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THE BASIC SCIENCE OF FETAL ALCOHOL SPECTRUM DISORDER: NOVEL MOLECULAR MECHANISMS AND POTENTIAL TARGETS FOR THERAPEUTIC INTERVENTION.

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In recent years, there have been tremendous advances in our understanding of the effects of ethanol on the developing brain. This symposium brought together a mixture of young and senior investigators from 3 different countries and 5 different nationalities to highlight these advances, which have led to a better understanding of the pathophysiology of fetal alcohol spectrum disorder and the identification of novel interventions that could be used to treat this disorder. The talks showed data obtained using a wide range of experimental approaches, including neurochemical, electrophysiological, optical imaging, histological and behavioral approaches. Drs. Reynold's and Valenzuela's talks focused on the effects of ethanol on neurotransmission. Dr. Naassila presented data indicating that exposure to ethanol during development results in persistent modifications in different brain areas ultimately increasing vulnerability to develop drug dependence. The final two talks concentrated on therapeutic interventions. Dr. Medina showed that the phosphodiesterase inhibitor vinpocetine restores ocular dominance plasticity in a ferret model of fetal alcohol exposure. Dr. Klintsova ended the symposium by talking about the effects of fetal ethanol exposure on the synaptic architecture of the motor and cerebellar cortices, and the therapeutic actions of complex motor skill training on these effects.

CHRONIC PRENATAL ETHANOL EXPOSURE ALTERS GLUCOCORTICOID REGULATION OF GLUTAMATE RELEASE IN THE HIPPOCAMPUS OF THE GUINEA PIG

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Chronic excessive maternal drinking of ethanol during pregnancy can produce teratogenic effects in children. These ethanol-induced birth defects are collectively termed fetal alcohol spectrum disorders (FASD), including fetal alcohol syndrome. The most debilitating feature of

ethanol teratogenicity is central nervous system (CNS) injury, which can manifest as intellectual, neurological and behavioural abnormalities. Clinical and experimental animal data indicate that the hippocampus is a major target site of ethanol CNS teratogenicity [Abel, 1985; Guerri, 1998; West and Pierce, 1986]. In view of the major role for the hippocampus in the processes of learning and memory, and behavioral regulation [Traub and Miles, 1991], morphological and/or functional changes in this brain region may be critical for the mental deficiency and hyperactivity of FASD.

Experimental animal studies have shown that the CNS teratogenic effects of ethanol in various mammalian species are qualitatively similar to those in the human [Zajac and Abel, 1992]. However, the mechanism of ethanol CNS teratogenicity appears to be multi-faceted and has not been fully elucidated. Several critical reviews [Abel, 1998; Abel and Hannigan, 1995; Guerri, 1998; Goodlett et al., 2005; Kimura et al., 2000; Reynolds and Brien, 1995; West and Pantazis, 1994], have focused on the many mechanisms that have been postulated. Of these, two that are receiving increasing attention for ethanol CNS teratogenicity are altered function of excitatory amino acid (primarily glutamate) receptors that can lead to abnormal neuronal development and/or neurodegeneration, and neuroendocrine disturbances that can have acute and long-term detrimental effects on brain structure and function. In particular, there is increasing evidence suggesting that dysregulation of stress responses, specifically involving the hypothalamic-pituitary-adrenocortical (HPA) axis, plays an important role in chronic prenatal ethanol exposure (CPEE)-induced brain injury [Vazquez, 1998]. Several studies conducted in the rat have linked CPEE to HPA axis hyper-responsiveness produced by a variety of stressful events [Lee et al., 1990; Taylor et al., 1982; Nelson et al., 1986; Weinberg, 1992; Weinberg and Gallo, 1982; Weinberg et al., 1995]. Moreover, recent evidence has demonstrated that in both non-human primate offspring [Schneider et al., 2004] and human infants [Jacobson, 1999], prenatal exposure to ethanol induces hyper-responsiveness of the HPA axis to activation by stress.

