vision and visual structures: Human and experimental studies

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The effects of exposure to metallic mercury have been evaluated in factories and the field using methods that enable sampling a large number of individuals. In contrast, we measure visual functions in individuals placed on disability retirement due to occupational mercury vapor exposure or in dentists in our laboratories using rigorous parametric procedures. Using clinical and psychophysical procedures, we found that these subjects had complex color vision alterations, decreased contrast sensitivity, and decreased central and peripheral visual fields. These deficits were persistent even years after cessation of exposure. Moreover, losses in visual functions were also revealed in electrophysiological experiments in which both retinal and cortical functions were probed using full-field electroretinography (ERG) and sweep visual evoked potential (VEP) techniques. We conclude that this rigorous battery of behavioral and electrophysiological tests were sensitive, revealed losses due to Hg exposure, and that the impairments were irreversible. An animal model of mercury intoxication was developed using different fish models. Retina of fish exposed to mercury showed alterations in electrophysiological responses, a dose-dependent decrease in bipolar and amacrine cell density and deposits of Hg in the photoreceptor layer, inner and outer nuclear layers, and plexiform layers.

NTX45

Mechanisms underlying ocular abnormalities in zebrafish embryos exposed to ethanol

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Human fetal ethanol exposure occurs world-wide and is a significant public health problem. It results in fetal alcohol spectrum disorder (FASD), which causes significant cognitive deficits and involves multiple organ systems. A common phenotype seen in FASD is microphthalmia (reduced eye size), generally accompanied with abnormalities in retinal function. We developed a zebrafish model for FASD whereby embryonic ethanol exposure over the time of retinal neurogenesis also results in microphthalmia. This microphthalmia is in part the consequence of reduced retinal cell differentiation, including that of photoreceptors. We showed that two signaling pathways implicated in other aspects of FASD pathogenesis, retinoic acid (RA), and Sonic hedgehog (Shh), are not involved in mediating the effects of ethanol on eye size, although RA is capable of restoring photoreceptor differentiation. Using time-series microarray analysis of gene expression within eyes of ethanol-treated embryos, we identified numerous differentially expressed genes, including several genes suggestive of a mis-regulated cellular stress response. Combined exposure to sub-threshold levels of ethanol and a morpholino targeting heat shock factor 1 (hsf1) mRNA resulted in significant microphthalmia, consistent with convergent molecular pathways. Ethanol-induced microphthalmia was partially prevented by a thermal preconditioning protocol that maintained Hsf-1 protein expression. Together our studies suggest potential therapeutic targets for ocular manifestations of FASD that are not related to RA or Shh signaling, but instead are related to the cellular stress response.

NTX46

The role of the age in mediating the efficacy of chelation therapy in lead poisoned young rats

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Background: Chelation therapy has been used as a main treatment for childhood lead poisoning for a long time, and graded chelation therapies are recommended based on the blood lead levels of lead-poisoned children. The child's age may affect his/her bone metabolism and may affect the efficacy of chelation therapy, but is not considered in current chelation plans.
Object To evaluate the efficacy of DMSA chelation on lead-poisoned young rats in different age groups.
Methods: Spagrue-Dawley young rats were fed with lead acetate during postnatal days (PND) 1- 21, PND 22-39, and PND 40-59 to respectively establish infant, childhood and adolescence lead-poisoning models. Each model were divided into 3 sub-groups to respectively receive pure water, one course, and two courses of DMSA chelation, to produce control, one-course-group and two-course-group. Blood lead and 4 kinds of bone lead (compact bone of tibia and femur, and spongy bone of tibia and femur) were tested.
Results: The blood and bone lead levels of lead-poisoned rats increased dramatically after being exposed to lead, about 10-50 times in blood lead and about 100 times in bone lead in different lead-exposed groups. For blood lead, after one course of chelation therapy, the decrease rates of blood lead levels in different age groups were: infant-hood groups> childhood groups >adolescence-hood groups, and the trend is same after the second course of chelation, and also same for the trends of 4 kinds of bone lead. In infanthood groups, the blood lead, spongy bone lead of tibia and femur, and compact bone lead of femur significantly decreased after one course of chelation(all $p<0.05$), and significantly decreased again after a second course (all $p<0.05$), however, in the adolescence-hood groups, one or two courses of chelation didn't significant decrease the compact bone lead of tibia and femur and spongy bone lead of femur (all $p>0.05$).
Conclusion: The age plays an important role in mediating the efficacy of chelation therapy in lead poisoned young rats, and its role should be paid attention in clinical chelation therapy.

NTX47

Relationship between prenatal mercury exposure and development of 18-month-old children

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Objectives: To investigate the impact of prenatal mercury exposure on the children's neurodevelopment at age of 18 months.
Methods: 305 cord blood lead were collected, the total mercury was measured by DMA-80 instrument. All the children were follow up at the age of 18 month. BSID-Ⅲ was used to assess neurodevelopment.
Results: The survey with completed data were 293 children. The median of cord blood levels of the 305 children was 1.92μg/L, geometric(standard deviation) was 2.00μg/L. There was no gender difference in cord blood mercury levels in all children(t = 0.193, P = 0.847).
The median cognitive composite score for children of 18 months was 105; and the mean of it was 106.23±9.93. The median and the mean of language composite score were 106, 105.84±10.36, respectively. The median and the mean of motor composite score were 103, 104.88±5.71, respectively. There was gender difference in children's cognitive (t = -2.973, P = 0.003), language (t = -4.763, P = 0.000) and motor development (t = -2.159, P = 0.032). Partial correlation analysis after adjusted for children's gender and age showed that cognitive development of 18 months old children correlated with maternal and paternal education levels, monthly income and family income per capita, inhabited site, consumption frequency of milk and yogurt, sea fish and shrimp during pregnancy, and meat and sea fish intake frequency during lactation. Multiple linear regression results indicated that paternal education levels, gender, age, sea fish intake frequency during pregnancy and cord blood mercury levels had an association with cognitive development; gender, paternal education levels, meat intake frequency during pregnancy, age and shrimp intake frequency during lactation had an association with language development; age had an association with motor development. Cord blood mercury concentration was a risk factor for cognitive development. No association was found between prenatal mercury exposure and language as well as motor development of 18-month-old children.
Conclusions
Cord blood mercury concentration was a risk factor for cognitive development of 18-month-old children.

NTX48

Fenazaquin aggravates tau pathology in p301s transgenic mice

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The P301S mutation in exon 10 of the tau gene causes a hereditary tauopathy. While mitochondrial complex I inhibition has been linked to sporadic tauopathies. Fenazaquin

4-[[4(1,1-dimethylethyl) phenyl] ethoxy] quinazoline, is a low dose emerging acaricide used to control mites attack in different vegetables. With the widespread use, long term persistence in the soil and the mitochondrial complex I inhibitory effects, fenazaquin has a possible link to some neurodegenerative disorders. The aim of this study was to determine whether there is a pathogenic interaction of the environmental toxin fenazaquin and the P301S mutation. Transgenic mice expressing human tau with the P301S-mutation (P301S+/+) and wildtype mice at 12 weeks of age were treated orally with vehicle (N=10 P301S+/+, N=10 wild-type) or fenazaquin (N=10 P301S+/+, N=10 wild-type mice) at a dose of 0.5 mg/kg/d for a period of 54 days . Tau pathology was measured by stereological counts of cells immunoreative with antibodies against phosphorylated tau (AD2, AT8, AT180, and AT100) and corresponding Western blot analysis. Fenazaquin significantly increased the number of phospho-tau immunoreactive cells in the cerebral cortex in P301S+/+ mice, but only to a variable and mild extent in wild-type mice. Furthermore, fenazquin led to increased levels of pathologically phosphorylated tau only in P301S+/+ mice. While we observed no apparent cell loss in the frontal cortex, the synaptic density was reduced by fenazaquin treatment in P301S+/+ mice. This study shows that exposure to fenazaquin aggravates the course of genetically determined tau pathology, providing experimental support for the concept of geneenvironment interaction in the etiology of tauopathies.

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NTX49

Role of glutamatergic receptors and associated signaling in arsenic induced neurotoxicity and protective efficacy of curcumin in rat primary cultured hippocampal neurons

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Earlier, we found that exposure to arsenic affects the hippocampal glutamatergic signaling in vivo and these changes were protected in rats simultaneously treated with curcumin. In continuation to this, studies have been carried out to investigate involvement of PI3K/Akt/MAPK neuronal survival pathway in arsenic induced glutamatergic dysfunctions and assess the protective efficacy of curcumin on specific targets in primary hippocampal cultured neurons. Primary cultured hippocampal neurons were treated with standardized dose of curcumin (20 µM) or arsenic (10 µM) alone or in combination. Expression of NMDA receptor subunits (NR1, NR2A, and NR2B) and post-synaptic signaling proteins (CAMKIIα, PSD-95, SynGAP) and those involved in PI3K/Akt/MAPK pathway (BDNF, TrkB, Akt, Gsk3β, Erk1/2) and CREB was studied by western blotting. Co-localization of CREB, GSK3β, Akt, SynGap and CamKIIα was assessed by immunofluorescence. Arsenic exposure in hippocampal cultures resulted to decrease expression of NR1, NR2A, pCaMKIIα, PSD95, pErk, BDNF, pAkt, pGsk3β and increased expression of SynGAP as compared to controls. No significant change in the expression off NR2B was observed on arsenic exposure. Treatment of hippocampal cultures with inhibitors specific for Erk1/2, GSk and Akt individually was found to inhibit expression of proteins associated with PI3K/Akt/MAPK pathway on arsenic exposure. The results exhibit involvement of PI3K/Akt/MAPK neuronal survival pathway in arsenic induced dysfunctions in glutamatergic signaling and post-synaptic proteins in hippocampal cultures and further suggests that simultaneous treatment with curcumin may protect these changes.
Pranay Srivastava is grateful to the CSIR, New Delhi for the award of research fellowship

NTX50

Gestational Exposure to Common Environmental Toxicants and Internalizing Symptoms among School-Age Children

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Approximately half of all Americans will experience a mental health disorder during their lifetime with the first onset usually occurring in childhood or adolescence. Children with mental health disorders are undertreated, and internalizing symptoms are often overlooked to focus on externalizing behaviors that are considered more disruptive in classroom and home settings. Elucidating the contributions of environmental toxicants to all mental health disorders in childhood is critical to establishing a clearer understanding of both the impact of specific chemical classes as well as the origins of disease.

Within a cohort of typically developing children enrolled in the HOME Study, followed since the second trimester of pregnancy, we examined associations between early life exposure to common environmental toxicants and internalizing symptoms, including anxiety, depression, and social impairment, measured at 8 years of age (n=239), utilizing both child self-report and parent report measures. We included the following toxicants in analyses: lead, cotinine (tobacco smoke), bisphenol-A, phthalates, polybrominated biphenyl ethers (PBDEs), and perfluorinated chemicals (PFCs). Toxicant concentrations were calculated as the mean of available maternal measures collected at 16 and 26 weeks gestation except for lead which consisted of the child's level at 2 years. Standard covariates in multivariable models were sex, race, and creatinine or lipids as appropriate for each toxicant. Additional demographic and socioeconomic variables were retained as covariates in the models when statistically significant. We conducted sex-stratified analyses for each exposure-outcome association, determined a priori, regardless of measured interactions.

In multivariable analyses of individual toxicants, maternal BPA and phthalate concentrations were not significantly associated with any internalizing symptoms. Child reported anxiety and depression scores were elevated among children whose mothers had higher PBDE and PFC concentrations during pregnancy and among children with higher lead levels. Parent reported internalizing symptom scores were elevated among children whose mothers had higher PBDE and PFC levels. Parent reported social impairment scores were elevated among children whose mothers had higher levels of cotinine and among children with higher lead levels. Some associations varied by child sex. Additional analyses will clarify the roles of singular and multiple toxicant exposures in childhood internalizing symptoms.

NTX51

Do peripheral inflammatory responses link early chronic low-level lead exposure and later psychiatric disease?

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Our studies have suggested that over half of minority children living in very low-income neighborhoods are chronically exposed to low-level environmental lead. Early chronic low-level lead exposure has been associated with poorer academic and neurocognitive function and neurobehavioral disorders. Few models have been proposed that suggest the causative pathways through which early chronic low-level lead exposure changes brain and behavior.
Lead exposure is associated with immune system dysfunction. Recently, neuropsychiatric studies have shown that abnormal proinflammatory immune responses are present in psychiatric patients with autism, depression and schizophrenia, as well as in those with a range of neurodegenerative diseases. Our laboratory has replicated and added to the literature on neurocognitive effects of low-level lead exposure in young children. In animal studies we have shown that, as compared with controls, C57BL6J mice chronically exposed to low-level lead had kidney glomerular hypertrophy and microglial abnormalities in dentate gyrus/hippocampus. Evidence from diverse research domains will be integrated to suggest a new model of early chronic low-level lead exposure in which changes in brain and behavior occur secondary to changes in organ function and peripheral immune response.

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NTX52

Neurotoxic Effects on Attention Deficit and Hyperactivity in Rodent Models

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Attention Deficit Hyperactivity Disorder (ADHD) is the most commonly diagnosed cognitive impairment of childhood. ADHD, as the nameimplies , is characterized by impairment of attentional function and locomotor hyperactivity. However, the symptoms of ADHD syndrome are multifaceted and also include impairments of behavioral control and disordered planning processes. There is considerable heterogeneity of symptoms within the diagnosis of ADHD. Some children primarily display attentional impairment without hyperactivity, while others exhibit both, and the ancillary symptoms may be present to greater or lesser extents. Neurotoxic exposures during development have been found to be associated with attentional impairment and higher rates of ADHD. In particular, maternal tobacco smoking has repeatedly been found to be significantly associated with higher ADHD rates in offspring. Animal models are useful for defining the direction of the causative arrow. We have recently shown that developmental exposure of pregnant rats to tobacco smoke extract (TSE) causes significant locomotor hyperactivity in the offspring. Impairment in novel object recognition, a low motivation test of attentional function, is also seen in the TSE offspring. In contrast, during the operant visual signal detection task, an appetitive, high motivation test of attentional function,the TSE offspring do not show impaired performance. Other toxicants such as heavy metals and pesticides have also been found to cause attentional impairments and locomotor hyperactivity after early life exposure. Animal models can provide key causative and mechanistic information concerning the relationship of early life neurotoxic exposure and long-term ADHD-like dysfunction.

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NTX53

Early life lead exposure and schizophrenia neuropathology: Effects on parvalbumin-positive GABAergic interneurons and subcortical dopaminergic activity.

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Environmental factors have been associated with psychiatric disorders and recent epidemiological studies suggest an association between prenatal lead (Pb2+) exposure and schizophrenia (SZ). Pb2+ is a potent antagonist of the N-methyl-D-aspartate receptor (NMDAR) and converging evidence indicates that NMDAR hypofunction plays a key role in the pathophysiology of SZ. The glutamatergic hypothesis of SZ posits that NMDAR hypofunction results in the loss of parvalbumin (PV)-positive GABAergic interneurons (PVGI) in the brain. Loss of PVGI inhibitory control to pyramidal cells alters the excitatory drive to midbrain dopamine neurons increasing subcortical dopaminergic activity. We hypothesized that if Pb2+ exposure in early life is an environmental risk factor for SZ, it should recapitulate the loss of PVGI and reproduce subcortical dopaminergic hyperactivity. We report that in postnatal day 50 (PN50) adolescence rats chronically exposed to Pb2+ from gestation through adolescence exhibit loss of PVGI in SZ-relevant brain regions. PV and glutamic acid decarboxylase 67kDa (GAD67) protein were significantly decreased in Pb2+-exposed rats with no apparent change in calretinin or calbindin protein levels suggesting a selective effect on the PV phenotype of GABAergic interneurons. We also show that Pb2+ animals exhibit a heightened locomotor response to cocaine and express significantly higher levels of dopamine metabolites and D2-dopamine receptors relative to controls indicative of subcortical dopaminergic hyperactivity. Our results show that developmental Pb2+ exposure reproduces specific neuropathology and functional dopamine system changes present in SZ. We propose that exposure to environmental toxins that produce NMDAR hypofunction during critical periods of brain development may contribute significantly to the etiology of mental disorders.

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NTX54

Developing and Evaluating AOPs for Research and Regulatory Application

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The conceptually identical concepts of Modes of Action (MOA) and Adverse Outcome Pathways (AOP) organize mechanistic knowledge at a range of levels of biological organization to facilitate its assimilation, integration and evaluation for research and regulatory application. There have been a number of recent developments internationally which are anticipated to contribute to increasing collective confidence in application of AOPs for specified purpose for both regulatory risk assessment and research. These include an update of the World Health Organization/International Programme on Chemical Safety (IPCS) mode of action/human relevance (MOA/HR) framework. The modified framework is incorporated within an iterative roadmap, encouraging continuous refinement of problem formulation and mode of action based (integrated) testing and assessment strategies, with increasing reliance on in vitro methods at lower levels of biological organization in preliminary assessment and testing strategies. Weight of evidence considerations for hypothesized MOAs/AOPs have been developed additionally in this update and more recently evolved as a basis to contribute to the revision of guidance and electronic tools for an international knowledge base on AOPs being developed for an initiative of the Organization for Economic Cooperation and Development (OECD). This includes simplification to facilitate application of modified Bradford Hill considerations as a basis to document support for linkages among key events and adverse outcomes, guidance on extent of support as a basis to delineate confidence for specified application and supporting templates to promote consistency and transparency. These developments will be reviewed and illustrated with an example AOP for neurotoxicity.

NTX55

Binding of Antagonist to NMDA Receptors During Brain Development (synaptogenesis) Induces Impairment of Learning and Memory Abilities

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It is well documented that learning and memory are processes that rely on functioning of the glutamate receptor N-methyl-D- aspartate (NMDAR), which is a postsynaptic channel protein permeable to Na+ and Ca2+. Pre-synaptically released glutamate binds to α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/glutamate receptor, which when repetitively activated depolarizes the postsynaptic cell, eventually leading to the relief of the Mg2+ block of the NMDAR. Activation of the NMDAR regulates neurodevelopment, results in long-term potentiation (LTP) and long-term depression (LTD) and affects neuronal synaptic plasticity. The crucial role of the NMDAR in synaptic plasticity is supported by the general scientific consensus on the effect of NMDAR blockade/deletion on LTP.

All functional NMDARs are tetrameric complexes, containing the essential subunit NR1 and one or more different NR2 types (NR2A, B, C and D). The necessary subunit NR1 needs to associate with other NR2 subunits that regulate channel gating and Mg2+ dependency. During synaptogenesis there is a switch from NR2B to NR2A expression in the cortex and hippocampus that will be relevant for the duration of channel opening. Switching from the NR2B to the NR2A subunit is thought to underlie functional alteration of the NMDAR during synaptic maturation. Consequently, alterations in the expression of the NR2A and NR2B subunits during synaptogenesis could affect the learning and memory processes.

Activation of the NMDAR also enhances brain derived neurotrophic factor (BDNF) release, which promotes neuronal survival, differentiation and synaptogenesis. Consequently, the blockage of NMDAR by chemical substances during synaptogenesis (critical process for brain development) disrupts neuronal network formation resulting in the impairment of learning and memory processes. In the proposed AOP binding of lead to NMDA receptor (Molecular Initiating Event) that triggers key cellular events causing impairment of learning and memory abilities will be discussed.

NTX56

Binding of Epigallocatechin Gallate to the laminin-β-integrin Binding Site Decreases Neural Progenitor Cell Adhesion and Migration: Adverse Outcome Pathway Framework Supporting Neurodevelopmental Toxicity Research and Risk Assessment.

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Identification of new Adverse Outcome Pathways (AOPs) leading to disturbed human brain development allows prevention of neurodevelopmental disorders by regulatory risk management. This study identifies that the major antioxidant in green tea, epigallocatechin gallate (EGCG), now commercialized in high doses as a food supplement, disturbs human neural progenitor cell development at concentrations achieved after maternal supplement intake. In vitro, as the molecular initiating event, EGCG directly binds to the extracellular matrix protein laminin thus masking the laminin-β1-integrin binding site, which causes impaired β1-integrin-laminin binding of human and rat neural progenitor cells (NPCs). This deficient macromolecular interaction is translated to the impacted key event (KE) of decreased NPC adhesion and migration. Other groups reported that β1-integrin knockout mice showed altered glial structure with lack of their orientation (due to deficient adhesion) leading as a causally linked KE to a lower density of neurons in cortical layers. In agreement with this mechanism in the β1-integrin KO animals, EGCG also altered GFAP+ processes orientation in human NPC in vitro. Moreover, maternal EGCG exposure decreases the number of 5-bromo-2-deoxyuridine positive cells in cortical layers of the offspring indicating that EGCG impairs neurodelvelopment also at the organism level. The effects of EGCG developmental exposure on glial development are currently under investigation. By establishing this novel AOP, we found a relevant molecular initiating event: binding of EGCG to laminin causing the KE of disturbance of β1-integrin function. We propose that DNT exerted by this AOP can easily be tested in human NPC for recognizing potential human DNT compounds, which thus helps to improve regulatory assessment of DNT.

This research was supported by a DFG GRK1427 grant (K.G.) and a Leibniz-DAAD Research Fellowship scholarship (M.B.).

NTX57

Adverse Outcome Pathway on: Binding of Pyrethroids to Voltage-gated Sodium Channels Induces Acute Neurotoxicity

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Pyrethroid insecticides have been used for pest control for over 50 years. Consequently, much is known regarding their acute neurotoxicity. In insects as well as mammals, two distinct poisoning syndromes (Adverse Outcomes) have been identified. Type I or T type syndrome, is characterized by hyperreactivity, aggressive sparing and tremor, while type II or CS syndrome is characterized by pawing and burrowing, choreoathetosis and salivation. These signs and symptoms, as well as the effects of pyrethroids on behaviour have been demonstrated in many different laboratories and have been extensively reviewed. This class of compounds has been well studied at several different levels of biological organization and there is a solid database of literature to support development of an adverse outcome pathway for neurotoxicity following acute exposure. By contrast, the developmental neurotoxicity of pyrethroids as a class is not as well understood, and there is not a sufficient database for the development of an AOP. Thus, the AOP to be described applies to acute neurotoxicity.

