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# ROLE OF CENTRAL NERVOUS SYSTEM INSULIN RESISTANCE IN FETAL ALCOHOL SPECTRUM DISORDERS

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## Abstract

Fetal alcohol spectrum disorder (FASD) is the most common preventable cause of mental retardation in the USA. Ethanol impairs neuronal survival and function by two major mechanisms: 1) it inhibits insulin signaling required for viability, metabolism, synapse formation, and acetylcholine production; and 2) it functions as a neurotoxicant, causing oxidative stress, DNA damage and mitochondrial dysfunction. Ethanol inhibition of insulin signaling is mediated at the insulin receptor (IR) level and caused by both impaired receptor binding and increased activation of phosphatases that reverse IR tyrosine kinase activity. As a result, insulin activation of PI3K-Akt, which mediates neuronal survival, motility, energy metabolism, and plasticity, is impaired. The neurotoxicant effects of ethanol promote DNA damage, which could contribute to mitochondrial dysfunction and oxidative stress. Therefore, chronic in utero ethanol exposure produces a dual state of CNS insulin resistance and oxidative stress, which we postulate plays a major role in ethanol neurobehavioral teratogenesis. We propose that many of the prominent adverse effects of chronic prenatal exposure to ethanol on CNS development and function may be prevented or reduced by treatment with peroxisome-proliferated activated receptor (PPAR) agonists which enhance insulin sensitivity by increasing expression and function of insulin-responsive genes, and reducing cellular oxidative stress.

### Keywords

Insulin signaling; mitochondria; oxidative stress; energy metabolism; insulin sensitizer; insulin resistance; central nervous system

## The Public Health Problem

Gestational exposure to alcohol is the leading preventable cause of mental retardation in North America. As many as 7 per 1000 women binge drink during pregnancy, and even higher percentages consume alcohol at various times during pregnancy. Binge drinking has not declined among women of child-bearing age in the USA.[1] The syndrome caused by maternal consumption of alcohol during pregnancy is termed, “Fetal Alcohol Spectrum Disorders” (FASD).[2] FASD is not one entity, but rather a collection of heterogeneous disorders that range broadly in terms of severity and outcomes.[3] Fetal alcohol syndrome (FAS) is the most severe form of FASD, and associated with intrauterine growth restriction, central nervous system (CNS) malformations, mental retardation, and craniofacial and skeletal defects, whereas less severe effects of prenatal alcohol exposure have been classified as alcohol-related birth defects and alcohol-related neurodevelopmental disorders.



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[4] The economic burden of FASD/FAS is high[5], and despite public health efforts, the incidence rates have not declined in the past decade.[6] Epidemiologic data indicate that in the US, FAS rates range from 0.2-1.5 per 1,000 live births, whereas alcohol-related birth defects and alcohol-related neuro-developmental disorders occur in approximately 0.9% of live births.[7]

The tendency to abuse alcohol during pregnancy could be consequential to heavy chronic or binge alcohol abuse during adolescence. In this regard, attendant sustained structural and functional abnormalities in the brain[8-11], including deficits in performing executive, visual-spatial[12-14], and working memory tasks[15], might contribute to poor judgment concerning alcohol and drug misuse during pregnancy. Increased tendency to abuse alcohol during child-bearing years, including its inadvertent misuse during pregnancy, could account for the high prevalence rates of attention deficit hyperactivity disorder (ADHD) in the United States.[16-18] Children with ADHD and deficits in visual-spatial, fine motor, and cerebellar learning are more challenged[8,19-21], and if their problems go unrecognized or ignored, the risk for engaging in aberrant, socially unacceptable behaviors, including drug and alcohol dependence and abuse as adolescents or young adults, also increases.[22-26] Experimentally, it has been demonstrated that alcohol misuse during adolescence does increase the propensity to consume alcohol as adults.[27] In essence, alcohol abuse in adolescents and young adults establishes a vicious cycle whereby impaired judgment and cognition increase the risk of causing pre-natal alcohol exposure. Long-term consequences of prenatal alcohol exposure range from behavioral abnormalities to learning disabilities and ADHD, to mental retardation.[19,20,28-31] Presumably, structural and functional CNS abnormalities mediate the increased tendency of adolescents and young people to participate in high-risk behaviors, including alcohol abuse during pregnancy.