In a previous study, we demonstrated that chronic maternal ethanol administration acts as a maternal stressor, causing activation of the HPA axis and leading to abnormal elevation in maternal cortisol concentration in early to mid gestation, but not in late gestation during the last week of pregnancy [Iqbal et al., 2005]. Moreover, CPEE induced hyper-responsiveness of the HPA axis to activation by swim stress in young adult guinea pigs [Iqbal et al., 2005], suggesting that the CPEE-induced perturbation of HPA function during fetal life causes a long-lasting reprogramming of HPA axis function in offspring. These observations led us to test the hypothesis that CPEE activates the near-term fetal HPA axis to produce high cortisol exposure in the fetal compartment. In the guinea pig, the near-term fetal adrenal gland is sufficiently developed to meet the cortisol needs of the fetus before birth [Dalle and Delost, 1980]. Moreover, since exposure to high glucocorticoid concentration may injure the developing brain, possibly via an excitotoxic mechanism involving excess glutamate [Mulholland et al., 2005], we examined the effect of CPEE on glucocorticoid regulation of amino acid neurotransmitter release in the hippocampus of the near-term fetal guinea pig, and on the expression of mRNA encoding for glucocorticoid and mineralocorticoid receptors, and for the NR1 subunit of the *N*-methyl-D-aspartate (NMDA) subtype of excitatory glutamate receptors.

Pregnant Dunkin-Hartley strain guinea pigs were assigned to two treatment groups: a) 4g ethanol (30% v/v) / kg maternal body weight / day with *ad libitum* access to food and water; and b) isocaloric sucrose (42% w/v) with pair-feeding to an ethanol-treated animal and *ad libitum* access to water. Daily treatments were administered as two equal divided doses separated by two hours from gestational day (GD) 2 to GD 63. At GD 63, pregnant guinea pigs were euthanized one hour after the second divided dose and the litter of each pregnant animal was delivered by cesarean section. Cortisol and adrenocorticotrophic hormone (ACTH) concentrations were determined in fetal plasma. Fetal hippocampal brain slices were prepared for the determination of basal and electrically-stimulated Glu release in the presence or absence of the selective glucocorticoid receptor agonist, dexamethasone. One hemisphere of each fetal brain was prepared for in situ

hybridization experiments to determine the levels of mRNAs coding for glucocorticoid and mineralocorticoid receptors, and for the NR1 subunit of the N-methyl-D-aspartate (NMDA) receptor.

At GD 63, individual near-term fetal body weight, brain weight, and hippocampal weight were lower in the CPEE group compared with the isocaloric-sucrose/pair-fed group (Table 1), establishing that the chronic maternal ethanol regimen used in this study was sufficient to induce CNS teratogenic effects in the near-term fetuses. On GD 63, one hour after the second divided dose of ethanol, the near-term fetal plasma concentrations of cortisol and ACTH were increased in the CPEE group compared with the isocaloric-sucrose/pair-fed group (Table 1).

In the absence of dexamethasone, there was no difference in basal glutamate or GABA release in the near-term fetal hippocampal slices between the CPEE and isocaloric-sucrose/pair-fed groups. However, when hippocampal slices were exposed to 3 μ M dexamethasone, there was a selective increase in basal glutamate release in the CPEE group compared with the isocaloric-sucrose/pair-fed group. There was no effect of dexamethasone on basal glutamate release in the near-term fetal hippocampal slices of the isocaloric-sucrose/pair-fed group. When hippocampal slices were electrically stimulated, there were significant main effects of maternal treatment regimen and dexamethasone concentration on glutamate release. Post-hoc analysis revealed that electrically stimulated glutamate release was greater in the hippocampal slices obtained from CPEE fetuses, and that low a (0.3 μ M), but not a high (3 μ M), concentration of dexamethasone inhibits electrically stimulated glutamate release in the near-term fetal hippocampus. In contrast to the glutamate release data, there was no effect of maternal treatment regimen or dexamethasone concentration on either basal or stimulated GABA release in the same near-term fetal hippocampal slices.

CPEE resulted in an increase in glucocorticoid receptor and NR1 subunit, but not mineralocorticoid receptor, mRNA expression in the near-term fetal hippocampus for the CPEE group compared with the isocaloric-sucrose/pair-fed group. For glucocorticoid receptor mRNA expression, there were significant main effects of maternal treatment regimen and hippocampal region, and a significant interaction between maternal treatment regimen and hippocampal region. Post-hoc analysis revealed that the interaction was due to the fact that, whereas the glucocorticoid receptor mRNA was increased in all three subfields of the hippocampus (CA1, CA3, and dentate gyrus), the change in glucocorticoid mRNA expression was greatest in the CA1 region of the hippocampus. NR1 mRNA expression was increased in all three subfields of the near-term fetal hippocampus (CA1, CA3, and dentate gyrus).