NTX58

The Developmental Neurotoxicity of Non-dioxin-like PCBs: Sensitization of Ryanodine Receptors Interferes with Neurodevelopmental Processes that Determine Neuronal Connectivity

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Polychlorinated biphenyls are ubiquitous environmental contaminants. Despite being banned from production in the 1970's PCBs remain a significant children's health concern because of legacy contamination and their inadvertent production by various industrial processes and the contamination of indoor air, especially in schools across the United States. Non-dioxin-like (NDL) PCB congeners with multiple ortho chlorine substituents are predominant constituents of environmental samples, and it is generally agreed that the behavioural deficits linked to perinatal exposure PCBs are mediated primarily by NDL PCB congeners. NDL PCB congeners are potent sensitizers of the ryanodine receptor (RyR). This presentation will present data supporting an adverse outcome pathway (AOP) linking RyR activation by PCBs, the molecular initiating event, to key molecular events (calcium-dependent signaling pathways) to key cellular events (dendritic arborization and synaptogenesis) to key events at the organismal level (deficits in learning and memory). Uncertainties, inconsistencies and data gaps in this proposed AOP will also be discussed.

NTX59

The neurobehavioral toxicity of FireMaster 550® in zebrafish (Danio rerio): Chronic developmental and acute adolescent exposures

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Firemaster® 550 (FM550) is the second most commonly used fire retardant product in consumer goods and has also been detected in household dust samples. However, the neurobehavioral effects of exposure to FM550 have not been substantially characterized. We investigated the effect of embryonic exposure on subsequent locomotor behavior of larvae as well as the persisting effects of this exposure on adolescent behavior in zebrafish. In addition we studied the acute effects of FM550 exposure during adolescence. Developmental exposure to 0, 0.01, 0.1 or 1 mg/L of FM550 was accomplished via immersion 0-5 days post fertilization. The behavioral consequences were tested on day 6 (larvae) and day 40 (adolescents). Acute adolescent exposure to 0, 1 or 3 mg/L was accomplished, via immersion, for 24 hrs, with testing 2 hr or 1 week later. Zebrafish behavior was characterized across several behavioral domains including larval activity, learning, social affiliation, sensorimotor function, predator escape, and novel environment exploration. Persisting effects of embryonic exposure manifested in adolescence as a significant ($p < 0.01$) reduction in social behavior among all exposure groups. This emerged despite normal larval activity and comparable motoric behavior on several adolescent assays. The effects of exposure acutely during adolescent were generally widespread when tested 2 hr after exposure, including locomotor hypoactivity and reduced social behavior (p's < 0.05). These effects were attenuated at the 1-week testing point. Taken together, these data indicate that FM550 causes persisting neurobehavioral alterations to social behavior in the absence of perturbations along other behavioral domains and that developmental exposures to FM550 are more costly to the organism than acute adolescent exposure.
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NTX60

Solvents and Parkinson Syndromes

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Certain organic solvents are well known for their chronic neurotoxic potential: some aliphatic and aromatic compounds induce peripheral (axonal) neuropathy, while solvent mixtures are linked to encephalopathy. Prolonged occupational exposures to carbon disulfide or to chlorinated solvents (notably trichloroethylene) appear to be risk factors for Parkinsonism or Parkinson disease (PD) [1, 2] but solvent-associated PD mortality was not found in a recent large population-based prospective cohort study [3].
We report 4 cases of PD related to a mixed solvent exposure in occupational settings. After clinical examination, all patients underwent neuropsychological testing, brain magnetic resonance imaging (1.5 or 3.0 Tesla) and single-photon emission computed tomography DaT SCAN. The EUROQUEST solvent questionnaire and polysomnography were administered in two cases. All 4 workers were car painters whose jobs required the use of solvent-containing paints, glues, lacquers, adhesives, and surface cleaners. Product access in one case suggested exposure to trichloroethylene, toluene, xylene and styrene.
One of the major challenges in solvent neurotoxicity research is that people are exposed to mixtures of different compounds, such that it is impossible to ascribe culpability to single or multiple interacting substances. While the clinical workup of these cases is consistent with Parkinson syndrome an etiological association with solvent neurotoxicity is guarded. Solvent exposure was assessed with an occupational exposure matrix and job register, not on analysis of solvent composition, breathing zone air concentration, or absorbed dose.
Our goal is to assess the link between occupational solvent exposure and Parkinson syndromes from a regulatory point of view. Scientific evidence supporting causation might lead to their recognition as an occupational disease in French Law. However, major questions remain: How can we improve assessment of cause and effect? How much weight should be placed on epidemiological association? Could some brain imaging techniques help? Are definitive controlled experimental studies needed to support clinical and epidemiologic data?

1. D.M. Gash et al., Ann. Neurol. 63:184, 2008
2. S.M. Goldman et al., Ann. Neurol. 71:776, 2012
3. M. Brouwer et al., Occup. Environ. Med. Feb 23, 2015

NTX61

Gestation-only trichloroethylene exposure induced differential brain region-specific neurotoxicity in male offspring

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Previous studies in our lab focused on neurotoxic effects of postnatal exposure to the organic solvent and environmental pollutant, trichloroethylene (TCE) in autoimmune-prone MRL+/+ mice. The purpose of this study was to examine the neurotoxic potential of TCE during prenatal exposure in male offspring since others have shown maternal exposure to chemicals during pregnancy induced a variety of functional abnormalities in the brain of the offspring. In the current study, TCE (~ 3 mg/kg/day) administered to the maternal drinking water during gestation increased locomotor activity and velocity (cm/s) in male offspring at when evaluated at 6 weeks of age. Interestingly, these effects were not observed at the high dose (29 mg/kg/day). Glial cells from whole brain of prenatally-exposed male mice were isolated, cultured, and stimulated with LPS ex vivo. Culture supernatants from the TCE-exposed mice had significantly increased levels of the proinflammatory cytokine, IL-6, relative to controls (213 vs. 480 pg/ml, 0 and 3 mg/kg/day, respectively). Neuroinflammation, oxidative stress and loss of neurotrophic support are closely linked with adverse behavior. Thus, oxidative-stress biomarkers and neurotrophins were evaluated in cerebellum and hippocampus. There was a significant increase in oxidized glutathione (GSSG) and the ratio of reduced/oxidized glutathione (GSH/GSSG) in hippocampus but not cerebellum indicating increased hippocampal oxidative stress. The dramatic decrease in cerebellar cysteine, the precursor of GSH in cerebellum further suggested the cerebellum was better able to regulate oxidative stress with TCE exposure. Relative mRNA expression of BDNF was also assessed in hippocampus and cerebellum. BDNF was significantly decreased in cerebellum and significantly increased in hippocampus. These findings suggested that the hippocampal region needed neurotrophic support to combat its increased vulnerability to oxidative stress. The effects of prenatal low-level TCE exposure were long lasting and altered behavior, and increased neuroinflammation and oxidative stress biomarkers in the hippocampal region. Future research will focus on epigenetic mechanisms of this transgenerational effect of TCE on hippocampal-specific behaviors.
This research was supported by the National Institutes of Health and the Arkansas Biosciences Institute (SJB)

NTX62

Combined exposure to impulse noise and styrene

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The neuropharmacological (rapid) and cochleotoxic (slow) effects of styrene can exacerbate the impact of noise on the peripheral auditory receptor. The mechanisms through which co-exposure to noise and styrene impairs hearing are difficult to identify particularly when the slowly developing cochleotoxic process is masked in the short-term by the rapid pharmacological effect on the central nervous system (CNS). It is clear that the neuropharmacological impact of the solvent on the acoustic (middle-ear and olivo-cochlear) reflexes can have a global effect on hearing.
The study was designed to evaluate the effects of a noise (continuous vs. impulse), and a low or high concentration (300ppm vs. 600ppm) of styrene on the peripheral auditory receptor, and on the CNS, and secondly, the auditory frequency range sensitive to noise, to styrene, and to noise and styrene combined. Male Brown-Norway rats were exposed either to styrene, or to an octave band noise centered at 8 kHz, or to both noise and styrene. The noise exposure was of two different types: impulse noise with a LEX,8h of 80 dB or continuous noise with a LEX,8h of 85 dB SPL. Hearing was tested using distortion product oto-acoustic emissions, and the receptors analyzed with histology.
Although the LEX,8h of the impulse noise was lower (80 dB SPL) than that of the continuous noise (85 dB SPL), it appeared more detrimental to the cochleae. Moreover, a co-exposure to styrene and continuous noise was less damaging than exposure to continuous noise alone, regardless of the concentration of styrene. In contrast, the traumatic effects of impulse noise on the organ of Corti were enhanced by co-exposure to styrene, even at the lower concentration of styrene. The results showed also that the noise spectrum defined the location of the cochlear trauma caused by combined exposure. The tonotopicity of the styrene-induced damage depended on the associated noise spectrum, what complicates diagnosis of styrene-related hearing loss with a tone-frequency audiometric approach. In conclusion, there is not a frequency specificity of impairments due to styrene. Based on the present results, the temporal structure of the noise should be reintroduced as a key parameter in hearing conservation regulations.

NTX63

Alteration of juvenile rat emotional behavior and social play following preweanling exposure to inhibitors of FAAH

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Repeated developmental exposure to the organophosphorus (OP) insecticide chlorpyrifos (CPF) results in the inhibition of fatty acid amide hydrolase (FAAH), the enzyme that metabolizes the endocannabinoid anandamide (AEA), and leads to the accumulation of AEA in the forebrain. At lower dosages, this occurs without measurable inhibition of cholinesterase (ChE), which is the canonical target of CPF. This suggests that the endocannabinoid system may be an important target in the developmental toxicity of OP insecticides. However, it is not clear if these biochemical changes during development will result in functional effects as the animal ages. To investigate this, rat pups were exposed daily by oral gavage to either 0.5, 0.75, or 1.0 mg/kg CPF from postnatal day (PND) 10-16 and behaviors related to the endocannabinoid system were monitored. In our initial experiment, rats were placed into a dark container in a novel open field and the latency to emerge from the container was measured. All CPF treated groups spent significantly less time in the dark prior to emerging as compared to control suggesting a decreased level of emotional reactivity induced by CPF exposure (PND25). In a subsequent experiment, an additional treatment group received 0.02 mg/kg PF-04457845, a specific inhibitor of FAAH. In the open field (PND23), the high CPF and PF-04457845 groups exhibited increased motor activity but no differences in the time spent in the field's center. In the elevated plus maze (PND29), all three CPF dosage groups and the PF-04457845 group had increased % entries into the open arms and % time spent in the open arms. On PND36, social behavior was monitored and all three CPF dosage groups and the PF-04457845 group spent more time playing than did controls. The similarities in behavior between PF-04457845 and CPF suggest that developmental inhibition of FAAH could be responsible for the altered behavior induced by developmental CPF exposure.

NTX64

Low-dose paraquat exposure inhibits cell proliferation and induced apoptosis in human neural progenitor cells

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Extensive evidence demonstrates exogenous chemicals could affect normal development of nervous system by interfering proliferation of neural stem cells, in which cell cycle and apoptosis play an important role. Paraquat (PQ) is a widely studied neurotoxicant that perturbs the normal structure/function of adult CNS. However, the impacts of PQ exposure on the developing nervous system remain unclear. In this study, we observed effects of oxidative stress caused by PQ on immortalized human embryonic neural progenitor cells (hNPCs) by treating them with various concentrations of PQ (1μM, and 10μM). We found that PQ had no adverse effect on cell viability but reduced NSC proliferation and altered the expression of cell cycle regulators (p53, p16 and p21). These changes were observed in cells directly exposed to PQ (parent cells) and in their daughter cells cultured under PQ-free conditions. In addition, PQ induced apoptosis, reactive oxygen species (ROS) production and increased the lipid peroxidation marker MDA level in a dose-dependent manner after 24h PQ treatment. Meanwhile, mediators of programmed cell death, caspase3 and caspase9, were increased significantly at 1μM and 10μM of PQ, respectively. Similarly, PQ triggered cytochrome C releases at the concentration of 10μM. These results suggest that PQ via enhanced oxidative stress could significantly reduce proliferation of hNPCs at the dose causing no decrease of cell viability and the inhibitation could last in daughter cells. Besides, PQ could induce early and late apoptosis by activation of mitochondrial pathway.

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NTX65

Neurodevelopmental effects of manganese and lead co-exposure: A case study of teeth as a novel exposure biomarker

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Our current understanding of the health effects of Pb and Mn co-exposure is based largely on metals levels measured in blood. However, there may be substantial exposure misclassification, given the short half-life of Mn in blood. A preferred biomarker would be an integrated exposure measure that reflects specific windows of development. The tooth biomarker is such a measure, which can provide information on exposure timing and can be used to identify critical windows to individual chemicals and mixtures.

We collected 125 teeth from Mexican children enrolled in a prospective birth cohort and analyzed teeth for Mn and Pb concentrations, reflecting prenatal through early postnatal (0-12 months) exposures. We measured visual motor ability, an aspect of cognitive ability, among these children using the Wide Range Assessment of Visual Motor Abilities (WRAVMA). Individual and interactive effects of Mn and Pb on visual motor ability were estimated using multivariable regression models and distributed lag models.

Tooth Mn measurements at prenatal time points were positively associated with visual motor ability (adjusted βs, 2nd trimester = 3.9 [-2.6, 10.4]; 3rd trimester = 2.3 [95% CI: -3.0, 7.5]). However, at postnatal time points, negative slopes were observed (adjusted βs, ≤6 months = -2.1 [-7.1, 2.9]; >6 months = -4.0 [-12.7, 2.9]). The Mn-visual motor ability association changed markedly when considering co-exposure to Pb: at high Pb levels (tooth Pb>median), the positive association with visual motor ability during the prenatal period was attenuated; postnatally, Mn was significantly inversely associated with visual motor ability in children who have higher Pb levels (p=0.003).

Associations between Mn exposure, measured in teeth, and visual motor ability varied by exposure timing, supporting the notion of critical developmental windows. The observed effect modification of the Mn-neurodevelopment association by Pb is consistent with previously published findings. Our data, however, are able to distinguish specific exposure windows that are important for individual and joint effects of metals on neurodevelopment. Future analyses will address higher-order chemical interactions and their associations with neurodevelopment.

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NTX66

Increased GABA levels in manganese-exposed welders correlate with exposure, brain manganese, cognitive function, and motor function

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Excessive manganese (Mn) exposure has been associated with decline in cognitive and motor function. This study explores the relationships between individual Mn exposure levels in a typical US occupational setting, brain GABA levels measured in vivo by magnetic resonance spectroscopy (MRS), brain Mn deposition measured by MRI, and basal ganglia function as measured by Simon and Flanker tasks.
28 welders and 19 controls were recruited from a US truck trailer manufacturer. Welders filled out a questionnaire of detailed work history and were monitored with personal air sampling to estimate individual exposure to airborne Mn. For each subject, a 3T GE Signa MRI scanner was used to acquire high-resolution T1 relaxation maps, an inverse indicator of Mn deposition. GABA spectra were acquired from the thalamus with a TE68 MEGA-PRESS sequence and quantified using LCModel. GABA levels were significantly higher in welders vs. controls [2.14±0.82 mM vs. 1.67±0.65 mM, $p<0.05$]. Increased thalamic GABA levels significantly correlated with (a) average exposure estimated for the previous three months before the MRI exam [$R=0.526$, $p<0.05$],with (b) decreased T1 relaxation time in the substantia nigra, denoting increased Mn deposition [$R=-0.396$, $p<0.05$] and c) with impaired basal ganglia function as tested by Simon [$N=32$, $R=0.692$,$p<0.01$]and Flanker tasks [$N=31$, $R=0.651$, $p<0.01$].
These results confirm elevated thalamic GABA levels in a typical US occupational setting. The significant correlations between increased GABA levels and recent exposure levels, as well as with brain Mn accumulation in the substantia nigra, suggest that GABA-edited MRS in conjunction with quantitative T1 relaxation MRI may serve as a biomarker of Mn exposure. The fact that increased GABA levels also correlate with impaired basal ganglia function indicates that GABA levels are involved in the mechanism of manganese induced cognitive impairment. (Supported by NIEHS R01 ES020529 and CDC/NIOSH T03 OH008615)

NTX67

Peripheral and central auditory dysfunction associated with solvent exposure in humans

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A number of studies have suggested that solvents may induce auditory dysfunction. However, there is still little knowledge regarding the main signs of solvent-induced hearing loss (SIHL) in humans. The aim of this research was to investigate the association between solvent exposure and adverse effects on peripheral and central auditory functioning with a comprehensive audiological test battery. Eighty workers exposed to a mixture of solvents (toluene, xylene and methyl ethyl ketone) and 80 non-exposed workers were selected to participate in the study. The test battery comprised pure-tone audiometry (PTA), transient evoked otoacoustic emissions (TEOAE), Hearing-in-Noise test (HINT), Dichotic Digits (DD), and Random Gap Detection (RGD) tests. Solvent-exposed subjects presented with poorer mean test results than non-exposed subjects. A bivariate and multivariate linear regression model analysis was performed. One model for each auditory outcome (PTA, TEOAE, HINT, DD, PPS, and RGD) was independently constructed. For all of the models solvent exposure was significantly associated with the auditory outcome. Age, hearing level, and gender also appeared significantly associated with some auditory outcomes. A discussion of these results and the utility of some hearing tests to evaluate SIHL will be addressed.

NTX68

Low dose tobacco smoke extract exposure during development causes long-term behavioral dysfunction in rats

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Tobacco smoke is a chemically complex form of air pollution including not only nicotine but also a wide variety of over 4,000 other compounds. Tobacco exposure during gestation has been associated with higher rates of attention deficit/hyperactivity disorder (ADHD) and other sorts of cognitive dysfunction. Exposure to nicotine alone during development has been shown to exhibit long-term behavioral dysfunction. However, the effects of exposure to the more complex, total tobacco chemical mixture have not been as well studied. This experiment assessed the effects of developmental exposure to nicotine and tobacco smoke extract (TSE) in Sprague-Dawley rats. Female rats were exposed to nicotine or TSE over a four-week period (sc) via osmotic minipump (Alzet, Model 2ML4) starting three days prior to mating. The pumps delivered 0, 0.2 or 2 mg/kg/day of nicotine based on pre-mating weight. The TSE group was administered a dose that contained 0.2 mg/kg/day of nicotine together with the remainder of the chemicals in TSE. Offspring of both sexes were assessed for locomotor hyperactivity, emotional dysfunction and cognitive impairment with lower and higher motivational incentives. Developmental exposure to the TSE with 0.2 mg/kg nicotine caused significant locomotor hyperactivity compared with groups treated with vehicle control solution or 0.2 mg/kg/day of nicotine alone. Developmental TSE exposure also caused a significant impairment in working memory relative to controls in the novel object recognition task (NOR), a low motivation cognitive task with only novelty as a motivator. In contrast no TSE induced impairments were observed in the radial-arm maze and operant signal detection tasks, which use appetitive motivation in food restricted rats. These results suggest that non-nicotine chemicals contained in tobacco appear to potentiate the effect of nicotine such that developmental exposure to all chemicals together produce locomotor hyperactivity in juvenile rats and impaired object recognition in young adults. The cognitive impairment is more clearly seen in a low motivation task. The neurochemical and epigenetic bases for this long-term behavioral dysfunction are being investigated.

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NTX69

Effects of environmental exposure to manganese on the visuoperception and visual memory in Mexican children.

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The Molango mining district located in the state of Hidalgo has important manganese (Mn) deposits. An ecosystem health approach has been used to study the factors that determine the population exposure using the interaction between social, environmental and health sciences. The objective of this study is to evaluate the effect of Mn exposure on visual perception and visual memory in school-aged children. Materials and methods. Cross sectional study. School-aged children between 7 and 11 years old were selected 148 children from two communities exposed in the Molango mining district and 116 children from unexposed communities with similar socioeconomic conditions. The Rey-Osterrieth Complex Figure (ROFC) was applied. The exposure to Mn was measured in hair (MnH). MnH was transformed to natural logarithm. To estimate the association between MnH and the ROFC were used multilevel regression models or hierarchical linear models, assuming that the effects of the predictor variables are fixed. A random term was used for analyzes the difference intergroup. These models were adjusted for confusing variables. Results: Mean arithmetical MnH was significantly different between exposed group (5.25 µg/g) and unexposed group (0.55 µg/g) $p<0.05$. We found statistically significant association between the lnMnH-age interaction and ROCF test. ROCF copy: for each increase lnMnH-age interaction there is an increment in the distortion error (β 0.016, CI 0.003 - 0.029), tangency errors (β 0.018, CI 0.005 - 0.031), overtracing (β 0.013, CI 0.000 - 0.027), overtracing (type A) (β 0.012, CI 0.002 - 0.022). ROCF Immediate Recall: For each increase lnMnH-age interaction there is an increment in the distortion error (β 0.027, CI 0.012 - 0.042), tangency errors (β 0.027 CI 0.013 - 0.041), incompletely draw (β 0.007 CI 0.001 - 0.013), angle error (β 0.019 CI 0.010 - 0.029), overtracing (β 0.011 CI 0.002 - 0.020), overtracing (type A) (β 0.010 CI 0.003 - 0.017). Conclusions: This study demonstrating that the environmental exposure to Mn has an association about the visuoperception and visual memory in school-aged children. The effects of environmental exposure to Mn about the visuoperception function and visual memory are greater in the girls.

NTX70

The effects of lead (Pb) and methylmercury (MeHg) on neurochemistry and behavior in chicken hatchlings

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Lead (Pb) and mercury (Hg) have been used by humans for thousands of years, despite their known toxicity. Both of these metals enter the environment through human activities. Hg is converted to more bioavailable methylmercury (MeHg) by bacteria in aquatic sediments. Ecosystems are often subjected to pollution from numerous sources, exposing wildlife to combinations of contaminants. Also heavy metal pollutants, including Pb and MeHg, commonly occur together worldwide and threaten the health of humans and wildlife. Birds are particularly susceptible to Pb and MeHg because of dietary exposure common in both water birds and scavengers. Our understanding of the developmental and neurological effects of these contaminants on avian development is still poor; and while we know Pb and MeHg individually affect avian behavior, their combined affects have not been previously assessed in birds. The objective of our study was to examine both the individual effects of Pb and MeHg, and their combined effects (Pb+MeHg) on the development, behavior, and neurochemistry of chicken hatchlings by injecting lead nitrate and MeHg into fertilized chicken eggs. The exposure were done at ecologically relevant concentrations. Chicken was used as a model to increase understanding of behavioral and neurotoxicity while also serving as sentinels for wildlife exposures. Mortality and developmental malformations were examined at hatching or during embryonic necropsy. Morphometric assessments were made 3 hours post-hatch. This was followed with behavioral assessments at 5 and 10 days post-hatch and neurochemical analysis for effects on NMDA and GABA receptor densities. Malformations were seen in embryos at high doses of Pb. Pb, MeHg, and Pb+MeHg changed the behavioral responses of hatchlings compared to non-exposed individuals. These data help support the notion that Pb can have damaging effects on population size and fitness of birds in the wild. Notably, the behavioral comparisons in this study among Pb, MeHg, Pb+MeHg and controls may reveal important interactions of Pb and MeHg.

