Title

DEVELOPMENTAL EXPOSURE TO NEAR-ROADWAY POLLUTION PRODUCES BEHAVIORAL AND HISTOLOGICAL PHENOTYPES RELEVANT TO NEURODEVELOPMENTAL DISORDERS

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mental health disorders affect brain development and functioning?; and 3) How do neural systems identified in animals inform the development of treatments?

Results: This Symposium briefly reviews past work in animal model systems that has advanced the field and presents on the following areas of ongoing work: 1) epigenetic and inflammatory mechanisms in an animal model of Rett syndrome; 2) epigenetic and inflammatory mechanisms contributing to ADHD-relevant phenotypes; 3) the effect of air pollution on neurodevelopment and behavioral phenotypes; 4) the effect of maternal proinflammatory cytokines on striatal development and autism spectrum disorder–relevant phenotypes; and 5) cortical developmental disruption due to maternal immune activation.

Conclusions: Research presented in this session illustrates how genetic variation and environmental exposures might contribute to neurodevelopmental outcomes through mechanisms involving epigenetic and inflammatory processes. Studying these mechanisms in animal models allows deeper understanding of the underpinnings of mental health disorders, which will facilitate the development of improved diagnosis, prevention, and treatment.

ANI, NEURODEV, R

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19.1 NEUROIMMUNE, EPIGENETIC, AND METABOLIC INTERACTIONS DURING SYMPTOM PROGRESSION IN A MOUSE MODEL OF RETT SYNDROME

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Objectives: Mutations in the X-linked gene *MECP2* cause Rett syndrome (RTT), a neurodevelopmental disorder in females. Because of the complex MeCP2 mutant/wild-type mosaicism observed in MeCP2 mutant female mice, the majority of RTT preclinical studies are performed only on MeCP2 null male mice lacking MeCP2 in all cells. But RTT patients are heterozygous females that exhibit delayed and progressive symptom onset beginning in late infancy, including neurologic as well as metabolic, immune, respiratory, and gastrointestinal phenotypes. Two differentially spliced isoforms of exons 1 and 2 (MeCP2-e1 and MeCP2-e2) contribute to the diverse functions of MeCP2, but only mutations in exon 1 are observed in RTT. We developed an MeCP2-e1–deficient mouse model based on a human RTT mutation that lacks MeCP2-e1, while preserving expression of MeCP2-e2.

Methods: We conducted a longitudinal assessment of neurologic, motor, and metabolic symptom development in MeCP2-e1 mutant females and males. Single-cell transcriptomics from hippocampus and hypothalamus at 4 time points are currently being integrated with weekly longitudinal measurements of immune dysfunction, metabolite, and gut microbiota from fecal samples.

Results: A delayed and progressive onset of motor impairments was observed in both female and male MeCP2-e1 mutant mice, including hind limb clasping and motor deficits in gait by 6 to 8 weeks of age. Fecal short-chain fatty-acid and untargeted lipid metabolomics revealed a significant increase in butyrate by 4 weeks and biogenic amine and lipid metabolites by 8 weeks of age in MeCP2-e1 mutant females but not males, compared to wild-type littermates. Fecal metabolite dysregulation occurred prior to the age-dependent elevation in body weight and body fat and increased triglycerides in the liver by 9 to 10 weeks.

Conclusions: These results implicate a distinct disease pathogenesis in a female RTT mouse model, suggesting novel X-linked epigenetic interactions between MeCP2-e1 mutation, gut absorbance of important metabolites, and progressive neurologic symptoms.

ANI, GS, NI

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19.2 ANIMAL MODELS AND THE ROLE OF EPIGENETICS IN ADHD

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Objectives: The biological mechanisms of ADHD have traditionally focused on the role of different gene variants of dopamine and other neurotransmitter-regulating proteins. *In utero* drug exposure, poverty, and early adverse life events have also been associated with increased risk for ADHD. This presentation will focus on animal models that have shed light on possible epigenetic mechanisms that connect neurobiological mechanisms and the risk for ADHD.