## Prenatal Ethanol Exposure Impairs CNS Growth and Development

Heavy gestational exposure to alcohol can be teratogenic, resulting in gross abnormalities in the developing CNS[2,17,32-35] including microencephaly, white matter hypomyelination, hydrocephalus, cerebellar hypoplasia, neuronal migration disorders, and neuroglial heterotopias.[5,32,36-43] Moderate levels of prenatal alcohol exposure tend to be less harmful, although they still lead to structural and functional abnormalities, including altered gene expression in the brain[40,44-46] and impairments in cognitive, behavioral, and motor functions.[47] Ethanol mediates its neurotoxic effects on proliferating and immature neuronal cells by causing permanent structural and functional abnormalities that promote cell death[41,48-50] and impair neurotransmission and plasticity.[27,51-55] Correspondingly, prenatal ethanol exposure causes sustained cognitive-motor deficits in children, adolescents, and young adults.[20,28 29]

Although the mechanisms are not entirely understood, based on the known targets of alcohol neurotoxicity and structure-function relationships, long-term cognitive deficits are likely due to impaired function of the hippocampus and anterior cingulate region of the frontal lobe. Sustained motor deficits could be caused by structural and functional impairments in the cerebellum. Behavioral abnormalities are probably caused by loss of neurons within hypothalamic-limbic brain structures. Our research has focused on the role of impaired insulin and insulin-like growth factor (IGF) signaling as mediators of neuro-developmental abnormalities caused by chronic gestational exposure to alcohol.[40,42,50,56] Others have examined the contributions of impaired IGF actions in relation to sustained deficits in neuro-cognitive function caused by prenatal alcohol exposure.[57-62] Herein, we review the importance of insulin and IGF signaling in relation to CNS neuronal function, and the mechanisms and potential consequences of ethanol-impaired insulin/IGF signaling in brain. While emerging data suggest alcohol-mediated epigenetic modifications in gene expression

may serve as underlying mechanisms of the brain structural abnormalities and associated aberrant behaviors[27,63-67], it is noteworthy that at least some of these effects are linked to perturbations in IGF, in particular IGF-2 expression and function in the CNS.[67,68]

## Insulin Regulates Growth, Viability and Function in CNS Neurons by Signaling through Insulin Receptor Substrate Molecules

