Published in final edited form as:

Alcohol. 2017 December ; 65: 19–24. doi:10.1016/j.alcohol.2017.07.001.

Proceedings of the 2016 Annual Meeting of the Fetal Alcohol Spectrum Disorders Study Group

Alexandre E. Medina^a, Jeffrey Wozniak^b, Anna Klintsova^c, and Derek Hamilton^d

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

^bDepartment of Pediatrics, University of Minnesota, School of Medicine, Minneapolis, MN

^cDepartment of Psychological and Brain Sciences, University of Delaware, Newark, DE

^dDepartment of Psychology, University of New Mexico, Albuquerque, NM

Abstract

The 2016 Fetal Alcohol Spectrum Disorders Study Group (FASDSG) meeting was titled “Rehabilitation in FASD: Potential Interventions and Challenges”. During the previous decades, studies with human subjects and animal models have improved much of our understanding of the mechanisms underlying FASD, putting the scientific community in a good position to test hypotheses that can lead to potential therapeutic interventions. During the conference, two keynote speakers addressed potential interventions used in different fields and their applicability to FASD research. The conference also included updates from several government agencies, short presentations by junior and senior investigators that showcased the latest in FASD research, and award presentations. The conference was closed by a talk by Dr. Charles Goodlett, the recipient of the 2016 Henry Rosett award.

The 2016 Fetal Alcohol Spectrum Disorders Study Group (FASDSG) annual meeting was held on June 25, in New Orleans, LA, as a satellite of the Research Society on Alcoholism meeting. Approximately 146 senior and junior investigators attended the meeting, including individuals from the United States (from 28 states), Canada, South Africa, and South Korea. The program included two keynote speaker presentations, and 13 FASD Data blitzes (one slide, 5-min presentations of original data). Most of the FASD presentations were given by graduate students and post-doctoral associates, and seven of them were supported by travel awards from the FASDSG.

In addition, two other trainees were selected to receive the Timothy A. Cudd and the Kenneth R. Warren Merit Awards. All trainees attending the meeting had the opportunity to interact with more senior researchers and clinicians at a networking lunch. Representatives 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 from these agencies.

The highlight of the meeting was the presentation of the Rosett Award, to recognize lifetime contributions, achievement, and service in FASD research.

The theme of the 2016 meeting was “Rehabilitation in FASD: Potential Interventions and Challenges”. After several decades of investigating the epidemiology and the mechanisms underlying FASD, the scientific community is in a position to evaluate potential interventions. Currently, there are approaches being tested, such as choline supplementation and behavioral interventions. This year we invited two keynote speakers to talk about two potential strategies of rehabilitation that have been applied, transcranial magnetic stimulation and sensory-motor enrichment, and to discuss different conditions that may have potential as an intervention for FASD.

Keynote presentations

The first keynote presentation was given by Dr. James “Cole” Galloway, P.T., Ph.D., Professor of Physical Therapy at the University of Delaware. His talk was titled: “Go Baby Go: Lifespan technology R&D for making brains, making buddies and getting into serious trouble!” Dr. Galloway’s work aims to devise strategies and technologies to improve mobility and provide environmental enrichment in subjects with neurodevelopmental conditions such as cerebral palsy and Down syndrome. Dr. Galloway started his talk by addressing the importance of mobility and environmental exploration during critical periods of brain development. Children with impairments that limit their sensory experience and the ways in which they interact with their peers may have long-lasting consequences on cognition and sociability. To ameliorate neurodevelopmental problems, interventions aiming to provide sensory enrichment or to improve mobility through physical therapy sessions are often used. While these types of interventions can be important, Dr. Galloway stressed that they have the limitation of being restricted to short periods of time, instead of engaging patients in their daily routines. With this in mind, Dr. Galloway devised a series of strategies aiming to bring to patients’ homes tools that can be used constantly to improve mobility and sensory experience. By doing so, this enriched environment would lead to neuronal plasticity changes that could be beneficial even to other behavioral aspects such as cognition and self-esteem. In order to improve mobility and sensory experience in children with different severities of cerebral palsy, Dr. Galloway used modified toy ride-on cars, harness-assisted mobility, and “super suits”, in collaboration with Dr. Michelle Lobo, also from the University of Delaware. Modified toy ride-on cars provide better mobility than conventional wheelchairs, can be built for a fraction of wheelchair cost, and can be used by children with minimal assistance. Harness-assisted mobility can be achieved by installing tracks throughout the patient’s house, allowing the subject to be mobile in their own environment. Finally, a “supersuit” is a combination of the concept of a wearable functional exoskeleton and fashionable clothing. This work, done in collaboration with Dr. Michele Lobo, ameliorates movement problems with a solution that is inexpensive, accessible, and esthetically pleasant.

The approach proposed by Dr. Galloway may be applicable to FASD research and potential treatment strategies. There are several studies providing evidence that enriched environments can ameliorate cognitive deficits in FASD (Hamilton et al., 2014; Hannigan, Berman, &

Zajac, 1993; Klintsova et al., 1998). Dr. Galloway's talk suggests that making an enriched environment as a part of the patient's daily life may produce better results.

