RESEARCH ARTICLE

Ethanol Exposure Induces Neonatal Neurodegeneration by Enhancing CB1R Exon1 Histone H4K8 Acetylation and Up-regulating CB1R Function causing Neurobehavioral Abnormalities in Adult Mice

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Abstract

Background: Ethanol exposure to rodents during postnatal day 7 (P7), which is comparable to the third trimester of human pregnancy, induces long-term potentiation and memory deficits. However, the molecular mechanisms underlying these deficits are still poorly understood.

Methods: In the present study, we explored the potential role of epigenetic changes at cannabinoid type 1 (CB1R) exon1 and additional CB1R functions, which could promote memory deficits in animal models of fetal alcohol spectrum disorder.

Results: We found that ethanol treatment of P7 mice enhances acetylation of H4 on lysine 8 (H4K8ace) at CB1R exon1, CB1R binding as well as the CB1R agonist-stimulated GTPγS binding in the hippocampus and neocortex, two brain regions that are vulnerable to ethanol at P7 and are important for memory formation and storage, respectively. We also found that ethanol inhibits cyclic adenosine monophosphate response element-binding protein (CREB) phosphorylation and activity-regulated cytoskeleton-associated protein (Arc) expression in neonatal and adult mice. The blockade or genetic deletion of CB1Rs prior to ethanol treatment at P7 rescued CREB phosphorylation and Arc expression. CB1R knockout mice exhibited neither ethanol-induced neurodegeneration nor inhibition of CREB phosphorylation or Arc expression. However, both neonatal and adult mice did exhibit enhanced CREB phosphorylation and Arc protein expression. P7 ethanol-treated adult mice exhibited impaired spatial and social recognition memory, which were prevented by the pharmacological blockade or deletion of CB1Rs at P7.

Conclusions: Together, these findings suggest that P7 ethanol treatment induces CB1R expression through epigenetic modification of the CB1R gene, and that the enhanced CB1R function induces pCREB, Arc, spatial, and social memory deficits in adult mice.

Keywords: cannabinoid receptor system, epigenetics, FASD, memory loss, synaptic signaling
Introduction

Alcohol consumption during pregnancy exposes fetal brains to ethanol that causes various birth defects (Jones and Smith, 1973) in humans, collectively known as fetal alcohol spectrum disorders (FASDs; Streissguth et al., 1990). The consequential neurological abnormalities (Goodman et al., 1999; Mattson et al., 1999) are understood to be one of the major causes of intellectual disability in Western nations (Mattson et al., 2011). The studies using developmental animal models have long established that fetal ethanol exposure is associated with enormous reduction in the number of neurons in numerous brain regions, including the hippocampus (Olney, 2004), in addition to long-lasting synaptic and memory deficits in adult rodents (Izumi et al., 2005; Wilson et al., 2011; Sadrian et al., 2012; Subbanna et al., 2013a). Several pathways appear to activate neuronal death by ethanol; however, recent studies suggest that the endocannabinoid system not only contributes to neurodegeneration (Hansen et al., 2008; Subbanna et al., 2013a), but also plays a significant role in the development of synaptic and learning and memory deficits in adult mice (Subbanna et al., 2013a).

The endocannabinoid system includes endogenous ligands, cannabinoid type 1 (CB1R) and 2 receptors, and synthesizing and degrading enzymes (Piomelli, 2003; Basavarajappa, 2007; Subbanna et al., 2013a). A substantial amount of previous research has demonstrated multiple ways in which the endocannabinoid system regulates synaptic events (Ohno-Shosaku et al., 2001; Wilson and Nicoll, 2001; Bacci et al., 2004) in the developing (Berghuis et al., 2007; Mulder et al., 2008; Subbanna et al., 2013a) and adult brain (see Basavarajappa et al., 2009). Research findings from animal and human studies imply that the endocannabinoid system is one of the most relevant biochemical systems mediating the action of ethanol in multiple brain regions (Basavarajappa et al., 1998, 2003, 2006, 2008; Basavarajappa and Hungund, 1999a, 1999b; Roberto et al., 2010; DePoy et al., 2013; Hirvonen et al., 2013a; Subbanna et al., 2013a; Ceccarini et al., 2014).

The CB1R is one of the most abundant inhibitory G-protein-coupled receptors expressed in the brain (Howlett et al., 1986; Herkenham et al., 1990) and is primarily located on presynaptic terminals, where it controls neurotransmitter release (Matyas et al., 2007). The ability of the CB1R to suppress neurotransmission allows endogenous cannabinoids such as anandamide (AEA) and 2-arachidonoylglycerol to prevent the recruitment of new synapses (Kim and Thayer, 2001), which has a profound impact on neuronal communication, learning, and memory (Castellano et al., 2003; Mechoulam and Parker, 2013; Subbanna et al., 2013a). Moreover, cannabis use during brain development induces several specific human developmental disorders (Stefanis et al., 2004), including fetal alcohol syndrome-like deficits (Wu et al., 2011), which are likely mediated through the activation of CB1Rs.

Recently, epigenetic alterations have been shown to play a role in both normal development and several human developmental disorders (Campuzano et al., 1996; Petronis, 2005; Makedonski et al., 2005; Ryu et al., 2006; Warren, 2007; Gavín and Sharma, 2010), and have been implicated in developmental ethanol effects (Kaminen-Ahola et al., 2010; Bekdash et al., 2013; Perkins et al., 2013), including neurodegeneration (Subbanna et al., 2013b; Subbanna et al., 2014). Although the detailed mechanisms are not yet clear, we have recently shown that P7 ethanol treatment increases AEA/CB1R signaling, results in neonatal neurodegeneration, and contributes to the development of synaptic and object recognition memory deficits relevant to FASD (Subbanna et al., 2013a). In the present study, we explored the epigenetic and CB1R-mediated signaling events that may directly cause the neurodegeneration in neonatal mice and spatial and social recognition memory deficits in adult mice.

