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Proceedings of the 2015 Annual Meeting of the Fetal Alcohol Spectrum Disorders Study Group

C. Fernando Valenzuela^a, Alexandre E. Medina^b, Jeffrey Wozniak^c, and Anna Klintsova^d

^aDepartment of Neurosciences, University of New Mexico Health Sciences Center, Albuquerque, NM

^bDepartment of Pediatrics, University of Maryland, School of Medicine, Baltimore, MD

^cDepartment of Psychiatry, University of Minnesota, School of Medicine, Minneapolis, MN

^dPsychology Department, University of Delaware, Newark, DE

Abstract

The 2015 Fetal Alcohol Spectrum Disorders Study Group (FASDSG) meeting was titled “Basic Mechanisms and Translational Implications”. Despite decades of basic science and clinical research, our understanding of the mechanisms by which ethanol affects fetal development is still in its infancy. The first keynote presentation focused on the role of heat shock protein pathways in the actions of ethanol in the developing brain. The second keynote presentation addressed the use of magnetoencephalography to characterize brain function in children with FASD. The conference also included talks by representatives from several government agencies, short presentations by junior and senior investigators that showcased the latest in FASD research, and award presentations. An important part of the meeting was the presentation of the 2015 Henry Rosett award to Dr. Michael Charness in honor of his achievements in research on FASD.

Keywords

fetal; alcohol; spectrum; disorder; brain; development

The 2015 Fetal Alcohol Spectrum Disorders Study Group (FASDSG) annual meeting took place on June 20, in San Antonio, TX as a satellite of the Research Society on Alcoholism meeting. Approximately 180 senior and junior investigators attended the meeting (34 of which were students or postdoctoral fellows), including individuals from the United States, Canada, and South Africa. The program included 14 brief, one-slide 5-min presentations of recent data. Thirteen of these presentations were given by graduate students and postdoctoral fellows (5 of these received travel awards). In addition, two other trainees were selected to receive the Timothy A. Cudd and the Kenneth R. Warren Merit Awards. Representatives

Corresponding Author: C. Fernando Valenzuela, MD, PhD, Department of Neurosciences, MSC08 4740, University of New Mexico Health Sciences Center, Albuquerque, NM 87131-0001, Telephone: +1 505 272 3128, FValenzuela@salud.unm.edu.

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from the National Institute of Alcohol Abuse and Alcoholism (NIAAA), the Interagency Coordinating Committee on Fetal Alcohol Spectrum Disorders (ICCFASD), Substance Abuse and Mental Health Services Administration (SAMHSA), and the Centers for Disease Control and Prevention (CDC) gave updates on FASD-related programs of these agencies. The trainees attending the meeting had the opportunity to interact with more senior researchers and clinicians in a networking lunch. The highlight of the meeting was the annual presentation of the Rosett Award, to recognize lifetime contributions, achievement, and service in FASD research.

The theme of the 2015 meeting was “Basic Mechanisms and Translational Implications”. The first keynote presentation by Dr. Kazue Hashimoto-Torii, principal investigator at the Children’s National Medical Center and assistant professor of pediatrics at George Washington University, was titled “The molecular defense mechanisms deployed by the developing brain against alcohol”. Dr. Hashimoto-Torii began her talk by emphasizing that susceptibility to neuropsychiatric disorders is determined by both genetic and environmental factors. Disorders such as autism, schizophrenia, dyslexia, and bipolar disorders are polygenic, involving numerous genetic mutations, some of which overlap among these disorders (Fromer et al., 2014). In addition, these disorders involve multiple epigenetic alterations that can be the result of environmental influences (e.g., nutritional deficiencies, prenatal infections, maternal stress, and medication use). Multiple environmental risk factors may target a common intracellular signaling pathway. Manifestation of phenotypes will depend on the balance between the pathological insult and defense mechanisms. An important defense mechanism during development is the heat shock pathway. It involves translocation of heat shock factor 1 (HSF1) from the cytoplasm to the nucleus and induction of expression of heat shock proteins (HSPs), which act as molecular chaperones that act, in part, by facilitating refolding of proteins that were damaged by cellular stressors (e.g., exposure to changes in temperature or ultraviolet light). Drugs that promote heat shock signaling are currently being investigated for the treatment of cancer and neurodegenerative disorders.