The second keynote presentation was given by Dr. Alexander Rotenberg, M.D., Ph.D., Associate Professor of Neurology, Boston Children's Hospital, Harvard Medical School. The title of his talk was "Measures and modifications of cortical excitability by non-invasive brain stimulation". Dr. Rotenberg started his presentation by giving an overview of the basic mechanisms and the applicability of transcranial magnetic stimulation (TMS), a non-invasive method to increase or decrease brain activity in a specific region. TMS is performed with a metal coil that generates an electric current, which in turn produces a magnetic field that can affect cortical responses in areas with a typical radius of 0.5 cm. Typically, three different types of stimulation protocols are used: a) Single pulse, b) Paired Pulse, and c) Repetitive. Single pulse (sTMS) is often used for brain mapping in pre-surgical planning or to detect thresholds for cortical excitability (Frye, Rotenberg, Ousley, & Pascual-Leone, 2008). In fact, using sTMS in selected areas of the motor cortex to calculate the amount of current necessary to produce a response in an EMG electrode, Dr. Rotenberg demonstrated how cortical excitability matures with age. Dr. Rotenberg called attention to animal studies that showed decreased myelination after developmental alcohol exposure (David & Subramaniam, 2017), and noted that it would be interesting to use the non-invasive sTMS approach to evaluate the state of the maturation of excitability and nerve conductivity in subjects with FASD. Another alteration of cortical excitability that is common in subjects with FASD is epileptic seizures (Bell et al., 2010; Nicita et al., 2014). Dr. Rotenberg presented data demonstrating the feasibility and efficacy of low frequency repetitive stimulation (0.5–1 Hz) in decreasing cortical excitability and in turn reducing seizures in different conditions (Gersner, Oberman, et al., 2016; Rotenberg et al., 2008, 2009). Interestingly, repetitive stimulation TMS (rTMS) was also used in combination with lorazepam (a commonly prescribed anticonvulsant), and it reduced the anticonvulsant dose required to suppress seizures (Gersner, Dhamne, Zangen, Pascual-Leone, & Rotenberg, 2016). What are the mechanisms underlying the positive effects of rTMS? One explanation is that low frequency rTMS protocols may induce long-term depression (LTD), a temporary but long-lasting reduction in synaptic strength that results in decrease of neuronal responses. In fact, both low frequency rTMS and LTD use similar stimulation protocols of 0.5–1 Hz (Lenz & Vlachos, 2016; Nakano, Yamada, Udagawa, & Kato, 2004; Sheng & Ertürk, 2013). In addition to changing neuronal excitability, TMS can also be used to evaluate the balance between excitation and inhibition. For instance, paired-pulse TMS (ppTMS) stimulation applied in the motor cortex results in two motor-evoked potentials (MEPs), in a way that the second MEP has a smaller amplitude than the first (Oberman et al., 2010). The decrease in amplitude is attributed to GABAergic neurotransmission, and therefore a ratio between these responses should reflect the balance between excitation/inhibition (Oberman et al., 2010). Using ppTMS, Dr. Rotenberg and colleagues showed a reduction in GABA-mediated inhibition after traumatic brain injury in a rodent model (Hsieh et al., 2016). This change of inhibition had been hypothesized to be indirectly caused by an increase in oxidative stress. According to the hypothesis, an increase in oxidative stress would result in damage to the perineuronal net that surrounds mainly inhibitory parvalbumin (PV) neurons. A decrease in the number of PV neurons would decrease inhibition, resulting in tissue that is more

excitable and prone to seizures (Hsieh et al., 2016). A parallel can be drawn to FASD, since it has been reported that developmental alcohol exposure can lead to an increase in oxidative stress (Brocardo, Gil-Mohapel, & Christie, 2011), a decrease in PV neurons (Smiley et al., 2015), and the development of seizures (Bell et al., 2010; Nicita et al., 2014).

In summary, TMS has great potential as a tool for evaluating cortical excitability and suppressing seizures in neurodevelopmental disorders such as FASD.