ACTH does not distribute across the guinea pig placenta [Schellenberg, 1997], and therefore both ACTH and cortisol were quantified in near-term fetal blood plasma to obtain a measure of fetal HPA axis activity. Our data demonstrate that CPEE activates the near-term fetal guinea pig HPA axis, as shown by increased ACTH and cortisol concentrations in fetal plasma. Studies conducted in the rat have demonstrated that exposure to high glucocorticoid concentration can exacerbate hippocampal injury induced by ischemia and seizures in the adult [McEwen, 2001], or ethanol withdrawal in neonatal hippocampal slice cultures [Mulholland et al., 2005]. In the hippocampus, exposure to high concentration of adrenocorticosteroid can increase extracellular glutamate concentration [Lowy et al., 1993; Moghaddam et al., 1994], and this effect also occurs with dexamethasone, a synthetic glucocorticoid receptor agonist [Abraham, 1996]. The mechanism by which glucocorticoids exacerbate hippocampal injury is proposed to be related to increased synaptic glutamate accumulation, thereby leading to over-activation of postsynaptic NMDA receptors and subsequent increased intracellular calcium ion concentration [Armanini et al., 1990; Mulholland et al., 2005].

The CA1 region of the guinea pig hippocampus, which is selectively vulnerable to CPEE-induced neuronal cell death in the perinatal period [Gibson et al., 2000; McGoey et al., 2003], has the highest expression of glucocorticoid receptor mRNA in the near-term fetus [Owen and

Matthews, 2003]. Our studies demonstrate that this region of the near-term fetal hippocampus also has the greatest CPEE-induced increase in glucocorticoid receptor mRNA expression. Coupled with the upregulation in expression of the NR1 subunit of the NMDA receptor, it is postulated that CPEE produces direct and cortisol-dependent NMDA-receptor-mediated neurodegeneration in the CA1 region of the fetal hippocampus. Studies that will directly test this postulate are currently underway in our laboratory.

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Table 1: CPEE-Induced Brain Injury and Altered HPA Axis Function in the GD 63 Fetal Guinea Pig.

Outcome Variable	Treatment	
	CPEE	Isocaloric-Sucrose/Pair-Feeding
Body Weight (g)	70 ± 3*	95 ± 2
Brain Weight (g)	1.59 ± 0.04*	2.12 ± 0.02
Hippocampal Weight (g)	0.084 ± 0.008*	0.124 ± 0.004
[Cortisol] (µg/dL)	38.8 ± 4.9*	25.4 ± 4.4
[ACTH] (ng/mL)	0.71 ± 0.08*	0.43 ± 0.08

Data are reported as group means ± SEM for 12-15 fetuses in each group. *, P < 0.05 compared with the isocaloric-sucrose/pair-fed treatment.

MODULATION OF DEVELOPING SYNAPSES BY ETHANOL: THE VIEW FROM THE SLICE.

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Ethanol (EtOH) severely affects the brain, producing neurobehavioral deficits later in life such as learning and memory alterations, which may be the result of hippocampal damage. The mechanism by which EtOH damages the hippocampus is not fully understood. EtOH acts as a depressant in the mature CNS and it has been assumed that this also applies to immature neurons. It has been proposed that EtOH damages developing neurons by directly inhibiting NMDA receptors and potentiating GABA-A receptors, leading to excessive inhibition of neuronal activity. We have found evidence that this is actually not the case in the CA1 and CA3 hippocampal subfields. In the CA3 region, we found that ethanol increases the frequency of giant depolarizing potentials (GDPs), a network-driven form of neuronal activity that is thought to be important for circuit maturation in this hippocampal region (Galindo et al., 2005). This effect appears to be driven by an increase in GABA release at interneuron-to-pyramidal neuron synapses. This increase in GABA release excites pyramidal neurons via activation of GABA_A receptors, which have excitatory actions during development because immature neurons have

higher intracellular Cl⁻ levels than mature neurons. This is a consequence of low levels of expression of the Cl⁻ exporter, KCC2, and high expression of the Cl⁻ importer, NKCC1.