NTX71

The adverse effects of pesticides on the central auditory nervous system in tobacco growers

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In Brazil, the plantation of tobacco occupies a very important place in the economy. In tobacco fields, the use of pesticides is massive which has a direct adverse effect on rural workers and their families' health. The aim of this study was to investigate the effects of exposure to agrochemicals on the central auditory system of growers. This was a cross-sectional study carried out between 2010 and 2012. All participants had normal hearing thresholds (for 500, 1000 and 200 Hz) and normal middle-ear function. A total of 20 growers exposed to agrochemicals (study group) and 21 participants who were not exposed to agrochemicals (control group) and with the same educational level as study group participants were selected. Participants were evaluated with two behavioural procedures to investigate the central auditory nervous system, the Random Gap Detection Test (RGDT, to evaluate temporal processing) and the Dichotic Digit Test in Portuguese (DD, to evaluate binaural integration). RGDT is a binaural test which is comprised of 4 subtests (500, 1000, 2000 and 4000 Hz). DD is a dichotic test and thus results are calculated for the right and left ears, and an average score between both ears is also obtained. No significant differences between groups for pure-tone thresholds were observed. A significant association between pesticide exposure and the results for RGDT and DD tests was found. Significant differences between pesticide-exposed and non-exposed subjects were found for RGDT 500 ($F= 4.7$, $p< 0.05$), RGDT 1000 ($F= 7.3$, $p< 0.05$), RGDT frequency average ($F= 5.7$, $p< 0.05$), and DD binaural average ($F= 5.0$, $p<0.05$) when including age and hearing level as covariates. Age was significantly associated with RGDT 500 ($F= 6.3$, $p<0.05$), RGDT frequency average ($F= 4.2$, $p< 0.05$), DD left ear ($F= 16.7$, $p<0.0001$), DD binaural average ($F= 6.8$, $p< 0.05$), and DD REA ($F= 10.7$, $p< 0.01$). Hearing level was not significantly associated with any of the test scores. The results showed that growers exposed to pesticides exhibited signs of central auditory dysfunction. This suggests that agrochemicals are associated with decrements in temporal processing and binaural integration processes/abilities in exposed individuals.

NTX72

Study of evoked otoacoustic emissions and suppression effect on workers exposed to pesticides and noise

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Scientific literature on the combined effect of exposure to noise and chemical agents on the auditory system is extensive. However, the effects of pesticides were more rarely examined. The purpose of study was to examine the effects of combined exposure to pesticides and noise in workers using objective measures of the auditory function. The sample consisted of 55 participants with hearing thresholds < 25 dBHL, with ages ranging from 18 to 35 years, divided into two groups: one group chronically exposed to noise and pesticides (NPG) and a control group (CG). The NPG was composed of 25 workers exposed to a representative daily level of occupational noise exposure and organophosphate pesticide. All NPG workers were involved in annual campaigns fighting against the dengue mosquito (100%) and exposed daily, during a 6-hour period, to an organophosphate pesticide commercially known as Malathion. The chemical substance is spread using motorized back atomizers, generating a moderate level of noise of 86 dBA when operated, which corresponds to their daily occupational exposure. In addition to hearing protection equipment, all NPG participants (100%) make use of other protection equipment, such as masks, pants, capes, boots, gloves, helmets, goggles. The CG was composed of 30 participants who were not exposed to noise or pesticides. All participants underwent Transient Evoked Otoacoustic Emissions (TEOAE) and Distortion Product Otoacoustic Emissions (DPOAE) assessments and a measure of the suppression effect which allowed examining the integrity of the medial olivocochlear efferent system. Student's *t* test and Fisher's test were applied to analyze the variability among TEOAE, DPOAE, and suppression effect averages between the NPG and the CG. The findings revealed significant differences for the TEOAE and DPOAE measures between NPG and CG groups. Workers in the NPG showed a reduced signal to noise ratio in both TEOAE and DPOAE measurement. A smaller general response level and a lower suppression value were also observed in the NPG. These findings suggest that chronic combined exposure to pesticides and noise may have altered the auditory function at the level of the medial olivocochlear efferent system.

NTX73

Assessment of the short-term neurobehavioral toxicity of a perinatal exposure to the HexaBromoCycloDoDecane (HBCDD) α-isomer in rats

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The present study aimed to investigate the developmental neurotoxicity of an early exposure to a-HBCDD through the ingestion of contaminated hen's egg in pregnant and lactating Wistar female rats. Eggs were obtained from hens after 3 months of daily feeding with a-HBCDD-contaminated feed at two levels of exposure, resulting in a content of a-HBCDD within the eggs of 30 and 100 ng/g of lipids, respectively. Female rats were daily administered p.o. with an appropriate volume of the whole egg from the day of fertilization (GD0) to the weaning day for pups (PND21). Thus, fetuses and pups were continuously exposed to HBCDD through the dam over a whole 42-day period including both gestation and lactation. Neurobehavioral development of pups was investigated from PND3 to PND 25 using various tasks including the righting reflex (PND3-5), the grasping reflex (PND4-6), the negative geotaxis (PND8-10) and the locomotor coordination test (PND19-21). Ultrasonic vocalizations of pups were also daily recorded from PND4 to PND16. After weaning, spontaneous motor activity and anxiety-related behavior were examined at PND25 in the open-field and in the elevated-plus maze, respectively.

Present results showed a significant decrease in body weight of both pups exposed to the lower HBCDD level from PND3 to PND25, whereas the weight of rat pups exposed to 100 ng/g of HBCDD was not different from controls. During the first 3 weeks of life, impairments in the motor maturation of pups were observed in a dose-dependent manner depending on the test, whereas no significant differences were reported between male and female pups. At PND25, the anxiety level of female rats exposed to the lowest dose of HBCDD (30 ng/g) was significantly reduced whereas it remained unchanged in males. No significant variations were measured in rats exposed to the higher level of HBCD (100 ng/g).

These results suggest the potent developmental neurotoxicity of an early chronic exposure to the HBCDD a-isomer through the ingestion of hen's eggs contaminated with this pollutant and question the long-lasting consequences of this exposure on behavior abilities and brain functioning in adulthood.

NTX74

Role of opioids in hemin-induced neurotoxicity

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Background: Intracerebral hemorrhagic (ICH) stroke is defined by the rupture of intracranial artery that leads to the formation of a hematoma. Neuronal damage is associated with the lysis of red blood cells, which releases hemoglobin. Hemoglobin and its breakdown product hemin are neurotoxic. Increasing evidence suggests that drug abuse in the form of opioids can precede and contribute to the pathology of ICH brain injury. The role of opioids and their cognate receptors in hemin-induced neurotoxicity remains unclear.

Hypothesis: We hypothesize that morphine may exacerbate and naltrexone may protect against hemin-induced toxicity.

Methods: Using neuronal and glial cultures, we investigated hemin-induced toxicity and the role of mu-opioid receptors. SK-N-SH and A172 cells were used to model neurons and astrocytes respectively. Cell viability following hemin (10.0 – 100μM) treatment for 18h was measured using LDH, Calcein AM and MitoTracker Red assays (n=6). To measure the role of the mu-opioid receptor in these cell types against hemin toxicity, receptor selective agonist, morphine (1 – 100μM) and antagonist, naltrexone (1 – 100μM) were used.

Results: Currently, preliminary experiments are being performed with hemin alone (vs. vehicle control), pre- and co-treatment of neurons with morphine and naltrexone with hemin.

Conclusion: Opioid abuse has reached epidemic levels across the USA, however it is not clear how opioid abuse affects the cell types including the neurovascular of the brain. Opioid abuse may damage neurons and glial cells that may contribute to reduced neuroplasticity therefore later in life, precede stroke. Understanding how exposure to neurotoxic substances during development leads to permanent plasticity problems is not only scientifically interesting but also of clinical relevance when prevention fails.

NTX75

Characterizations of 3’ splice variants of Acetylcholinesterase (AchE) gene in rat: Implications for neurotoxicology studies

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Acetylcholinesterase (AchE) is often used as a molecular marker in neurotoxicology studies. In mammals, AchE gene produces three splice variants (AchE-S, AchE-H, and AchE-R) by alternate use of 3'-end exons. The "synaptic" splice variant (AchE-S) and "read-through" variant (AchE-R) are particularly relevant to adverse response events in neural tissues. Earlier studies have described the transcriptional perturbations of these variants following exposure to neurotoxic chemicals. We assessed the expression of a battery of developmentally and toxicologically-relevant genes in developing rat brain, in rat primary cerebellar granule cells (CGCs) and in an established rat cell line (PC12). While attempting to assess the expression of AchE-S and AchE-R splice variants in these rat samples, we realized that the annotated sequences of these variants in rats were not available in GenBank database and that the exon-intron organization of AchE was incompletely characterized due to gaps in rat genomic sequence. Given that such information is fundamental for reliable and reproducible assessment of gene expression, we proceeded to the characterization of these splice variants in rat. First, we sequenced rat AchE genomic regions covering the sequence gaps. This information allowed us to complete the characterization of rat AchE exon-intron structure, to perform comparative sequence analysis of AchE across mammalian species and to identify and annotate orthologous exons in rat. We then confirmed the Open Reading Frames (ORF) of these splice variants by sequencing of their mRNA. Finally, differential expression patterns for AchE splice variants were observed in rat brain at different developmental stages, in various brain regions and in different adult tissues. In contrast to the view that AchE-R splice variant is scarce or short-lived, we observed moderate expression of this splice variant during normal brain development and in bone marrow, and a low level of expression in different regions of adult brain. The information on the sequences and expression patterns of AchE splice variants will help us to better assess the transcriptional response of CGCs to neurotoxic chemicals, hopefully leading to a better understanding of mechanisms involved in neurotoxicity and improved developmental neurotoxicity risk assessments.

NTX76

Tremor and movement disorders from carbon monoxide exposure - Case report and review of the literature

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Neurological effects are well known consequences of carbon monoxide (CO) exposure. Parkinsonism occurs in 10% of those exposed to CO. Patients have reported difficulty with cognition and all have had impaired gait and rigidity. Parkinsonism may reveal itself in one of two clinical presentations: (1) mild to moderate neurocognitive symptoms immediately after acute exposure with development of abnormal neurological exam findings; and/or (2) severe short term symptoms followed by some recovery, then onset of delayed neurological syndrome that may include Parkinsonism and/ or critical illness. Parkinsonism is accompanied by tremor in 25% and has been characterized as having intention or kinetic, but not resting tremor. No patients, however, have been reported in the literature to have resting or intention tremor as their primary neurological finding however, dystonia, chorea and myoclonus have been reported. We present an unusual case of a hyperkinetic movement disorder and literature review on tremor, movement disorder and Parkinsonism as a result of CO poisoning.

A 52-year-old male, suffered dizziness, headache, and tremulousness following use of a gas-powered concrete saw in an unventilated space for 30 minutes. The patient presented at a local ER with a carboxyhemoglobin (HbCO) of 19% and treatment was initiated. Soon after, he had complaints of a right upper extremity tremor and was noted to have an unchanging large amplitude pronation supination type tremor while at rest, and with outstretched arms. Past medical history, labs, MRI and Magnetic Resonance spectroscopy were unremarkable. Occupational Neurology and University Movement Disorder consultation acknowledged tremor diagnosis and agreed that this was likely secondary Parkinsonism from CO exposure. Further neuropsychological assessment revealed evidence for mild deficits in visuospatial function, information processing and cognitive flexibility as well as severe deficits in executive functioning. Tremor and neurological exam remained non-progressive several years post exposure, and during this time the patient did report some improvement with Sinemet but later discontinued this medication. The patient currently reports his tremor is improved.

CO associated movement disorders are complex and isolated tremor is a rare occurrence. Improving public health awareness of the dangers of CO will reduce these sometime catastrophic neurological consequences.

NTX77

Maturation dependent susceptibility to the herbicide paraquat in 3D rat brain cell cultures

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Exposure to environmental toxicants during vulnerable windows of brain development is suspected to raise the prevalence for neurological dysfunctions at later stages in life.

The underlying reasons that might render a developing brain more susceptible to neurotoxicants than an adult are extensive differentiation processes and changes in morphology, as well as a lack of physiological barriers. However, also the intrinsic capacity of cells to combat toxicant induced cellular stress might differ between an immature and mature brain.

In order to study whether this intrinsic protection capacity differs between immature and maturated brain cells we chose to study the maturation-dependent adverse effects of the known neurotoxicant Paraquat Dichloride (PQ) in 3D rat brain cell cultures. This in vitro system consists of all brain cell types, and over the time in culture cells undergo differentiation and maturation into a tissue-like organization.

PQ was applied repeatedly over ten days in the sub-micromolar range, and adverse effects were evaluated on different cell types. We observed that despite a higher PQ-uptake in mature cultures, its deleterious effects on glutamatergic-, GABAergic- and dopaminergic neurons were more pronounced in immature cultures. This was associated with a stronger astrogliosis in immature- versus mature cultures, as well as perturbations of the glutathione-mediated defense against oxidative stress. Furthermore, we observe microglial activation only in mature cultures, whereas immature cultures appear to down-regulate markers of a neuroprotective M2–microglial phenotype upon PQ-exposure.

Taken together our results indicate that immature brain cell cultures have a lesser intrinsic capacity to combat cellular stress as compared to mature cells and that PQ could possibly be considered as a developmental neurotoxin.

NTX78

Neuronal cell models and methods simulating nervous system function to screen for neurotoxic compounds

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There is a great variety of neurotoxins with many different modes of actions. Some of them induce cell death, others interfere with neurite growth or disturb neuronal signal processing and transmission. These effects are usually examined with animal testing that is expensive, time consuming and controversial with respect to animal welfare. To date, several available neuronal cell models have been developed with the focus to replace animal testing. These models could emulate specific functions of the nervous system, however, they are still barely characterized and the emulated functions are often limited. Therefore, the aim of our study is to establish and refine a cell based test battery to identify neurotoxic effects based on: neuronal viability, structure and function. For this purpose, embryonic stem cell derived neurons (ESCN) were compared with primary rat and mouse cortical neurons. Then, model neurotoxic test substances were tested in these models and the changes in cell viability and neuronal cell structure (neurite outgrowth) were analyzed in all cell types. In addition, the neuronal function was analyzed using live cell calcium imaging (Ca-Im) to identify effects of model compounds on neurotransmitter and voltage induced calcium responses. While primary cortical neurons of both rodent species only respond to glutamate and GABA stem cell derived neurons formed a more heterogeneous population of neurons responding to a broad variety of neurotransmitter stimuli. With respect to the effects of tri-ortho-cresyl phosphate (ToCP) we found ESCNs being less sensitive than primary neurons. On the other hand we found similar effects after exposure to acrylamide in all cell systems. The ToCP results indicate that glu-GABA-only cells might be more sensitive for some compounds. Currently we are evaluating the added value of the application of Ca-Imaging as part of a testing battery together with micro-electrode array (MEA) to assess a) the neurotoxic potential and b) to determine the mode of action of test substances considering single cells and networks of cell populations of different heterogeneity.

NTX79

DNTox-21c 3D brain models to predict DNT and study neurodegeneration

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Broader testing of substances is crucial to respond to surging neurodevelopmental problems, including autism and ADHD. Therefore, our principal goal is to develop a human-relevant, quality-assured, medium-throughput testing strategy to prioritize chemicals for developmental neurotoxicity (DNT) testing. Recently, we developed two 3D human neuronal models: (1) human iPSC-derived 3D mini-brains and (2) LUHMES 3D dopaminergic neuronal model.

iPSC-based mini-brains much more closely closer recapitulate the in vivo situation than traditional monolayer cultures since they a) consist of all neural cell types of CNS (neurons: GABAergic, glutaminergic, dopaminergic; NPCs, astrocytes and oligodendrocytes), b) are electrically active, showing spontaneous action potential, and c) have myelinated axons. We have shown the efficient differentiation and maturation in 3D using immunocytochemistry, RT-PCR, MEAs, flow cytometry. Importantly, this model allows the study of inter-individual differences in response to environmental stress by generating mini-brains from different iPSC-donors.

LUHMES is a neuroprogenitor cell line, which can be differentiated within few days into mature dopaminergic neurons. We optimized the protocol for 3D differentiation and extensively characterized the model by immunocytochemistry, RT-PCR, flow cytometry. 3D cultivation of differentiating LUHMES increased cell survival and maturation (synaptogenesis and neurite arborization). Using LUHMES ubiquitously expressing turbo-RFP we optimized confocal imaging in 3D and are establishing the method to quantify neurite integrity after toxicant treatment. The most important advantages of this model are (a) the model is homogenous (100% dopaminergic neurons). This allows us to deduce the response from a single cell type and compare it with the response from more complex systems such as iPSC-mini-brains. (b) Rapid differentiation allows fast compound screening. (c) Prolonged cultivation up to 21 days allows studying cellular resilience after short-term, pulse exposures.

These two models complement each other in our DNT and neurodegenerative disease studies, e.g. Parkinson disease. Rotenone, a well-known neurotoxicant was used to establish toxicological endpoints in aforementioned models: apoptosis, neurite integrity, ROS production, mitochondria activity as well as metabolomics, transcriptomics and microRNA profiling.
Notably, human brain models will represent a versatile tool to study CNS physiology and pathology, and neurological disorders. This innovative approach will strongly contribute to the paradigm shift in Toxicology (Tox-21c).

NTX80

Chronic Solvent-induced Encephalopathy: Course and prognostic factors

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Worldwide millions of people are occupationally exposed to organic solvents. When workers are exposed to solvents during many years, some workers develop a syndrome of Chronic Solvent induced Encephalopathy (CSE). Although CSE is a relatively mild disease, many patients experience extreme impact on activities in daily life. To date, no cure for CSE is available. A better understanding of the prognostic factors influencing the course of CSE would be helpful in educating and advising CSE patients and could provide clues for new treatment and rehabilitation strategies and supportive actions.

The objectives of this study were to describe the course of CSE and to obtain prognostic factors for the course of CSE.

The study subjects are patients from the two Dutch multidisciplinary diagnostic teams for CSE (Solvent Teams). From 2001-2011, 2009 patients were referred to the Solvent Teams for a first diagnostic evaluation. After an occupational history interview and exposure assessment, 1012 patients meeting the WHO criteria entered the second step in the diagnostic procedure, consisting of an extensive neuropsychological, psychiatric and neurological assessment. Out of 1012, only 530 patients passed both performance validity tests. These patients were included in this study. 161 patients were diagnosed with CSE, and 86 patients were diagnosed with CSE in combination with another minor somatic or psychological diagnosis (CSE+). 105 CSE patients and 78 CSE+ patients showed up for follow-up assessment 1,5 – 2 years after initial diagnosis.

Possible prognostic factors resulting from a systematic review are considered in the data analysis: initial severity of neuropsychological impairment, age, history of peak exposure, depression and anxiety, medication affecting CNS. As previous research indicates that CSE is a non-progressive disease when exposure to organic solvents is ceased, cessation of exposure will also be considered as a potential prognostic factor for the course of CSE.

NTX81

Performance validity in patients suspected of Chronic Solvent-induced Encephalopathy

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Worldwide millions of people are long term occupationally exposed to organic solvents, some of those workers develop a syndrome of Chronic Solvent induced Encephalopathy (CSE). The diagnostic process for this neurotoxic disease is complex and relies heavily on the exclusion of differential diagnosis and substantiating the cognitive complaints by neuropsychological assessment. Neuropsychological assessment is crucial for the diagnosis, therefore it is important that the neuropsychological test results reliably represent the cognitive capacity of the assessed patient. Recent European consensus guidelines on neuropsychological assessment of CSE recommend to assess at least one stand-alone performance validity test and to document implicit signs of performance invalidity. In case of suspected invalid performance, test results must be considered inconclusive and a re-test may be offered at least six months after the initial test.

The two Dutch multidisciplinary diagnostic teams for CSE (Solvent Teams) are confronted with many patients with invalid performance. This leaves the patient and the diagnostic team with the problem of an unestablished diagnosis. After a year a re-assessment is offered, hoping test results will be valid and a diagnosis can be made. But is this clinical practice helpful to the patient? How many patients alter their performance from invalid to valid? Are there prognostic factors for valid test results a year later?

The study subjects are patients from the Solvent Teams. From 2001-2011, 2009 patients were referred to the Solvent Teams for a first diagnostic evaluation. After an occupational history interview and exposure assessment, 1012 patients meeting the WHO criteria entered the second step in the diagnostic procedure, consisting of a neuropsychological, psychiatric and neurological assessment. Out of 1012, 355 patients performed below cut-off on at least one of two performance validity tests. 186 patients chose to conduct a re-assessment a year later. These patients are the focus of this study.

NTX82

Perinatal hypothyroidism and ultrasonic vocalization in rat pups

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Introduction: Perinatal hypothyroidism causes irreversible damage to auditory system functions in rats. Hearing loss has potential to affect communication behavior. We used rat pups as an animal model for communication behavior because they emit about 40 kHz ultrasonic vocalizations (USVs) under the separation from their mother rats. We examined whether perinatal hypothyroidism affects USV emissions under maternal separation.

Methods: Pregnant rats were treated with the antithyroid drug methimazole (MMI) from gestational day 15 to postnatal day (PND) 21 via drinking water. The concentrations of MMI (w/v) were 0 % (control), 0.01 % (low-dose), and 0.015 % (high-dose). On PND 5, 10, 15, and 20, the pups were individually separated from their mother rats for 8 min and their USV emission was recorded for the last 3 min by using ultrasonic microphones and Sonotrack system (Metris, Hoofddorp, the Netherlands).

Results: On PND 15, both the high and low dose groups exhibited increased number of USV emissions and longer USVs in duration compared with the control group. The control group displayed USV emissions with maximum durations of 400 ms, whereas the high-dose group displayed those with maximum durations of 700 ms. The frequency of USVs was not different among the three dose groups. On PND 20, there was few emissions of USV in the three dose groups on PND 20.

Discussion: Rat pups emit 40-50 kHz USVs under the separation from their mother rat. Subsequently, the mother rat approaches to the pups and retrieves them to the nest. Thus, pup's USV emissions under maternal separation are considered as communication behavior. In this study, the MMI-treated pups indicated increased number of USV emissions and longer USVs in duration. Perinatal hypothyroidism damages the cochlear structure. The tectorial membrane of the cochlear canal is distorted and many of the outer hair cells are lost. The auditory thresholds to induce prepulse inhibition and auditory brainstem response are elevated. The rat pups might not be able to hear USVs emitted by themselves due to hearing loss. We conclude that perinatal hypothyroidism has potential to affect communication behavior by means of USV emissions.