Methods

Animals and Treatment

Male C57BL/6J, CB1R wild type (WT), and knock out (KO) mice (Subbanna et al., 2013a) on a C57BL/6J background were generated from a heterozygous breeding colony at NIKI. C57BL/6J, CB1RWT, and KO mice were housed in groups under standard laboratory conditions (12 h light/dark cycle) with food and water available ad libitum. Animal care and handling procedures followed institutional (NIKI IACUC) and National Institutes of Health guidelines. Ethanol (2.5 g/kg s. c. at 0 h and again at 2 h) and [N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamidohydrochloride][SR141716A] (SR, 1 mg/kg, 10 μg body weight) treatment and determination of ethanol levels were carried out as previously described by our laboratory (Subbanna et al., 2013a; Subbanna et al., 2013b). Three- to four-month-old mice were used for several analyses, as described below. For independent experiments, 5–8 animals were used.

ChIP Assay

Chromatin immunoprecipitation (ChIP) assay was performed as described before (Subbanna et al., 2014). For the ChIP assay, pups were sacrificed by decapitation and the hippocampus and neocortex were dissected 8 h after the first saline or ethanol injection. Tissue (25 mg) was fixed by 1% formaldehyde, homogenized, and subjected to DNA shearing; the amount of sample was normalized to contain equivalent protein amounts. Chromatin was immunoprecipitated with anti-acetyl histone H4K8 (# 07–328; Millipore) and anti-H3K9me2 (#4658; Cell Signalling) antibodies. As a negative control, samples were immunoprecipitated with rabbit IgG (Millipore). Immune-complexes were collected and processed as described before (Subbanna et al., 2014) using primers for mouse CNR1 (CB1R) exon I (mouse CNR1 219 F 5′-AGAGACAAACAACACATTACA-3′, mouse CNR1 277 P 5′-AAGGGAAAACTTCCAGTGTTGG-3′, and mouse CNR1 307 5′-TAGAACCTCCATGCCTAAAT-3′). Relative quantification for acetylated and methylated histone-associated genes in saline and ethanol groups was calculated by the ΔΔCt method (Schmittgen and Livak, 2008).

Protein extraction, Electrophoresis, and Immunoblotting

For Western blot analysis, homogenates from the flash frozen hippocampus (HP) and neocortex (NC) from neonates and adult (P 90) was processed to prepare nuclear and total extracts (Basavarajappa et al., 2014; Basavarajappa and Subbanna, 2014) as described previously (Lubin and Sweatt, 2007; Subbanna et al., 2013b). The samples were prepared in a sample buffer as described below. For independent experiments, 5–8 animals (Subbanna et al., 2013a) on a C57BL/6J background were generated from a heterozygous breeding colony at NIKI. C57BL/6J, CB1RWT, and KO mice were housed in groups under standard laboratory conditions (12 h light/dark cycle) with food and water available ad libitum. Animal care and handling procedures followed institutional (NIKI IACUC) and National Institutes of Health guidelines. Ethanol (2.5 g/kg s. c. at 0 h and again at 2 h) and [N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamidohydrochloride][SR141716A] (SR, 1 mg/kg, 10 μg body weight) treatment and determination of ethanol levels were carried out as previously described by our laboratory (Subbanna et al., 2013a; Subbanna et al., 2013b). Three- to four-month-old mice were used for several analyses, as described below. For independent experiments, 5–8 animals were used.

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Homogenates (20 μg of protein) were incubated with 0.1 nM GTP\(^\gamma\)S binding was performed as previously described\([\text{Basavarajappa et al.}, 1998, 2006]\). The percentage of social investigation were previously described\([\text{Subbanna and Basavarajappa, 2014}]\). The percentage of social investigation was calculated by dividing the investigation time during the second exposure by the initial investigation time \(\times 100\).

**Statistics**

A statistical comparison of the data was performed by either a student's t-test, one-way analysis of variance (ANOVA), or two-way ANOVA with Bonferroni's post hoc test. In all of the comparisons, \(p < 0.05\) was considered to indicate statistical significance. The statistical analyses were performed using Prism software (GraphPad).

**Results**

Neonatal Exposure to Ethanol Enhances H4K8 Acetylation and Dimethylates H3K9 at CB1R Exon1

We used ChiP assay to determine whether CB1R transcriptional activation involves specific epigenetic modification of histone proteins in exon1 of the CB1R gene. The results indicated that ethanol treatment increased acetylated H4K8 levels (Figure 1a and b); \(p < 0.001\) and reduced dimethylated H3K9 (Figure 1c and d) at CB1R exon1 in the HP (\(p < 0.01\)) and NC (\(p < 0.05\), which is correlated with increased CB1R transcription\([\text{Subbanna et al.}, 2013a}]\).

Enhanced CB1R Binding and CB1R Agonist-Stimulated GTP\(^\gamma\)S Binding During Ethanol-Induced Neurodegeneration in the Developing Brain

Administration of ethanol to mouse pups at P7 resulted in an ethanol level of \(-0.47 \pm 0.25\) g/dL at 3h that was gradually reduced to \(0.27 \pm 0.07\) g/dL at 9h following injection. This ethanol paradigm has been shown to produce a widespread pattern of neurodegeneration throughout the forebrain, including the HP and NC, as indicated by the formation of CC3 in ethanol-exposed brains.

Our current results demonstrated that P7 ethanol treatment significantly enhanced the specific binding of CP-55,940 in a time-dependent manner both in the HP and NC (\(p < 0.05\); Figure 1e). Furthermore, to examine the function of CB1Rs after P7 ethanol treatment, we examined CP-55,940-stimulated...
Figure 1. Enhanced H4K8 acetylation and reduced H3K9 dimethylation at exon1 of the CB1R gene regulate postnatal ethanol-induced expression of CB1R. ChIP analysis of the CB1R exon1 gene in hippocampal (HP) and neocortical (NC) tissues from the saline and ethanol groups (n = 8 pups/group) with anti-acetylated H4K8 (a and b) or anti-H3K9me2 (c and d) antibodies and levels of CB1R exon 1 chromatin enrichment in the IPs were measured by quantitative PCR. *p < 0.05, **p < 0.01, ***p < 0.001; compared with respective saline group; student's t-test. Error bars = SEM. (e) CB1R binding was performed with PM (20 mg protein/ml) at 30°C for 60 min using [3H]CP-55,940 as the labeled ligand and 30μM unlabeled CP-55,940 to define non-specific binding. Each point is the mean ± SEM (n = 12/group). [35S] GTPγS binding was performed with various concentrations (f) or 2 μM (g) of CP-55, 940. Non-specific binding was determined in the presence of unlabeled GTPγS (30 μM). Data are expressed as the percentage of basal [35S] GTPγS binding. Basal [35S] GTPγS binding in the absence of CP-55,940 was ranged between 4.4 ± 0.50 (NC) and 5.0 ± 0.2 (HP) pmol/mg protein. Error bars = SEM (n = 12/group). (h) Mice pre-treated for 30 min with SR141716A (SR; 1 mg/kg) or vehicle were exposed to ethanol, and CC3 levels were determined by a Western blot analysis (***p < 0.001 vs. S+V; #p < 0.001 vs. E+V). (i) CB1R WT and KO mice were exposed to ethanol, and CC3 levels were determined by a Western blot analysis. β-actin was used as a loading control. Each point is the mean ± SEM. ***p < 0.001 vs S+CB1RWT; #p < 0.001 vs E+CB1RWT.
Ethanol Exposure in Neonatal Mice Reduces Arc Protein Expression