In the CNS, neuronal survival, energy metabolism, and plasticity are critical for maintaining cognitive and motor functions, and regulated through the actions of insulin and IGF types 1 and 2. Insulin, IGF-1 and IGF-2, and their corresponding receptors are abundantly expressed in various cell types throughout the brain, including neurons.[69-71] The highest brain levels of insulin and IGF polypeptide and receptor expression are distributed in the hypothalamus, temporal lobe, and cerebellum, which notably represent major targets of ethanol neurotoxicity. Insulin promotes neurite outgrowth, protein synthesis, neuronal cytoskeletal protein expression, and nascent synapse formation.[72] The stimulatory effects of insulin are mediated through complex intracellular signaling pathways, beginning with ligand binding and activation of the intrinsic receptor tyrosine kinase (RTK).[69,72-78] Insulin RTK phosphorylates specific cytosolic molecules, including one of its major substrates, insulin receptor substrate, type 1 (IRS-1). Tyrosyl phosphorylated IRS-1 (PY-IRS-1) transmits intracellular signals that mediate growth, metabolic function, and viability by interacting with downstream src-homology 2 (SH2)-containing molecules through specific motifs located in the C-terminal region of IRS-1. Phosphorylation of tyrosine residue 897 within the YVNI motif of IRS-1 ($^{897}$YVNI) enables binding to the growth-factor receptor-bound protein 2 (Grb2) adapter molecule, whereas phosphorylation of tyrosine 1180 ($^{1180}$YIDL motif) enables IRS-1 interaction with Syp protein tyrosine phosphatase, and phosphorylation of tyrosine residues 613 and 942 ($^{613}$YMPM and $^{942}$YMKM motifs) leads to IRS-1 binding to the p85 subunit of phosphatidylinositol-3 kinase (PI3K). Grb2 binds to PY-IRS-1 via its SH2 domain, and to a prolinerich region of son-of-sevenless (SOS) through its SH3 domain. Sos complexed with PY-IRS-1 and Grb2, interacts with Ras-GDP, catalyzes a GDP/GTP exchange on Ras, which promotes sequential activation of p21$^{ras}$, mitogen-activated protein kinase kinase (MAPKK), and MAPK. Erk MAPK activation directly contributes to growth factor-stimulated mitogenesis, neuritic sprouting, and gene expression. Tyrosyl phosphorylated Syp acts as an adapter protein between the Grb2-SOS complex and the epidermal growth factor (EGF) or platelet-derived growth factor (PDGF) receptor, whereas catalytically inactive (non-phosphorylated) Syp inhibits MAPK activity. Therefore, the catalytic substrates of Syp may help regulate mitogenic signals and cell cycle progression. The binding of PY-IRS-1 to p85 stimulates glucose uptake and inhibits apoptosis by signaling through Akt/Protein kinase B. In addition, insulin signaling through PI3K phosphorylates and thereby inactivates glycogen synthase kinase 3β (GSK-3β). GSK-3β has roles in energy metabolism, cell survival, and phosphorylation of neuronal cytoskeletal proteins. A very similar signaling cascade exists for IGF-1.

## Ethanol Inhibits Neuronal Responses to Growth Factors

Ethanol-induced developmental arrest in the CNS may be due to impaired responses to various growth factors.[56,58,61,79,80] For example, ethanol inhibits bFGF-, PDGF-AA-, PDGF-BB-, NGF, and IGF-1-stimulated proliferation and cell cycle progression in neural cells.[61,81-83] Ethanol inhibition of neuronal proliferation can be mediated by reduced levels of growth factor receptor expression, growth factor stimulated receptor autophosphorylation, abolishment of the association between growth factor receptor and Ras GTPase-activating protein (Ras-GAP), and inhibition of Erk MAPK activation.[82] Major consequences of ethanol-impaired neuronal responses to growth factor stimulation include reduced viability (increased cell death), motility, adhesion, mitochondrial function, and acetylcholine

homeostasis. [40,54,83-88] Importantly, many of these adverse effects of ethanol are mediated by reduced phosphorylation and consequently increased activation of GSK-3β.[42,50, 89-93]