NTX83

The association of early exposure to phenols and neuro-behavior development in school-aged children

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Neuro-behavior problems were found in an increasing number of children worldwide in recent one decade. Few studies showed that early exposure to environment hormones, which is transmitted from mothers' exposure to these substances during pregnancy, can disrupt children's development and their behaviors. Some studies have been done to find out the effect of phenols' exposure in prenatal period on the development of neuro-behavior, but there has been no conclusive results yet. This study aims to explore the association of prenatal exposure to phenols (bisphenol A, nonylphenol, and octylphenol) and neuro-behavior development in school-aged children.

Methods: We conducted a prospective Taiwan Birth Panel Cohort Study in which 483 mother-child pairs were enrolled. We analyzed the association between phenols concentration in umbilical cord blood and the scores of SNAP-IV(Swanson, Nolan, and Pelham, Version IV), CBCL (Childhood Behavior Checklist)and SDQ (Strengths and Difficulties Questionnaires) rated by children's caregivers when they were 7 years old. Finally, 149 child's neuro-behavior development ratings scales were collected. We used Taiwan's standard norm to interpret the original scores. As for correlation analysis, multiple linear regression was adopted to adjust the potential confounders, including maternal education years, family's annual income, postnatal environmental to tobacco smoke exposure and gender.

Results: The association of early exposure to phenols and neuro-behavior development in school-aged children was only significant in few domains.

The level of BPA in umbilical cord blood was significantly correlated with the oppositional domain in SNAP-IV(β=-0.08; 95% confidence interval (CI) : -0.286-0.1261; p=0.041) and the thought problem domain in CBCL(β=-0.268,; 95% CI : -0.567-0.0314; p=0.0446). The level of octylphenol in umbilical blood was significantly correlated with the peer interaction domain in SDQ, (β=0.1552; 95% CI :-0.217-0.5269; p=0.0308).

Conclusion: This study shows that the association between prenatal exposure to phenols and neuro-behavior development in school-aged children is not significant in most domains of SNAP, CBCL, and SDQ rating scales.

NTX84

Solvents effects on the stapedial reflex

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There are two reflexes involved in the physiology of hearing. The stapedial reflex (SR) protects the cochlea by contracting the middle-ear muscles, while the olivocochlear reflex is mainly dedicated to high-frequency discrimination. In this study, the effects of the olivocochlear reflex were negligible compared to those of the SR. Anesthetics are known to inhibit the SR, and aromatic solvents can counterbalance the inhibitory effects of anesthetics on this reflex. However, the mechanism of interaction between these compounds remains unknown. We studied the mechanisms of the interaction between anesthetics and solvents to develop a model capable of predicting a possible synergy between noise and chemical effects on hearing. This type of model could be useful in screening for chemicals likely to potentiate noise effects. The hearing of Brown Norway rats was tested by measuring the amplitude of distortion product emissions. The SR was triggered for 3 s every 30 s by a contralateral stimulation at 95 dB SPL. The SR amplitude was stabilized at 1.8 dB-SPL by adjusting the depth of anesthesia with an ip-administered ketamine & xylazine mixture. Six solvents(toluene, styrene, ethylbenzene, the 3 xylenes) were tested at a constant concentration intratracheally on anesthetized animals . The first results showed that the brain concentrations of the solvents did not correlate directly with the Log (Kow). For example, p-xylene increased the SR amplitude, whereas o-xylene did not. The structure of the solvent appears to be the parameter most contributing to the SR in anesthetized animals. These first results thus suggest a specific action of the solvent on membrane receptors, rather than an effect on the phospholipids of the neuronal membrane. This pharmacological effect will be analyzed to screen aromatic solvents as a function of their impact on the SR. Then, the model will be used to assess how a chloride solvent affects the reflex, and thereby to predict the effects of co-exposure to a chlorinated chemical and noise.

NTX85

Role of the PON_{1Q192R} polymorphism in the cognitive performance of agricultural workers exposed to organophosphate pesticides in the north of Chile (Coquimbo Region)

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Organophosphate pesticides (OPs) are widely used worldwide. Studies on chronic or acute exposure have revealed health effects attributed mainly to the inhibition of the enzyme acetylcholinesterase (AChE). Besides the peripheral physiological function of this enzyme, AChE is also involved in cognitive processes. On the other side, it has been described that the paraoxonase 1 (PON1) enzyme involved in the detoxification route of OPs, displays polymorphisms that account for human susceptibility to OP exposure. Although the physiological and toxicological role of PON1 has been well described, its role in the cognitive impairment observed in people chronically exposed to OPs has not been well established In this study we evaluated the effect of chronic exposure to OPs on cognitive performance and on the activities of the enzymes used to evaluate acute poisoning (erythrocyte AChE and plasma cholinesterase, ChE) of 93 agricultural workers and 85 non-exposed people and correlate these data with the $PON1_{Q192R}$ polymorphism. The neuropsychological battery consisted of 31 tests that evaluated general mental state, memory, attention, praxis, executive functions, motor coordination, language and mood.

AChE and ChE activities from the exposed group did not differ from those in the unexposed group. In the exposed group scores in 21 tests were lower compared to the scores of the unexposed group. Furthermore, individuals who showed a performance under the normal scores in the 90% of the tests belong to the exposed group. The analysis of the sample after stratifying the population according to 4 levels of impairment reveals that only the activity of AChE shows less catalytic activity in the individuals with higher deterioration. Finally, our results do not show a role of $PON1_{Q192R}$ polymorphism in global cognitive impairment; however, individuals with QQ genotype (or Q allele carriers) showed a higher percent of impairment in areas such as attention, executive function and motor coordination. This work provides information of great importance in terms of occupational health and environmental toxicology, as the Q allele (present in about 60% of the studied population) is less efficient than the R allele for metabolizing chlorpiryfos, the main OP pesticide used in the study area.

NTX86

Effect of dichlorvos in spatial learning and memory during the ontogeny of Sprague-Dawley rats

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Dichlorvos (DDVP) is an organophosphate (OP) that has been used as a pesticide and also as a cognitive enhancer due to its inhibitory effect on acetylcholinesterase (AChE). However recent studies have shown that this drug may act through alternative mechanisms to AChE inhibition. Previous studies have shown that when the enzyme acylpeptide hydrolase (ACPH) is selectively inhibited by low doses of DDVP positive changes in neural plasticity and cognitive performance are observed. Nevertheless we have found that the same DDVP dosage given during the ontogeny of rats produces biphasic response triggering beneficial pharmacological effect in young rats through the enhancement of learning and memory and a toxicological effect in old rats.

In order to determine the DDVP doses displaying nootrophic or toxic effects in learning and memory we chronically injected during 28 days Sprague-Dawley rats of 1 and 3 month-old and 1 year-old with a range of DDVP doses: 0.03, 0.1, and 2 mg/kg per day. After the treatment, rats were tested in the Morris water maze to assess spatial learning during five training days and memory 24h after training. After this, rats were sacrificed and AChE and ACPH activities were assessed in homogenized hippocampus. Synaptic plasticity parameters were also measured ex vivo (not shown).

Chronic treatment of DDVP 2 mg/kg had no effect in 1 month-old rats, a deteriorating effect in 3 month-old rats and a toxic effect in 12 months-old rats in learning and memory. Treatment with DDVP 0.1 mg/kg produces an improvement in learning and memory in 1 month-old rats. However no effect and a deteriorating effect is observed in 3 and 12 months-old rats respectively. Finally the treatment with DDVP 0.03 mg/kg showed no effect in 12 months rats. Currently we are testing DDVP 0.03 mg/kg in 1 and 3 months old rats and DDVP 0.01 mg/kg in 12 months old rats. At all DDVP doses, only hippocampal ACPH activity was specifically inhibited, remaining AChE activity unaffected. Taken together, these results indicate that the magnitude of ACPH inhibition could be used as a predictor of pharmacological or toxicological effects when learning and memory are used as endpoints.

NTX87

Assessing exposure to organophosphate pesticides, biomarkers and neuropsychological outcomes in rural populations of Chile

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The increased agricultural activity that Chile has experienced in recent years has resulted in increased use of pesticides. The pesticide family mainly involved in episodes of acute poisoning corresponds to organophosphates, causing a 39% of acute intoxications. While acute poisonings are easily diagnosed, chronic exposure often goes unnoticed. The main problem for monitoring chronic exposure is the lack of highly sensitive biochemical biomarker capable of being monitored in a body fluid. Biomarkers available today only serve to diagnose acute poisoning. The project sought to determine whether the activity of the erythrocyte enzyme called acylpeptidehydrolase (ACPH) serves as a biomarker for biomonitoring of human populations exposed to organophosphate pesticides; correlating its catalytic activity with cognitive performance. The project recruited a total of 268 volunteers of which 87 were occupationally exposed, 81 were environmentally exposed and 100 unexposed. The population was homogeneous in age range, alcohol intake, drugs consumption and smoking habits; however there were differences in gender and educational level, being the indirectly exposed group those presenting a higher number of women and educational level than the other two groups. The interview consisted in a neuropsychological evaluation and a blood sampling for measuring erythrocyte acetylcholinesterase, plasma cholinesterase and erythrocyte acylpeptide hydrolase activities. Neuropsychological assessment included general mental state, memory, language, attention, praxis, executive function, motor coordination and mood. The results of the blood tests indicated that the enzyme activities found to be inhibited by high environmental burden of organophosphates (during fumigation) were acylpeptide hydrolase in the environmentally exposed group and plasma cholinesterase in the occupational exposed group. Regarding to cognitive performance, both exposed groups showed abnormal results in most of the areas evaluated; being the most affected memory, executive function and motor coordination. Regarding to the relation of enzyme activities with cognitive functions, six predictive models were generated for impaired memory, executive function and motor coordination during fumigation period using random forest analysis. The variable that shows a higher contribution to the model was the "exposure index" which depends on the years of labor or environmental exposure reported by individuals; while the three enzymes studied delivered little contribution to the model.

NTX88

Delayed neurobehavioral effects caused by zebrafish embryonic exposure to low levels of PCB-126

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There is increasing recognition that early-life stages are most sensitive to stress and that toxicant exposure during early development may result in adverse health effects later in life. Dioxin-like polychlorinated biphenyls (PCBs) are highly toxic persistent organic pollutants causing toxicity through the aryl hydrocarbon receptor (AHR) pathway. Despite regulations and intensive remediation efforts, humans are continuously and ubiquitously exposed to dioxin-like PCBs, primarily through consumption of contaminated food. Epidemiological studies show that perinatal exposure to dioxin-like PCBs is associated with neurodevelopmental toxicity in children. Yet, the full potential for later-life health effects that result from early-life low level exposure to dioxin-like compounds is not well understood. Zebrafish are excellent tools for studying later life effects of embryonic exposure for several reasons; their short generation time is ideal for full embryo-to-adult experiments in relevant time-scales, their external development and transparent embryos allow for easy evaluation of exposure levels that do not cause immediate overt effects, their easy maintenance and breeding and high fecundity allow high throughput experimentation with many replicates. Moreover, zebrafish have recently become a highly popular tool for the study of developmental neurotoxicity, utilizing a rapidly growing list of behavioral tests, developed for both juvenile and adult fish, which allow the assessment of particular effects. We are using such tests to assess the developmental neurotoxicity of PCB-126 (3,3',4,4',5-pentachlorobiphenyl), the most toxic dioxin-like PCB congener. We exposed zebrafish embryos to either vehicle control (DMSO) or low concentrations of PCB-126 (0.3, 0.6, 1.2 nM) for 20 hours (4-24 hours post fertilization, hpf), and then reared the fish to adulthood (3 months) in clean water. We conducted behavior tests at several larval stages and after the fish reached adulthood. Our study shows that early, embryonic exposure to PCB-126 causes adult behavioral changes that are not apparent at the larval stages. Furthermore, we are exploring possible underlying neurodevelopmental mechanisms leading to the behavioral phenotype, looking at the AHR and neurogenic pathways.

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NTX89

Screening for potential developmental neurotoxicity based on changes in the ontogeny of activity in rat cortical neural networks using multi well microelectrode arrays

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Thousands of chemicals in the environment have not been characterized for their potential to cause developmental neurotoxicity (DNT). The need for a method to screen compounds rapidly for their DNT potential is a high priority. Multi-well microelectrode arrays (MEA) simultaneously measure the spontaneous activity (action potential spikes and bursts) of electrically excitable cells at multiple sites in a network. Typically, MEAs have been used for acute toxicity studies, but can also be used to study network ontogeny. Primary cultures of cortical cells are comprised of both excitatory (glutamatergic) and inhibitory (GABAergic) neurons as well as glial cells. The current study examined the ontogeny of spontaneous activity in cortical neurons from Long Evans rat pups (0-1 day old) exposed to 18 ToxCast compounds (0-30 µM) over the first 12 days in vitro (DIV). The cells were exposed to compound 2 hours after seeding on a 48 well MEA plate and 15 minute recordings of activity were made on DIV 2, 5, 7, 9, and 12. Cells experienced a full media change and re-dose on DIV 5 and 9. On DIV 12 total LDH and Cell Titer Blue were used to measure viability. When considering 3 endpoints: mean firing rate (MFR), number of active electrodes (#AE) and viability, 9 of 18 compounds (aldicarb, carbaryl, cypermethrin, flusilazole, heptachlor epoxide, imidacloprid, lactofen, lindane, and mevastatin) decreased MFR at concentrations that were not cytotoxic. For 5 of these compounds (aldicarb, carbaryl, cypermethrin, heptachlor epoxide, and lindane), evidence of in vivo DNT has been published. Four flame retardant compounds: triphenyl phosphate, tris(1,3-Dichloro-Isopropyl) phosphate, 3,3',5,5'- tetrabromobisphenol A, and tris(2-chloroethyl) phosphate appeared to have no effect on the parameters measured. These results demonstrate that this method may be able to identify DNT compounds as well as distinguish them from non DNT compounds.

This abstract does not reflect US EPA policy

NTX90

Screening the ToxCast Phase I and II libraries for acute neurotoxicity using cortical neurons grown on multi-well microelectrode array (mwMEA) plates

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The development of multi-well MEA (mwMEA) systems have increased the throughput of traditional MEAs making them an effective *in vitro* screening method to detect and characterize drugs, chemicals, and particles as potential neurotoxicants. Previous experiments established a multiplexed approach which allows for simple and rapid characterization of compound effects on both neurophysiological and cellular viability endpoints within the same network. The present experiments used this multiplexed approach to examine 894 compounds in EPA's ToxCast Phase I and II libraries to determine effects on spontaneous neural activity and cell health in primary cortical cultures grown on 48 well MEA plates. On DIV 13, baseline activity (40 min) was recorded prior to exposure to each compound at 40 µM. DMSO and $GABA_A$ antagonist bicuculline (BIC) were included as controls on each mwMEA plate. Changes in spontaneous network activity (mean firing rate; MFR) and cell viability (lactate dehydrogenase; LDH and CellTiter Blue; CTB) were assessed within the same well following 40 min compound exposure. Activity calls were established using the 85th and 15th percentiles of the chemical-induced change in MFR (medians of triplicates) across all tested chemicals. Following exposure, 266 compounds from altered MFR beyond one of these thresholds. This included metals (mercury), pesticides (e.g. carbamates) and compounds known to act on the $GABA_A$ (e.g. lindane, abamectin, fipronil) and nicotinic receptors (e.g. nicotine, imidacloprid), voltage-gated sodium channels (allethrin, tetramethrin, DDT). Of all 266 active compounds, less than 10% also decreased cellular viability. All of these hits, as well as a subset of ~40 inactive compounds will be rescreened in a concentration-response experimental design to confirm activity and determine potency. This will allow for the data on acute neurotoxicity obtained on MEAs to be compared to the results of other ToxCast assays. (This abstract does not reflect EPA policy).

NTX91

Early-life exposure to organophosphate flame retardants alters behavior in adult zebrafish: A comparison with organophosphate pesticides

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Exposure to organophosphate flame retardants (OPFRs) is widespread in humans, and the extent of their toxicity and whether it is comparable to the neurotoxicity of organophosphate (OP) insecticides is poorly characterized. This study seeks to examine whether developmental exposure to one of two OPFRs, TPP and TDCPP, can produce behavioral abnormalities later in adulthood, and to compare these toxicities to those of a well-studied OP insecticide, chlorpyrifos. Zebrafish embryos were exposed to 0.03 or 0.3 μM of chlorpyrifos, TPP, or TDCPP from 0-5 days post fertilization (dpf). DMSO (.01%) served as a vehicle control. Solutions were changed daily until 5dpf, at which point embryos were put into aquarium water for 24 hours. At 6dpf embryos were transferred to an aquarium system, where they were reared to 12 weeks of age. Adult fish were tested on a tap-startle habituation test and on a novel environment dive/exploration test. Embryonic OP exposure altered adult zebrafish behavior in the tap-startle test, with a significant interaction of OP exposure and tap number on locomotor activity in the 5 seconds after the startle stimulus. OP exposure also disrupted later-life behavior in the novel environment exploration test, with a significant interaction and a significant linear trend of OP exposure and minute on the dive response, measured as distance from the bottom of the tank, with fish exposed to 0.3 μM of TPP exhibiting an abnormal dive behavior relative to controls. Additionally, the novel environment test revealed a main effect of OP exposure on total locomotor activity, where exposure to 0.3 μM of TDCPP resulted in fish hyperactive relative to controls. Future behavioral and neurochemical testing will further characterize the prolonged effects, and affected neurotransmitter systems, of early-life exposure to organophosphate flame retardants.

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NTX92

Neurobehavioral and physiological effects of manganese exposure in welders

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Welders are especially vulnerable to the neurotoxicity of manganese (Mn) due to the exposure to micro- and nano-sized metal particles in welding fumes. By this inhalational exposure the normal homeostatic Mn regulation in the gastrointestinal tract can be bypassed. Mn accumulates in various brain regions including the basal ganglia (BG). This subcortical brain structure is characterized by a fine-tuned interplay of various neurotransmitters initiating and modulating various cognitive functions. Action cascading processes and response inhibition are such functions. They have not been studied in welders yet and therefore the present study aims at the investigation of manganese related effects on tasks assessing these functions.

Action cascading was assessed with a stop-change paradigm and response inhibition with a modified flanker task. The different task conditions were modeled as within-subject factors reflecting the particular involvement of BG functions. 39 welders (mean age = 58,5, SD = 9,5) and 37 controls (mean age = 52, SD = 5,1) participated in the study. The respirable fraction of airborne Mn exposure of the welders was measured by personal air sampling in the breathing zone during a regular shift. Additionally, blood manganese of all participants was determined in whole blood samples. Response speed and accuracy as well as event-related potential derived from EEG recordings were used as dependent variables.

In comparison to the control group welders showed less accurate responses in the stop-change task especially under task-conditions that are modulated in the globus pallidus. These results were further supported by the analyses of the EEG data. In both tasks the response speed of welders was positively correlated with the estimated cumulative airborne Mn exposure.

By means of specific tasks that have been validated in neuroscience the neurotoxicity of Mn can be assessed very precisely and effects of low-level exposures can be detected. Moreover, in combination with electrophysiological measures such tasks are capable to explore the neurotoxic mechanism of Mn in the BG more precisely.

NTX93

Domoic acid targets developing oligodendrocytes to potentially mediate toxicity in the nervous system

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Harmful algal blooms (HABs) have become increasingly prevalent over the last few decades, in part due to human activities. HABs release toxins that accumulate in seafood and affect human health. While regulations prevent the harvest of seafood at acutely toxic levels, emerging research shows that even low-level exposure during the vulnerable stages of development can have long-term consequences. However, the underlying cellular and molecular mechanisms are unknown. To understand how the HAB toxin domoic acid (DA) affects the developing nervous system, the goal of this study was to identify a specific cell type targeted by DA, and to characterize subsequent effects on behavior. We investigated how DA affects the development of oligodendrocytes, which insulate axons and enable signal transmission. We injected zebrafish embryos with 1.42ngDA, 2.96ngDA, or 7.1ngDA during the 2-8 cell stage. Oligodendrocyte precursor cells (OPCs) were quantified at 4 days post fertilization (dpf), the degree of myelination was measured at 5dpf, and startle responses were tested at 7dpf. Exposure to 7.1ngDA disrupted oligodendrocyte precursor formation in 4dpf larvae, as shown by a 3.5-fold reduction in the number of OPCs forming along the dorsal spinal cord (p=.0002). However, quantitative gene expression analyses showed that exposure to DA did not reduce the overall expression of *myelin basic protein*, suggesting that DA can perturb oligodendrocyte development but does not decrease overall myelination. Nonetheless, DA exposure was shown to alter whole animal behavior, as measured by the larval startle response. Larvae exposed as embryos to DA (1.42, 2.6 and 7.1ng DA) were less likely to respond to vibrational taps (14V, 18V and 22V intensity) than water-injected controls. Furthermore, the long-latency startle response (12ms after stimuli) of larvae exposed to 7.1ngDA had a smaller bend angle during their first c-bend (p=.005) and were significantly slower (p=.03) in executing their counter turn compared to water-injected controls. We conclude that developmental exposure to DA alters startle response in a dose-dependent manner, while oligodendrocyte development is only perturbed at the highest dose (7.1ng DA). Further analyses will determine which processes in oligodendrocyte development are perturbed, and whether altering OPCs is related to the aberrant startle responses.