Dr. Hashimoto-Torii then provided an overview of her work on the developmental effects of ethanol on cortical development. She used both human slice cultures from 15–18 gestational week fetuses exposed to ethanol *in vitro* for 24 h and fetal cerebral cortical samples obtained from pregnant mice (gestation days 14–16) injected with 2 g/kg of ethanol (Hashimoto-Torii, Kawasawa, Kuhn, & Rakic, 2011). Microarray analyses revealed that multiple HSPs were upregulated by ethanol exposure (e.g., heat shock 70 kDa proteins [HSP70] 1A and 1B). Similarly, exposure of pregnant mice at gestational day 14 to other environmental stressors (pentylenetetrazol-induced maternal kindling or methylmercury) also increased HSP70 expression in the fetal cerebral cortex (Hashimoto-Torii et al., 2014). In homozygous *Hsf1*KO mice, prenatal exposure to environmental stressors caused cortical heterotopias (at embryonic day 14), and reduced cortical layer thickness (at postnatal day 25), and these effects were not seen in heterozygous *Hsf1*KO mice (Hashimoto-Torii et al., 2014). Homozygous *Hsf1*KO mice prenatally exposed to pentylenetetrazol-induced maternal kindling, ethanol, or methylmercury also exhibited an increase in chemically induced seizure susceptibility at postnatal days 21–25 (Hashimoto-Torii et al., 2014). At embryonic day 15,

these mice also exhibited greater reductions in neuronal cell proliferation and higher levels of cell death in response to ethanol exposure (Hashimoto-Torii et al., 2014). These disturbances in cortical development at embryonic stages may result in morphological abnormalities (i.e., heterotopia and the reduced cortical thickness) observed in adulthood. Using reporter mice that express red fluorescent protein driven by HSF1, it was determined that prenatal exposure to ethanol and other environmental stressors does not produce activation of the HSP pathway equally across all brain regions and also other organs (liver, heart, eye, limb bud, arteries, and spinal cord). Environmental stressors activate the HSP pathway at different levels even under conditions of identical cellular microenvironment and genetic background, suggesting that the heterogeneous effect of ethanol could be a consequence of a stochastic process. Computer simulation studies suggest that this process is indeed probabilistic. Overexpression of Hsf1 via *in utero* electroporation at embryonic day 14 inhibits cortical neuronal migration at postnatal day 14. Overall, these studies suggest that heterogeneous activation of the HSP pathway may lead to variable effects on brain development. For example, in cells exhibiting low activation of HSP, prenatal ethanol exposure may result in reduced cell proliferation and increased apoptosis. However, in cells with high HSP activation, ethanol exposure would result in arrested migration. Dr. Hashimoto-Torii concluded her talk by stating that stabilizers of the stress responses could play a role in the treatment of neurodevelopmental disorders (for example, the antipsychotic aripiprazole, which stabilizes dopamine signaling). She also pointed out that homogeneous up/down changes in gene expression may not account for the complex phenotypes observed in neurodevelopmental disorders. Since the pathological effects of ethanol can occur in a probabilistic manner, this supports the recommendation that there is no safe level of alcohol drinking during pregnancy.

The second keynote presentation was given by Dr. Julia Stephen, MEG/EEG Core Director, associate professor of translational neuroscience, The Mind Research Network, and was titled “Identifying atypical brain development based on altered timing: Insights from MEG”. Dr. Stephen focused on the effects of prenatal ethanol exposure in infants, preschool children, and adolescents. The overall goal of her research is to better identify individuals with atypical brain development. For her studies, she uses magnetoencephalography (MEG), a technique that measures electrical activity in the brain. In contrast to electroencephalography (EEG), which measures volume currents that are distorted when they pass through the skull, MEG measures magnetic fields that are not subjected to this type of distortion. Therefore, the area of the brain where signals originate can be better determined using MEG. MEG has several additional advantages, including that signals are measured at a distance using a helmet rather than sensors attached to the scalp, reducing the difficulties with acquiring data in children with sensory sensitivities. MEG and EEG detect responses in the millisecond range allowing for the detection of neuronal responses with excellent temporal resolution. In contrast, functional magnetic resonance imaging of blood flow changes in response to increases in neuronal activity occur over seconds as opposed to milliseconds, 3 orders of magnitude slower than MEG and EEG. However, MEG signals need to be corrected for head movement to avoid smearing of the signal and improving the signal-to-noise ratio. After correction for head movement, MEG’s spatial resolution has been shown to be 2–4 mm.