To further assess the contribution of intracellular signaling events to the action of ethanol on the developing brain, we determined the levels of CREB phosphorylation by Western blot analysis using specific phospho-CREB antibodies. P7 ethanol treatment significantly reduced the pCREB but not the total CREB protein levels in the HP (F3, 28 = 56, p < 0.001) or the NC (F3, 28 = 60, p < 0.001; Figure 2a) at 8h and 24h time points.

Impaired pCREB and Arc are Long-Lasting to Adulthood and Neuroprotective Effects of CB1R Blockade Involves pCREB and Arc Pathway

To elucidate the downstream intracellular pathways involved in the protective effects of the CB1R blockade, we studied the involvement of the pCREB and Arc pathway, a key regulator of cell survival (Luijkart et al., 2008) and synaptic plasticity (Caroni et al., 2012). We investigated whether pre-treatment of SR, which prevents ethanol-induced neurodegeneration, could rescue these ethanol-induced pCREB and Arc deficits. Our results suggest that CREB phosphorylation, as well as Arc protein expression, were rescued by SR pre-treatment (compared with the ethanol group) in neonatal (HP: pCREB, F3, 28 = 55, p < 0.01, Arc, F3, 28 = 30, p > 0.01; NC: pCREB, F3, 28 = 35, p > 0.01, Arc, F3, 28 = 45, p > 0.01, two-way ANOVA; Figure 3a and 4a) and adult mice tissues (HP: pCREB, F3, 28 = 40, p > 0.01, Arc, F3, 28 = 25, p > 0.01; NC: pCREB, F3, 28 = 45, p > 0.01, Arc, F3, 28 = 55, p > 0.01, two-way ANOVA; Figure 5a and 6a). We found that the total CREB protein levels were not altered in the ethanol-treated samples compared with the saline samples. In addition, SR did not alter the CREB protein levels in either the ethanol or saline samples of neonatal (Figure 3a) and adult (Figure 5a) rats. Similarly, CB1RKO mice, which do not exhibit ethanol-induced neurodegeneration, provided protection against P7 ethanol-induced inhibition of CREB phosphorylation and Arc expression in the neonatal brain (compared with the ethanol group) in neonatal (HP: pCREB, F3, 28 = 56, p < 0.001, Arc, F3, 28 = 60, p < 0.001; Figure 1h) and adult mice tissues (HP: pCREB, F3, 28 = 42, p < 0.001, Arc, F3, 28 = 52, p < 0.001; NC: pCREB, F3, 28 = 58, p < 0.001, Arc, F3, 28 = 62, p < 0.001, one-way ANOVA; Figure 3b and 4b).
Figure 3. Pharmacological blockade or genetic ablation of CB1Rs provides protection against ethanol-induced inhibition of CREB phosphorylation in the neonatal mouse brain. (a) Hippocampal and neocortical nuclear extracts from the four treatment groups (S+V, E+V, S+SR, and E+SR) were subjected to Western blot to analyze the levels of pCREB and CREB [n = 10 pups/group; ***p < 0.001 vs. S+V; #p < 0.001 vs. E+V]. (b) Additional Western blot analyses were performed to determine the levels of pCREB and CREB in the hippocampal and cortical nuclear extracts obtained from the saline and ethanol-treated P7 CB1RWT and KO mice. The representative blots are shown for the hippocampal and cortical nuclear extracts [n = 10 pups/group; ***p < 0.001 vs. S+CB1RWT; @p < 0.001 vs. E+ CB1RWT; #p < 0.001 vs. S+CB1RWT]. β-actin was used as a loading control. Two-way ANOVA with Bonferroni’s post hoc tests was used for statistical analysis. Each point is the mean ± SEM. HP, hippocampus; NC, neocortex.
adult mice tissues (HP: pCREB, F₃, 20 = 22, p < 0.01, Arc, F₃, 20 = 32, p < 0.01; NC: pCREB, F₃, 20 = 38, p < 0.01, Arc, F₃, 20 = 32, p < 0.01, one-way ANOVA; Figure 5b and 6b). In addition, neonatal and adult CB1RKO mice also exhibited enhanced CREB phosphorylation and Arc levels compared to their WT littermates (p < 0.01; Figure 5b and 6b).

Figure 4. Pharmacological inhibition or genetic deletion of CB1Rs provides protection against ethanol-induced inhibition of Arc expression in the neonatal mouse brain. (a) Hippocampal and neocortical total extracts from the four treatment groups (S+V, E+V, S+SR, and E+SR) were processed for Western blot to analyze the levels of Arc protein (n = 10 pups/group; *** p < 0.001 vs. S+V; # p < 0.001 vs. E+V). (b) To determine the Arc protein levels in saline and ethanol-treated CB1RW T and KO P7 mice samples, the hippocampal and neocortical total extracts were subjected to Western blot analyses. The representative blots are shown for the hippocampal and cortical cytosolic extracts (n = 10 pups/group; *** p < 0.001 vs. S+CB1RW T; # p < 0.001 vs. E+CB1RW T; @ p < 0.001 vs. S+CB1RW T). Two-way ANOVA with Bonferroni’s post hoc tests was used for statistical analysis. Each point is the mean ± SEM. HP, hippocampus; NC, neocortex.
Pharmacological Blockade or Genetic Deletion of CB1Rs Before P7 Ethanol Treatment Rescues Memory Loss in Adult Mice