Kenneth R. Warren Merit Award

Bodnar, T., Rainecki, C., & Weinberg, J. (University of British Columbia) *Effects of prenatal alcohol exposure on immune function across the lifespan*. Ms. Bodnar was awarded the Kenneth R. Warren Merit Award for work that is part of her doctoral dissertation. In her talk, she described a range of immune effects of prenatal alcohol exposure in humans and animals, including increased susceptibility to infection, abnormalities in the development and adaptability of the immune system, inflammatory diseases, susceptibility to cancers, and problems transferring immunity from mother to offspring through lactation. Research in this field and in this lab has often employed a “two-hit” model with PAE being the first hit and a later “challenge” such as chronic or acute stress representing the second hit. The first study that Ms. Bodnar presented examined female rats who were offspring of mothers exposed during gestation to ethanol, or pair-fed, or given a control diet. Environmental stressors were applied between days P31 and P41 and the rats were later injected with Complete Freund’s Adjuvant to increase the likelihood of arthritis during adulthood. The PAE rats showed significantly higher rates of arthritis compared to controls or pair-fed rats and they also tended to maintain inflammation longer. Bone and cartilage cross sections revealed long-lasting inflammation and damage in the PAE rats. Ms. Bodnar presented a second study using a more acute stress paradigm (adequate or low bedding material in the cage). The same three groups were included, but both males and females were studied. All three groups showed increased levels of C-reactive protein (CRP), but the PAE group showed the greatest elevation. As adults, the rats were injected with lipopolysaccharide (LPS) or saline. Animals with PAE and the inadequate bedding condition showed abnormal responses from lymphocytes and monocytes. All animals exposed to the poor rearing environment showed abnormal cytokine response. Examining the brain at day P8 revealed elevated cytokines in hippocampus and prefrontal cortex but reduced cytokines in the hypothalamus. Lastly, Ms. Bodnar showed data from a CIFASD dataset (T. Chambers) demonstrating high cytokine levels in alcohol-consuming Ukrainian mothers who had children with FASD compared to drinking mothers whose children were not diagnosed with FASD. Different cytokine patterns were seen in mothers in the control group.

Timothy A. Cudd Award

Montag, A., Jones, K., Hull, A., Wertelecki, W., Yevtushok, L., Zymak-Zakutnya, N., Chambers, C. and the CIFASD (Univ. of California – San Diego) *Second trimester ultrasound as a tool in early detection of fetal alcohol spectrum disorders (FASD)*. Dr. Montag was awarded the Timothy A. Cudd Award for her study on new potential methods of detection of FASD using ultrasound. She described a relatively small existing literature

showing that ultrasound was sensitive to reduced frontal cortex volume, cerebellar volume, and head-to-body size. Prenatal identification of FASD could facilitate earlier intervention and better outcome for affected children. Second-trimester ultrasound data were collected from a Ukrainian cohort in which women were screened prenatally for alcohol use. The cohort included women who consumed alcohol during pregnancy and a control group who did not. Neurobehavioral measures, including the Bayley Scales of Infant Development, were administered at 6 and 12 months. A number of linear measurements were obtained from the two-dimensional ultrasound data, including brain measures and orbital measures. Of 98 fetuses with PAE, 15 were eventually diagnosed with FASD (4 with full FAS). There were 135 controls. Outcome measurements were adjusted for maternal age and smoking status. Longer occipital-frontal diameter was associated with specific cardinal facial features of FASD. Greater intra-orbital distance was associated with FASD. Larger orbital diameter on ultrasound was unexpectedly associated with shorter palpebral fissures, perhaps reflecting delayed eye development. Neurodevelopmental delays in the child were associated with differences in a number of measures, including fronto-thalamic distance and intra-orbital distance. Ultrasound measures accounted for less than 10% of the variance, but adding alcohol-exposure data increased the predictive validity substantially to the point where it may provide a degree of clinical utility in the future.

FASt Data Presentations

Kodali, V. (Wayne State University) *Abnormal cortical gyrification mediates effects of prenatal alcohol exposure on arithmetic, executive function, and recognition memory*. Mr. Kodali presented MRI data from a Capetown, South Africa cohort of children prenatally exposed to alcohol, examining cortical gyrification (folding). The study included 9 children with FAS, 15 heavily exposed non-syndromic individuals, and 16 controls. Cortical gyrification (global sulcal index and cortical fold opening) was quantified using a semi-automated image analysis pipeline. Associations between these measures and amount of prenatal drinking were observed. In addition, cortical fold opening was associated with cognitive outcome (working memory, verbal learning, reading comprehension, math, and verbal fluency switching). Mediation analysis demonstrated that the relationship between prenatal alcohol exposure and cognitive outcome was mediated by cortical fold opening (for example, right parietal sulcal opening mediated the relationship between alcohol exposure and math scores).

Lam, V. (Univ. of British Columbia) *Effects of prenatal alcohol exposure and chronic unpredictable stress on ventral hippocampal mineralocorticoid receptor mRNA levels*. Ms. Lam presented a rat model examining the impact of prenatal alcohol exposure together with chronic stress on behavior and mineralocorticoid receptor mRNA levels in the ventral hippocampus. Control, pair-fed, and ethanol groups were included and animals were either subjected to stress or not subjected to stress. Behavioral testing and brain measures were done either immediately or 2 weeks post-stress exposure. Females showed greater immobility (a “depressive-like” response) during a forced-swim test when they were stressed vs. non-stressed, and the effect was most prominent when the behavioral tests were administered at 2 weeks post-stress. Mineralocorticoid receptor mRNA levels were found to be higher for the ethanol group when stressed and with the 2-week delay. This type of

increase could potentially be associated with alterations in the hypothalamic-pituitary axis tone.

Doyle, L. (San Diego State University) *Cognitive predictors of communication impairment in youth with histories of heavy prenatal alcohol exposure*. Ms. Doyle presented a study in which she examined cognitive functioning and practical communication skills in 142 children with prenatal alcohol exposure and 162 controls who were part of the CIFASD multi-site set of projects. Using multiple regression, measures of spatial working memory, inhibitory skill, systematic reasoning, and word generation were tested for their ability to “predict” practical communication skill (as rated by the parent/caregiver on a measure of adaptive functioning). Spatial working memory and inhibition predicted communication skill in both groups. Abstract reasoning skills predicted practical communication skills in the controls but not in alcohol-exposed children. Word generation predicted practical communication in the alcohol-exposed children but not the control children. The results have implications for understanding the neurocognitive origins of communication deficits in this patient population.