Dr. Stephen then presented an overview of her studies of visual pro-saccades in adolescents with FASD (Coffman et al., 2013). These studies evaluated the visual cortex response to central and peripheral stimuli. The latencies of M100 occipital cortex responses to both central and peripheral stimuli were significantly increased in the FASD group, and this effect was more pronounced for peripheral stimuli. Interestingly, saccade times were not altered in individuals with FASD in this study, suggesting that a mechanism had compensated for the deficits in visual cortical responses prior to the oculomotor response. An increase in gamma band (30–100 Hz range) oscillations were detected in subjects with FASD relative to controls, and it is hypothesized that this could perhaps explain the lack of behavioral alterations in these individuals (Stephen, Coffman, Stone, & Kodituwakku, 2013). Dr. Stephen has also characterized auditory sensory processing in preschoolers with and without prenatal alcohol exposure. In preschool children with FASD, an increase in the latency of auditory cortical responses was observed relative to controls (Stephen et al., 2012).

Dr. Stephen addressed the potential utility of MEG for early detection of alterations in brain development. Sensorimotor μ rhythm development can be evaluated in infants and preschoolers. The frequency of this rhythm increases linearly with age from approximately 4 Hz at 10 weeks of age to 8 Hz at 45 weeks (adult μ rhythm oscillation is in the 10-Hz range) (Berchicci et al., 2011). Therefore, this increase in μ rhythm frequency can be used to assess neurodevelopmental trajectory in a variety of conditions, including preterm birth; future studies should determine if it can also be useful in FASD cases. MEG and EEG are also being used to identify neurophysiological indices of sensorimotor deficits in 6- and 20-month-old infants prenatally exposed to opioids with and without alcohol co-exposure (ENRICH cohort). Preliminary results with 6-month-old infants suggest that there are alterations in theta band oscillations (associated with alertness) in alcohol-exposed infants.

Finally, Dr. Stephen discussed the use of joint individual component analysis linking MEG and diffusion tensor imaging (DTI) data (Stephen, Coffman, Jung, et al., 2013). Fractional anisotropy (FA) measured with DTI provides information on diffusion properties of water in white matter, reflecting overall tissue integrity. FA measures, which reflect myelination and other microstructural factors, can be correlated to alterations in neuronal responses in a brain region of interest. Ongoing studies are evaluating the relationship between structural changes in white matter and functional changes as measured by MEG to determine the interplay between the health of white matter networks and cortical functioning. Dr. Stephen concluded her talk by emphasizing that MEG provides good spatial resolution with high temporal resolution, which can be useful in identifying altered cortical development. Her work also shows that alterations in sensory and motor paradigms could be early markers of atypical brain development, and this may be linked to white matter abnormalities such as myelination deficits. Combining imaging technologies appears to be critical to fully understand and treat developmental disorders. Assessing oscillations with MEG could identify laminar alterations in the developing brain, and this could be useful in designing novel therapeutic interventions against FASD.