In our first behavioral test, adult mice treated with saline, ethanol (with vehicle), SR, or ethanol + SR at P7 were tested using spontaneous alternation in the Y maze. P7 ethanol, SR, or ethanol + SR treatment had no significant effect on exploratory activities assessed by the number of arm entries (Figure 7a) and time spent (Figure 7b) in each arm during Y-maze testing. Two-way ANOVA revealed that the ethanol-treated mice exhibited significantly reduced spontaneous alternation performance compared to saline-treated mice and that SR rescued these deficits ($F_{3,21} = 10$, $p < 0.001$; Figure 7c). Treatment with SR alone at P7 had no significant effect on spontaneous alternation performance. P7 ethanol treatment had no significant effect on the number of arm entries (Figure 7d) or the time spent in each arm (Figure 7e; exploratory activity) in either CB1RWT or KO mice. CB1RKO mice exhibited significantly enhanced spontaneous alternation behavior ($p < 0.01$) compared to WT mice. Notably, ethanol treatment at P7 failed to induce a spatial working memory deficit in the Y-maze test in adult CB1RKO mice ($p > 0.05$; Figure 7f).

In our second behavioral test, we examined spatial recognition memory using the Y-maze. Two-way ANOVA revealed that saline- and SR-treated mice entered more frequently into (Arm Entry: 1 h, $F_{3,21} = 21$, $p < 0.01$; 24 h, $F_{3,21} = 26$, $p < 0.01$) and spent more time in (Dwell Time: 1 h, $F_{3,21} = 61$, $p < 0.01$; 24 h, $F_{3,21} = 22$, $p < 0.01$) the novel, previously unvisited arm of the maze. In contrast, P7 ethanol-treated mice showed a reduced preference toward the novel arm ($p < 0.01$) and spent less time (Dwell Time: $p < 0.01$) in the novel arm compared to P7 saline-treated mice in both the 1 h (Figure 8a and b) and 24 h (Figure 8c and d) retention trials. SR pre-treatment rescued ethanol-induced impairments in the preference for the novel arm ($p < 0.01$) and time spent ($p < 0.01$) in the novel arm in both the 1 and 24 h retention trials. Although all saline- and SR-treated mice (combined 1 and 24 h) selected the novel arm as the first choice, ethanol-treated animals showed a reduced preference for the novel arm (Figure 8e),
which this was prevented by SR pretreatment ($F_{3,45} = 50$, $p < 0.01$). While CB1RKO mice showed an enhanced preference for the novel arm (Arm Entry, $p < 0.001$) and spent more time in the novel arm (Dwell Time, $p < 0.001$) compared to WT mice in both the 1h (Figure 8f and g) and 24h retention trials (Figure 8h and i). In addition, all saline- and ethanol-treated CB1RKO mice (combined 1 and 24h) selected the novel arm as the first choice (Figure 8j).

The social investigation results revealed that ethanol-treated mice exhibited significantly-reduced short-term (Figure 9a) and long-term (Figure 9b) SRM performance compared to salinel-treated mice. Two-way ANOVA revealed that SR pretreatment rescued ethanol-induced short-term ($F_{3,21} = 18$, $p < 0.01$) and long-term ($F_{3,21} = 14$, $p < 0.01$) SRM deficits compared to ethanol-treated mice. In addition, SR alone had no significant effects ($p > 0.05$) on SRM, and these mice exhibited normal SRM. Ethanol failed to impair SRM in CB1RKO mice, and KO mice exhibited normal SRM (Figure 9c and d).

**Discussion**

In this study, we demonstrate for the first time that transcriptional activation of CB1R followed by widespread neurodegeneration in the neonatal brain (Subbanna et al., 2013a) involves specific increases in H4K8 acetylation and demethylation of H3K9 at exon 1 in the CB1R gene. Dimethylation of histone H3K9 correlates with transcriptional silencing, whereas the acetylation of histone H4 at lysine 8 (H4K8ace) is linked to active transcription (Jenuwein and Allis, 2001). Our findings are consistent with postnatal ethanol-induced enhancement of active transcription of G9a gene (Subbanna et al., 2014). Demethylation of H3K9 found at the CB1R gene may be due to the global loss of the H3K9me2 mark secondary to postnatal-ethanol-induced caspase-3 mediated H3K9me2 degradation (Subbanna et al., 2013b). While studies related to epigenetic changes at the CB1R gene are either primitive (Wang et al., 2008) or have not yet been conducted, our unprecedented initial studies suggest that the epigenetic mark which regulates active gene transcription (Subbanna et al., 2013a; Subbanna et al., 2013b; Subbanna et al., 2013a) also regulates the CB1R gene expression (Subbanna et al., 2013a) triggered by neonatal ethanol neurotoxicity. Ethanol-induced CB1R protein expression (Subbanna et al., 2013a) also reflected increases in CB1R levels in the PM preparations as shown by specific binding of CP55,940. In addition, our studies suggest that ethanol-enhanced CB1R proteins are functionally active at the PM because CP55,940-stimulated GTP-$\gamma$S binding also enhanced in parallel with CB1R levels. Although the studies on the consequences of enhanced CB1R function in neonatal mice are limited (Hansen et al., 2008; Subbanna et al., 2013a), our previous studies suggest that postnatal ethanol-enhanced CB1R function induces neurodegeneration in neonatal mice that leads to long-lasting deficits in long-term potentiation and object recognition test (ORT) in adult mice (Subbanna et al., 2013a).
Consistent with this observation, elevation of endogenous AEA through inhibition of fatty acid amide hydrolase during postnatal development, as observed with postnatal ethanol (Subbanna et al., 2013a), leads to impaired working memory in adult mice (Wu et al., 2014). CB1R has been shown to regulate the generation and maturation of excitatory and inhibitory neurons during brain development (Keimpema et al., 2013) and has also been shown to inhibit the release of glutamate and gamma aminobutyric acid (GABA) in matured neurons (Wilson and Nicoll, 2002). However, in neonatal mice, it appears that the ethanol blocking action at NMDARs and its stimulatory action at GABAARs are mainly responsible for its neurodegenerative responses (Ikonomidou et al., 2000). While more research is warranted to understand the specific effects of ethanol-activated CB1R on NMDA and GABA-mediated neurotransmission in neonatal mice, it appears that GABA is excitatory during the early stages of brain development and becomes inhibitory later on as adulthood approaches (Ben-Ari, 2002). Therefore, dysregulation of the CB1R pathway which regulates NMDA and GABA neurotransmission may have long-lasting consequences on synaptic function.