## Ethanol Inhibits Insulin and IGF1 Signaling Through IRS-1 in Immature Neuronal Cells

Since insulin and IGFs have significant roles in regulating many functions in the immature brain, understanding how ethanol inhibits the corresponding signaling pathways will likely provide important mechanistic clues regarding the pathogenesis of FASD and its associated CNS developmental abnormalities. Previous studies demonstrated that ethanol inhibits phosphorylation and activation of insulin and IGF RTKs, as well as down-stream signaling through IRS-1.[94-98] In addition, ethanol inhibits G-protein expression [99], cyclic AMP-dependent signaling[100], IRS-1-associated PI3 kinase[94,98,101,102], and second messenger cascades such as calcium phospholipid-dependent protein kinases (PKC).[103] PI3 kinase is important for signaling cell survival through Akt/protein kinase B (PKB).[104] Therefore, inhibition of insulin signaling through PI3 kinase could account for the increased apoptosis observed in ethanol-exposed CNS cells. Apart from it's inhibitory effects on PI3K-Akt signaling, ethanol promotes neuronal apoptosis by increasing intracellular $Ca^{++}$ release[105], activating pro-death signaling through Bax, Bad, GSK-3β, and caspases, or by inhibiting survival signaling through Bcl-2.[46,50,93,98,101,106,107] Since the signaling networks corresponding to a number of trophic factor receptors expressed in brain converge downstream to modulate MAPK, PI3K-Akt, Bax, Bad, Bcl-2, GSK-3β, and caspases, ethanol's adverse effects on neuronal growth, survival, energy metabolism, and plasticity could be mediated by inhibition of one or more different receptors, including insulin and IGF-1. Studies utilizing immature brains (mainly cerebella) and cultured neuronal cells demonstrated that ethanol inhibits insulin and IGF signaling at multiple points within the cascade, beginning at the receptor level and extending downstream through pathways that regulate growth, survival, energy metabolism, neuronal migration, and plasticity (Figure 1).[88,101,108-111] Ethanol's inhibitory effects on insulin/IGF stimulated neuronal survival through blockage of downstream signaling through PI3 kinase-Akt[50,88,108,112] promote both apoptosis[88,106,108] and mitochondrial dysfunction.[50,84,85,108,112] At proximal points, ethanol inhibition of insulin and IGF stimulated survival signaling in brain is mediated at the receptor level by two distinct mechanisms: 1) reduced receptor binding and attendant activation of receptor tyrosine kinases (RTK) and corresponding downstream signaling through PI3 kinase-Akt[40]; and 2) increased activation of phosphatases that negatively regulate RTK (PTP-1b) and PI3 kinase (PTEN) and increase GSK-3β activity.[56,98,102] Therefore, chronic ethanol exposure causes insulin/IGF resistance in the developing brain.

## Ethanol Impairs Mitochondrial (Mt) Function

Ethanol toxicity perturbs the structural and functional integrity of mitochondria in brain.[50,112-118] Experimental chronic ethanol feeding results in oxidative modification of MtDNA, manifested by increased 8-hydroxydeoxyguanosine (8-OHdG) incorporation, reduced MtDNA content, and increased MtDNA single-strand breaks.[84,112,119-122] Ethanol-induced MtDNA damage and impaired Mt function increase cellular sensitivity to toxins, and promote Mt permeability transition resulting in necrosis or apoptosis.[84,112,121,122] These adverse effects of ethanol are likely mediated by increased oxygen free radical production, lipid peroxidation, and inhibition of Mt glutathione.[116,123-126] Ethanol metabolism by the microsomal monoxygenase system, involving the alcohol-inducible cytochrome P450 2E1 could contribute to oxidative cellular injury through hydroxylethyl radical formation.[127,128] Therefore, with regard to the CNS, we hypothesize that ethanol-induced MtDNA damage and impaired Mt function cause defects in energy metabolism and oxidative phosphorylation, leading to reduced neuronal viability, as well as compromised

activities required for synaptic plasticity and cognitive/motor functions. In this regard, experimental results demonstrated that chronic ethanol exposure during development results in significantly reduced expression of mitochondria-encoded cytochrome c oxidase and ATP synthase, and increased expression of NADPH oxidase.[50,84,112] These effects lead to reduced ATP production, and increased indices of oxidative stress, including DNA damage and lipid peroxidation in the developing brain.[84,115,118] In addition to impairing mitochondrial function, chronic ethanol exposure increases cellular stress by promoting free radical generation, endoplasmic reticulum stress, and pro-apoptosis signaling, and inhibiting survival pathways.[50,54,92,112,115-117,125,129-132]

The extent to which these mechanisms mediate the teratogenic effects of prenatal alcohol exposure has been demonstrated through the use of anti-oxidants to minimize the adverse effects of ethanol on brain development and function.[116,117,120,126,130,133]

## Downstream Consequences of Ethanol-Impaired Insulin/IGF Signaling in the CNS

Neuronal integrity and function in the CNS are highly influenced by insulin and IGF signaling. For example, CNS neuronal survival, energy metabolism, and plasticity, which are critical for maintaining cognitive and motor functions, are regulated through the actions of insulin and IGF types 1 and 2.[72] In this regard, insulin promotes neurite outgrowth, protein synthesis, neuronal cytoskeletal protein expression, and nascent synapse formation.[134,135] Insulin and IGF-1 regulate the expression and phosphorylation of *tau* [136,137], an important neuronal cytoskeletal protein, while ethanol-impaired signaling through insulin or IGF-1 increases GSK-3β phosphorylation of *tau*, which is pathologic and contributes to neurodegeneration.[138,139] Insulin and IGF-1 signaling also regulates choline acetyltransferase (ChAT)[40,140,141], which is required for acetylcholine biosynthesis.