We also characterized the effects of prolonged EtOH exposure on GDPs (Galindo and Valenzuela, 2006). Hippocampal coronal slices from neonatal rats were exposed to control artificial cerebrospinal fluid (ACSF) or ACSF plus 50 mM EtOH for 3-4 h. Recordings from CA3 pyramidal neurons did not show any evidence of tolerance development to the GDP stimulating effects of EtOH did not occur after continuous exposure. We also exposed neonatal rats to air or air plus 1.9 g/dl EtOH in vapor chambers for 4h/day for 1 or 3 days (neonatal peak blood EtOH concentration = 40-45 mM). Slice electrophysiological studies performed 24 h after the end of EtOH exposure revealed no statistically significant difference in the acute effect of 50 mM EtOH on GDP frequency in samples from neonates exposed to air or air plus EtOH. Thus, EtOH persistently stimulates network-driven oscillatory activity in the developing hippocampus and this could be in part the mechanism by which EtOH alters circuit formation in this brain region.

We next focused our attention on characterizing EtOH's effects on glutamatergic transmission in the CA3 hippocampal region (Mameli et al., 2005). Studies suggest that EtOH damages this region, in part, via alterations in glutamatergic transmission. Surprisingly, we found that acute ethanol exposure depresses AMPA receptor but not NMDA receptor-mediated currents evoked by exogenous agonist application in CA3 pyramidal neurons. Ethanol significantly decreased the amplitude of both AMPA and NMDA receptor-mediated EPSCs evoked by stimulation of the perforant path-mossy fiber axons. EtOH also increased the paired-pulse ratio and decreased the frequency of miniature EPSCs recorded in the presence of KCl. Omega-conotoxin-GVIA occluded the effect of ethanol on NMDA EPSCs, indicating that ethanol decreases glutamate release via inhibition of N-type voltage-gated Ca²⁺ channels. In more mature rats, these effects were not observed; however, we detected acute inhibition of NMDA-mediated currents by EtOH. Collectively, these findings indicate that postsynaptic NMDA receptors are not the primary targets of ethanol early in development.

Finally, we investigated the effects of ethanol on glutamatergic transmission in CA1 pyramidal neurons (Mameli and Valenzuela, 2006). We found that EtOH exposure strengthens AMPA receptor-mediated transmission by reducing the failure rate of low efficacy synapses. This effect is age-dependent and is occluded by application of the neurosteroid pregnenolone sulfate (PREGS). Importantly, an anti-PREGS antibody scavenger and blockade of PREGS synthesis prevented the effect of EtOH. These data indicate that the deleterious effects of EtOH on hippocampal development are mediated in part by alterations in neurosteroid production, which results in premature stabilization of excitatory synapses in the CA1 region.

We conclude that the mechanism by which EtOH modulates glutamatergic and GABAergic transmission is age-dependent and synapse-specific, and challenge the view that postsynaptic NMDA and GABA_A receptors are the main targets of EtOH early in development.

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EFFECTS OF EARLY LIFE ETHANOL EXPOSURE ON VULNERABILITY TO DRUGS OF ABUSE.

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Prenatal alcohol exposure can cause Fetal Alcohol Syndrome (FAS), first described by the french clinician Paul Lemoine in 1968. Prenatal and postnatal growth retardation, facial dysmorphology and CNS dysfunction are the defining feature of FAS.

Recently several clinical studies have indicated that prenatal alcohol exposure is a risk factor for the development of drinking problems, drugs dependence and mental illness in humans (Famy et al., 1998; Alati et al., 2006). These studies also indicated that offspring drinking problems were predicted more effectively by prenatal ethanol exposure than family history of alcoholism. Thus clinical and epidemiological findings provide evidence in establishing a direct link between early life ethanol exposure and subsequent patterns of ethanol intake. Only few preclinical studies investigated the effects of early life ethanol exposure on later vulnerability to ethanol and drugs of abuse. Data regarding neurobiological bases of this potential vulnerability induced by early life ethanol exposure are scarce. Many of the physical anomalies and behavioural abnormalities observed clinically are mirrored in animal models of FAS. This epidemiological evidence that prenatal exposure to ethanol predicts ethanol use in adolescence and early adulthood in humans has also been suggested in animal studies.