Darbinian, N. (Temple University) *Prenatal ethanol exposure dysregulates early facial biomarkers of FAS and inhibits normal fetal eye development in a human in-vivo model*. Dr. Darbinian presented a study of human fetal brain and eye tissue from pregnancies that were electively terminated between weeks 1 and 11. There was significant alcohol exposure in one group (n = 6) and no alcohol exposure in the other group (n = 6). Eye diameter and pupil diameter were found to be smaller in the alcohol-exposed group and there was an association with the amount of alcohol exposure. An association with caspase-3 activation suggested that the reduced eye size might have been related to apoptosis. In brain, BDNF and PMP22 were downregulated in the alcohol-exposed group relative to controls. These data suggest that myelination would have been affected further downstream in development in these individuals.

Bohora, S. (University of Oklahoma) *Using multilevel latent transition analysis to model transitions of alcohol use profiles in women at risk for alcohol-exposed pregnancies*. Mr. Bohora reported on a randomized controlled trial of a brief physician intervention (focusing on both alcohol use and contraception) in attempting to prevent prenatal alcohol exposure in a population of women in Russia. The intervention consisted of a 5–10-minute conversation between physician and patient. A total of 767 non-pregnant women, ages 18–44, were included. The outcome of interest was transition from heavy drinking to lighter patterns of drinking. The physician intervention proved to be associated with a greater likelihood of moving out of the heavy drinking category into a lower level of drinking and a lesser likelihood of moving from a lighter drinking category into a heavier use pattern. Those who moved from the heavy drinking category did not tend to move into the binge-drinking category, but instead to a less risky drinking pattern.

Dou, X. (Harvard University) *Ankyrin-G binding to L1 regulates L1 sensitivity to ethanol by inside-out signaling*. Dr. Dou reported on research examining the molecular mechanisms by which ethanol inhibits L1-mediated cell adhesion, specifically by actions at a binding site in the L1 extracellular domain. L1 mutant constructs that prevented ankyrin-G binding to L1

resulted in the uncoupling of L1 from the spectrin-actin cytoskeleton, abolishing L1's sensitivity to ethanol, but preserving L1's sensitivity to methanol. This indicates a shift in the alcohol cutoff of the binding pocket from four carbons to one. A mutation that prevents MAPK phosphorylation of L1-1248 abolished L1 sensitivity to ethanol, but not methanol, and uncoupled L1 from ankyrin-G. An ethanol-insensitive L1-expressing clonal cell line showed reduced L1 binding to ankyrin-G and the actin-spectrin cytoskeleton, compared to an ethanol-sensitive clonal cell line. The findings suggest that phosphorylation events in the L1 cytoplasmic domain change the conformation of the L1 extracellular domain by uncoupling L1 from the spectrin-actin cytoskeleton. These data have implications for understanding the toxic effects of ethanol on the developing nervous system.

Williams, S. (University of New Mexico) *Feasibility of estimating prenatal alcohol exposure from residual newborn screening*. Ms. Williams presented data on a prenatal alcohol exposure surveillance project using residual newborn screening blood spot cards. Phosphatidylethanol (PEth), a direct alcohol metabolite, provides information about alcohol exposure during a 1–3-week window prior to delivery. PEth was evaluated in 499 dry blood spot cards from the Texas Newborn Screening Repository. Cards that were less than 2 months old were obtained from 11 public health regions covering the state of Texas. The prevalence of alcohol exposure at a cutoff of >20 ng/mL was 9.2%. At a more conservative cutoff of >28 ng/mL, the estimated prevalence was 6.6%. There were significant regional variations in the prevalence rates across the public health regions. No prevalence differences were observed across gender of the baby nor race/ethnicity of the baby.

Chastain, L. (Rutgers University) *Epigenetic factors in the effects of neonatal alcohol exposure on hypothalamic microglia in rats*. Ms. Chastain presented a study examining the role of microglia (which serve an immune function in the brain) in the neurodevelopmental effects of prenatal alcohol exposure. Microglia overactivation in response to alcohol exposure has been shown to negatively affect hypothalamus development. In an *in vivo* study, three groups of rat pups were studied: alcohol-fed (2.5 g/kg), pair-fed, and controls. A separate cell culture study of isolated microglia with 50-mM ethanol exposure for 24 h was conducted. With alcohol exposure, a significant increase in pro-inflammatory transcripts were seen (TLR-4, MCP-1, and CSFR-1), indicating an increase in pro-inflammatory gene expression in the hypothalamus. Microglial activation (IBA-1, CX3CR1, IFN gamma, and MOR) was also increased in the *in vivo* model. Epigenetic regulators of microglia were altered by alcohol exposure. DNA methylation was also decreased in the alcohol-exposed animals. Similar results were also seen in the cell culture experiment. Results suggest possible epigenetic mechanisms by which microglia in the developing hypothalamus may become “programmed” to become more pro-inflammatory, thus contributing to abnormal neurodevelopment in those exposed to alcohol prenatally.