Acetylcholine is a major neurotransmitter that mediates cognitive-motor functions in the brain and is deficient in brains of chronic ethanol-exposed animal models.[40,129,142-145] Although ethanol has demonstrated inhibitory effects on many neurotransmitter systems that modulate neuronal activity and plasticity[51-53,129,146-150], herein we emphasize the adverse effects of ethanol on cholinergic systems because of their widespread distribution in the brain and their regulation by insulin/IGF signaling.[40,72,151]

In aggregate, the data suggest that an important mechanism by which ethanol impairs neuronal survival, growth, neurotransmitter function and plasticity is to inhibit the actions of insulin and IGFs in the developing brain. On a cellular basis, compromise of insulin/IGF signal transduction networks leads to increased neuronal apoptosis, mitochondrial dysfunction with deficits in energy metabolism, oxidative stress, activation of pro-death and pro-stress signaling, and deficits in cholinergic function, all of which are features of FASD.[40,56,84] Therefore, it is likely that ethanol inhibition of insulin and IGF signaling in the brain significantly contributes to the cognitive and motor impairments associated with FASD. Together, these observations led us to the hypothesis that insulin sensitizer agents such as peroxisome-proliferator activated receptor agonists that both enhance expression and function of downstream targets of insulin/IGF signaling and reduce cellular stress may have therapeutic application in the context of FASD.

## Peroxisome-proliferator Activated Receptors (PPAR)

PPAR-α, δ, and γ, are nuclear hormone receptors that bind to DNA and regulate gene transcription in a broad range of cells and tissues.[152-155] PPARs are regulated by ligand binding and mediate their effects by heterodimerizing with the retinoid × receptor.[152-155]

PPARs have important roles in regulating adipocyte growth and differentiation, insulin responsiveness, and cardiovascular function.[152-155] For example, the enhanced insulin sensitivity imparted by PPAR-γ agonists led to their current use in the treatment of type 2 diabetes mellitus.[156] In addition, PPAR agonists can modulate vascular function, resulting in vasorelaxation and lowered blood pressure through increased release of nitric oxide.[157,158] Correspondingly, dominant-negative mutations in the PPAR-γ gene result in early onset hypertension and insulin resistance in humans.[159] In addition to their actions on endocrine signaling, PPARs help protect cells from the adverse effects of lipid peroxidation.[154,155,160] Lipid peroxidation is a recognized consequence of oxidative stress in the brain, and increased levels of lipid peroxidation products have been detected in brains with neurodegeneration[161-164], as well as in disease states that cause mitochondrial dysfunction and oxidative stress.[84,165]

## Potential Therapeutic Role for PPAR Agonists in FASD

To begin examining potential therapeutic effects of PPAR agonists in relation to ethanol-induced neuro-cognitive deficits, we generated an in vivo model in which Long Evans rat pups were treated with ethanol (3 mg/g) or vehicle by intraperitoneal injection.[166,167] (50 μl) on postnatal days (P) 4, P6, and P8. Rats in both groups were also treated with vehicle or a PPAR-delta (δ agonist (L-165,041; 2 μg/Kg) by i.p. injection on P5, P7, and P9. We previously showed that L-165,041 treatment could prevent insulin resistance-mediated neurodegeneration[141] and alcohol-induced liver disease[168] in vivo. On P16 the rats were evaluated by rotarod tests of latency to fall using an incremental fixed speed protocol (10 trials, 1-5 rpm)[169], and data from Trials 7-10 (speed= 5 rpm) were grouped and analyzed by two-way ANOVA with the Bonferroni post-hoc test. From P27 to P30, rats were evaluated by Morris Water Maze testing as previously described.[141] In brief, rats were subjected to 3 daily trials in which the latencies (seconds) required to locate and land on the platform were recorded. On Day 1, the platform was visible, but on Days 2-4, the platform was submerged. On Days 3 and 4, the water entry quadrants were randomized for each trial. Morris water maze data were grouped and analyzed by repeated measures mixed models ANOVA (diet × treatment × trial day) using area-under-the-curve (AUC) calculations corresponding to performance over the 3 trials each day [141]. For both studies, 8 male rats were included in each sub-group.