NTX94

Role of lead-induced Src activation in regulation of occludin expression level and the permeability of brain barriers

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Lead (Pb) exposure is known to result in disruption of brain barriers, i.e. the blood brain barrier (BBB) and blood cerebrospinal fluid barrier (BCB);however, the underlying mechanisms are still unclear. In this study, RBE4 and Z310 cell lines were chosen to establish the *in vitro* BBB and BCB models respectively. We found that Pb exposure increased the permeability of BBB and BCB to dextran in transwell, accompanying with decreased levels of occludin and ZO-1. Meanwhile, Pb exposure resulted in a robust increase in Src phosphorylation and inactivation of Src pathway reversed Pb-induced down-regulation of occludin, but not ZO-1 in both RBE4 and Z310 cells. Pb exposure altered both the expression and distribution of GRP78 in RBE4 cells and knockdown of GRP78 using its specific siRNA attenuated Pb-induced Src phosphorylation and occludin reduction. Interestingly, involvement of GRP78 on cell surface in regulating Src phosphorylation were not observed following Pb exposure, suggesting that translocation of GRP78 to cell surface is not essential for Pb-induced Src activation and occuldin reduction. In Z310 cells, however, the GRP78 level remained unchanged following Pb exposure, and thus GRP78 may not regulate Src phosphorylation and TJPs. Taken together, this study revealed a novel linkage of Src phosphorylation to the down-regulation of occludin and BBB and BCB disruption following Pb exposure,

NTX95

Developmental dopamine D2 receptor effects on interneuron development and behavior

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The neurotransmitter dopamine (DA) participates not only in the regulation of the mature brain, but also in the formation and assembly of functional circuits within the developing brain. DA-induced alterations in neurodevelopmental trajectory permanently alter brain circuits and cellular signaling, and contribute to the developmental origins of brain disorders. The objective of the current study is to analyze the cognitive and motor effects produced by early postnatal administration of quinpirole, a high affinity dopamine D2-like receptor agonist. Male and female mice were treated postnatally (P1-P17, 1.0 mg/kg, b.i.d.) with quinpirole, and we examined the D2R agonist-induced effects on interneuron number in the cerebral cortex and striatum. Assessments were performed at the juvenile age, P21 (weaning), and the number of GAD67+ and PV+ positive interneuron cells in the medial frontal cortex were dramatically increased. Preliminary data suggest that the change may be sex-dependent, with female mice potentially protected from the effect. We also assessed locomotor, cognitive and anxiety- and depression-like behaviors following neonatal quinpirole. A behavioral battery of tests, including the elevated zero maze, Y-maze, and open field were used to distinguish if chronic activation of D2Rs within this sensitive period alters behavioral phenotypes. There was a significant decrease in locomotor activity within male mice treated with quinpirole compared with controls. Similar to the immunohistochemical observations, female mice appear to be protected from these effects. These findings demonstrate that early postnatal D2 receptor activation influences biobehavioral development. These studies may illuminate new mechanisms by which brain structure is permanently altered following developmental drug exposures, and lead to novel therapeutic approaches to restore normal neurodevelopmental trajectories.

NTX96

Structural Abnormalities and Learning Impairments Induced by Low Level Thyroid Hormone Insufficiency: A Cross-Fostering Study

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Severe reductions in thyroid hormones (TH) during development alter brain structure and impair learning. Uncertainty surrounds both the impact of lower levels of TH disruption and the sensitivity of available metrics to detect neurodevelopmental deficits of this disruption. We have demonstrated dose-dependent impairments in fear conditioning (FC) in offspring of rat dams exposed to graded levels of the goitrogen propylthiouracil (PTU) throughout gestation and lactation. Similarly, we have observed a structural anomaly in the brain, a subcortical band heterotopia (SBH), of exposed offspring. The present study was designed to determine the window of exposure critical for the induction of these two phenotypic outcomes. Pregnant rats (n=32) were administered PTU via drinking water (0 or 3ppm) from gestational day (GD) 6 to postnatal day (PN) 14. On PN2, half the offspring from each control litter was crossfostered to a PTU nursing dam, and reciprocally half of each PTU litter was crossfostered to control dams, creating 4 treatment groups: pups receiving PTU primarily prenatally (PTU-CON); postnatally (CON-PTU), not at all (CON-CON), or throughout the pre- and postnatal period (PTU-PTU). Blood was sampled from dams via tail bleeds during pregnancy (GD10,15,20) and lactation (PN5,10,14,18), and trunk blood was collected on PN21 at weaning of the pups. Pups were sacrificed and blood and brains collected on days corresponding to dam tail bleeds. Brains were fixed and prepared for immunohistochemistry for detection of SBH. One male and female pup from each litter/treatment were tested as adults (PN55-65) on FC. We observed the presence of SBH in brains of male and female neonates on PN14 and PN18 exposed prenatally (PTU-CON and PTU-PTU) but not with exposure limited to the postnatal period (CON-PTU). FC deficits were seen in male but not female offspring, and were limited to animals exposed throughout gestation and lactation (PTU-PTU). These findings indicate that distinct phenotypes are produced when TH insufficiency occurs over different periods of brain development. They are consistent with previous findings of a greater susceptibility to behavioral impairments in male offspring, and suggest that behavioral deficits appear independent of the formation of a SBH. *Does not reflect EPA policy*

NTX97

Thyroid Hormone-Dependent Formation of a Subcortical Band Heterotopia (SBH) in the Neonatal Brain is not Exacerbated Under Conditions of Low Dietary Iron

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Thyroid hormones (TH) are critical for brain development. Modest TH insufficiency in pregnant rats induced by propylthiouracil (PTU) results in formation of a structural abnormality, a subcortical band heterotopia (SBH), in brains of offspring. PTU reduces TH by inhibiting the synthesis enzyme, thyroperoxidase (TPO). Dietary iron deficiency (FeD) also inhibits TPO and reduces serum TH. This study aimed to determine if FeD alone was sufficient to induce a SBH and if a paired insult (FeD + PTU) would augment its formation. Pregnant SD rats were administered 0 1 3 or 10ppm PTU via drinking water starting on gestational day (GD) 6. Dietary FeD was induced in a 2nd set of dams beginning on GD2. A 3rd set received FeD on GD2 plus either 1 or 3ppm PTU on GD6. Serum was taken from pups on postnatal day (PN) 15 and dams on PN21. One pup per litter was sacrificed on PN18, the brain fixed and prepared for immunohistochemical detection of SBH. Dose-dependent reductions in T4 were seen in dams and pups exposed to PTU. FeD reduced pup T4 but not dam T4. Neither did FeD combined with PTU reduce dam T4 any further than PTU alone, in fact T4 levels were higher in FeD+PTU than PTU dams. In contrast, pup T4 was reduced more than dam T4, and FeD alone and in combination with PTU produced more severe T4 reductions than with either treatment alone. Consistent with previous results, SBH size and incidence increased with dose of PTU. No SBH was detected in the offspring of FeD dams. Counter to our hypothesis, neither was the presence or size of the SBH further exacerbated with combined treatments of FeD+PTU. Although limited by sample size, combining FeD with PTU lead to an apparent *reduction* rather than *increase* in both SBH incidence and size than that seen with comparable doses of PTU alone. As such, T4 levels in dams appear to be a better predictor of structural insult associated with TH insufficiency than those of pups, findings that are consistent with a prenatal origin of SBH formation. *Does not reflect EPA policy*

NTX98

Impact of Shift Work on Attention and Female Estrous Cycling: Initial Findings in a Rat Model

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Shift work perturbs circadian rhythms and is associated with impaired cognition, including inattention. Among other problems, female shift workers are at risk of reduced fertility and irregular menstruation. We modeled shift work using Long-Evans rats testing on the 5-choice serial reaction time task during their daily light or dark period 4 hours after lights turned on or off. We hypothesized that rats of both sexes tested during the light phase would be less attentive due to misaligned behavioral rhythms, and that female rats tested during the light phase would have disrupted reproductive cycles. Animals were food restricted and sucrose pellets were used as reinforcers during testing. Control animals were not tested but were given sucrose pellets. Circadian activity was assessed using running wheel activity in home cages. We determined that light-phase tested females made fewer incorrect responses during testing than the dark-phase tested ones. This suggests that females tested during light phase were more attentive, which is contrary to our hypothesis. Both light- and dark-phase tested animals showed anticipatory activity before testing. The light-phase tested animals aligned their daily rhythms to the phase of testing. Control animals showed anticipatory activity around the time they received their sucrose pellets, but most of their activity remained at night, indicating that rats performing the task were entrained to the test and not food. Also, tested and control female light-phase rats spent more time in estrous and had more irregular estrous cycles. We are also examining cholinergic signaling in the prefrontal cortex, striatum, and suprachiasmatic nucleus using immunohistochemistry and western blots to quantify expression of cholinergic proteins, and will compare protein expression with task performance and activity during the light and dark phases.

NTX99

Chronic MPTP treatment produces hyperactivity in male mice which is not alleviated by concurrent trehalose treatment

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The chronic MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)+probenecid treatment paradigm has been used in rodents to successfully model the neurochemical, neuropathological, and behavioral effects associated with Parkinson's Disease. Here, adult male C57Bl/6 mice were injected ip twice weekly with saline or 25 mg/kg MPTP with 250 mg/kg probenecid (MPTPp) for 5 weeks (i.e., 10 injections). Behavioral assessments included motor coordination, grip strength, spatial learning/memory, locomotor activity, and anhedonia which were repeated for up to 8 weeks post-treatment. In a subsequent experiment, adult male mice were treated with saline or MPTPp as described above and one-half of each group was allowed access to 1% trehalose in the drinking water. Trehalose is a naturally occurring disaccharide thought to stabilize proteins and promote autophagy, which serve as a preventative in neurodegenerative diseases. An earlier study in our laboratory (Sarkar et al., 2014) indicated oral trehalose treatment attenuated the decrease in striatal dopamine levels and tyrosine hydroxylase and dopamine transporter immunoreactivity in the caudate-putamen and substantia nigra produced by MPTPp treatment. Here, trehalose intake averaged 1.90-2.34 g/kg/day. Behavioral assessments in this subsequent experiment included motor coordination, grip strength, locomotor activity, olfaction, and exploratory behavior which were repeated 4 weeks post-treatment. The strongest MPTPp effect was hyperactivity as exhibited in the open field. This increased activity was apparent in both experiments and occurred at all time points post-treatment. Assessments of grip strength, water maze performance, olfaction, anhedonia, and exploratory behavior did not indicate MPTPp-related alterations in either experiment. A somewhat easier motor coordination test in the subsequent experiment indicated deficits exhibited by the MPTPp, the MPTPp+trehalose, and the trehalose groups: the addition of trehalose did not attenuate any of the MPTPp-induced alterations. Although MPTPp-induced hyperactivity might appear unusual, other chronic MPTPp treatment paradigms have described similar results (e.g., Luchtman et al., 2009, 2012). These results provide a more comprehensive description of the behavioral alterations resulting from the chronic MPTPp treatment regimen.

NTX100

Perinatal exposure to polychlorinated biphenyls alters cocaine behavioral sensitization and dopamine transporter (DAT) expression in the striatum and medial prefrontal cortex of Long-Evans rats

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PCB exposure alters brain dopamine (DA) function by directly affecting the expression of the Dopamine transporter (DAT). This alteration can lead to changes in the reinforcing properties of psychostimulants that act upon the dopamine system and in particular the DAT. Long Evans rats that were perinatally exposed to PCBs were used in a behavioral sensitization paradigm that examined locomotor activity following chronic cocaine (COC) intraperitoneal injections (IP). Western blot analysis was used to determine the DAT expression in the medial prefrontal cortex (mPFC) and striatum. Rat dams were exposed to 0 (control), 3, or 6 mg/kg/day PCBs throughout gestation and lactation. Behavioral sensitization to COC was assessed in one adult male and female/litter. On PND 90, each rat was placed in a locomotor testing chamber and given 30 min to habituate. Next, an IP injection was administered followed by 60 min of activity testing. On day 1, the IP injection was saline while on days 2-7 and 14 it was 10.0 mg/kg COC, and on day 21 it was 20.0 mg/kg COC. No PCB-related differences were observed following the first injection of COC. Behavioral sensitization in the 6 mg/kg/day PCB rats occurred just after the second COC injection which was earlier then in the control group. Significant differences between these exposure groups were seen following the second COC injection in males, and third COC injection for females. All exposure groups exhibited similar COC behavioral sensitization by the fifth COC injection. After behavioral testing, the mPFC and striatum were extracted for Western blot analysis. Results demonstrated that 6 mg/kg/day PCB rats had more DAT expression in comparison to the 3 mg/kg/day group and control group in both mPFC and striatal samples. Thus, perinatal PCB-exposure can alter DAT expression in manner that appears to increase the locomotor activating effects of cocaine and promote earlier behavioral sensitization to cocaine during adulthood.

NTX101

A Study of the Object-in-place Visual Recognition Paradigm for Measuring Memory Impairment in Young C57BL6J Mice with Early Chronic Low-level Lead Exposure.

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Early chronic low-level lead exposure has been associated with diminished memory in young children. The mechanisms by which lowest level lead exposure might alter memory function in young children are not known, and animal behavior models that are sensitive to the effects of early chronic low-level lead exposure are needed. This study examined the possible effects on memory of early chronic low-level lead exposure in young mice. Fifty-two C57BL/6J mice were bred and exposed to 0 ppm (control), 30 ppm (low-dose), or 430 ppm (high-dose) lead acetate via dam´s drinking water from post-natal day (PND) 0 to 28. On PND 28 mice were tested on the object-in-place visual recognition paradigm which separately assesses spatial and object recognition memory. Data were collected using the SMART video-software system (Harvard Panlab). Data were analyzed using SAS version 6.0. Recognition of novel spatial locations and the novel object were tested with repeated measures ANOVA; possible group differences by trial were examined with ANOVA. Sex (fixed effect) and litter (random effect) were included in all models. Significant differences in exploration of familiar and novel spatial location and object were observed for all groups suggesting that this task provides a valid measure of memory in young C567BL6J mice. Group differences were not observed however, suggesting that this task was not sensitive to the possible effects on recognition memory of early chronic low-level lead exposure in young C57BL6J mice. Secondary exploratory analyses suggested significant differences by lead exposure group with regard to measures of exploratory activity, suggesting that these differences might influence outcomes on the memory task. Strategies for future studies are discussed.

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NTX102

Gestational exposure to diethylstilbestrol does not elicit alterations in anxiety- and depressive-like behaviors in C57Bl/6 mice

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It has been shown that developmental exposure to diethylstilbestrol (DES), a potent synthetic estrogenic compound, can elicit numerous harmful effects. Prescribed to millions of pregnant women from the 1930s to the 1970s, DES exposure has been found to cause significant reproductive organ abnormalities and dysfunction in both men and women (DES sons and daughters, respectively) who were exposed in utero. A high incidence of anxiety and depression disorders has also been reported, primarily in adult DES daughters. Because of estrogen's influence on emotion, it has been postulated that exposure to DES may be able to disturb the normal regulation of emotion-related behaviors that are modulated by estrogen and estrogen receptors. The present study investigated the effects of gestational DES exposure on anxiety- and depressive-like behaviors during adulthood. C57Bl/6 mouse dams were exposed to either vehicle or one of three doses of DES (.1, 1.0, or 10.0 µg/kg/day) from gestational day 11 to 17 via oral gavage. Three females and one male from each litter were retained for behavioral testing at weaning. When the female offspring reached adulthood, one was ovariectomized (OVEX), one had a sham ovariectomy (SHAM), and one did not have surgery (INTACT). Two weeks after surgery (or at a similar age in the intact offspring), anxiety- and depression-like behaviors were assessed using the Elevated Zero Maze, Open Field, Tail Suspension, and Forced Swim Tests. Experiment 1 served as a comparison between the INTACT females and males. Experiment 2 compared the behavioral results of the OVEX and SHAM females. Results revealed that exposure to DES during late gestation did not provoke a significant influence over anxiety- and depressive-like behaviors in the INTACT female or male offspring (experiment 1) or result in a differential effect on the same behaviors in the OVEX and SHAM females (experiment 2). Thus, gestational DES exposure does not appear to interact with naturally circulating estrogen levels during adulthood to alter these emotion-related behaviors. These results support more recent epidemiological studies reporting no significant differences in rates of psychiatric diagnoses in women exposed to DES in utero compared to their unexposed sisters and/or clinical control groups.

NTX103

The impact of enrichment on spatial memory in Long Evans rats exposed to ethanol

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Cognitive enrichment has been used to attenuate long term memory deficits as experienced with injury or neurodegeneration (Dahlqvist, et al., 2004; Berardi, et al., 2007). The present study sought to investigate the role of cognitive enrichment, not on long term memory problems, but on transient memory deficits as seen with ethanol exposure. Twelve male, Long Evans rats were separated into an enriched or restricted group. Rats in the restricted condition were housed individually without enrichment. Rats in the enriched group were pair-housed, received paper tube toys in their home cage, and were taken to a play area every other day to interact with the other enriched rats for thirty minutes 15 days prior to the beginning of testing. Following this 15-day enrichment period, rats trained for 4 days in the Morris Water Maze (MWM) task, a widely used procedure used in behavioral neuroscience research to study spatial memory, in which the goal for the animal is to use various spatial cues within the circular tank to swim to the location of a hidden platform under the water's surface. Following acquisition training, rats were exposed to both a saline control and a low dose of ethanol on alternating days to investigate the role of enrichment on transient memory impairments caused by ethanol exposure. Escape latency and overall velocity of the rat were analyzed using EthoVision XT tracking software (Noldus Information Technology). During acquisition, enrichment had a significant effect on average swim velocity, but no apparent effect on latency to the platform. During drug exposure, enrichment also had a significant effect on velocity, but not latency.

NTX104

The effect of adolescent nicotine exposure on Morris Water Maze spatial learning and retention in the adult male Long-Evans rat: A pilot study

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This preliminary study was designed to determine whether deficits in adult spatial learning and retention exist in response to adolescent nicotine exposure as assessed by the Morris Water Maze (MWM). To test whether nicotine given to adolescent rats affects spatial learning in adulthood, male adolescent Long-Evans rats (n=12) were injected intraperitoneally with 1.0 mg/kg/day nicotine or saline control from postnatal days 25-59 (PN25-PN59), but not thereafter. Beginning on PN65, adult rats entered acquisition training in the MWM, consisting of four trials per day for five days. One week following acquisition training, retention was tested (PN77). In the acquisition phase, there was no significant difference between adolescent nicotine and control conditions, as measured by latency to platform data. In the retention phase, however, rats exposed to nicotine during adolescence had significantly higher latency to platform times than the control group. These effects converge with other findings in the field and reinforce the concern that adolescent nicotine exposure poses an important threat to cognitive capacity in adulthood (Trauth et al., 2000; Cheeta, et al., 2001; Slawecki, et al., 2003; Rezvani & Levin, 2004; Smith, et al., 2006; Fountain et al., 2008; Iniquez, et al., 2009; Pickens, et al., 2013; Renaud, et al., 2015). Further, this preliminary study suggests a specific deficit in spatial memory retention ability in adulthood following adolescent nicotine exposure. Further research is needed to better characterize the nature and persistence of this impairment.

NTX105

Effects of Adolescent Nicotine Exposure on Memory Precision in Middle-Aged Female Rats

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Nicotine exposure during adolescence has been found to cause deficits in learning and memory when tested in early adulthood in rats. In a previous experiment, our lab found that nicotine exposure impaired memory precision in young adult rats (postnatal day 190). Additionally, aging has also been shown to impair learning and memory of contextual cues in rats. Memory precision is conceptually defined as the ability to discriminate between an initial fear conditioning context and a neutral context that is distinctly different from the original when tested at a later time. To test the effects of adolescent nicotine and age on memory precision, a passive avoidance task was used. In this task, a rat that has memory precision should exhibit a short cross latency at test in a neutral context. The current experiment examined whether nicotine during adolescence would cause memory precision deficits in 52 middle-aged 11-month-old female rats. Rats were given intraperitoneal injections of 2.0 mg/kg of nicotine or saline during adolescence from postnatal day 21 to postnatal day 55 (P21-55). The rats were aged to P343-349, and then trained in a passive avoidance chamber in a distinctive context. 24-hours later, rats were tested for memory precision in either the same context as training or in a neutral context. Results show that loss of memory precision occurred in both control and nicotine groups. This loss of memory precision was indicated by long cross latencies in neutral contexts. Loss of memory precision at this time would point to a memory deficit that is not seen in young adult rats. This result contrasts with our previous study showing that only the rats that received adolescent nicotine exposure demonstrated loss of memory precision. In the current study, adolescent nicotine rats did not perform worse than control rats at P190. This could mean the effects of aging are greater than the deficits caused by adolescent nicotine exposure. Thus, adolescent nicotine exposure did not cause a significant deficit in memory precision at P343-349, like that observed in young adult rats at P190, that could be distinguished from aging effects.

NTX106

Sex-specific differences in the persistence of cognitive impairments caused by adolescent nicotine exposure

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Adolescent nicotine exposure causes sex-specific impairments in adult serial pattern learning in male and female rats when testing occurs in early adulthood starting on postnatal day 95 (P95) (Pickens et al., 2013). The current study aimed to assess the persistence of impairments caused by adolescent nicotine exposure and determine if the pattern of effects on element acquisition endure. Thus our experiment extended on prior findings by testing male (N=26) and female (N=26) rats in middle adulthood (P200) and male rats (N=30) in later adulthood (P320). To replicate Pickens et al., rats were given once-daily i.p. injections of either 1.0 mg/kg nicotine (as free base) or saline for 35 days during adolescence (P25-59). After a delay to either mid or later adulthood, rats were trained in the serial multiple choice (SMC) task to nose poke receptacles on the 8 walls of an octagonal chamber in the pattern, 123-234-345-456-567-678-781-818, where digits represent the clockwise position of successive correct receptacles in the circular array and dashes indicate brief pauses. The pattern consisted of three element types, namely, within-chunk, chunk-boundary, and violation elements, used to assess different learning mechanisms. Repeated-measures ANOVAs were performed on rats' acquisition data for each element type at each time point. Results indicate that female rats exposed to nicotine in adolescence and tested at P200 performed as well as saline rats in the SMC task. At P200, male rats had impaired acquisition of chunk-boundary elements but not within-chunk and violation elements, consistent with findings from Pickens et al. (2013) at P95. When testing occurred in later adulthood at P320, male rats exposed to nicotine in adolescence performed as well as saline rats, indicating that adolescent exposure to nicotine impaired serial pattern learning in the SMC task in early and middle adulthood (P95 and P200) but not in later adulthood (P320). Thus, adolescent nicotine induced cognitive impairments have been observed in both sexes in early adulthood, persist into middle adulthood in males, but do not appear to be permanent for either sex. This evidence indicates that the persistence of cognitive impairments caused by adolescent nicotine is sex-specific.