Petrenko, C. (University of Rochester) *Short-term findings from the Families on Track preventive intervention pilot trial with children with FASD and their families*. Dr. Petrenko presented early findings from a family-based intervention designed to address/prevent secondary conditions in children with FASD. The intervention (Families on Track) is for children ages 4–8. It consists of an in-home caregiver component via the empirically supported “Families Moving Forward” program (15–17 sessions), together with an

additional empirically supported weekly child-focused group called PATHS (30 weeks). Families Moving Forward uses motivational interviewing and cognitive behavioral strategies to address parenting needs and child behavior. PATHS, the child group, focuses on emotion understanding, self-control, problem solving, self-regulation, self-esteem, and peer relationships. The study design incorporated randomization to either Families on Track or a referral group (standard clinical intervention referral). Thirty families were randomized and intermediate and 6-month follow-ups were done. The intervention was associated with significant improvements in emotion regulation, self-esteem, family needs being met, and knowledge of FASD. Overall, families reported satisfaction with the intervention.

Sanchez, J. (University of New Mexico) *Determining the long-term effects of prenatal alcohol exposure on spinal glial-immune responses underlying neuropathic touch hypersensitivity*. Mr. Sanchez reported on a study examining the impact of prenatal alcohol exposure on spinal astrocyte and microglial responsivity and, in turn, on sensitivity to light touch (allodynia). Prenatal alcohol exposure is known to activate glial cells, and it is hypothesized that glial activation potentiates pathological pain. Pregnant Long Evans rats consumed ethanol daily (or saccharin as a control), and the male offspring were examined at 4.5 months or 1 year. Hindpaw light touch withdrawal was evaluated in offspring who were exposed to a sciatic chronic constriction surgery or sham surgery. Prenatal alcohol exposure enhanced the allodynia in those with the induced injury compared to controls. Heightened spinal glial sensitivity was observed in ethanol-exposed animals who had the sciatic injury in the form of alterations in GFAP and IBA1 (observed with immunohistochemistry). These data suggest that prenatal alcohol exposure “primes” glial cells such that they are increasingly responsive following a “second hit”, such as a mechanical injury.

Wagner, J. (University of New Mexico). *Moderate prenatal alcohol exposure-induced deficits in the extinction of contextually conditioned fear learning in male & female rats*. Dr. Wagner presented data from a set of experiments designed to examine the effect of moderate prenatal alcohol exposure on behaviors that are sensitive to frontal cortical injury. Frontal cortical injury is known to be associated with abnormalities in contextual fear conditioning. A voluntary drinking paradigm was used in Long Evans rats who consumed 5% ethanol solution for 4 h per day throughout pregnancy. At 8 to 9 months of age, the offspring were subjected to fear conditioning (pairing a 30-sec tone with a foot shock) for 2 days followed by 8 days of extinction. Moderate prenatal alcohol exposure did not impair acquisition of the fear conditioning itself but abnormalities did emerge in the alcohol group during the extinction phase. Specifically, freezing behavior tended to persist longer during extinction in the alcohol-exposed animals than in the controls.

Inkelis, S. (San Diego State University). *Reduced basal ganglia and cerebellum volumes in adolescents and young adults with FASD*. Ms. Inkelis presented a study utilizing MRI to examine structural abnormalities in basal ganglia in participants with prenatal alcohol exposure vs. controls who were ages 13 to 30 years old. In general, data of this type in adults are underrepresented in the literature. Automated segmentation and parcellation were utilized to obtain caudate, putamen, pallidum, and nucleus accumbens volumes as well as the cerebellar cortical volume and cerebellar white matter volume. Group differences (prenatal alcohol-exposed vs. controls) were tested along with sex differences (male vs.

female) and an age. All basal ganglia regions were smaller in the alcohol-exposed group compared to controls. When total brain volume was included in the model, caudate and putamen volumes remained significant. Cerebellar white matter also remained significantly smaller in the alcohol-exposed group after controlling for total brain volume. An examination of the age term revealed significant negative associations between caudate and putamen volumes and age (older ages were associated with smaller volumes), but there were no group differences in these age trajectories.