Kenneth R. Warren Merit Award

L. Glass, E.M. Moore, E.P. Riley, S.N. Mattson (San Diego State University). Leila Glass' presentation focused on the academic achievement (math, reading, and writing) of school-age children with prenatal alcohol exposure. Several factors can influence academic achievement in these children, including quality of education, household environment, cognitive abilities, demographic variables, access to services, and behavioral problems. The aims of this study were: 1) to identify and characterize academic difficulties in children with prenatal alcohol exposure, 2) to determine the prevalence of academic deficits (according to several operationalized definitions), and 3) to determine if there are any neural correlates associated with these performances. School-age children (8–16 years) were assessed using the Wechsler Individual Achievement Test (2nd edition), the Wechsler Intelligence Scale of Children (4th edition), and the Child Behavior Checklist. Patterns of performance were examined in children with heavy prenatal alcohol exposure ($n = 67$) and controls ($n = 61$). Data were analyzed with repeated-measures multivariate analysis of covariance. Exposure group was the between-subjects variable, and academic domain (word reading, spelling, numerical operations, math reasoning) was the within-subject variable. Neural correlates were explored in a subset of subjects with structural magnetic resonance imaging at 13–16 years of age using FreeSurfer-v5.3. Relations between cortical surface area and performance were evaluated, controlling for age and sex. Demographic variables were not significantly different between the groups, with the exception of intelligence quotient, which was lower in the prenatal alcohol-exposed group. Alcohol-exposed children performed worse on word reading, spelling, numerical operations, and math reasoning compared to the control group. Children with heavy prenatal alcohol exposure performed significantly worse on math reasoning in comparison to their performance on the other three domains. Performance on the numerical operations subtest was significantly lower than word reading and spelling, and the latter two were not significantly different from each other. Regarding the prevalence of academic difficulties in children with heavy prenatal ethanol exposure, it was found that 58.2% of them exhibited low achievement on one or more academic domains, in contrast to 16.4% of control children. Academic deficits remain significant after accounting for age, intelligence quotient, and the presence of behavioral problems. In control subjects, imaging analyses revealed an inverse relationship between math scores and cortical surface area of the left superior parietal and right lateral/middle occipital cortices. However, this relationship was absent in alcohol-exposed children, possibly due to atypical neurodevelopment in this group. In conclusion, children prenatally exposed to high levels of alcohol have deficits in several academic performance domains, with particular weaknesses in math functioning. Structural alterations in the parietal and occipital cortices may contribute to these deficits. These findings advocate for the development, implementation, and dissemination of evidence-based interventions in this population (i.e., MILE program) (Kable, Taddeo, Strickland, & Coles, 2015).

Timothy A. Cudd Award presentation

K.A. Donald, J. Ipser, F.M. Howells, A. Roos, J-P. Fouche, E.P. Riley, N. Koen, R.P. Woods, B. Biswal, H.J. Zar, K.L. Narr, D.J. Stein. Inter-hemispheric functional brain connectivity in neonates with prenatal alcohol exposure (University of Cape Town). This

study aimed to test whether developmental alcohol exposure alters the establishment of functional brain networks in the first weeks of life. They used resting-state functional MRI (RS-fMRI) to detect these alterations and tested whether these anomalies could be predictive of neurobehavioral outcome. Results showed that alcohol exposure was associated with significantly increased connectivity between the brainstem and the anterior motor network ($p = 0.024$), and between the striatum and both the anterior and posterior motor networks ($p < 0.01$ for both). A trend was observed for an increase in connectivity between the brainstem and the left somatosensory network ($p = 0.056$), and for a reduction in inter-hemispheric connectivity between left and right somatosensory networks ($p = 0.066$). These findings suggest that RS-fMRI studies in infants have much to contribute to our knowledge of the etiology of FASD.

FASt presentations

E.L. Wong, G.O. Sipe, C.E. Lamantia, A.K. Majewska. Synaptic dynamics and microglial phenotype in adolescent mice with deficient activity-dependent plasticity after early postnatal high binge ethanol exposure (University of Rochester). Ms. Wong, a graduate student in the Majewska lab, is investigating the underlying mechanisms of the enduring deficits in experience-dependent synaptic plasticity caused by alcohol exposure during the third-trimester equivalent of human gestation. It is well established that plasticity in the visual cortex is dependent on dendritic spine dynamics and can be modulated by microglial function. Therefore, Ms. Wong evaluated neuronal spine stability, microglial motility, and microglial response to focal injury to evaluate if an effect of alcohol on these factors could underlie the observed decrease in neuronal plasticity. Using *in vivo* two-photon microscopy, she assessed spine turnover, but did not find any evidence of an alcohol effect. An evaluation of microglial morphology and response to cortical tissue injury also did not reveal an effect of alcohol. Therefore, it is unlikely that alcohol-induced changes in the dynamics of microglial contacts with dendritic spines underlie the deficits in activity-dependent plasticity seen in this model.

K.E. Boschen, K.J. Criss, M.J. Ruggiero, V. Palamarchouk, T.L. Roth, A.Y. Klintsova. Neuroprotection in the developing brain: BDNF and microglial response following postnatal alcohol exposure in rats (University of Delaware). Ms. Boschen, a graduate student in the Klintsova lab, investigated whether a third-trimester equivalent binge-like alcohol exposure induces a neurotrophic and neuroimmune response in the developing rat hippocampus. She exposed Long-Evans rats to 5.25 g/kg/day of alcohol via intra-gastric intubation from postnatal day (P) 4–9 and collected the hippocampus at P10. Results were compared to those obtained from animals that were intubated but received no liquid (sham) or undisturbed controls. They observed that BDNF levels were increased in both alcohol- and sham-intubated animals in comparison with undisturbed controls. Therefore, this effect cannot be attributed only to alcohol exposure, since the stress caused by the intubation procedure was a possible contributor. In contrast, TrkB protein, a receptor for BDNF, was increased only in the alcohol group, suggesting that this effect may be specific to ethanol exposure.