NTX107

Effects of Acute Nicotine on Larval Zebrafish Exploratory Behavior in a Complex Environment

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The larval zebrafish is emerging as a useful model to assess neurobehavioral toxicity. Behavioral assays have been developed to characterize normal behavior and the acute and chronic effects of a variety of compounds in larval zebrafish. To date, such behavioral assays have been limited to relatively simple behavioral measures (e.g., swimming activity in a single well). The present experiment describes methodology to assess exploratory behavior in 5 days-post-fertilization (5dpf) larval zebrafish using a six-chamber, complex well-plate. In addition, the effect of acute nicotine exposure on exploratory behavior in this complex environment was examined. 5dpf TU strain larvae were treated with either 0, 16 or 48μM nicotine and were observed for 15 minutes in a six-chamber, complex well-plate. Chamber Transitions, Thigmotaxis (outer zone preference), and Distance Traveled were measured using a Noldus tracking system. Larvae dosed with 16μM nicotine (16μM NIC) exhibited significantly more chamber transitions than controls (CON) and larvae treated with 48μM nicotine (48μM NIC) (CON = 7.3±1.6; 16μM NIC = 13.8±1.4; 48μM NIC = 7.5±1.1). 16μM nicotine induced significantly more thigmotaxis compared to CON and 48μM NIC (CON = 62±2; 16μM NIC = 73±2; 48μM NIC = 62±2). These preliminary results demonstrate (1) the utility of this novel testing methodology, (2) that the low dose of nicotine increased exploratory behavior in a complex environment and (3) that the low dose of nicotine resulted in increased thigmotaxis, suggesting altered control of a specific type of exploratory behavior as compared to a general increase in behavioral activation. This study (1) describes a novel methodology that can be used to study complex behavior in larval zebrafish and (2) reports a difference in the effects of a low and high dose of nicotine on larval zebrafish behavior in a complex testing environment.

NTX108

Does administration of thimerosal-containing vaccines to infant rhesus macaques result in an autism-like neuropathology?

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Thimerosal, an ethylmercury-based preservative, was routinely included in most US pediatric vaccines and many other medical products for several decades. Its use has now been phased out of most pediatric vaccines, although it can still be found in multi-dose influenza vaccines included in the current US pediatric immunization schedule. While the overwhelming majority of research has concluded that there is no evidence of toxicity from thimerosal in vaccines, parental perceptions that thimerosal-containing vaccines (TCVs) are associated with the onset of neurodevelopmental disorders, such as autism, continue to impact vaccine uptake. Post-mortem brains from children with autism have shown significant reductions in the number of cerebellar Purkinje cells, in the number of cells in the lateral nucleus of the amygdala, and in the size of CA1 hippocampal cells.

In this study, we examined whether administration of multiple TCVs to non-human primate infants resulted in a similar neuropathology as observed in children with autism. Using infant male rhesus macaques, we administered TCVs following the pediatric schedule from the 1990's (n=12) and an expanded vaccine schedule from 2008 (n=8), which is similar to that used today. Control animals received saline injections (n=16). At 18 months of age, we examined the number of Purkinje cells, amygdala cells, and CA1 hippocampal cells, as well as hippocampal neurogenesis by stereological analysis. Protein levels were analyzed by immunoblot.

No neuronal cellular or protein changes were observed in vaccinated animals compared to controls. These data suggest that administration of TCVs to infant macaques does not result in neuropathological abnormalities like those observed in children with autism.

NTX109

Sleep disturbance as detected by actigraphy in juvenile monkeys receiving therapeutic doses of fluoxetine.

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Sleep disturbance and behavioral activation are both reported side effects of antidepressant drugs in children. Using actigraphy, two 48 h sessions of activity were recorded in the home cage environment of juvenile male rhesus monkeys during a two year period of treatment with a therapeutic dose (1.6-2.4 mg/kg) of the selective serotonin reuptake inhibitor (SSRI) fluoxetine. Young monkeys were active 92% of light period and 57% of the dark period of the 12:12 light cycle. The fluoxetine-treated group demonstrated sleep fragmentation as indexed by a greater number of active-inactive transitions compared to controls. In addition fluoxetine led to lower activity during the day as indexed by the duration of inactive periods and the level of activity during these periods. Hyperactivity as indexed by greater overall average or maximum activity scores was not influenced by fluoxetine. In the absence of diagnosed psychopathology or behavioral disorders, fluoxetine therapy led to sleep disturbance with apparent consequences for daytime motor activity in this nonhuman primate model of childhood SSRI therapy. Supported by NIH grants HD065826 (to MSG), RR019970 (to John Capitanio), OD011107 (to Harris Lewin).

NTX110

Treatment with the antidepressant fluoxetine increases peer social interaction in juvenile rhesus monkeys.

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Antidepressants are known to increase social interaction but it is not clear whether this effect is secondary to decreased depression or a direct effect on social behavior. Male rhesus monkeys 1 to 3 years of age were treated with a therapeutic dose of fluoxetine (1.6-2.4 mg/kg/d) and observed for social interaction during 3 30-min sessions with their familiar and like-treated cagemate over a 2 year dosing period. Fluoxetine treated animals spent 30% more time in social interaction than vehicle treated controls (n=16/group). Fluoxetine significantly increased the duration of quiet forms of socialization, cling and groom, the most common type of interaction, and also of immature sexual behavior typical of rhesus in this age group. Active play behaviors were not affected, but play time was lower in the fluoxetine group in the first session. While fluoxetine generally increased the duration of socialization, specifics depended on monoamine oxidase A (MAOA) genotype of the animal and its social partner. Subjects were genotyped for MAOA VNTR polymorphisms and categorized for polymorphisms with high or low transcription rates (hi-MAOA, low-MAOA). Cagemate dyads were categorized as hi-hi MAOA, low-low MAOA and hi-low MAOA. Social invitation and initiation behaviors were higher in hi-MAOA genotype monkeys receiving fluoxetine than in controls with the same genotype. Grooming episodes were longer in the low-low MAOA dyads and were increased by fluoxetine. Mildly aggressive facial and vocal expressive behaviors used in social situations were most frequent in members of the low-low MAOA dyads and were increased by fluoxetine. Fluoxetine may facilitate social interaction in children independent of remediation of psychopathology, but specific behaviors affected may depend on common genetic variants affecting the serotonin system. Supported by NIH grants HD065826 (to MSG), RR019970 (to John Capitanio), OD011107 (to Harris Lewin).

NTX111

Neurodevelopmental Outcome Following Prenatal Exposure to Anti-Depressant Medications

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While research on the adverse effects of late gestational use of SSRIs on neonatal behavioral adaptation has been undertaken, less research has been devoted to the long-term neurodevelopmental impact of prenatal exposure to SSRIs. Some studies have suggested behavioral alterations in children following prenatal SSRI exposure. This exploratory study investigates whether children with prenatal exposure to SSRI monotherapy or multi-drug therapies for the treatment of affective disorders have reduced performance on measures of mental ability compared to unexposed children. We identified 3 groups of women recruited through the MothertoBaby California cohort: SSRI monotherapy (n=80); polytherapy (n=28), or unexposed, healthy women (n=63). The polytherapy group included exposures to an SSRI plus other commonly used medications for the treatment of mood disorders. The Wechsler Intelligence Scale for Children (WISC-IV) and Wechsler Preschool and Primary Scale of Intelligence (WPPSI) were used to measure verbal (VIQ), performance (PIQ) and general mental ability (full-scale IQ - FSIQ) in 4-8 year old children of singleton births. Using regression analysis, controlling for socioeconomic status (based on the Hollingshead Four-Factor Index) and sex of the child, we found no differences between groups on FSIQ, VIQ, or PIQ, although the scores were consistently lower in the polytherapy group. Consistent with general guidelines to minimize the number of exposures during pregnancy, these results add further support to the advantages of monotherapy. Further research on a larger number of children is needed to determine optimal treatment approaches with respect to the impact on long-term child outcome. In light of the current practice of reduction or discontinuation of SSRI treatment in late gestation, we plan to examine the behavioral characteristics of infants treated throughout pregnancy compared to those with reduced exposure during late gestation.

NTX112

Prenatal Exposure to Acetaminophen and Child Neurodevelopment using a Maternal Self-Report Questionnaire

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Acetaminophen is the recommended drug for pain and fever reduction during pregnancy. A recent Danish National Birth Cohort study showed that children born to mothers who used acetaminophen during pregnancy had an increased risk for hyperkinetic disorders and ADHD-like behaviors[1]. We aimed to investigate whether children with prenatal exposure to acetaminophen perform worse on a maternal self-reported questionnaire of neurodevelopment. Participants were recruited from MotherToBaby California, a prospective open-cohort, and categorized into exposed to acetaminophen (N=59) and unexposed to acetaminophen (N=87). Maternal self-reports of neurodevelopment were collected when the child was between 12-16 months of age and 24-28 months of age with the Ages and Stages Questionnaire (ASQ). 48 children were evaluated at both time points. The ASQ assesses five domains of function (i.e. Communication, Fine Motor, Gross Motor, Problem Solving and Personal Social Skills). Only children with no exposure to known or suspected CNS teratogens were used for this analysis. Linear regression was used to examine data from the Ages and Stages Questionnaire (ASQ) using the software package SAS version 9.2. No statistically significant associations were observed between acetaminophen use during pregnancy and ASQ scores in any of the five domains at 12-16 months of age or at 24-28 months of age, after adjusting for age and sex. More research is needed to determine the impact of acetaminophen in pregnancy on child neurodevelopment. We are now examining the effects of dose, duration, and stage.

[1]Liew Z et al.. Acetaminophen Use During Pregnancy, Behavioral Problems, and Hyperkinetic Disorders. *JAMA Pediatr.* 2014;168(4):313-320

NTX113

Childhood and adolescent fish consumption and adult neuropsychological performance: An analysis from the Cape Cod Health Study

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Background: Methylmercury (MeHg) forms in aquatic environments when inorganic mercury from natural and anthropogenic sources is methylated by aquatic microorganisms, resulting in the contamination of fish and seafood. Numerous studies have shown that prenatal exposure to MeHg from fish has an adverse effect on the developing nervous system. However, certain fish and seafood are also rich in long-chain polyunsaturated fatty acids (PUFAs) providing a significant nutritional benefit to brain and vision development.
Objective: To examine the effect of childhood and adolescent fish consumption on adult neuropsychological performance.
Methods: The Cape Cod Health Study, a retrospective cohort study, assessed fish consumption from age 7 to 18 years via questionnaire. A sample of 65 participants underwent an extensive battery of neuropsychological tests.
Results: No statistically significant associations were observed between fish consumption patterns and performance in the domains of academic achievement, language, visuospatial, executive function, motor, or mood. Consuming fish at least twice per month was associated with better performance on tests of visual learning and memory and attentional abilities.
Conclusion: The results suggest that consumption of fish during childhood and adolescence is a relevant exposure period potentially effecting adult neuropsychological performance and future studies should be conducted examining this time period of exposure.

NTX114

Prenatal exposure lead and manganese and the intelligence of 7 year-old children.

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Background: Exposure to environmental levels of lead (Pb) and manganese (Mn) has been associated with detrimental effects to neurodevelopment. However, it is not clear that association between prenatal lead and manganese co-exposure and intelligence in childhood. The purpose of this study is to understand prenatal exposure affect the intelligence of 7 year-old children.

Methods: We recruited 153 mother-child pairs from Taiwan Birth Panel Study (TBPS). We collected their cord blood for measuring lead and manganese levels by an Agilent 7500C ICP-MS. To assess the intelligence outcome in children at 7 year-old, Comprehensive the Wechsler intelligence scale 4th edition (WISC-IV) were used, whose indicates full scale IQ (FSIQ), verbal comprehension index (VCI), perceptual reasoning index (PRI), working memory index (WMI) and processing speed index (PSI).We examined the association between lead and manganese co-exposure and child intelligence by linear regression and mixed-effect models.

Results: The median concentrations of manganese, and lead in cord blood were 50.7672455μg/L(SE= 15.6077787 ,17.8772447 - 92.4919211),and 1.2721023μg/L(SE= 0.7068022 ,0.0190237 - 4.1364812)in this study, respectively . After adjusting for maternal education, infant gender, parity, and cotinine in cord blood, there were significantly higher risks of having lower quartile FSIQ (OR=-5.56178873, p-value =0.0414) in higher manganese level .We found that under the higher manganese level, and higher lead level in cord blood had a significantly adverse association with the IQ (B=-7.9716579, p-value = 0.0198). The association between lead and manganese co-exposure affects child intelligence.

Conclusion: Lead and manganese prenatal exposure may have an effect intelligence on child. Mechanistic studies are needed to elucidate the causal relationship.

NTX115

Prenatal Exposure to Environmental Tobacco Smoke and Attention Deficit/Hyperactivity Symptoms in Children at 7 Years of Age

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Background: The association between maternal exposure of environmental tobacco smoke (ETS) and the developmental impact on children have been studied past. However, there is little report discuss the relationship between maternal ETS exposure and Attention deficit/hyperactivity symptoms. This study aims to find the association between the cotinine levels in umbilical cord blood and ADHD symptoms at 7 years of age of children.

Methods: The study recruited 191 mother- child pairs from the Taiwan Birth Panel Study (TBPS), a cohort group. We analyzed the umbilical cord blood of cotinine level, indicating the prenatal ETS levels, by the high-performance liquid chromatography (HPLC) with tandem mass spectrometric (MS/MS) and its detected limit demonstrated 0.05ng/mL. In order to assess the children's behavior when they were 7 year-old, we used different questionnaires, Chinese version of Swanson, Nolan, and Pelham, version IV scale (SNAP-IV), Strengths and Difficulties Questionnaires (SDQ) and Child Behavior Checklist (CBCL), which had been reported by their parents/caregivers. We used multiple linear regression to adjust potential confounders, involving maternal education level, annual income, Gender, and postnatal environmental tobacco smoke exposure.

Results: The geometric mean of cotinine level in cord blood are -1.56 ng/mL. According to continuous or categorical measures, we found that negative effects in CBCL, which are internalizing problems, externalizing problems and total problems domains, and furthermore the attention problems (β=1.45, p=0.01) that higher risk group has difficulty concentration or poor schoolworks and the rule-breaking behavior (β=0.71, p=0.04) which concerns lies/cheats, steal and sex problems of the CBCL test were significantly.

Conclusion: This study shows that prenatal exposure to environmental tobacco smoke has significantly negative association with the attention problems
and rule-breaking in CBCL, but in SNAP-IV and SDQ are insufficient.

NTX116

Effects of prenatal exposure to cigarette smoke on adiposity and metabolism: preliminary evidence of attenuated energy metabolism

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Introduction: Evidence of prenatal environments having persistent downstream effects on the health of the child is found in a recent meta-analysis indicating that in utero exposure to cigarette smoking is associated with childhood and adolescent obesity defined by elevated body mass index. However, it remains unclear whether prenatal exposure to smoking is related to differences in body composition and/or persistent biological alterations in energy metabolism. The purpose of our research is to examine the association of prenatal maternal smoking status and adiposity, and to further examine whether an association exists with smoking status and resting metabolic rate (RMR) and the thermic effect of feeding (TEF), i.e., the metabolic rate required to process food.

Methods: A cohort of 9 non-smoking males (n=4 control and n=5 smoking exposed) with a mean age of 20 (±2) years was recruited from a sample of 190 offspring followed as part of a longitudinal prenatal marihuana and smoking study (Ottawa Prenatal Prospective Study). For this follow-up, subjects arrived at the laboratory in the morning after an overnight fast where body weight (Standard Scale, Tanita), height (Stadiometer, Tanita), body composition (DEXA, GE Systems), and RMR and TEF (Deltatrac II Cart, SensorMedics) were measured.

Results: Due to the pilot nature of the data, comparisons between groups were assessed using effects sizes (ES) presented with means ± standard deviations, which were adjusted for birth weight. Relative to controls, subjects in late adolescence/early adulthood who were exposed to smoking during pregnancy exhibited greater percent body fat (30.5±9.8 vs. 20.4±9.8%, ES= 1.02) and lower relative RMR (20.9±24.4 vs. 24.4±2.6 kcal/kg, ES= -1.35) and TEF (2.1±0.2 vs. 2.31±0.2 kcal/kg/120min, ES- -1.10).

Conclusions: Preliminary findings from this young adult cohort suggest that prenatal exposure to smoking may not only be associated with elevated adiposity, but also altered energy metabolism evidenced by an attenuated RMR and TEF. These findings led us to perform a similar study that we are currently running with the same dependent variables in children aged 5-11 and we will be presenting both sets of data aimed at identifying potential mechanisms by which prenatal smoking may lead to downstream child obesity.

NTX117

Effects of prenatal cocaine exposure on early sexual activity: Gender difference in externalizing behavior as a mediator

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Objective: To assess: 1) the effects of prenatal cocaine exposure (PCE) on early sexual behavior and 2) whether the earlier effect of PCE on externalizing behavior mediates the effects of PCE on early sexual activity differently by gender.

Methods: Adolescents (N=354; 180 PCE, 174 NCE; 192 girls, 142 boys), primarily African-American and of low socioeconomic status, were prospectively enrolled in a longitudinal study at birth (88% retention). At the 15 year follow-up visits, age at first time of sexual intercourse was asked using items from the Youth Risk Behavior Surveillance System (YRBSS). Early sexual behavior was defined as any sexual intercourse before 15 years of age. Externalizing behavior was assessed at 12 years using the Youth Self-Report (YSR). Blood lead levels were measured at ages 2 or/and 4 years. Adolescents-reported violence exposure and parental monitoring were also assessed at 12 years. Logistic regression was conducted to assess the effects of PCE on early sexual activity and to test mediation controlling for covariates including other prenatal drug exposures, blood lead levels, parental monitoring, and violence exposure.

Results: Adolescents with PCE (n=69, 38%) were 2.3 times more likely (95% CI= 1.2 - 4.2, $p < .008$) to report sexual intercourse before age 15 than adolescents without PCE (n=49, 28%) after controlling for covariates. Blood lead was also related to a greater likelihood of early sexual intercourse (OR=2.6, 95% CI=1.4 - 4.7, $p < .002$). There was no PCE by gender interaction on early sexual behavior. However, significant gender by externalizing behavior interaction was noted ($p < .05$). Separate logistic regressions indicated that externalizing behavior assessed at 12 years fully mediated the effects of PCE on early sexual intercourse in girls, but not in boys. For boys, greater violence exposure was associated with early sexual behavior (OR=1.6, 95% CI=1.1 - 2.2, $p < .02$).

Conclusions: PCE is related to early sexual intercourse, and externalizing behavior problems mediate PCE effects in female adolescents. Interventions targeting externalizing behavior will reduce early sexual initiation and thereby reduce HIV risk behaviors and early, unplanned pregnancy in girls with PCE.

NTX118

Measuring the Impact of Diet and Environment on Infant Metabolism and the Microbiome

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The long- and short-term health benefits of breast-feeding have long been recognized. Indeed, breast-feeding is associated with lower incidences of necrotizing enterocolitis and diarrhea in early life, in addition to lower incidences of inflammatory bowel diseases, type-2 diabetes, obesity, and cardiovascular disease later in life. The mechanism by which breast-feeding imparts these protective measures is poorly understood partly due to a lack of available analytical methods to measure the comprehensive effects of feeding practices on infant metabolism. Previously, we reported profound differences between breast-fed and formula-fed infants on growth trajectory, immunological development, succession of the gut microbiome and metabolism that suggests that early imbalances in the pediatric microbiome may influence the development of diseases and disorders in adulthood. It is unknown whether the difference between breast feeding and formula feeding is related to the specific diet used, or whether other diets or formula additives will cause the same effect. To investigate the response of different diets on the gut microbiome and host metabolism, fecal microbial ecology, measured through 16s rRNA sequencing, and comprehensive metabolic profiling of serum, urine, and feces measured through ^{1}H NMR metabolomics, have been analyzed in the context of high and low protein formula diets, and with the addition of probiotics. These results will be discussed in an effort to highlight the links between diet, development of the infant microbiome, host metabolism, and health.

NTX119

Impact of Intrapartum Antibiotic Prophylaxis and Other Perinatal Interventions on the Infant Gut Microbiome

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Up to 40% of newborns are exposed to perinatal antibiotics, either directly with intravenous ampicillin and gentamicin for early-onset sepsis, or indirectly with administration of maternal intrapartum antibiotic prophylaxis (IAP). In Canada and the US, these treatments patterns adhere to clinical practice guidelines for the prophylaxis of vaginal Group B Streptococcus (GBS) and caesarean section (CS) delivery. With rising rates of CS delivery and GBS colonization during pregnancy, IAP has become a routine part of the birthing process in North America. However, these practices are not universal in Norway, Denmark, Australia and the UK. While effective in preventing early-onset neonatal sepsis, maternal GBS prophylaxis has been linked to amoxicillin-resistant late-onset *E. coli* infections in infants. Longer-term, infant antibiotic use has been associated with childhood obesity, asthma, and allergy, conditions linked to gut microbiota aberrancies during early life. The presentation will draw from data on gut microbiota profiles of 198 healthy term infants in the Canadian Healthy Infant Longitudinal Development (CHILD) pregnancy cohort study. In the CHILD cohort, maternal IAP exposures and birth method were documented from hospital records and breastfeeding was reported by mothers. Infant gut microbiota were characterized by Illumina 16S rRNA sequencing of faecal samples at 3 and 12 months. IAP for Group B Streptococcus prophylaxis or pre-labour rupture of membranes was administered to 21% of mothers; another 23% received IAP for elective or emergency CS. Infant gut microbiota community structures at 3 months differed significantly with all IAP exposures, and differences persisted to 12 months for infants delivered by emergency CS. Taxon-specific composition also differed, with the genera Bacteroides and Parabacteroides under-represented, and Enterococcus and Clostridium over-represented at 3 months following maternal IAP exposure. Compositional differences were especially evident following IAP with emergency CS, with some changes persisting to 12 months, particularly among nonbreastfed infants. Intrapartum antibiotics in CS and vaginal delivery are associated with infant gut microbiota dysbiosis, and breastfeeding modifies some of these effects.

NTX120

Maternal Stress and the Neonate Gut Microbiome: Effects on Early Life Programming and Neurodevelopment

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The neonate is exposed to the maternal vaginal microbiota during parturition, providing the primary source for normal gut colonization, host immune maturation, and metabolism. These early interactions between the host and microbiota occur during a critical window of neurodevelopment, suggesting early life as an important period of cross talk between the developing gut and brain. As perturbations in the prenatal environment such as maternal stress increase neurodevelopmental disease risk, disruptions to the vaginal ecosystem could have significant and long-term consequences for the offspring. During this talk, I will describe a series of experiments to examine the hypothesis that changes in the vaginal microbiome are associated with effects on the offspring gut microbiota and on the developing brain. Using multivariate modeling, we identified broad changes to the maternal vaginal environment that influence offspring microbiota composition and metabolic processes essential for normal neurodevelopment. Maternal stress altered proteins related to vaginal immunity and microbiota composition. Transmission of a stress-altered vaginal microbiota altered colonization in neonates and resulted in long-term disruption of gut microbiota composition in these offspring. Further, altered microbiota composition in the neonate gut corresponded with changes in metabolite profiles involved in energy balance, and with region- and sex-specific disruptions of amino acid profiles in the developing brain. Lastly, I will discuss results from experiments examining whether the early prenatal stress phenotype is transferrable to unexposed offspring by seeding with stress-altered vaginal microbiota. Taken together, these results identify the vaginal microbiota as a novel mode of transmission by which maternal stress may contribute to reprogramming of the developing brain that may predispose individuals to neurodevelopmental disorders.