Gavin, D. (University of Illinois – Chicago). *Neuronal PARP activity in fetal alcohol spectrum disorders*. Dr. Gavin presented data testing the hypothesis that prenatal alcohol exposure has long-term effects on the developing organism via effects on factors that maintain gene expression over the long term. Poly-ADP Polymerase (PARP) enzymes represent one such factor, in that they participate in histone post-translational modifications as well as DNA methylation/demethylation and are known to be highly inducible by ethanol. The experiment involved treating rat pups with 5.25 g/kg ethanol per day via intragastric intubation during post-natal days 4–9 (third trimester human exposure equivalent). Animals were sacrificed and examined at day 40. PARP activity was increased significantly in male offspring and at a trend level in female offspring. The same effect was observed in cultured neurons, and the effect was able to be eliminated with a PARP inhibitor. PARP1, PAR group, and PPAR γ promoter binding were measured via chromatin immunoprecipitation (ChIP). Promoter 5methylcytosine (5MC) and 5hydroxymethylcytosine (5HMC) were measured using methylated DNA immunoprecipitation (MeDIP) or hydroxymethylated MeDIP (hMeDIP). The enzyme that removes PAR groups (Parg) was reduced in the alcohol-exposed group – essentially the equivalent of increased PARP activity. Alterations in genes associated with neuronal survival were also seen (Klf4, c-Myc, and Dbnf9a). Similar changes were observed in the cell cultures. In summary, ethanol may induce neurodevelopmental changes and long-term abnormalities via PARP-related impacts on gene expression.

NOFAS “Stamp out Stigma” campaign. Communication by Dr. Charness, M. (Harvard University & Boston VA) & Dr. Wozniak, J. (University of Minnesota)

Dr. Charness and Dr. Wozniak provided an overview of the National Organization on Fetal Alcohol Syndrome’s recent campaign on stigma in FASD. The campaign is encouraging a shift in the language that we use in discussing FASD in an attempt to reduce the stigma for mothers and encourage more open conversations about prenatal alcohol exposure. Dr. Charness and Dr. Wozniak provided examples of how subtle changes in language (when speaking or writing about FASD) can shift the emphasis, reduce shaming and blaming of mothers, and provide a more accurate picture of the complex conditions that actually lead to prenatal alcohol exposure. Clinicians and scientists were encouraged to think about the potential impact of their own use of language as they continue their work in this field.

Rosett Award

This year the Rosett Award was given to Dr. Charles Goodlett, PhD (Department of Psychology at Indiana-Purdue University). Dr. Goodlett started his presentation by stressing the importance of collaborative work and mentioned how many other scientists had influenced his research. However, Dr. Goodlett's work has also influenced and inspired a generation of scientists interested in fetal alcohol spectrum disorders research. During his lecture, he shared some of the important lessons that he learned throughout his life as a researcher that can be useful for students, young investigators, and even more senior ones. Among them are: the importance of experimental design, the need to use the right model to ask the right questions, and the need to be aware that the mechanisms of action of alcohol can be very different depending on the dose and timing of the developmental exposure.

Dr. Goodlett was one of the first investigators to detect poor spatial memory in an animal model of FASD (Goodlett, Kelly, & West, 1987). In a series of classic papers, he showed that exposing rodent pups to alcohol impaired their performance in the Morris water maze as adults. Moreover, Dr. Goodlett showed that these results were dependent on timing, dose, and regimen of the alcohol exposure and the gender of the animal (females more susceptible than males) (Kelly, Goodlett, Hulsether, & West, 1988; Goodlett, Kelly, & West, 1987; Goodlett & Peterson, 1995). Importantly, Dr. Goodlett's studies were corroborated by Dr. Derek Hamilton and by Dr. Dan Savage and colleagues, who showed that children with FASD are impaired in a virtual Morris water task (Hamilton, Kodituwakku, Sutherland, & Savage, 2003).

Dr. Goodlett also showed that the "third trimester equivalent of human gestation" is a period when the cerebellum is particularly susceptible to the effects of alcohol (Goodlett, Marcussen & West, 1990). In addition to showing that developmental alcohol exposure can lead to cell death in the cerebellum, his studies were paramount in establishing the importance of the pattern and timing of exposure on ethanol effects (Goodlett & Eilers, 1997; Goodlett & Lundah, 1996; Marcussen, Goodlett, Mahoney, & West, 1994). In addition, with Dr. Jennifer Thomas, Dr. Goodlett showed that the effect of alcohol on Purkinje cell loss is correlated with deficits in motor performance (Thomas, Goodlett, & West, 1998).

In another series of classic studies, Dr. Goodlett in collaboration with Dr. Mark Stanton showed that animal models of FASD are impaired in the eyeblink conditioning (EBC) test, a test that has been extensively used to evaluate cerebellar-dependent motor learning in animal models (Stanton & Goodlett, 1998). Importantly, the effects on the EBC test were highly correlated with the cerebellar cell loss caused by alcohol (Green, Tran, Steinmetz, & Goodlett, 2002). More recently, impaired EBC was observed in children with FASD, confirming the predictive validity of the animal models (Jacobson et al., 2008).

Having described the effects of pattern and timing of alcohol exposure on cerebellar anatomy and function, Dr. Goodlett was one of the pioneers in proposing rehabilitative strategies to ameliorate neurobehavioral deficits in FASD. Together with Dr. Anna Klintsova and Dr. William Greenough, Dr. Goodlett showed the great potential of motor training for

reversing the effects of developmental alcohol exposure on cerebellar-dependent behavior (Klintsova et al., 1998, 2017; Wagner, Klintsova, Greenough, & Goodlett, 2003).