In the present work, we have investigated the long-term effects of pre- and post-natal ethanol exposure on drugs vulnerability in rats. Ethanol-treated dams received as sole drinking fluid a 10% (v/v) ethanol solution, for 4 weeks before mating and had unlimited access to standard rat chow, as previously described (Naassila and Daoust, 2002; Dubois et al., 2006). The ad libitum control group had unlimited access to standard rat chow and water. After successful matings, the ethanol-treated group was maintained on 10% ethanol solution throughout gestation and lactation. Since genetic factors are involved in drugs of abuse vulnerability we used two rat strains, Sprague-Dawley and Long-Evans rats. We have analyzed the behavioural responses (drugs consumption, sensitization, sensitivity to the locomotor and rewarding effects of different drugs of abuse (alcohol, cocaine, amphetamine and morphine) in young adult rat offspring. We have also investigated if the observed persistent modifications of behavioural response to drugs of abuse were associated to modifications of both gene and protein expression, in different brain areas (hippocampus, hypothalamus/thalamus, striatum, cerebellum and frontal cortex). Using quantitative real time PCR we analyzed the expression of some candidate genes involved in synaptic function, cellular signaling, neuronal growth and protein trafficking. Differential protein expression was assessed by the two-dimensional gel electrophoresis in both hippocampus and striatum.

Our results show that progeny exposed to early life ethanol displayed increased consumption of both ethanol and morphine solutions and increased sensitivity to the rewarding effects of cocaine assessed in the conditioned place preference test. The increased sensitivity to cocaine-induced conditioned place preference was observed in both Sprague-Dawley and Long Evans rats. This increase in drug consumption was related neither to anhedonia nor anxiety. In addition, the sensitivity to hypothermic effects of ethanol and ethanol metabolism were not altered by early life ethanol exposure. In contrast, the sensitization to psychostimulants was facilitated after early life ethanol exposure while no difference of sensitivity was observed after a single injection in

Sprague-Dawley rats. In contrast, in Long Evans strain, early life ethanol-exposed rats were more sensitive to the locomotor effect of both amphetamine and cocaine and this difference was observed in both male and female rats.

Our gene expression analysis reveals that early life ethanol exposure altered expression of a large number of genes in the brain regions that are involved in the reinforcing effects of drugs of abuse (frontal cortex, hippocampus, striatum, thalamus/hypothalamus and cerebellum). This long term alteration of expression of genes involved in synaptic transmission, plasticity, development and of behavioural responses to ethanol and other drugs of abuse may confer an increased liability for addiction in exposed offspring. Results of our proteomic study indicate that level of several hippocampal proteins was altered by early life ethanol exposure. Interestingly, some of these proteins have been involved in neurodegenerative diseases such as Parkinson's disease and have also been shown to be altered in brain of alcoholics.

Our results indicate that early life ethanol exposure has long-term effects on behavioural response to alcohol and other drugs of abuse and that these modifications are associated to persistent neurochemical alterations at both gene and protein expression levels.

In a context where research is needed to understand the mechanisms underlying the increased vulnerability to drugs abuse disorders induced by early life ethanol exposure, our rat model of pre- and postnatal ethanol exposure (Naassila and Daoust, 2002; Dubois et al., 2006) will be useful to investigate the neurobiological bases of this increased vulnerability to drugs of abuse.

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RESTORING NEURONAL PLASTICITY IN A MODEL OF FETAL ALCOHOL SPECTRUM DISORDER

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Fetal Alcohol Spectrum Disorder (FASD) is characterized by a constellation of behavioral and physiological abnormalities including learning and sensory deficits. There is growing evidence that abnormalities of neuronal plasticity underlie these deficits (Medina et al., 2003; Medina and Ramoa, 2005; Rema and Ebner, 1999; Richardson et al., 2002; Stanton and Goodlett, 1998). However, the cellular and molecular mechanisms by which prenatal alcohol exposure disrupts neuronal plasticity remains elusive.

Much of our knowledge on the basic mechanisms of neuronal plasticity came from studies in the visual cortex. Classic studies showed that if the eyelid of one eye is closed during a period of development (monocular deprivation), neurons in the ocular dominance column that should receive visual information from the deprived eye become instead wired to the experienced eye. As

a result, ocular dominance columns representing the deprived eye shrink while the columns representing the experienced (open) eye expand (Hubel et al., 1977; Hubel and Wiesel, 1970; Wiesel and Hubel, 1965). Ocular dominance plasticity has been widely used to study the mechanisms that underlie neuronal plasticity in general and shares common mechanisms with learning and memory, such as dependence on the activation of the NMDA receptor (Bear et al., 1990; Roberts et al., 1998) and the transcription factor CREB (Mower et al., 2002).