NTX121

Microbiota-Gut-Brain Axis: From Neurodevelopment to Behavior

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There is a growing appreciation of the relationship between gut microbiota, and the host in maintaining homeostasis in health and predisposing to disease. Bacterial colonisation of the gut plays a major role in postnatal development and maturation of key systems that have the capacity to influence central nervous system (CNS) programming and signaling, including the immune and endocrine systems. Individually, these systems have been implicated in the neuropathology of many CNS disorders and collectively they form an important bidirectional pathway of communication between the microbiota and the brain in health and disease. Over the past five years substantial advances have been made in linking alterations in microbiota to brain development and even behaviour and the concept of a microbiota-gut brain axis has emerged. Moreover, it has become clear that diet is one of the most potent ways to modify microbiota composition. Animal models have been essential in moving forward this frontier research area. In order to assess such a role we use studies involving, germ free mice and early-life microbiota manipulations and finally probiotic administration in adulthood. We assess neurochemical, molecular, and behavioural effects following these manipulations. Our data show that the gut microbiota is essential for normal stress, antidepressant, and anxiety responses. Moreover, microbiota is essential for both social cognition and visceral pain. Finally, there are critical time-windows early in life when the effects of microbiota on brain and behaviour appear to be more potent. Manipulation of the microbiota in early life by cesarean delivery, antibiotics, or stress leads to long-lasting effects on brain and behavior. Our data also demonstrates that these effects may be mediated via the vagus nerve, spinal cord, or neuroendocrine systems. Such data offer the enticing proposition that specific modulation of the enteric microbiota by dietary means may be a useful “psychobiotic”-based strategy for both stress-related and neurodevelopmental disorders ranging from depression to autism.

NTX122

Neurobiological Consequences of Nicotine Exposure During Adolescence: Mechanisms of Short and Long-Term Effects

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As individuals age from adolescence into adulthood, the brain undergoes significant changes in structure and function that contribute to age-dependent differences in addictive behaviors. Research in both humans and laboratory animals has suggested that normal developmental processes are perturbed by exposure to drugs of abuse, such as nicotine. Moreover, nicotine exposure during this time may have adverse consequences on behavior (reward processing, learning, and decision making) that persist into adulthood. In her presentation, Dr. O'Dell will discuss the effects of adolescent exposure to nicotine with the overall goal of identifying factors that contribute to risk in developing addictive behaviors as well as neuroadaptations that can be targeted for therapeutic intervention. We will discuss our recent findings showing that adolescent rats display high levels of nicotine- and food-intake as well as weight gain during chronic access to nicotine self-administration sessions as compared to adult rats. Our results also revealed that exposure to nicotine during adolescence blocked the food- and weight-suppressant effects of nicotine later in adulthood as compared to naïve adults that displayed robust weight suppressant effects of nicotine. The clinical implications of this work will be discussed as it applies to treating adolescent smokers with nicotine replacement therapy. These issues are important to consider, as nicotine exposure during adolescence may produce detrimental long-term consequences on food intake and weight control later in adulthood.

NTX123

Age and sex differences in starting nicotine self-administration in early, mid or late adolescence vs. adulthood: Cause and effect relationships determined in a rat model

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The large majority of people who become addicted to tobacco start during adolescence. It has long been known that people who start earlier have a greater risk of lifelong addiction. However, in human studies it is not possible to tease apart whether the same genetic and environmental factors that cause greater risk for addiction also cause earlier onset or whether tobacco use during adolescent brain development causes lasting damage that increases long-term addiction risk. With rat models of nicotine self-administration (SA), we can randomly assign groups of rats to start nicotine SA at different ages. Male and female Sprague-Dawley rats were tested for the age threshold for the adult-like nicotine SA and to determine sex differences. Nicotine access was started at 4-8 weeks of age in an FR1 schedule for IV nicotine (0.03 mg/kg/infusion) in 45-minute sessions for two weeks and then one week of resumed access after one week of enforced abstinence. Nicotine SA was increased in adolescent vs. adult rats and that the effect was more pronounced in adolescent males, but the increased nicotine SA was more persistent in adolescent-onset females. The age threshold for adult-like behavior was 6-7 weeks of age. Female rats that had begun nicotine SA during adolescence showed exaggerated increases in nicotine SA after a switch back to FR1 from FR8, indicating a lessened control over their self-administration even when they became adults. Starting during adolescence causes greater amount of nicotine to be self-administered than starting during adulthood in both sexes. Males have a greater increase than females during adolescence, but females have a more persistent extension of higher SA into adulthood.

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NTX124

Understanding adolescent E-cigarette use behaviors: Implications for tobacco regulatory efforts

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Electronic-cigarettes (ECIGS), which deliver nicotine in vapor form are rapidly gaining popularity among younger smokers and there is concern that experimentation with these nicotine delivery devices will lead to nicotine addiction, continued use of ECIGS and other tobacco products. It is critical to develop a better understanding of use behaviors among youth. We used focus groups and surveys to examine use rates and perceptions about e-cigarettes among adolescents in high schools and middle schools in CT. Results from a 2013 survey in Connecticut (CT) observed that 3.5% of MS and 25.2% of HS students reported lifetime use of e-cigarettes. Furthermore, among those who had never tried e-cigarettes, 26.4% of MS and 31.7% of CT HS students reported being susceptible to future use. Focus groups conducted in 2013 with middle and high school adolescents, identified experimentation themes (i.e., curiosity, flavors, family/peer influence, easy access, and perceptions of e-cigarettes as "cool" and as a healthier/better alternative to cigarettes) and discontinuation themes (i.e., health concerns, loss of interest, high cost, bad taste, and view of e-cigarettes as less satisfying than cigarettes). Top reasons for experimentation were curiosity (54.4%), appealing flavors (43.8%), and peer influences (31.6%) and the top reasons for discontinuation were responses related to losing interest (23.6%), perceiving e-cigarettes as "uncool" (16.3%), and health concerns (12.1%). Evidence from a subsequent survey conducted in Spring 2014 observed that among those who had indicated trying an e-cigarettes (23%), the top sources of e-cigarette acquisition were friends (37.2%), tobacco shops (12.6%), gas stations (8.4%), and online (6.4%). Adolescent lifetime e-cigarette users reported using e-cigarettes primarily at home (51.9%), in locations where smoking was not allowed (24.6%) and at school (23.4%). Of the lifetime e-cigarette users who tried to purchase e-cigarettes from a physical store (n=393) or an online store (n=319), 23.1% and 3.1% were refused sale, respectively. Among all adolescents, 82.3% reported that they would not try e-cigarettes if they could not be used indoors. This evidence suggests that regulatory efforts to reduce e-cigarette use among minors are urgently needed.

NTX125

The waterpipe: A new way of hooking youth on nicotine

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Tobacco smoking continues to be the number one preventable cause of morbidity and mortality worldwide. Several evidence-based interventions and policies have been successful in reducing cigarette smoking in developed countries. Globally however, many beginning smokers are introduced to tobacco by means other than cigarettes. In particular, waterpipe smoking (a.k.a. hookah, narghile, shisha) has been dramatically increasing among youth worldwide. In this short review I will introduce the audience to this emerging tobacco use method and focus on its addictive properties, and how this pertains to the development of effective interventions to curb its spread. Waterpipe smoking is likely to be associated with much of the harmful effects of cigarette smoking, is addictive, and can serve as a bridge to cigarettes. Due to its unique features, waterpipe-specific interventions and policies are needed to curb the global waterpipe epidemic.

NTX126

Identification of conserved developmental pathways targeted by methylmercury in Drosophila melanogaster

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Methylmercury (MeHg) is a persistent environmental toxicant present in seafood that is known to target the developing nervous system. Differences in genetics and diet are likely factors that contribute to the wide variation in outcomes that occur among individuals in a population who are similarly exposed. Furthermore, characterization of genes involved in these processes are an inroad to identifying adverse outcome pathways specific to toxicants such as MeHg. To examine how genetic variation and dietary factors impact MeHg toxicity we assess developmental tolerance to MeHg in *Drosophila melanogaster* as measured by eclosion rate (larval to adult stage development) on various concentrations of MeHg containing food medium. Using a newly developed resource, the fully genome sequenced Genetic Reference Panel (DGRP) of inbred flies, genome wide association (GWA) analyses can be conducted in a relatively rapid and statistically rich manner. Genes associated with a particular trait, such as MeHg tolerance and the protective effects of caffeine, can therefore be identified in an unbiased query of the genome leading to effective hypothesis generation for testing novel pathways engaged in toxicity mechanisms. Our genome-wide association (GWA) analysis for MeHg tolerance has identified candidate genes that fall into several gene ontology categories, with enrichment for genes involved in muscle and neuromuscular development. Additional genes associated with caffeine abatement of MeHg toxicity are also found to group in muscle development categories. We will discuss the outcomes of this GWA and subsequent functional assays of manipulating expression of genes in the context of developing muscle in transgenic flies. It is likely that the effects of MeHg observed in *Drosophila* can be generalized across phyla since fundamental developmental pathways are highly conserved. These studies have the potential to elaborate hitherto unrecognized adverse outcome pathways in MeHg toxicity while also shedding light on dietary manipulations that may offer protection against the deleterious effects of MeHg exposure.

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NTX127

The Role of skn-1 in Methylmercury-induced Latent Dopaminergic Neurodegeneration.

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Mercury (Hg) is a persistent environmental bioaccumulative metal, with developmental exposure to methylmercury (MeHg) resulting in long-term health effects. We examined the impact of early-life exposure to MeHg and knockdown of skn-1 on dopaminergic (DAergic) neurodegeneration in the nematode Caenorhabditis elegans. SKN-1, a the major stress-activated cytoprotective transcription factors, promotes the transcription of enzymes that scavenge free radicals, synthesizes glutathione and catalyzes reactions that increase xenobiotic excretion. Deletions or mutations in this gene suppress stress resistance. Thus, we hypothesized that the extent of MeHg's toxicity is dependent on intact skn-1 response; therefore skn-1 knockout (KO) worms would show heightened sensitivity to MeHg-induced toxicity compared to wildtype worms. In this study we identified the impact of early-life MeHg exposure on Hg content, stress reactivity and DAergic neurodegeneration in wildtype, and skn-1KO C. elegans. Hg content, measured by Inductively Coupled Plasma Mass Spectrometry, showed no strain-dependent differences. Reactive oxygen species generation was dramatically increased in skn-1KO compared to wildtype worms. Structural integrity of DAergic neurons was microscopically assessed by visualization of fluorescently-labeled neurons, and revealed loss of neurons in skn-1KO and MeHg exposed worms compared to wildtype controls. Dopamine levels detected by High-performance liquid chromatography were decreased in response to MeHg exposure and decreased in skn-1KO worms, and functional behavioral assays showed similar findings. Combined, these studies suggest that knockdown of skn-1 in the nematode increases DAergic sensitivity to MeHg exposure following a period of latency.

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NTX128

Avian species as alternate models to understand the neurodevelopmental effects of methylmercury

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Methylmercury is a neurodevelopmental toxicant in a number of species, including birds. Here we describe work in our and other labs demonstrating that birds are excellent alternate sentinel species to characterize the neurodevelopmental effects of early-life methylmercury exposure. We describe in vitro and in vivo studies from wild birds, juveniles and adults, and those exposed in ovo via egg-injection. Tissues obtained from birds harvested in the field (e.g., common loons, bald eagles, herring gulls) indicate that mercury exposures are related to significant changes in brain neurochemistry (e.g., increased muscarinic receptor levels; decreased NMDA receptor levels). To better resolve the mechanisms by which methylmercury affects avian neurodevelopment (so as to understand inter-species differences, establish risk thresholds, etc.) experimental studies were performed in the laboratory. In vitro studies using cell-free methods (on cerebrum tissues from five species - bald eagle, zebrafinch, Japanese quail, mallard, chicken) determined that key enzymes and receptors that mediate the neurotransmission of dopamine, GABA, glutamate and acetylcholine can be impaired by methylmercury and mercuric salts, with IC50 values often approaching values considered ecologically relevant. In ovo studies in which fertilized chicken eggs were injected with methylmercury and mercuric chloride were revealing. As expected methylmercury was assimilated into many tissues (mainly brain, kidney, liver, and feather) but mercuric chloride was not. Further, tissue concentrations rose throughout embryonic development especially after yolk absorption in the hatchlings. Second, despite rather high developmental exposures studies on hatchlings up to 10 days of age revealed no significant mercury-associated effects on brain neurochemistry (see aforementioned markers), brain lesions, or neurobehavioral effects (e.g., angled beam balance, startle response) that were consistent though consistent changes in righting reflex were found. Third, studies in which chickens were developmentally exposed to mixtures of methylmercury and lead are not yielding any conclusive outcomes (despite suggestive findings from nature). We will also elaborate on other experiments that also show mixed results. In summary, there is substantial evidence that ecologically relevant levels of methylmercury can affect avian neurodevelopment though there is tremendous variability in the findings, some of which is likely related to inter-species differences in metabolism and lifestage of exposure and latencies.

NTX129

Neural Stem Cells Provide New Insights Into the Mechanisms of MeHg Developmental Neurotoxicity

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Neural stem cells (NSCs) play a critical role in the developing nervous system, therefore have been proposed as relevant models to study neurodevelopmental toxicity *in vitro*. Using NSCs from rodents, we have investigated the short-term direct and long-term inherited effects of very low concentrations (nanomolar) of methylmercury (MeHg). Our results showed that MeHg reduces NSC proliferation, inhibits spontaneous neuronal differentiation, and modifies the expression of cell cycle regulators (p16 and p21) and senescence-associated markers. In addition, we found a decrease in global DNA methylation in the exposed cells, indicating that epigenetic changes are involved in the mechanisms underlying MeHg-induced effects. Interestingly, these changes were observed in cells directly exposed to MeHg (parent cells) as well as in their daughter cells cultured under MeHg-free conditions. In agreement with the *in vitro* findings pointing to a programing effect of MeHg, cell proliferation was also decreased in the hippocampal neurogenic region (the subgranular zone) of adult mice exposed to low levels of MeHg during the pre- and perinatal period. This impaired proliferation had a measurable impact on the total number of neurons in the hippocampal dentate gyrus. Moreover, it was associated with depression-like behavior and changes in the expression of brain-derived neurotrophic factor. MeHg has been suspected to exert endocrine disrupting effects, but the mechanisms are still unclear. We therefore investigated possible intearactions with steroid hormone receptors that could be linked to the alterations observed in NSCs. All togheter, our studies provide novel mechanisms behind the programming effects induced by MeHg in the developing nervous system and support the use of NSC cultures as alternative models to expand our knowledge on the mechanisms of developmental neurotoxicity.

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NTX130

Developmental toxicity of methylmercury is associated with reduced antioxidant status and cofilin phosphorylation

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Environmental exposure to methylmercury (MeHg) during development is of concern because it is easily incorporated in children both pre- and post-natal, it has actions at several levels of neural pathways (mitochondria, cytoskeleton, neurotransmission) and it causes behavioral dysfunction in childhood. The main mechanisms that have been identified include oxidative stress, changes in intracellular calcium, mitochondrial changes, inhibition of glutamate uptake, of protein synthesis and disruption of microtubules.

We have studied the effects of prolonged exposure to 50 - 300 nM MeHg on these neural pathways by using primary cultures of mice cortical (CCN) and of cerebellar granule cells (CGC) that were exposed during their differentiation period.

MeHg-induced cell death was prevented by antioxidants like probucol, ascorbic acid, trolox and resveratrol. MeHg-induced reduction of antioxidant defenses, lipid and protein oxidation were prevented by the anti-oxidant and anti-hyperlipidemic drug probucol. The neurotransmitter dopamine but not norepinephrine, serotonin or GABA protected cortical neurons from methylmercury-induced toxicity.

Exposure to MeHg reduced the amount of phosphorylated cofilin in both neuronal types. Non-phosphorylated cofilin was translocated from cytosol to mitochondria in CGC, but not in CCN, whereas actin was in both cell types. CGC also showed increased lysosomal cathepsin D activity in the cytosol. All these effects were prevented by probucol. On the other hand, the cathepsin D inhibitor pepstatin A did not avoid MeHg-induced actin and cofilin translocation into mitochondria in CGC.

During human fetal development methylmercury is transferred to the fetus through the placenta and accumulates in the fetus. The placenta also has transporters for glutamate thus preventing the accumulation of glutamate in the fetus. We have determined in a reduced number of human placentas (n=10) from a Spanish birth cohort whether prenatal methylmercury exposure correlated with altered antioxidant defenses, cofilin phosphorylation and glutamate transport. Although not statistically significant we found a trend for decreased *i*) activity of glutathione reductase and glutathione peroxidase, and *ii*) phosphorylation of cofilin in the fetal side of placental samples from the highest methylmercury-exposed group.

NTX131

Enhanced reproductive, endocrine and behavioral deficits induced by maternal exposure to a mixture of low dose endocrine disrupting chemicals

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Understanding the toxicity of low dose, endocrine disrupting chemical (EDC) mixtures is of paramount importance given recent studies demonstrating that mixtures, differentially-acting on androgens, can produce additive male reproductive dysfunction, and little is known about CNS effects. To assess how low dose developmental EDC exposure alters reproductive, endocrine and behavior phenotypes, pregnant female mice were exposed to four EDCs, Atrazine (ATR: 10mg/kg), Perfluorooctanoic acid (PFOA: 0.1 mg/kg), Bisphenol-A (BPA: 50µg/kg), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD: 0.25µg/kg) and their mixture (MIX) or vehicle, from gestational day 7 until weaning. Adult offspring were exposed to three behavioral tests to assess fear-related behaviors, sociality, and temporal discrimination learning: elevated plus maze (EPM), social conditioned place preference (CPP) and fixed interval (FI) reinforcement schedule performance. During the EPM, male-specific deficits were seen, as MIX, BPA, TCDD and ATR exposed males showed decreased time in the closed arms, a typically favorable condition for mice. The CPP demonstrated MIX males showed a unique preference for isolation, with 80% choosing the location in which they were alone, compared to 80% of control males choosing the location with their cage-mate. A replicated, enhanced effect of increased FI response rates was found in MIX males during the acquisition of FI behavior, suggesting an inability to inhibit early responding. Increased response rates were not seen in any other treated group until more than 25 sessions occurred, after which BPA male FI rates also increased. MIX exposed females did not show behavioral deficits; however, TCDD alone increased FI response rates dramatically. Given the critical role of testosterone in sexually-dimorphic behavior, we measured testosterone levels at birth, a critical period of testosterone sensitivity. Testosterone levels were uniquely elevated in MIX exposed males, with no changes occurring in females. Consequently, anogenital distances (AGD) at weaning and adult reproductive organ weights were measured. The enhanced effect of the MIX was also seen on male reproductive physiology, with MIX males having longer AGDs and greater penile weights. Taken together, reproductive, endocrine and behavioral deficits occurred after exposure to relatively low maternal doses of EDCs in mice, with enhanced effects seen in MIX males. P30 ES001247 & T32 ES007026-36.

NTX132

Epigenetic and neurobiological consequences of prenatal exposure to bisphenol A

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Early life exposure to bisphenol A (BPA) can have lasting consequences for health and development. Studies of in utero exposure to BPA in humans suggest that high levels of this endocrine disruptor are associated with increased behavioral problems in children suggesting an impact on neurodevelopment. Interestingly, these BPA-associated effects are sex-specific, with different behavioral profiles emerging in males versus females in response to exposure level. One potential mechanism that may mediate the long-term consequences of early life exposures is epigenetic modulation of gene expression. Using a murine model of gestational exposure to environmentally relevant doses of BPA, we have explored the epigenetic, neurobiological, and behavioral consequences of BPA for male and female offspring. Analyses of gene expression patterns in the hypothalamus suggest a sex-specific curvilinear dose-response curve in response to in utero BPA, which is particularly evident on measures of estrogen receptor and DNA methyltransferase mRNA levels. DNA methylation levels of the estrogen receptor also vary as a function of BPA exposure, with the direction of effect dependent on sex and brain region (hypothalamic versus cortical). Analyses of the expression of brain-derived neurotrophic factor (BDNF) within the hippocampus indicates that high in utero exposure leads to reduced BDNF mRNA in male offspring and increased DNA methylation within the BDNF gene promoter may account for this decreased expression. This sex-specific epigenetic effect may impact learning and memory. The epigenetic variation observed in our murine model is also observed in human cord blood samples of high versus low BPA exposed infants, suggesting the translational significance of these findings. Finally, analyses of postnatal interactions between mothers and offspring suggest that modulation of the social environment may have moderating roles in determining BPA-associated effects.

NTX133

Maternal smoking during pregnancy and offspring methylation: Preliminary data from a case-crossover design

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Maternal smoking during pregnancy (SDP) remains a considerable public health concern with adverse consequences to the health and well-being of the fetus. From a developmental perspective, the prenatal period is one of the most critical windows during which adverse intrauterine conditions and exposures, such as SDP may influence the growth and development of the fetus as well as its future postnatal health and behavior. Research is beginning to suggest that one possible mechanism by which exposure to SDP may affect key pathways crucial for proper fetal growth and development is due to epigenetic signatures that affect gene expression. Thus, we will examine methylation patterns in a sample of full-sibling pairs discordant for exposure to maternal smoking during pregnancy in order to address the question whether children exposed to SDP differ in their methylation patterns relative to their unexposed siblings (i.e., does exposure to SDP modify methylation?). We will also explore whether these methylation signatures differ between discordant sibling pairs of heavier smokers vs those of lighter smokers. This discordant sibling model offers some methodological control for shared genetic and environmental influences and thus could provide a powerful means of examining epigenetic effects of prenatal smoking exposure.