Rotarod performance differed significantly among the groups as demonstrated by two-way ANOVA ($F=4.98$, $P=0.028$ for interaction of group by treatment; $F=4.754$, $P=0.031$ for treatment effect; $F=3.04$, $P=0.084$ for group effect). The Bonferroni post-hoc test demonstrated that vehicle-treated ethanol exposed rats had a significantly shorter mean latency to fall compared with all other groups ($P<0.05$; Figure 2A). In essence, L-165,041 treatment of ethanol-exposed rats increased the mean latency to fall, resulting in an overall performance that was similar to controls treated with either vehicle or the PPAR-δ agonist. With regard to the Morris water maze tests, all groups exhibited gradual improvements in latency required to locate the platform. The repeated measures mixed model ANOVA test demonstrated highly significant inter-group performance differences due to treatment effects ($F=20.83$, $P<0.0001$) and trial day/time ($F=95.72$, $P<0.0001$). Furthermore, Bonferroni post-hoc tests revealed significant differences in mean latency for locating the platform between the Control+Vehicle and Ethanol+Vehicle groups on Trial Days 1 ($P<0.05$) and 4 ($P<0.01$), between the Control+PPAR-δ and Ethanol+Vehicle groups on Trial Days 1 ($P<0.001$), 2 ($P<0.001$), 3 ($P<0.05$), and 4 ($P<0.01$), and between the Control+PPAR-δ and Ethanol +PPAR-δ groups on Trial Days 1 ($P<0.001$), 2 ($P<0.01$), and 4 ($P<0.01$). In essence, ethanol treatment significantly impaired performance on the Morris water maze tests relative to controls that were treated with either vehicle or the PPAR-δ agonist. Importantly, PPAR-δ agonist treatment improved Morris water maze performance in ethanol exposed rats such

that, on Trial Days 3 and 4 when the platform was hidden and the entry points were randomized, their mean latencies needed to locate the platform were similar to controls, and significantly shorter than in the ethanol+vehicle group (Figure 2B).

These findings suggest that early treatment with a PPAR-δ agonist can reduce the severity or prevent ethanol-mediated cognitive-motor deficits. Moreover, these results support our main hypothesis.

## HYPOTHESIS

The experimental evidence to date suggests a dual mechanism underlies ethanol-mediated CNS neuronal death during development, namely: 1) impaired survival signaling through brain insulin (IRβ) and probably also IGF-1 receptors, as evidenced by reduced levels of tyrosine phosphorylated (PY) IRβ, IRβ tyrosine kinase (TK) activity, PY-insulin receptor substrate-1 (IRS-1), and PI3 kinase-Akt; and 2) increased oxidative stress that is principally mediated by the combined effects of mitochondrial dysfunction (Figure 3), increased generation of reactive oxygen species (ROS), lipid peroxidation, and DNA damage. Our working hypothesis is that chronic gestational exposure to ethanol produces long-lasting and progressive abnormalities in CNS structure and function due to persistent impairments in insulin signaling caused by CNS insulin resistance. We propose that by circumventing upstream problems in the insulin signaling cascade, i.e. bypassing receptor functions, activating downstream pathways, and altering gene expression, we will be able to therapeutically rescue CNS neuronal cells from ethanol-mediated injury/degeneration.