Dr. Goodlett closed his presentation by commenting on the original vision of Dr. Henry Rosett and the importance of fostering new insights from a diverse pool of experts, including clinicians and basic scientists. This vision can be well illustrated by Dr. Goodlett's work, the success of his collaborations, and his effort in translating his findings in animal models to human subjects.

Acknowledgments

Funding for this conference was made possible in part by funds from NIAAA and NICHD (R13 AA015661). The views expressed in written conference materials or publications, and by speakers and moderators do not necessarily reflect the official policies of the Department of Health and Human Services, nor imply endorsement by the U.S. government.

References

- Bell SH, Stade B, Reynolds JN, Rasmussen C, Andrew G, Hwang PA, et al. The remarkably high prevalence of epilepsy and seizure history in fetal alcohol spectrum disorders. *Alcoholism: Clinical and Experimental Research*. 2010; 34:1084–1089. DOI: 10.1111/j.1530-0277.2010.01184.x
- Brocardo PS, Gil-Mohapel J, Christie BR. The role of oxidative stress in fetal alcohol spectrum disorders. *Brain Research Reviews*. 2011; 67:209–225. DOI: 10.1016/j.brainresrev.2011.02.001 [PubMed: 21315761]
- David P, Subramaniam K. Prenatal alcohol exposure and early postnatal changes in the developing nerve-muscle system. *Birth Defects Research. Part A, Clinical and Molecular Teratology*. 2017; 73:897–903. DOI: 10.1002/bdra.20190
- Frye RE, Rotenberg A, Ousley M, Pascual-Leone A. Transcranial magnetic stimulation in child neurology: current and future directions. *Journal of Child Neurology*. 2008; 23:79–96. DOI: 10.1177/0883073807307972 [PubMed: 18056688]
- Gersner R, Dhamne SC, Zangen A, Pascual-Leone A, Rotenberg A. Bursts of high-frequency repetitive transcranial magnetic stimulation (rTMS), together with lorazepam, suppress seizures in a rat kainate status epilepticus model. *Epilepsy & Behavior*. 2016; 62:136–139. DOI: 10.1016/j.yebeh.2016.05.021 [PubMed: 27467275]
- Gersner R, Oberman L, Sanchez MJ, Chiriboga N, Kaye HL, Pascual-Leone A, et al. H-coil repetitive transcranial magnetic stimulation for treatment of temporal lobe epilepsy: A case report. *Epilepsy & Behavior Case Reports*. 2016; 5:52–56. DOI: 10.1016/j.ebcr.2016.03.001 [PubMed: 27114902]
- Goodlett CR, Eilers AT. Alcohol-induced Purkinje cell loss with a single binge exposure in neonatal rats: a stereological study of temporal windows of vulnerability. *Alcoholism: Clinical and Experimental Research*. 1997; 21:738–744.
- Goodlett CR, Kelly SJ, West JR. Early postnatal alcohol exposure that produces high blood alcohol levels impairs development of spatial learning. *Psychobiology*. 1987; 15:64–74.
- Goodlett CR, Lundah KR. Temporal determinants of neonatal alcohol-induced cerebellar damage and motor performance deficits. *Pharmacology, Biochemistry, and Behavior*. 1996; 55:531–540.
- Goodlett CR, Marcussen BL, West JR. A single day of alcohol exposure during the brain growth spurt induces brain weight restriction and cerebellar Purkinje cell loss. *Alcohol*. 1990; 7:107–114. [PubMed: 2328083]
- Goodlett CR, Peterson SD. Sex differences in vulnerability to developmental spatial learning deficits induced by limited binge alcohol exposure in neonatal rats. *Neurobiology of Learning and Memory*. 1995; 64:265–275. DOI: 10.1006/nlme.1995.0009 [PubMed: 8564380]
- Green JT, Tran T, Steinmetz JE, Goodlett CR. Neonatal ethanol produces cerebellar deep nuclear cell loss and correlated disruption of eyeblink conditioning in adult rats. *Brain Research*. 2002; 956:302–311. [PubMed: 12445699]