Our previous studies suggested the presence of a remarkable specificity involving the AEA/CB1R/ERK pathway, but not the AKT pathway (Young et al., 2008), in the regulation of ethanol-induced neonatal neurodegeneration (Subbanna et al., 2013a). Our current findings suggest that reduced CREB phosphorylation could be rescued by blockade or genetic deletion of CB1R in neonatal mice exposed to ethanol because the protein kinase A/cAMP/ERK pathway has been shown to phosphorylate CREB on Ser133; ethanol-induced inhibition of this pathway may be responsible for the observed deficits in CREB phosphorylation. It should be noted that CREB has been shown to mediate adaptive responses of neurons to several stimuli to regulate neuronal survival in the developing brain (Bonni et al., 1999). Interestingly, CB1R neonatal KO mice exhibit high levels of CREB phosphorylation similar to adult CB1RKO mice compared to their WT littermates (Basavarajappa et al., 2014; Basavarajappa and Subbanna, 2014). Activated CREB has been shown to regulate the expression of many genes involved in numerous cellular functions, including neuronal survival, synaptic plasticity, and learning and memory (Nonaka, 2009; Benito and Barco, 2010; Sakamoto et al., 2011). Therefore, ethanol-caused dysregulation of this pathway during postnatal development to adulthood may significantly contribute to long-term neurobehavioral deficits commonly associated with FASD (Izumi et al., 2005; Wilson et al., 2011; Sadrian et al., 2012; Subbanna et al., 2013a).
While the precise signaling cascades involved in Arc transcription are not well defined, one study has shown that PKA/MAPK cascades regulate Arc expression (Waltereit et al., 2001) and that the MAPK/CREB pathway is also essential for Arc expression (Ying et al., 2002; Nonaka et al., 2014). Given the impaired ERK1/2 and pCREB in postnatal ethanol-exposed neonatal mice, Arc protein levels were also significantly reduced in ethanol-exposed neonatal as well as adult mice and blockade or genetic deletion of CB1R prior to P7 ethanol treatment—restored Arc protein levels in neonatal and adult mice. Interestingly, both neonatal and adult CB1R KO mice expressed high levels of Arc compared to their WT littermates. It is therefore possible that the neuroprotective role of the CB1R antagonist in neonatal mice may be due to the activation of Arc expression through CB1R-mediated CREB pathway. Arc expression is very tightly controlled by
neuronal activity downstream of multiple signaling pathways (Bramham et al., 2008; Shepherd and Bear, 2011). Therefore, several molecules responsible for mediating neural activity, such as NMDA and the P/Q-type voltage-dependent Ca$^{2+}$ channel, also regulate Arc expression (Kakizawa et al., 2000; Hashimoto et al., 2011). To our knowledge, this is the first study to suggest that the regulation of Arc expression through CB1R activity in neonatal and adult mice. Arc transcription is also regulated by voltage-sensitive calcium channels (Adams et al., 2009), which are negatively regulated by CB1R (Basavarajappa and Arancio, 2008). Our findings suggest that the ERK1/2-pCREB-Arc pathway is involved in neuronal survival downstream of the CB1Rs in the developing brain and is compromised by ethanol treatment. It is possible that ethanol-induced suppression of the ERK1/2-pCREB-Arc signaling pathway might disrupt the fine-tuning of neuronal circuits, leading to persistent synaptic and memory dysfunction (Subbanna et al., 2013a). Consistent with this notion, CB1RKO mice do not exhibit P7 ethanol-induced neurodegeneration during the neonatal period (Hansen et al., 2008; Subbanna et al., 2013a) or deficits in long-term potentiation and ORT during adulthood (Subbanna et al., 2013a).

The current findings also revealed significant deficits in learning and memory in adult mice exposed to ethanol at P7 compared to controls, as tested in several domains (spontaneous alternation, spatial, and social recognition). These findings are in general agreement with previous reports showing that an acute dose of ethanol at P7 impairs synaptic plasticity in the HP (Izumi et al., 2005; Sadrian et al., 2012; Subbanna et al., 2013a) and olfacto-HP (Wilson et al., 2011; Sadrian et al., 2012) circuits as well as ORT (Subbanna et al., 2013a) in adult mice. Most importantly, we have shown that the blockade of CB1Rs before ethanol exposure is sufficient enough to rescue ethanol-induced neuronal deficits in every paradigm we have examined. Several other rodent models of FASD also show impaired learning and memory in adult rodents exposed to acute or chronic ethanol at pre- or postnatal stages of development (Girard et al., 2000; Savage et al., 2002; Christie et al., 2005; Iqbal et al., 2006; Thomas et al., 2008). There is also growing evidence that heavy prenatal alcohol exposure leads to widespread cognitive deficits in children across several domains, including memory and social and adaptive functioning (Norman et al., 2015).

The findings from SRM studies are in general agreement with other studies in which acute exposure to ethanol on P12 caused pronounced and permanent deficits in social behavior throughout ontogeny (Mooney and Varlinskaya, 2011). Similar SRM deficits were also found in another animal model of FASD (Shirasaka et al., 2012), as well as in the CD38 KO model of autism (Jin et al., 2007). Most importantly, SR pretreatment and genetic deletion of CB1R provided protection against ethanol-induced deficits in SRM in adult mice. It has been suggested that retaining normal SRMs throughout ontogeny would help to establish relationships within a group or between partners, besides developing the ability to recognize families (Cushing and Kramer, 2005). Evidence suggests that the two brain regions, the olfactory system (Sanchez-Andrade and Kendrick, 2009) and the limbic system (Brothers et al., 1990; Baron-Cohen et al., 1994) regulate social behavior. In ethanol-treated P7 mice, improper processing of socially relevant olfactory stimuli might produce the observed deficit in SRM in adult mice. Early ethanol exposure damages olfactory neuroanatomy and physiology in both humans and rodents (Wilson et al., 2011). The olfactory system provides a major input to the HP formation (Wilson and Sullivan, 2011), and this structure is involved in integrating the complex stimuli necessary for the recognition process (Alvarez et al., 2002; Ross and Eichenbaum, 2006), therefore the SRM might be regulated also by HP structure (Kogan et al., 2000). Consistent with this notion, our previous findings suggest that ethanol treatment of P7 mice significantly impairs olfacto-HP system function in adult mice (Wilson et al., 2011; Sadrian et al., 2012).