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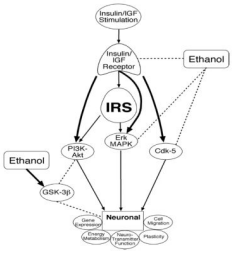


**FIG. 1.**

Schematic of ethanol's adverse effects on insulin/IGF signaling in the brain. Ethanol inhibits phosphorylation and activation of insulin/IGF-1 receptor tyrosine kinases, as well as downstream mediators of neuronal growth, survival, plasticity, energy metabolism, migration, and neurotransmitter function. In addition, ethanol stimulates GSK-3β activity through inhibition of PI3K and activation of PTEN phosphatase.

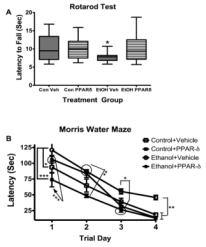


**FIG. 2A & 2B.**

Therapeutic benefit of PPAR-delta (δ treatment in an ethanol exposure model. Long Evans rat pups were treated with ethanol (3 mg/g) or vehicle (Veh) by i.p. injection (50 μl) on postnatal days (P) 4, 6, and 8. Rats were also treated with vehicle or L-165,041 (2 μg/Kg), a PPAR-δ agonist by i.p. injection on P5, P7, and P9. Eight male rats were included in each sub-group. (A) On P16, the rats were evaluated by rotarod testing of latency to fall using an incremental (1-5 rpm) fixed speed protocol. Rotarod data from trials 7-10 (speed= 5 rpm) were grouped and analyzed by two-way ANOVA ($F=4.98$, $P=0.028$ for interaction of group by treatment; $F=4.754$, $P=0.031$ for treatment effect; $F=3.04$, $P=0.084$ for group effect). The Bonferroni post-hoc test revealed that the mean latency to fall was significantly shorter for the Ethanol (EtOH) +Veh group compared with all other groups ($P<0.05$). (B) From P27 to P30, rats were evaluated by Morris Water Maze testing with 3 trials per day. On Day 1, the platform was visible, but on Days 2-4, the platform was submerged. On Days 3 and 4, the water entry quadrant was randomized. The latency for locating and landing on the platform was recorded. Data were analyzed using area-under-the-curve (AUC) calculations corresponding to performance over the 3 trials each day. The graph depicts the mean ± S.E.M. of AUC latencies for each group on each day of testing. Inter-group comparisons were made using repeated measured mixed model ANOVA ($F=20.83$, $P<0.0001$ for treatment; and $F=95.72$, $P<0.0001$ for Trial Day). Post-hoc Bonferroni tests demonstrated significant differences between Control+Vehicle and Ethanol+Vehicle on Trial Days 1 ($P<0.05$) and 4 ($P<0.01$), between Control+ PPAR-δ and Ethanol+Vehicle on Trial Days 1 ($P<0.001$), 2 ($P<0.001$), 3 ($P<0.05$), and 4 ($P<0.01$), and Control+PPAR-δx and Ethanol +PPAR-δ on Trial Days 1 ($P<0.001$), 2 ($P<0.01$), and 4 ($P<0.01$). (*$P<0.05$; **$P<0.01$; ***$P<0.001$)

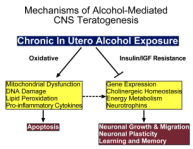


**FIG. 3.**

Dual mechanisms hypothesis of alcohol-mediated CNS teratogenesis. Chronic gestational exposure to ethanol causes oxidative stress and insulin/IGF resistance in the brain. The oxidative stress is caused by mitochondrial dysfunction, which promotes DNA damage, lipid peroxidation, pro-inflammatory cytokine activation, and apoptosis. Insulin/IGF resistance in brain impairs gene expression required for neuronal genesis, myelin maintenance, cell migration, neurotransmitter function, and energy metabolism. Oxidative stress and pro-inflammatory cytokine activation exacerbate the adverse effects of insulin/IGF resistance. Together, these abnormalities lead to deficits in neuronal plasticity, learning and memory.