Methods: Studies of dopamine regulation were conducted in an animal model of ADHD, dopamine D4 receptor (D4R) knockout mice. High-performance liquid chromatography with electrochemical detection and voltammetry with carbon fiber electrodes allowed for measures of dopamine and dopamine metabolites in the mouse striatum and nucleus accumbens. Epigenetic models of nicotine use in males compared mice with and without exposure to nicotine in drinking water. Adverse life events were modeled with social isolation in postweaning mice. Age-related epigenetic regulation of the dopamine transporter was studied in the Long Evans rat.

Results: *In vivo* electrochemistry showed that potassium-evoked dopamine release was diminished in the nucleus accumbens of the D4R knockout mice. First-generation progeny of the nicotine-exposed male mice showed decreased attention and deficits in dopamine receptor mRNA expression. Analysis of their sperm showed changes in DNA methylation in the promoter regions of the dopamine D2 receptor gene. The dopamine transporter (DAT) inhibitor, methylphenidate, was able to reverse behavioral consequences of social isolation in male mice. Long Evans rats showed age-related methylation changes in the dopaminergic transcription factors Nurr1 and Pitx3 within the DAT promoter.

Conclusions: These animal model studies demonstrate that dopamine regulation plays a role in the neurobiology of ADHD. Dopamine dysregulation can result not only from direct gene variations but also from epigenetic influences from parents, *in utero* exposure, and early life stressors. Implications of these animal studies for the risk of ADHD and its prevention and treatment will be discussed.

ANI, ADHD, GS

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19.3 DEVELOPMENTAL EXPOSURE TO NEAR-ROADWAY POLLUTION PRODUCES BEHAVIORAL AND HISTOLOGICAL PHENOTYPES RELEVANT TO NEURODEVELOPMENTAL DISORDERS

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Objectives: Epidemiological studies consistently implicate traffic-related air pollution (TRAP) and/or proximity to heavily trafficked roads as risk factors for developmental delays and neurodevelopmental disorders (NDDs); however, there are limited preclinical data demonstrating a causal relationship. To test the effects of TRAP, pregnant rat dams were transported to a vivarium adjacent to a major freeway tunnel system in Northern California where they were exposed to TRAP drawn directly from the tunnel or filtered air (FA).

Methods: Offspring remained housed under the exposure condition into which they were born and were tested in a variety of behavioral assays between postnatal days 4 and 50. To assess the effects of near-roadway exposures, offspring of dams housed in a standard research vivarium were tested at the laboratory. An additional group of dams was transported halfway to the facility and then back to the laboratory to control for the effect of transport stress.

Results: Near-roadway exposure delayed growth and development of psychomotor reflexes and elicited abnormal activity in open field locomotion. Near-roadway exposure also reduced isolation-induced 40-kHz pup ultrasonic vocalizations, with the TRAP group having the lowest number of call

emissions. TRAP affected some components of social communication, evidenced as reduced neonatal pup ultrasonic calling and altered juvenile reciprocal social interactions. Histologic analyses showed that TRAP exposure increased microglial infiltration in the CA1 region of the hippocampus, decreased astrogliosis in the dentate gyrus, and increased markers of hippocampal neurogenesis in both male and female rats. Microglia isolated from tunnel rats were also collected for transcriptome and methylome analysis and are currently being examined.

Conclusions: These behavioral and anatomical findings confirm that living in close proximity to traffic during early life alters neurodevelopmental outcomes and brain growth and that these exposures may increase the risk of NDDs in genetically susceptible individuals.

ANI, RF, NEURODEV

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19.4 THE EFFECT OF MATERNAL INFLAMMATION ON STRIATAL DEVELOPMENT AND AUTISM SPECTRUM DISORDER–RELEVANT PHENOTYPES

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Objectives: Neuropsychiatric disorders of childhood have disruptions of striatal-dependent learning. Embryonic development of the striatum underpins all subsequent striatal function. There are gaps in knowledge about factors that disrupt striatal development and the mechanisms by which striatal developmental changes may affect learning. The striatum is often enlarged in individuals with autism spectrum disorder (ASD); we have pilot data suggesting that prenatal maternal stress causes overproduction of striatal neurons. Our objective here is to determine which maternal stress factors impact striatal development and phenotypes relevant to ASD.