In conclusion, CB1R gene transcription is regulated by specific epigenetic modification, associated with active transcription leading to enhanced CB1R function. Our current findings directly pinpoint the participation of CB1R signaling during early brain development (Figure 10) leading to long-lasting pCREB-Arc impairments and neurobehavioral abnormalities. Currently, effective treatment for individuals suffering from FASD is not available. The CB1R-pCREB-Arc mediated molecular mechanisms in the effect of postnatal ethanol on abnormalities in neuronal survival and its long-lasting influence on synaptic plasticity, learning, and memory, including SRM, may eventually lead to the development of drugs to improve specific aspects of the symptomatology of ethanol-induced neurobehavioral teratogenicity.

Figure 10. A schematic diagram of the proposed mechanism of action by which epigenetically enhanced CB1R function in neonatal mice regulates ethanol-induced neurodegeneration, leading to neurobehavioral abnormalities in adult mice. P7 ethanol enhances specific H4K8ac of CB1Rs promoter to enhance CB1R expression and function, which causes caspase-3 activation as well as pERK1/2 (Subbanna et al., 2013a), pCREB and Arc deficits and leads to neonatal neurodegeneration (>). These mechanisms during postnatal development may disrupt the refinement of neural circuits (Wilson et al., 2011; Sadrian et al., 2012) and lead to long-lasting deficits in synaptic plasticity (Subbanna et al., 2013a), learning, and memory, including social recognition memory in adult animals. The inhibition of CB1Rs (——> rescue pERK1/2, pCREB, and Arc deficits as well as neonatal neurodegeneration (caspase-3 cleavage), which results in normal neurobehavioral function in adult mice. The genetic ablation of CB1Rs (——> provides protection against ethanol-induced pCREB, Arc deficits, neonatal neurodegeneration, synaptic (Subbanna et al., 2013a), learning, and memory, including social recognition memory deficits in adult mice. Hence, the putative CB1R/pERK1/2/pCREB/Arc signaling mechanism may have a potential regulatory role in neuronal function during brain development and may be a valuable therapeutic target for FASD.
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Statement of Interest

The authors declare no conflicts of interest.

References

Adams JP, Robinson RA, Hudgins ED, Wissink EM, Dudek SM (2009) NMDA receptor-independent control of transcription factors and gene expression. Neuroreport 20:1429–1433.

Alvarez P, Wendelken L, Eichenbaum H (2002) Hippocampal formation lesions impair performance in an odor-odor association task independently of spatial context. Neurobiol Learn Mem 78:470–476.

Bacci A, Huguenard JR, Prince DA (2004) Long-lasting self-inhibition of neocortical interneurons mediated by endocannabinoids. Nature 431:312–316.

Basavarajappa BS, Cooper TB, Hungund BL (1998) Chronic ethanol administration down-regulates cannabinoid receptors in chronic ethanol exposed mouse. Brain Res 815:89–97.

Basavarajappa BS, Hungund BL (1999b) Down-regulation of cannabinoid receptor agonist-stimulated [35S]GTP-S binding in synaptic plasma membrane from chronic ethanol exposed mouse. Brain Res 815:89–97.

Basavarajappa BS, Cooper TB, Hungund BL (1998) Chronic ethanol increases the Cannabinoid Receptor Agonist, Anandamide and its Precursor N-Arachidonoyl phosphatidyl ethanolamine in SK-N-SH Cells. J Neurochem 72:522–528.

Basavarajappa BS, Subbanna S (2014) CB1 Receptor-Mediated Signaling Underlies the Hippocampal Synaptic, Learning and Memory Deficits Following Treatment with JWH-081, a New Component of Spice/K2 Preparations. Hippocampus 24:178–188.

Basavarajappa BS, Cooper TB, Hungund BL (1998) Chronic ethanol administration down-regulates cannabinoid receptors in mouse brain synaptic plasma membrane. Brain Res 793:212–218.

Basavarajappa BS, Subbanna S, Arancio O (2008) NMDA receptor-independent control of transcription factors and gene expression. Neuroreport 20:1429–1433.

Basavarajappa BS, Saito M, Cooper TB, Hungund BL (2003) Chronic ethanol inhibits the anandamide transport and increases extracellular anandamide levels in cerebellar granule neurons. Eur J Pharmacol 466:73–83.

Basavarajappa BS, Nagre NN, Xie S, Subbanna S (2014) Endocannabinoids in hippocampal neurons. J Neurochem 107:1001–1013.

Basavarajappa BS, Nixon RA, Arancio O (2009) Endocannabinoid system: emerging role from neurodevelopment to neurodegeneration. Mini Rev Med Chem 9:484–462.

Basavarajappa BS, Yalamanchili R, Cravatt BF, Cooper TB, Hungund BL (2006) Increased ethanol consumption and preference and decreased ethanol sensitivity in female FAAH knockout mice. Neuropharmacology 50:834–844.

Bkedash RA, Zhang C, Sarkar DK (2013) Gestational Choline Supplementation Normalized Fetal Alcohol-Induced Alterations in Histone Modifications, DNA Methylation, and Proopiomelanocortin (POMC) Gene Expression in beta-Endorphin-Producing POMC Neurons of the Hypothalamus. Alcohol Clin Exp Res 37:1133–1142.

Ben-Ari Y (2002) Excitatory actions of gaba during development: the nature of the nurture. Nat Rev Neurosci 3:728–739.

Benito E, Barco A (2010) CREB’s control of intrinsic and synaptic plasticity: implications for CREB-dependent memory models. Trends Neurosci 33:230–240.

Berghuis F, Rajnicke AM, Morozov YM, Ross RA, Mulder J, Urban GM, Monery K, Marsigiano C, Matteoli M, Canty A, Irving AJ, Katona I, Yanagawa Y, Rakic P, Lutz B, Mackie K, Harkany T (2007) Hardwiring the brain: endocannabinoids shape neuronal connectivity. Science 316:1212–1216.