R.D. Risbud, T.T. Taggart, J.D. Thomas. Neonatal Alcohol Exposure Alters the Effects of Subsequent Adolescent Alcohol Exposure on Emotional Development (San Diego State

University). The study aimed to test the hypotheses that prenatal alcohol exposure exacerbates the consequences of alcohol consumption during adolescence on cognitive, motor, and emotional domains. One group of Sprague-Dawley rats received alcohol (2.5 g/kg/day) or sham intubations on postnatal days (P) 4–9, mimicking drinking during the last months of gestation in humans. Another group of animals received alcohol (4 g/kg/day) or sham intubation in a 2-day-on/1-day-off pattern during adolescence (P28–P42). Finally, another group of animals received both postnatal plus adolescent exposure to alcohol. Both regimens resulted in blood alcohol concentrations of approximately 200 mg/dL. After P45, animals were tested in a battery of behavioral tests that included open field, Morris water maze, and trace fear conditioning. Combined exposure did not increase the deficits in the cognitive domains of the behavioral tests performed when compared to the single exposure (only first week postnatal or during adolescence). However, animals subjected to the combined alcohol exposure were more affected in emotional domains such as a reduction in thigmotaxis in both water maze and open field. These findings suggest that combined ethanol exposure during the third-trimester equivalent followed by ethanol exposure during adolescence can result in more severe emotional alterations than individual exposure during either of these periods.

T. Jirikovic, L.Y. Hsu, S.W. McCoy, M.A. Ciol, R. Price, D.K. Kartin. Virtual reality-based sensorimotor training improves balance and motor responses in children with FASD (University of Washington). Dr. Jirikovic tested the potential of a novel rehabilitation system based on virtual reality in improving balance and motor abilities in children with FASD. This system is named Sensorimotor Training to Affect Balance, Engagement and Learning (STABEL). STABEL allows manipulation of visual and somatosensory information by subjects standing on a flexible surface while playing a computer game. Twenty-three children with FASD (6 tested in the lab, 9 tested at home, and 8 not trained) were tested. Children with FASD who completed a 1-month training showed an improvement in balance and motor skills compared to those that were not trained. No differences were seen between children that were trained at the lab or at home. These findings suggest that interventions such as STABEL have a good potential for rehabilitation of children with FASD.

C.B. Lovely, T. Henegar, J.L. Norrie, M.E. Swartz, J.K. Eberhart. Zebrafish genetic screens identify ethanol susceptibility loci (University of Texas, Austin). Dr. Lovely presented his work conducted in Dr. Eberhart's lab, on the interactions between genes and developmental alcohol exposure. Using a zebrafish model, they investigated the effect of subteratogenic doses of ethanol in causing craniofacial anomalies in different mutants. Results showed that the bone morphogenetic protein (BMP) pathway was particularly susceptible to alcohol exposure. Alcohol-treated BMP mutants showed craniofacial defects such as lower jaw hypoplasia and malformation of the palatal skeleton. These malformations are compatible with some malformations seen in FASD. These findings stress the importance of the interaction between alcohol exposure and genes. A better understanding of these interactions may contribute to the identification of subjects that are more likely to develop FASD.

P. Trindade, B.H. Hampton, A.C. Manhaes, and A.E. Medina. Long-lasting change on astrocyte secretome following early alcohol exposure (University of Maryland, Baltimore).

It is well established that acute alcohol exposure can disrupt astrocyte function. It is also established that the effects of developmental alcohol exposure can persist throughout one's lifespan. Dr. Trindade tested the hypothesis that early alcohol exposure can lead to an enduring effect on the profile of proteins secreted by astrocytes. He exposed ferrets to 3.5 g/kg of alcohol or control saline in a time equivalent to the last months of human gestation, which is postnatal in the ferret, and then made astrocyte cultures from these animals. After undergoing multiple cell divisions during several weeks in culture, these astrocytes became confluent. Using a proteomic technique (SILAC combined with LC/MS), they identified the proteins secreted in the astrocyte-conditioned media and compared alcohol- and saline-treated groups. They found that even long after the period of alcohol exposure, the conditioned media from the alcohol group displayed remarkable differences from the saline-treated group. For instance, the conditioned media from astrocytes cultured from alcohol-treated animals showed a marked decrease in proteins related to the extracellular matrix (such as laminin and proteoglycans). These findings show that alcohol exposure can have an enduring effect on the astrocyte secretome.