Using a combination of electrophysiology and optical imaging of intrinsic signals we recently showed that ocular dominance plasticity is permanently impaired in a ferret model of third trimester alcohol exposure (Medina et al., 2003; Medina and Ramoa, 2005). Ferrets were treated with alcohol between post natal day (P) 10 to P30 and examined following a prolonged (15-20 days) alcohol-free period. In these animals, single-unit recordings revealed a large number of neurons that still responded to stimulation of the visually deprived eye and optical imaging revealed that ocular dominance columns were not affected by the period of deprivation. The impairment of ocular dominance plasticity in alcohol-exposed ferrets probably reflects the poor capacity of their brains to reorganize neuronal connections. Interestingly, ocular dominance plasticity was not impaired when alcohol exposure was delayed (P20-P40) or shortened for just 5 days (P10-P25), indicating that timing and duration of alcohol exposure is crucial for its effects on cortical plasticity. These findings provided us with a novel approach to investigate what mechanisms underlie neuronal plasticity deficits in FASD and how it can be restored.

Phosphodiesterase (PDE) inhibitors have been considered good candidates as plasticity enhancers in animal models and human subjects. PDE inhibitors prevent breakdown of cAMP to 5'-AMP, maintaining activation of protein kinases and the transcription factor CREB leading to the expression of plasticity-related genes (Beavo, 1995). Vinpocetine treatment has been shown to facilitate LTP (Molnar et al., 1994; Molnar and Gaal, 1992), enhance the structural dynamics of dendritic spines (Lendvai et al., 2003), improve memory retrieval (DeNoble, 1987), and enhance performance on cognitive tests in humans (Hindmarch et al., 1991). However little is known about the effectiveness of this drug to improve neuronal plasticity in cases of mental disorders. Recently, we showed that vinpocetine restores the impairments on ocular dominance plasticity caused by early alcohol exposure (Medina et al., 2006). Alcohol-treated ferrets (third trimester equivalent exposure) received vinpocetine (20 mg/kg, i.p. daily) or vehicle solution during the period of monocular deprivation (around P45, way after the period of alcohol exposure). Single unit recordings or optical imaging of intrinsic signals showed that as expected, the ocular dominance shift was markedly reduced in animals that had been exposed to alcohol during the third trimester equivalent of human gestation. In contrast, the ocular dominance distribution was shifted towards the experienced eye in the visual cortex of alcohol-exposed ferrets treated with vinpocetine. This shift was similar to that observed in saline-exposed ferrets, demonstrating that vinpocetine restored ocular dominance plasticity in alcohol-exposed animals (Medina et al., 2006). Our findings may be potentially significant from a clinical standpoint and suggest that PDE inhibitors have a role to play in restoring neuronal plasticity in FASD. We propose that plasticity enhancement may be an effective approach to ameliorate neurological problems caused by alcohol exposure during development.

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PLASTICITY OF DIFFERENT SYNAPTIC TYPES AND GLIA IN CEREBELLUM AND CORTICAL AREAS AFTER NEONATAL BINGE-LIKE ALCOHOL EXPOSURE AND ADULT INTERVENTION

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Alcohol exposure in neonatal rats, in a model of binge-drinking during the third trimester of human pregnancy, permanently reduces the number of cerebellar neurons and impairs motor performance. Rats exposed to alcohol (5.25 g/kg/day) from postnatal days 4-9, maintain experience-induced neuronal plasticity in adulthood when the experience is targeted to a specific skill, such as complex motor training. Twenty days of complex motor skill training eliminated motor performance deficits induced by neonatal binge alcohol exposure, and increased the number of parallel fiber synapses per Purkinje cell in the cerebellar cortex, as well as the number of excitatory synapses per neuron in layer II/III of the motor cortex. In addition, coordinated changes in glia (especially glial peri-synaptic processes) in cerebellar cortex are elicited by the complex motor task learning in both suckle control and alcohol exposed treatment groups ($F_{1,32}=8.01$, $p<.01$). Current studies examine changes in the climbing fiber synapses on Purkinje cells after binge-like neonatal alcohol exposure and motor skill learning. Our data suggests that motor learning-associated synaptogenesis is supported by enhanced plasticity of glial processes that persists in cerebellum and cortical areas of the AE rats.

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