Bonni A, Brunet A, West AE, Datta SR, Takeasu MA, Greenberg ME (1999) Cell survival promoted by the Ras-MAPK signaling pathway by transcription-dependent and -independent mechanisms. Science 286:1358–1362.

Bramham CR, Worley PF, Moore MJ, Guzowski JF (2008) The immediate early gene arc/arg3:1 regulates, mechanisms, and function. J Neurosci 28:11760–11767.

Brothers L, Ring B, Kling A (1990) Response of neurons in the macaque amygdala to complex social stimuli. Behav Brain Res 41:199–213.

Campuzano V et al. (1996) Friedreich’s ataxia: autosomal recessive disease caused by an intronic GAA triplet repeat expansion. Science 271:1423–1427.

Caroni P, Donato F, Muller D (2012) Structural plasticity upon learning: regulation and functions. Nat Rev Neurosci 13:478–490.

Castellano C, Rossi-Arnaud C, Cestari V, Costanzi M (2003) Cannabinoids and memory: animal studies. Curr Drug Targets CNS Neurol Disord 2:389–402.

Cecchinato J, Hompes T, Verhaeghen A,asteels C, Peuskens H, Bormans G, Claes S, Van Laere K (2014) Changes in cerebral CB1 receptor availability after acute and chronic alcohol abuse and monitored abstinence. J Neurosci 34:2822–2831.

Christie BR, Swann SE, Fox CJ, Froc D, Lieblach SE, Redilla V, Webber A (2005) Voluntary exercise rescues deficits in spatial memory and long-term potentiation in prenatal ethanol-exposed male rats. Eur J Neurosci 21:1719–1726.

Cushing BS, Kramer KM (2005) Mechanisms underlying epigenetic effects of early social experience: the role of neuropeptides and steroids. Neurosci Biobehav Rev 29:1089–1105.

Dellu F, Mayo W, Cherkaoui J, Le Moal M, Simon H (1992) A two-trial memory task independently of spatial context. Neurobiol Learn Mem 78:470–476.

Dember WN, Fowler H (1958) Spontaneous alternation behavior. Psychol Bull 55:412–428.

DeFoy L, Daut R, Brigham JL, MacPherson K, Crowley N, Gunduz-Cinar O, Pickens CI, Cinar R, Saksida LM, Kunos G, Lovinger DM, Bussey TJ, Camp MC, Holmes A (2013) Chronic alcohol produces neuroadaptations to prime dorsal striatal learning. Proc Natl Acad Sci USA 110:14783–14788.

Gavin DP, Sharma RP (2010) Histone modifications, DNA methylation, and schizophrenia. Neurosci Biobehav Rev 34:882–888.
Girard TA, Xing HC, Ward GR, Wainwright PE (2000) Early postnatal ethanol exposure has long-term effects on the performance of male rats in a delayed matching-to-place task in the Morris water maze. Alcohol Clin Exp Res 24:300–306.

Goodman AM, Delis DC, Mattson SN (1999) Normative data for 4-year-old children on the California Verbal Learning Test-Children’s Version. Clin Neuropsychol 13:274–282.

Hansen HH, Krutz B, Sifringer M, Stefovska V, Bittigau P, Pragt F, Marsicano G, Lutz B, Ikonomidou C (2008) Cannabinoids enhance susceptibility of immature brain to ethanol neurotoxicity. Ann Neurol 64:42–52.

Hashimoto K, Tsujita M, Miyazaki T, Kitamura K, Yamazaki M, Shin HS, Watanabe M, Sakamura K, Kano M (2011) Postsynaptic P/Q-type Ca2+ channel in Purkinje cell mediates synaptic competition and elimination in developing cerebellum. Proc Natl Acad Sci USA 108:9987–9992.

Herkenham M, A.B.L, Little MD, Johnson MR, Melvin LS, de Costa BR, Rice KC (1990) Cannabinoid receptor localization in brain. Proc Natl Acad Sci USA 87:1932–1936.

Hirvonen J, Zanotti-Fregonara P, Umhau JC, George DT, Rallison FR, Pragst F, Wozniak DF, Zorumski CF (2005) A single day of ethanol exposure can be overcome by non-spatial pre-training. Neuropsychopharmacology 30:719–727.

Holcomb L, Gordon MN, McGowan E, Yu X, Benkovic S, Jantzen BR, Rice KC (1990) Cannabinoid receptor localization in brain. Proc Natl Acad Sci USA 87:1932–1936.

Izumi Y, Kitabayashi R, Funatsu M, Izumi M, Yuede C, Hartman RE, Watanabe M, Mackie K, Katona I, Freund TF (2007) Molecular architecture of the cannabinoid signaling system in the core of the nucleus accumbens. Ideggogy Sz 60:187–191.

Kim D, Thayer SA (2001) Cannabinoids inhibit the formation of new synapses between hippocampal neurons in culture. J Neurosci 21:RC146.

Kogan JH, Frankland PW, Silva AJ (2000) Long-term memory underlying hippocampus-dependent social recognition in mice. Hippocampus 10:47–56.

Lubin FD, Swett JD (2007) The ikappaB kinase regulates chromatin structure during reconsolidation of conditioned fear memories. Neuron 55:942–957.

Luikart BW, Zhang W, Wayman GA, Kwon CH, Westbrook GL, Parada LF (2008) Neurotrophin-dependent dendritic fipolo- dial motility: a convergence on PI3K signaling. J Neurosci 28:7006–7012.

Makedonski K, Abuhatzira L, Kaufman Y, Razin A, Shemer R (2005) MeCP2 deficiency in Rett syndrome causes epigenetic aberrations at the PWS/AS imprinting center that affects UBE3A expression. Hum Mol Gen 14:1049–1058.

Mattson SN, Crocker N, Nguyen TT (2011) Fetal alcohol spectrum disorders: neuropsychological and behavioral features. Neuropsychol Rev 21:81–101.

Mattson SN, Goodman AM, Caine C, Delis DC, Riley EP (1999) Executive functioning in children with prenatal alcohol exposure. Alcohol Clin Exp Res 23:1808–1815.