- Hamilton DA, Kodituwakku P, Sutherland RJ, Savage DD. Children with Fetal Alcohol Syndrome are impaired at place learning but not cued-navigation in a virtual Morris water task. *Behavioural Brain Research*. 2003; 143:85–94. [PubMed: 12842299]
- Hamilton GF, Jablonski SA, Schiffino FL, St Cyr SA, Stanton ME, Klintsova AY. Exercise and environment as an intervention for neonatal alcohol effects on hippocampal adult neurogenesis and learning. *Neuroscience*. 2014; 265:274–290. DOI: 10.1016/j.neuroscience.2014.01.061 [PubMed: 24513389]
- Hannigan JH, Berman RF, Zajac CS. Environmental enrichment and the behavioral effects of prenatal exposure to alcohol in rats. *Neurotoxicology and Teratology*. 1993; 15:261–266. [PubMed: 8413080]
- Hsieh TH, Lee HH, Hameed MQ, Pascual-Leone A, Hensch TK, Rotenberg A. Trajectory of Parvalbumin Cell Impairment and Loss of Cortical Inhibition in Traumatic Brain Injury. *Cerebral Cortex*. 2016; [epub ahead of print]. doi: 10.1093/cercor/bhw318
- Jacobson SW, Stanton ME, Molteno CD, Burden MJ, Fuller DS, Hoyme HE, et al. Impaired eyeblink conditioning in children with fetal alcohol syndrome. *Alcoholism: Clinical and Experimental Research*. 2008; 32:365–372. DOI: 10.1111/j.1530-0277.2007.00585.x
- Kelly SJ, Goodlett CR, Hulsether SA, West JR. Impaired spatial navigation in adult female but not adult male rats exposed to alcohol during the brain growth spurt. *Behavioural Brain Research*. 1988; 27:247–257. [PubMed: 3358862]
- Klintsova AY, Cowell RM, Swain RA, Napper RM, Goodlett CR, Greenough WT. Therapeutic effects of complex motor training on motor performance deficits induced by neonatal binge-like alcohol exposure in rats. I. Behavioral results. *Brain Research*. 1998; 800:48–61. [PubMed: 9685583]
- Klintsova AY, Scamra C, Hoffman M, Napper RM, Goodlett CR, Greenough WT. Therapeutic effects of complex motor training on motor performance deficits induced by neonatal binge-like alcohol exposure in rats: II. A quantitative stereological study of synaptic plasticity in female rat cerebellum. *Brain Research*. 2017; 937:83–93.
- Lenz M, Vlachos A. Releasing the Cortical Brake by Non-Invasive Electromagnetic Stimulation? rTMS Induces LTD of GABAergic Neurotransmission. *Frontiers in Neural Circuits*. 2016; 10:96.doi: 10.3389/fncir.2016.00096 [PubMed: 27965542]
- Marcussen BL, Goodlett CR, Mahoney JC, West JR. Developing rat Purkinje cells are more vulnerable to alcohol-induced depletion during differentiation than during neurogenesis. *Alcohol*. 1994; 11:147–156. [PubMed: 8204201]
- Nakano M, Yamada S, Udagawa R, Kato N. Frequency-dependent requirement for calcium store-operated mechanisms in induction of homosynaptic long-term depression at hippocampus CA1 synapses. *The European Journal of Neuroscience*. 2004; 19:2881–2887. DOI: 10.1111/j.0953-816X.2004.03390.x [PubMed: 15147321]
- Nicita F, Verroti A, Pruna D, Striano P, Capovilla G, Savasta S, et al. Seizures in fetal alcohol spectrum disorders: evaluation of clinical, electroencephalographic, and neuroradiologic features in a pediatric case series. *Epilepsia*. 2014; 55:e60–66. DOI: 10.1111/epi.12638 [PubMed: 24815902]
- Oberman L, Ifert-Miller F, Najib U, Bashir S, Woolacott I, Gonzalez-Heydrich J, et al. Transcranial magnetic stimulation provides means to assess cortical plasticity and excitability in humans with fragile X syndrome and autism spectrum disorder. *Frontiers in Synaptic Neuroscience*. 2010; 2:26.doi: 10.3389/fnsyn.2010.00026 [PubMed: 21423512]
- Rotenberg A, Bae EH, Takeoka M, Tormos JM, Schacter SC, Pascual-Leone A. Repetitive transcranial magnetic stimulation in the treatment of epilepsy partialis continua. *Epilepsy & Behavior*. 2009; 14:253–257. DOI: 10.1016/j.yebeh.2008.09.007 [PubMed: 18832045]
- Rotenberg A, Muller P, Birnbaum D, Harrington M, Riviello JJ, Pascual-Leone A, et al. Seizure suppression by EEG-guided repetitive transcranial magnetic stimulation in the rat. *Clinical Neurophysiology*. 2008; 119:2697–2702. DOI: 10.1016/j.clinph.2008.09.003 [PubMed: 18977170]
- Sheng M, Ertürk A. Long-term depression: a cell biological view. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*. 2013; 369:20130138.doi: 10.1098/rstb.2013.0138

- Smiley JF, Saito M, Bleiwas C, Masiello K, Ardekani B, Guilfoyle DN, et al. Selective reduction of cerebral cortex GABA neurons in a late gestation model of fetal alcohol spectrum disorder. *Alcohol*. 2015; 49:571–580. DOI: 10.1016/j.alcohol.2015.04.008 [PubMed: 26252988]
- Stanton ME, Goodlett CR. Neonatal ethanol exposure impairs eyeblink conditioning in weanling rats. *Alcoholism: Clinical and Experimental Research*. 1998; 22:270–275.
- Thomas JD, Goodlett CR, West JR. Alcohol-induced Purkinje cell loss depends on developmental timing of alcohol exposure and correlates with motor performance. *Brain Research. Developmental Brain Research*. 1998; 105:159–166. [PubMed: 9541734]
- Wagner JL, Klintsova AY, Greenough WT, Goodlett CR. Rehabilitation training using complex motor learning rescues deficits in eyeblink classical conditioning in female rats induced by binge-like neonatal alcohol exposure. *Alcoholism: Clinical and Experimental Research*. 2003; 37:1561–1570. DOI: 10.1111/acer.12122.