P.K. Goh, L.R. Doyle, L. Glass, K.L. Jones, E.P. Riley, S.N. Mattson. Developing a decision tree for clinical identification of children affected by prenatal alcohol exposure I: model development (San Diego State University). The clinical identification of children presenting with FASD remains a challenge. This study proposed a model that can be useful as a diagnostic screening tool. A combination of neuropsychological, neurobehavioral, and dysmorphology data was collected from 146 alcohol-exposed and 288 non-exposed subjects as part of the Collaborative Initiative on Fetal Alcohol Spectrum Disorders phase 2. The non-exposed group included subjects with ADHD, and lower IQs (but not alcohol exposure). The diversity of the control group was important to test the specificity and sensitivity of the model. The decision tree had two possible entries, one through an initial analysis by a psychologist or psychiatrist (Entry 1) or an alternate one, with the initial analysis made by a dysmorphologist/pediatrician (Entry 2). Data were analyzed using a logistic regression to obtain the discriminatory ability of each step of the tree. Both Entry 1 and 2 presented accuracy higher than 80%. These findings demonstrate that this novel model can differentiate alcohol-exposed children from non-exposed ones with good sensitivity and specificity.

C.J. Fontaine, J.L. Dunbar, J.B. Munich, **A.R. Patten**, B.R. Christie. Neonatal odor learning impairments following prenatal ethanol exposure (University of Victoria). This study tested whether there is an association of reduced olfactory learning caused by developmental alcohol exposure and decreased levels of the antioxidant glutathione. Animals were exposed to alcohol throughout gestation, and were tested at postnatal day (P) 6 in an odor discrimination learning test. This test is based on pairing a particular odor with tactile stimulation mimicking maternal care. As a result, when placed in an arena where two different odors are presented on opposed sides, pups tend to move toward the odor that was previously associated with the tactile stimulation. The data showed that animals previously exposed to alcohol show a poor performance in this test when compared to both pair-fed and chow controls. At P7, the olfactory bulb and the anterior piriform cortex, regions associated with olfactory learning, were dissected to assess levels of glutathione, an antioxidant that has

been associated in the past with deficits in hippocampal plasticity. They found that glutathione levels were reduced in both alcohol- and pair-fed animals when compared to chow controls. No differences were seen in glutathione levels between alcohol- and pair-fed animals. These findings suggest that early alcohol exposure disrupts olfactory learning, but the glutathione pathway may not be the mechanism underlying this deficit.

P. Muralidharan, S. Sarmah, J.A. Marrs. Ethanol-induced defects in zebrafish retinal development rescue by nutritional supplements (Indiana University-Purdue University). This study used the zebrafish model to investigate the effects of developmental alcohol exposure on development of the retina and optic nerve. Animals were exposed to alcohol (100 and 150 mM) 2–24 h post-fertilization, during the gastrulation period. This type of exposure resulted in obvious microphthalmia and hypoplasia of the optic nerve, and altered numbers of rod and cones, features commonly seen in fetal alcohol syndrome. Remarkably, supplementation with retinoic acid or folic acid was able to ameliorate these deficits. These findings support the potential of using simple nutritional supplements to protect the visual system against the effects of early alcohol exposure.

S. Tamana, G. Andrew, J. Pei, C. Rasmussen. Executive function in children with FASD and mental health comorbidity (University of Alberta). The study aimed to test executive functioning in children with FASD who have co-morbid externalizing disorders (attention-deficit hyperactivity disorder, oppositional defiant disorders) to those who have co-morbid internalizing disorders (depression, anxiety); a group of children with FASD that did not present externalizing nor internalizing disorders was also included. A total of 24 children with FASD were tested based on neurobehavioral assessments and caregiver reports. No significant differences were found between groups; however, the FASD + externalizing disorders and FASD + internalizing disorders groups showed a trend to have poorer performance in executive function and working memory, respectively. These findings support the idea that the presence of co-morbidity may influence behavioral profiles in FASD.