NTX134

Brain epigenetic and telomere alterations associated with early-life adversity

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Child maltreatment negatively impacts brain development, often producing transgenerational continuity of abusive parenting and increased risk for a range of psychiatric disorders. Epigenetic alterations and telomere shortening have emerged as biomarkers for measuring the impact of stress and as important mechanisms by which early-life adversity could interact with DNA to affect brain function and physical and mental health outcomes. In my presentation, I will discuss our studies using rats that are exposed to caregiver maltreatment during the first week of life and then later examined for alterations in DNA methylation, histone modifications, and telomere length within the brain. We have observed significant epigenetic alterations and changes in telomere length across behaviorally-relevant brain regions, with different patterns observed in males and females. These alterations also parallel disturbances in behavior (for example maltreated-females mistreat their own offspring). As our understanding of the role brain epigenetic programming and telomere dynamics play in brain function and behavior is still evolving, continued investigation is necessary to better understand their role in mediating the long-term consequences of maltreatment on brain development and mental health.

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NTX135

Epigenetic effects of drugs on early human neural development

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The phenotype and function of cells is dependent on the sets of genes that are active or silenced in a given cell type. One important determinant of gene activity are signaling processes that lead to the activation of transcription factors. However, gene activity is also determined by the local chromatin structure. Epigenetic modifications, such as the methylation of DNA bases, or the posttranslational modification of histone proteins strongly affect the chromatin. Such chromatin changes have been little considered yet as target of neurotoxicants. We examined here, how the developmental neurotoxicant valproic acid, and other histone deacetylase inhibitors affected epigenetic modifications at the promoters of key developmental genes (e.g. Pax6). As model system, we used human pluripotent stem cells developing to neural precursor cells, i.e. a developmental stage corresponding to the early neural tube in fetuses. We identified epigenetic changes related to transient drug effects (histone acetylation), and other histone modifications (lysine methylation) that correlated well with permanent developmental disturbances. The findings of persistent methylation changes after drug exposure offer an explanation for persistent drug effects on neurodevelopment even after initial drug exposure has ended.

NTX136

DNA methylation mediating the impact of exposure on behavior

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Early life stress and prenatal stress experienced during pregnancy are well-known to be associated with later life behavior and disease patterns. DNA methylation is one of the epigenetic mechanisms considered to be responsible for such long term effects. Indeed, significant changes of pivotal genes have been found in human brains and experimental animals as potential link between chemical exposure and delayed effects on behavior and health.

NTX137

Alzheimer's Disease: Environmental Risk Factors and Epigenetic Mechanisms

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Alzheimer's disease (AD) affects about 5 million patients in the USA. Recent data suggests that AD maybe the third leading cause of death in the USA. The etiology of this disease, particularly, the sporadic form of AD, which affects 95% of patients, remains unknown. The late onset pattern and the absence of a genetic causation factor for sporadic AD, suggest an environmental involvement. Another unknown for the etiology of the disease is the period of onset. Does AD result from old age or does it have an earlier beginning? In this presentation, we will discuss the various environmental, dietary, and metabolic risk factors that may contribute to AD pathogenesis. We will explore potential mechanisms that can transmit the impact of environmental exposures across the lifespan. We will particularly focus on gene environment interactions, namely epigenetic pathways.

NTX138

Long-lasting cognitive deficits in rhesus monkeys after neonatal general anesthesia induced by isoflurane plus nitrous oxide

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It was shown previously that 24 hours of ketamine-induced general anesthesia during the first week of life causes significant increases in neuronal cell death and seemingly permanent cognitive deficits in rhesus monkeys. In the present study, 8 monkeys were exposed on PND 5 or 6 to isoflurane (1%)/nitrous oxide (70%) anesthesia (ISO/N2O; maintenance of a light surgical plane) for 8 hrs. Eight control animals were unexposed. At 7 months of age all animals began training to perform a series of cognitive function tasks as part of the National Center for Toxicological Research (NCTR) Operant Test Battery (OTB). Tasks included those for assessing aspects of learning, motivation, color discrimination, and short-term memory. Subjects responded for banana-flavored food pellets by pressing response levers and press-plates during daily (M-F) test sessions (50 min) and were assigned training scores based upon their individual task performance. Beginning as early as 8 months of age-and continuing for at least the following 20 months--control animals earned more reinforcers in the appetitive motivation task than animals exposed to ISO plus N2O. At about 14 months of age, controls also began outperforming ISO/N2O animals in the OTB learning and color discrimination tasks: exposed animals responded more slowly in the color discrimination task and completed less of the learning task--, responding more slowly and less accurately--and these effects have continued until the present (for at least 15 months). Performance in the short-term memory task is no different between the groups. These long-term cognitive impairments seen after an 8hr ISO/N2O exposure, while slightly different from those noted in our previous ketamine studies, provide additional evidence that general anesthesia of sufficient duration and occurring during a sensitive period of brain development, can result in very long-lasting deficits in brain function in primates.
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NTX139

Social behavior in non-human primate infants and juveniles following administration of thimerosal-containing vaccines

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In the 1990s, thimerosal (sodium ethylmercurithiosalicylate) was used as a preservative in most pediatric vaccines. While there is currently only one thimerosal-containing vaccine (TCV) included in the routine US pediatric immunization schedule, parental perceptions that TCVs are associated with the onset of neurodevelopmental disorders still continue to impact vaccination rates. In this study, we examined whether administration of multiple TCVs to non-human primate infants increased the incidence of negative behaviors, such as fear-disturbance, rock-huddle-self-clasp, and stereotypies.

Vaccines were administered to 6 groups of infant male rhesus macaques (n=12-16/group) using a standardized thimerosal dose. Study groups included the recommended 1990s pediatric vaccine schedule (which included TCVs given at birth, 2, 4, 6, 12 and 52 months), an accelerated 1990s primate schedule with or without the measles-mumps-rubella (MMR) vaccine (given at birth, 2, 4, 6, 12 and 52 weeks), the MMR vaccine only, and the expanded 2008 pediatric vaccine schedule (which remains very similar to that used today). Saline injections were administered to age-matched control animals (n=16). Behavior was evaluated in 40-min daily playroom sessions for each peer group from 1-12 and 13-18 months of age representing pre- and post-social living, respectively. Scoring was conducted by a blinded observer in 5-min focal periods using a coding system of mutually exclusive and exhaustive behaviors. Scored behaviors included passive, explore, withdraw, fear-disturbance, rock-huddle-self-clasp, stereotypy, play, sex and aggression, and could be scored as either a social interaction or a non-social behavior. Data were analyzed using multi-level modeling.

Analyses of social and non-social interaction data indicated that there were no significant differences in behaviors in animals in the vaccinated groups relative to controls at both 12 months (end of pre-social living) and 18 months (after 6 months of post-social living). TCVs did not affect the development of typical social behaviors expected for laboratory-reared macaques of this age. Of particular relevance under the hypothesis that TCVs may impact behavior, there were very few instances of negative behaviors, such as rocking, self-clasping, and stereotypy, reported across the entire infancy period for all groups.

NTX140

Sex-specific Effects of Prenatal Exposure to VPA: Behavioral and Anatomical Evidence

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Compelling evidence points to prenatal environment influences as an etiological factor in autism. Fetal exposure to valproic acid (VPA), a commonly prescribed anticonvulsant and mood-stabilizer, results in birth defects and behavioral abnormalities similar to those seen in autism spectrum disorder (ASD), including diminished social interaction and heightened anxiety. Mechanistically, studies in humans suggest that norepinephrine (NE) and serotonin (5-HT) are atypical in autistic children. The aim of the study was to correlated changes in behaviors characteristically altered in ASD with morphological changes in the amygdala, a structure involved in social bonding and processing of fearful stimuli. Pregnant Sprague-Dawley rats were injected on gestational day 11.5, with 500 mg/kg of sodium valproate, i.p, or saline. Offspring were weaned at PND 21 and housed as singlets until testing. At PND 33-34 males and females were tested with a same-sex and -age conspecific, for a variety of social interactions for fifteen minutes. At PND 60, anxiety-like behavior was tested in the elevated plus maze. Sex-specific alterations in both social behaviors and anxiety were observed in VPA offspring. Decreased play dominant behavior was observed only in females. A similar findings occurred for partner sniffing, an index of social investigation. In males only play solicitation was decreased, and only during the first five minutes. Submissive play variables (being pinned and escape behavior), and comfort behaviors (social grooming) did not show significant treatment effects. Differences in measures of anxiety were observed only in males; VPA showed heightened anxiety, spending significantly less time in the open arms of the maze than control counterparts. No treatment effects were seen for either the number of open or closed arm entries, or the percent number of open arm entries for either sex. Neuroanatomical results suggest that the development of NE-containing projections to the central nucleus of the amygdala (CEA) were disrupted. Control animals exhibited a robust sex difference in fiber densities which was abolished by prenatal VPA treatment. These data suggests the sex-specific behavioral phenotypes induced by fetal VPA exposure reported here may reflect in part perturbations on sexually dimorphic innervation of the CEA by noradrenergic systems.

NTX141

Behavioral effects in male and female mice following high-dose taurine consumption during adolescence

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Energy drink sales are a multi-billion dollar industry in the United States with more than half of all energy drinks consumed by adolescents and young adults. Common energy drinks contain high levels of the amino acid taurine which is abundant in the brain and can be neuroprotective. However, recent studies indicate high levels of taurine can be neurotoxic. To assess the effects of chronic, high-level taurine consumption in the developing brain, we treated male and female C57BL/6J mice with drinking water containing 0.12% taurine from postnatal day (PND) 30 to PND 60. Treated and control males and females then went through a battery of behavioral tests. We found no significant effects of taurine treatment on baseline locomotor activity or exploration in the zero maze, although there was a significant sex difference. There was a trend ($P = 0.07$) for a sex x treatment interaction in marble burying with taurine-treated females burying fewer marbles while taurine-treated males buried more. The most striking differences were in tests of learning and memory. We found a significant main effect of treatment ($P < 0.05$) in novel object recognition with control mice spending a greater percentage of time exploring the novel object compared with taurine-treated mice. There was a significant sex x treatment interaction in the Morris water maze with taurine-treated females having shorter path lengths on Days 5 and 6 of the most difficult Shift-reduced phase while taurine-treated males had longer path lengths compared with controls ($P < 0.05$). To investigate the effect of caffeine, which is another major ingredient in energy drinks, we conducted a stimulant challenge following behavioral testing. There was a significant main effect of caffeine ($P < 0.001$) and a significant treatment x sex x caffeine interaction ($P < 0.05$). Taurine-treated males had a greater response to caffeine compared with control males. In summary, consumption of high-dose taurine during adolescence induces non-spatial learning deficits in adult mice and sex-dependent changes in behavior and Morris water maze performance.

NTX142

Development of an in vitro co-culture model of the chicken Hypothalamic-Pituitary-Gonadal-Liver (HPG-L) axis to study neuroendocrine disruption

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Exposure to Endocrine Disrupting Chemicals (EDCs) such as pesticides and industrial chemicals can cause changes in neuroendocrinology, reproductive physiology and metabolism. The vertebrate reproductive pathway is controlled by the brain via a tightly regulated Hypothalamic-Pituitary-Gonadal-Liver (HPG-L) axis. Disruptions in this pathway can cause severe endocrine imbalance and sex-ratio disturbances, which could have population level impacts. Dopamine (DA) and γ-aminobutyric acid (GABA) are some of the primary brain neurotransmitters that, respectively, inhibit and stimulate the HPG-L axis. While field- and whole-organismal studies have shown that EDCs adversely affect avian species such as chickens, quails and bald eagles, only a small proportion of research has examined the effect of EDCs on neuroendocrine processes. And while offering compelling evidence of harm, these studies involve sacrificing a large number of animals, are time consuming and expensive. In recent years there has been great interest in developing *in vitro* systems to study biologically relevant disturbances in interconnected pathways composed of complex biochemical interactions and small molecules that control communication between cells. Among the various approaches being explored, tissue explants show great promise as an alternate *in vitro* model. The objective of this study is to establish an explant co-culture of the chicken HPG-L axis to mimic *in vivo* interactions. Hypothalamus, pituitary, gonads and liver were dissected from day 19 white leghorn chicken embryos. Each component from one individual was sectioned, weighed and cultured together at 37°C and 5% CO2. The co-cultures were either fed DMEM (control), or DMEM with DA, or DMEM with DA followed by GABA (n=3). At the end of the culture tissues were weighed and frozen. Currently, gene expression experiments to study key neurohormones and sex hormones such as Gonadotropin Releasing Hormone, Gonadotropins, Estrogen and Testosterone are ongoing. Following the establishment of the co-culture, tissues will be dosed with a range of EDCs. Understanding the effects of contaminants on the neurohormonal brain-pituitary-gonadal communication systems is important in determining the detrimental effects that EDCs have on humans and wildlife at organismal levels, and this *in vitro* model could serve as a valuable screening tool to study these interactions.

NTX143

Short- and long-term neurobehavioral toxicity of fluorene after a nose-only exposure during the lactating period (14 days) in F1 Wistar rats.

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Among polycyclic aromatic hydrocarbons (PAHs), fluorene is a highly volatile compoundbeing one of the most abundant in air. Because significant fluorene concentrations have been measured in the breast milk of non-smoking women, the risk for the developing brain of a breast-feeding exposure to this compound has to be evaluated. Wistar female rats were nose only exposed to 1.5 or 150 ppb of fluorene from postnatal day 3 (PND3) to PND16 (6h/day, 14 days) whereas the controls were inhaled with clean air over the same period. Rat pups were assessed for their sensory-motor development during the first three weeks of life and for anxiety (elevated-plus maze, EPM), activity (open-field) and spatial learning (Y maze, eight-arm maze) at adulthood. Results of the tests performed before weaning showed transient delays in the maturation of motor and sensory abilities of fluorene-exposed pups compared to controls at both levels of exposure (1.5 and 150 ppb) whereas the maternal behavior of the dams towards the pups was exacerbated. At the adult stage, persistent behavioral disturbances were observed in fluorene-contaminated rats. Thus, a locomotor hyperactivity and a concomitant reduction in the level of anxiety were observed in rats exposed to 150 ppb of fluorene in the EPM and in the open-field. On the opposite, the anxiety level of rats exposed to 1.5 ppb of fluorene was significantly increased in the EPM whereas no significant modifications were detected in the open-field. In both groups, the learning abilities of the animals remained unaffected. These results show the same behavioral defects as those reported in adult rats directly exposed to fluorene (Peiffer et al., 2013) and confirm the potential neurotoxicity of this highly volatile PAH. The same exposure was performed during gestation and showed slight behavioral defects, suggesting a significant contribution of the period of exposure in the toxicity of this compound.

Peiffer J., Cosnier F., Grova N., Nunge H. Salquèbre G., Decret MJ., Cossec B., Rychen G. Appenzeller BMR., Schroeder H. (2013) Neurobehavioral Toxicity of a Repeated Exposure (14 Days) to the Airborne Polycyclic Aromatic Hydrocarbon Fluorene in Adult Wistar Male Rats. PlosOne, 8(8): e71413. doi:10.1371/journal.pone.0071413.

NTX144

Nicotine - cadmium exposure alters working memory, motor function and increased anxiety in adolescent female mice

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This study investigates spatial and non spatial working memory, anxiety related behavior, and motor activities in cadmium and/or nicotine exposed female adolescent mice. P28 female adolescent mice (albino strain) were divided into four groups of five (n = 5) mice each. A set of mice (Nic) received subcutaneous nicotine (2.0 mg/kg) while a separate set (Cd) was treated with 2.0 mg/kg cadmium (subcutaneous). For the combined treatments of cadmium and nicotine, we administered 2.0 mg/kg Nicotine and 2.0 mg/kg of Cd. Subsequently, a separate group of animals (n = 5; control) received normal saline. The total duration of treatment for all groups was 28 days (P28-P56).At P56, the treatment was discontinued, after which the animals were examined in behavioural tests. Nicotine and cadmium increased the metabolism and food intake in the female adolescent mice. This also corresponded to an increase in weight when compared with the control. However, a combined nicotine-cadmium treatment induced a decline in weight of the animals versus the control. Also, nicotine administration increased the motor function, while cadmium and nicotine - cadmium treatment caused a decline in motor activity. Both nicotine and cadmium induced a reduction in memory index; however, nicotine-cadmium treatment induced the most significant decrease in non-spatial working memory.

NTX145

Brain GABA concentrations and their relation to exposure, movement and cognition in occupational manganese exposure

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In the past years significantly elevated in vivo levels of the neurotransmitter gamma-aminobutyric acid (GABA) have been reported in the thalamus of metal workers from China, suggesting GABA levels measured by Magnetic Resonance Spectroscopy mac act as early biomarker of effect for Mn toxicity. A currently ongoing longitudinal study on a cohort of active welders in the US is investigating the relationship between elevated brain GABA levels and Mn exposure, brain Mn levels as measured by MRI T1 mapping, subtle movement deficiencies and neurobehavioral measures. The baseline measures of this study confirm elevated thalamic GABA levels, which correlate with measures of Mn exposure, also in a typical welding environment in the United States. In this presentation we will discuss the relation of in vivo GABA levels to cumulative Mn exposure, brain and toenail Mn levels, movement disorders as measured by the UPDRS-III and basal-ganglia specific neurobehavioral measures. Comparing all these measures to a group of patients with idiopathic Parkinson Disease as positive controls allows discussing similarities and differences to the GABA-ergic mechanistic components of manganism. [This work is supported by grant number ES020529].

NTX146

Motor and verbal learning and naming slowing of active welders in relation to manganese exposure and MRI imaging results

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Active welders are rarely evaluated during their welding years on verbal learning and naming in addition to motor function, which is more common. Thirty-two production welders, primarily white welders age 42 (±10.4yrs) were evaluated for their cumulative lifetime, past-year and past 7-12 months exposure to manganese (Mn), using a model combining work histories and air sampling. Welder's Finger Tapping speed declined both at 7-12 month prior exposure, past year and cumulative exposure (p=0.001). These findings of deficits in neuropsychological tests (NPT) of motor speed and fine tactile manipulative dexterity are typically reported in welders exposed to Mn exposure. Toenail Mn also showed significance for lower nondominant Finger Tapping speed. Additionally to the NP testing, brain manganese deposition was tested using T1-weighted magnetic resonance images (MRI), analyzed in preselected bilateral brain areas of the basal ganglia, caudate, substantia nigra and the frontal lobes. Higher signal intensity reflected higher manganese deposition. Although Mn and motor inefficiencies are commonly tested and reported, verbal learning and category naming decrements from Mn have been largely overlooked. In this study verbal NPT of category naming and verbal learning were administered. The category naming tests and first two verbal learning trials were significantly correlated with shortened T1 relaxation times in left frontal cortex, indicating higher accumulation of Mn. A trial measuring verbal intrusion was associated with shortened T1 values in the red nucleus. This suggests the need for inclusion of verbal testing in assessing early Mn exposure in active welders and not only tests of motor function. [This work is in part supported by grant number ES020529].

NTX147

Manganese-induced parkinsonism does not involve degeneration of nigrostriatal dopaminergic neurons: Evidence from genetic mutations and environmental exposure in humans and non-human primates

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It is now well established that occupational and environmental exposures to excess levels of manganese (Mn) results in a neurological syndrome with neuropsychological abnormalities and an atypical form of parkinsonism that is not responsive to drug therapy used in idiopathic Parkinson's disease. We have published neuroimaging and neuropathological studies in non-human primates indicating that chronic exposure to Mn does not result in the loss of dopamine neuron terminals in the striatum but produces dopamine neuron dysfunction since there is a marked decrease of *in vivo* dopamine release (Guilarte et al., 2008). Since our original studies were published, new evidence has emerged from two different types of human studies indicating that chronic Mn exposure does not cause dopamine neuron degeneration. First, studies from Eastern European countries have provided evidence that young individuals using the Mn-containing psychoactive drug ephedrone, express clinical parkinsonism that is not responsive to L-dopa therapy and neuroimaging studies show no evidence of dopamine terminal loss in the striatum. Secondly, humans with mutation in the gene *SLC30A10*, now recognized a Mn transporter, express parkinsonism with dystonia and high levels of Mn in the brain and blood. These individuals with clinical parkinsonism are not responsive to L-dopa or dopamine agonist drugs. Further, SPECT imaging of dopamine transporter levels in the striatum were normal indicating a lack of dopamine terminal degeneration in individuals expressing the *SLC30A10* mutation. These studies provide strong evidence in humans and non-human primates that Mn-induced parkinsonism may be the result of dopamine neuron dysfunction, i.e. inhibition of dopamine release, in conjunction with downstream effects on neurons intrinsic to the striatum [This work is supported by grant number ES010975]

NTX148

Pre- and post-synaptic dopaminergic function in Mn-exposed humans

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To investigate the integrity of the dopamine system in Mn-exposed humans, we have performed molecular imaging with positron emission tomography (PET) with the following radioligands: [18F] FDOPA which measures dopamine decarboxylase and [11C]DTBZ which measures VMAT2, and [11C]NMB which measures D2 receptors. Compared to controls, we have found that Mn-exposed humans with minimal clinical signs have lower uptake of [18F] FDOPA and [11C]DTBZ binding potential in the caudate nucleus but relatively normal values in the putamen. These same subjects had higher [11C]NMB binding potentials in caudate and putamen, consistent upregulation of dopamine receptors in response to pre-synaptic dopaminergic dysfunction. In addition, we have found that subjects with clinical parkinsonism have reduced uptake of [18F] FDOPA in caudate and putamen, suggesting that as Mn-exposed subjects develop more motor signs, there is most involvement of the putamen. There is currently no known human disease in which reductions in [18F] FDOPA uptake or [11C]DTBZ binding potentials are reversible. Nevertheless, demonstrating progression of dopaminergic dysfunction on follow-up imaging with these radioligands would provide additional evidence of Mn as a mediator of dopaminergic degeneration.

NTX149

Decreased brain volumes in manganese-exposed welders

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A great deal of research has been devoted to identifying subclinical functional brain abnormalities in manganese (Mn)-exposed welders. However, no previous study has investigated morphological brain abnormalities, such as changes in brain volume, in welders. This study evaluates morphological changes in brain volume among welders, and investigates the relationship between structural brain abnormalities and subclinical dysfunction in this population. We used voxel-based morphometry (VBM) to assess differences in gray and white matter brain volumes between 40 welders with chronic Mn exposure and 26 age-matched control subjects. Correlation analyses were used to investigate the relationship between brain volume changes and decreased performance on neurobehavioral tests. Brain volumes in the globus pallidus and cerebellar regions were significantly diminished in welders with chronic Mn exposure compared to controls (FDR-corrected $P < 0.05$). These changes in brain volume were negatively correlated with cognitive performance and grooved pegboard scores. There are measurable brain volume reductions in the globus pallidus and cerebellum of welders chronically exposed to Mn, and these volume reductions correlate with cognitive and motor neurobehavioral deficits. Our findings therefore indicate that volumetric measurement could be a useful subclinical marker among welders that show no signs of manganism.