Methods: We used mouse models of repetitive prenatal restraint, stress hormone (corticosterone) exposure, and prenatal inflammation (recombinant mouse interleukin-6 [IL-6]) 3 times/day from embryonic day 12 (E12) to E18, to target the intensive period of striatal neurogenesis. We evaluated maternal serum, placenta, embryonic striatal primordium, adult striatum, and adult striatal-dependent learning behavior. We assessed histological, protein-level, transcriptional, and behavioral outcomes with ANOVA and *t* test comparisons, as appropriate.

Results: Maternal serum had elevated levels of IL-6 during stress, but embryonic brain did not, suggesting that maternal inflammation induced secondary mechanisms, possibly through placental oxidative stress, to impact the embryonic brain. We found that maternal stress as well as IL-6, but not corticosterone, resulted in increased production of medium spiny neurons in the striatum by histological and transcriptional assessments. These excess cells persisted across all ages assessed, from the embryonic period to adulthood. We also found that maternal stress altered striatal-dependent learning in adult offspring; behavioral assessments of offspring from mothers exposed to IL-6 also suggest learning deficits.

Conclusions: Striatal neuron populations in the embryonic brain are increased after maternal inflammation and placental oxidative stress. These increased populations persist into adulthood of these offspring, disrupting normal striatal structure and striatal-dependent learning, phenotypes similar to those found in individuals with ASD. Buffering maternal inflammation may protect healthy brain development *in utero*.

ANI, NEURODEV, STRESS

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19.5 ACUTE AND LASTING IMPACTS OF MATERNAL IMMUNE ACTIVATION ON PRENATAL CORTICAL DEVELOPMENT

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Objectives: Maternal immune activation (MIA) has emerged as a key environmental risk factor for neurodevelopmental disorders (NDDs) and mental health disorders, including autism spectrum disorder (ASD) and schizophrenia. Animal models of MIA provide an opportunity to identify molecular pathways that initiate disease processes and lead to NDD-related neuropathology and behavioral deficits. In this work, we sought to explore how poly(I:C)-driven MIA alters cortical development by identifying molecular pathways that initiate disease processes and lead to neuropathology and investigating how changes in gene co-expression during cortical development drive molecular pathology.

Methods: We injected pregnant female mice with either polyinosinic-polycytidylic acid (poly[I:C]) or saline (vehicle) at day 12.5 postconception and applied transcriptional profiling through RNA sequencing (RNA-seq) and neuroanatomical characterization across multiple embryonic time points during cortical development.

Results: MIA induced strong transcriptomic signatures, including induction of genes associated with hypoxia, immune signaling, and angiogenesis. The acute response was followed by precocious changes in proliferation, neuronal and glial differentiation, and cortical lamination that emerged at embryonic day 14.5 (E14.5) and peaked at E17.5. Neuroanatomical studies confirmed RNA-seq differential expression, identified cortical cell types altered in poly(I:C)-exposed cortex at E17.5, and revealed a shift in developmental timing toward late embryonic brain development following MIA. Specifically, we identified asynchronous patterns in proliferation, differentiation, and cortical lamination. Finally, MIA-induced transcriptional changes were largely suppressed by maternal interleukin-6 inhibition.

Conclusions: The transcriptomic maps and neuroanatomical changes identified in this work provide novel insights into molecular and developmental processes linking MIA and neurodevelopmental sequelae, highlighting an acute transcriptional response followed by neurodevelopmental asynchrony and subtler, lasting expression changes. MIA transcriptomic signatures, supported by altered neuroanatomical patterns, overlap significantly with perturbations identified in NDDs, potentially revealing new targets for the development of novel approaches for earlier diagnosis and treatment of these disorders.

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SYMPOSIUM 20

SUPPORTING INFANT MENTAL HEALTH THROUGH EARLY INTERVENTIONS WITH CAREGIVERS WHO EXPERIENCED TOXIC STRESS AND EARLY ADVERSITY

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Objectives: Parents who experienced toxic stress and early adversity are at high risk of presenting pre- and postnatal psychological distress, mother-infant bonding impairments, and parenting difficulties. Early in development, their offspring are correspondingly more likely to present biological, developmental, and affective risk indicators than offspring of parents without histories of trauma. Therefore, there is a critical need for timely detection of the most vulnerable parents to provide effective preventive interventions. This Symposium will introduce participants to 4 programs aiming to support infant