S. Bohora, T. Balachova, B. Bonner, G. Isurina, L. Tsvetkova, E. Volkova, Y. Zhao. Does the effect of FASD education brochures on women's alcohol consumption differ among smokers and non-smokers? (University of Oklahoma). The study evaluated effects of education brochures designed to reduce the risk for an alcohol-exposed pregnancy among women who smoke and women who do not smoke. In a previous study, the authors showed that an education brochure with positive messages had higher effect on knowledge, attitudes, and reduction in alcohol use among women compared to a brochure with negative messages about alcohol use during pregnancy and a general pregnancy health information brochure. Using area under the curve regression, the authors showed that the effect of brochures differed among smokers and non-smokers. Among smokers, the positive brochure had greater effect on women's alcohol use compared to the general health material, while among non-smokers both brochures had a similar effect to reduce women's alcohol use.

M. Cabrera, P. Shrivastava, L.G. Chastain, D.K. Sarkar. Fetal alcohol exposure induces microglial activation and alters opioid receptor expression in the hypothalamus (Rutgers University). There is evidence that alcohol exposure can induce microglia to kill neurons

that produce beta-endorphin (BEP). This peptide acts via mu- (MOR) and delta- (DOR) opioid receptors. These studies tested the hypothesis that since BEP, an opioid peptide, acts via MORs and DORs, these receptors are involved in the communication between BEP neurons and microglia during ethanol toxicity. The study used a rat model of neonatal alcohol exposure via oral gavage compared to pair-fed or untreated controls. Immunohistochemistry for IBA-1 revealed an increase in microglia in alcohol-treated animals. This augmentation was accompanied by an increase in inflammatory markers such as TLR-4. The authors showed a co-localization of MORs and DORs in IBA-1 positive cells, confirming that these receptors are present in microglia. Western blotting analyses showed that alcohol led to an increase in DORs but not in MORs, suggesting that alcohol has a specific effect in a subset of opioid receptors. It was concluded that fetal alcohol exposure induces microglial activation and proinflammation, and alters opioid receptor expression in the hypothalamus. Co-localization of opioid receptors on microglia suggests that changes in opioid receptor expression observed in the hypothalamus may also occur on microglia following fetal alcohol exposure. Thus, opioid receptors may play a role in developmental alcohol-exposure induced microglial activation.

T. Bodnar, W. Yu, J. Weinberg. Prenatal alcohol exposure: evidence for an early-life immune signature? (University of British Columbia). In this study the authors hypothesized that prenatal alcohol exposure would alter the balance of cytokines during development, making subjects more susceptible to inflammation. To test this, they exposed Sprague-Dawley rats to a liquid ethanol diet throughout gestation (PAE), to an isocaloric liquid control diet (PF), or to a pelleted version of the control diet (C). The authors found that overall, spleen weights were increased over the pre-weaning period in PAE offspring, and on postnatal day 8 (P8), spleen weight was positively correlated with maternal alcohol intake. Interestingly, on P8, cytokine levels were increased in the plasma (IL-13, IFN- γ , TNF- α) and hippocampus (IFN- γ , IL-1 β , IL-4, IL-2, IL-5, TNF- α) of PAE offspring, but the opposite profile of decreased cytokines (IL-13, IL-5, IL-10, IL-6, IL-4, IL-2) was detected in the spleen. These findings suggest that prenatal alcohol exposure can affect the expression of cytokines in different compartments and this may underlie the altered susceptibility to inflammation in individuals with FASD.

V. Macht, S.J. Kelly. Impact of chronic low-dose amphetamine treatment during juvenile development on catecholamine neurons in a model of FASD (University of South Carolina). Attention-deficit hyperactivity disorder is often observed in children with FASD; however, it is sometimes refractory to conventional treatments. Using a third-trimester binge-drinking model of FASD, the authors looked at the effects of 0.5 mg/kg amphetamine or vehicle from postnatal day P26 to P41. During this period, animals were tested in an open field, and at P42 animals were euthanized for tissue collection. Results did not show an effect of alcohol on amphetamine-induced locomotor activity in the open-field behavior. Moreover, amphetamine treatment increased expression for dopamine beta-hydroxylase, a marker for noradrenergic axons, in the prefrontal cortex to a similar extent in control and ethanol-treated animals. These findings suggest that catecholaminergic neurons are relatively resistant to third-trimester equivalent alcohol exposure.

Henry Rosett Award

The 2014 Henry Rosett Award recipient was Dr. Michael E. Charness (VA Boston Healthcare System, Harvard Medical School, and Boston University School of Medicine). Dr. Charness was introduced by Dr. Edward Riley (San Diego State University). Dr. Riley started by giving some background on Dr. Henry Rosett, who was a faculty member at Boston University School of Medicine. In 1974, Dr. Rosett conducted a study of problem drinking by pregnant women and demonstrated the benefits of a reduction in alcohol consumption in this patient population. Dr. Rosett co-authored more than 50 scientific articles and a book titled: "Alcohol and the Fetus". Dr. Riley then gave an overview of Dr. Charness' career. Dr. Charness got his MD degree from Johns Hopkins University and completed residency training in neurology at University of California, San Francisco. Dr. Charness is an accomplished scientist who has worked in the field of FASD for 23 years. He has served as an advisor on many occasions for NIAAA and is the scientific director of the Collaborative Initiative on FASD (CIFASD). He is a world-renowned scientist for his work on the impact of fetal alcohol exposure on cell adhesion molecules.

Dr. Charness' presentation was titled "What molecules and cells can tell us about genetic risks for FASD". He started by giving background information on the L1 adhesion molecule, which plays roles in several developmental processes including neuronal migration, neurite extension, axon fasciculation, growth cone motility and path finding, and neuronal survival. The brains of children with fetal alcohol syndrome share some characteristics with those of children with L1 genetic mutations (CRASH syndrome) (Charness, Safran, & Perides, 1994; Ramanathan, Wilkemeyer, Mittal, Perides, & Charness, 1996). These include intellectual disability, corpus callosum hypoplasia, hydrocephalus, and cerebellar dysplasia. Dr. Charness initially tested the effect of ethanol on L1 in cultured NG108-15 cells, finding that pharmacological concentrations of this agent inhibited cell adhesion induced by bone morphogenetic protein type 7 (Charness et al., 1994). In fibroblasts transfected with the human version of L1, ethanol potently inhibited adhesion (half maximal effect at 5 mM) (Ramanathan et al., 1996). Studies with alcohols of different chain lengths showed that methanol, ethanol, 1-propanol, and 1-butanol were effective at inhibiting L1; however, n-alcohols longer than 1-pentanol did not have an effect (Wilkemeyer, Sebastian, Smith, & Charness, 2000). Interestingly, agents such as 1-octanol and 3-butanol-1-ol blocked the L1 inhibitory effects of ethanol and 1-butanol, respectively (Wilkemeyer, Menkari, & Charness, 2002; Wilkemeyer et al., 2000). These studies suggested that ethanol binds to a pocket of specific size in the L1 molecule. Photoaffinity labeling studies identified amino acid residues where alcohols bind on L1 (Arevalo et al., 2008). Experiments with whole mouse embryos in culture revealed that 1-octanol prevented cell death and birth defects induced by ethanol exposure (Chen, Wilkemeyer, Sulik, & Charness, 2001). Two peptides known as NAP and SAL were also shown to potently block ethanol-induced inhibition of L1 and to prevent ethanol-induced neural tube defects in mouse embryos (Chen, Charness, Wilkemeyer, & Sulik, 2005; Wilkemeyer et al., 2003). In an elegant series of experiments, Dr. Charness and his team investigated whether there are intracellular signaling pathways that regulate L1 sensitivity to ethanol. It was demonstrated that phosphorylation of L1 by extracellularly regulated kinase2 (ERK2) was required for ethanol-induced inhibition of L1

(Dou et al., 2013). Collectively, these findings indicate that the L1 adhesion molecule plays a central role in the pathophysiology of FASD, emphasizing its importance as a target for the development of therapeutic interventions against this disorder.

Dr. Charness ended his presentation by acknowledging his mentors and collaborators, as well as financial support from several agencies (e.g., NIAAA and the Department of Veterans Affairs). He also thanked patients with FASD and their families who are struggling with the consequences of this disorder and provide inspiration for our work in this field.

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Highlights

- Focus on basic mechanisms of prenatal alcohol exposure and translational implications
- The role of heat shock pathway on ethanol-induced damage was addressed
- The potential uses of magnetoencephalography in this area of research was also discussed
- The 2015 Henry Rosett award presentation focused on L1 adhesion molecule