Matyas F, Watanabe M, Mackie K, Katona I, Freund TF (2007) Cannabinoid receptor binding in alcohol dependence measured with positron emission tomography. Mol Psychiatry 18:916–921.

Ohno-Shosaku T, Maejima T, Kano M (2001) Endogenous cannabinoids mediate retrograde signals from depolarized postsynaptic elements and presynaptic 1 transgenes. Nat Med 4:97–100.

Perkins A, Lehmann C, Lawrence RC, Kelly SJ (2013) Alcohol exposure during development: impact on the epigenome. Int J Dev Neurosci 31:391–397.

π-Endocannabinoid signals in the developmental programming of delayed-onset neuropsychiatric and metabolic illnesses. Biochem Soc Trans 41:1569–1576.
Roberto M, Cruz M, Bajo M, Siggins GR, Parsons LH, Schweitzer P (2010) The endocannabinoid system tonically regulates inhibitory transmission and depresses the effect of ethanol in central amygdala. Neuropsychopharmacology 35:1962–1972.

Ross BS, Eichenbaum H (2006) Dynamics of hippocampal and cortical activation during consolidation of a nonspatial memory. J Neurosci 26:4852–4859.

Ryu H, Lee J, Hagerty SW, Soh BY, McAlpin SE, Cormier KA, Smith KM, Ferrante RJ (2006) ESET/SETDB1 gene expression and histone H3 (K9) trimethylation in Huntington’s disease. Proc Natl Acad Sci USA 103:19176–19181.

Sadrian B, Subbanna S, Wilson DA, Basavarajappa BS, Saito M (2012) Lithium prevents long-term neural and behavioral pathology induced by early alcohol exposure. Neuroscience 206:122–135.

Sakamoto K, Karelina K, Obrietan K (2011) CREB: a multifaceted regulator of neuronal plasticity and protection. J Neurochem 116:1–9.

Sanchez-Andrade G, Kendrick KM (2009) The main olfactory system and social learning in mammals. Behav Brain Res 200:323–335.

Sarnyai Z, Sibille EL, Pavlides C, Fenster RJ, McEwen BS, Toth M (2004) Impaired hippocampal-dependent learning and functional abnormalities in the hippocampus in mice lacking serotonin1A receptors. Proc Natl Acad Sci USA 97:14731–14736.

Savage DD, Becher M, de la Torre AJ, Sutherland RJ (2002) Dose-dependent effects of prenatal ethanol exposure on synaptic plasticity and learning in mature offspring. Alcohol Clin Exp Res 26:1752–1758.

Schmittgen TD, Livak KJ (2008) Analyzing real-time PCR data by the comparative C(T) method. Nat Protoc 3:1101–1108.

Shirasaka T, Hashimoto E, Ushigome T, Ishii T, Tateno M, Saito T (2012) Stem cell therapy: social recognition recovery and memory. J Neurosci 26:4852–4859.

Schipper TD, Livak KJ (2008) Analyzing real-time PCR data by the comparative CT method. Nat Protoc 3:1101–1108.

Shepherd JD, Bear MF (2011) New views of Arc, a master regulator of synaptic plasticity. Nat Neurosci 14:279–284.

Shirasaka T, Hashimoto E, Ushigome T, Ishii T, Tateno M, Saito T (2012) Stem cell therapy: social recognition recovery in a FASD model. Transl Psychiatry 2:e188.

Stefanis NC, Delespaul P, Henquet C, Bakoula C, Stefanis CN, Van Os J (2004) Early adolescent cannabis exposure and positive and negative dimensions of psychosis. Addiction 99:1333–1341.

Streissguth AP, Barr HM, Sampson PD (1990) Moderate prenatal alcohol exposure: effects on child IQ and learning problems at age 7 1/2 years. Alcohol Clin Exp Res 14:662–669.

Subbanna S, Basavarajappa BS (2014) Pre-administration of G9a/GLP inhibitor during synaptogenesis prevents postnatal ethanol-induced LTD deficits and neurobehavioral abnormalities in adult mice. Exp Neurol 261:34–43.

Subbanna S, Nagre NN, Shivakumar M, Umapathy NS, Psychoyos D, Basavarajappa BS (2014) Ethanol induced acetylation of histone at G9a Exon1 and G9a-mediated histone H3 dimethylation leads to neurodegeneration in neonatal mice. Neuroscience 258:422–432.

Subbanna S, Shivakumar M, Psychoyos D, Xie S, Basavarajappa BS (2015a) Anandamide-CB1 receptor signaling contributes to postnatal ethanol-induced neonatal neurodegeneration, adult synaptic and memory deficits. J Neurosci 35:8350–8366.

Subbanna S, Shivakumar M, Umapathy NS, Saito M, Mohan PS, Kumar A, Nixonc RA, Verin AD, Psychoyos D, Basavarajappa BS (2013b) G9a-mediated histone methylation regulates ethanol-induced neurodegeneration in the neonatal mouse brain. Neurobiol Dis 54:475–485.

Thomas JD, Sather TM, Whinery LA (2008) Voluntary exercise influences behavioral development in rats exposed to alcohol during the neonatal brain growth spurt. Behav Neurosci 122:1264–1273.

Thor DH, Wainwright KL, Holloway WR (1982) Persistence of attention to a novel conspecific: some developmental variables in laboratory rats. Dev Psychobiol 15:1–8.

Waltereit R, Dammermann B, Wulff F, Scafield J, Staubli U, Kaulemann G, Bundman M, Kuhl D (2001) Arg3/1/Arg mRNA induction by Ca2+ and cAMP requires protein kinase A and mitogen-activated protein kinase/extracellular regulated kinase activation. J Neurosci 21:5484–5493.

Wang D, Wang H, Ning W, Backlund MG, Dey SK, DuRois RN (2008) Loss of cannabinoid receptor 1 accelerates intestinal tumor growth. Cancer Res 68:6468–6476.

Warren ST (2007) The epigenetics of fragile X syndrome. Cell Stem Cell 1:488–489.

Wilson DA, Sullivan RM (2011) Cortical processing of odor objects. Neuron 72:506–519.

Wilson RI, Nicoll RA (2001) Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. Nature 410:588–592.

Wilson RI, Nicoll RA (2002) Endocannabinoid signaling in the brain. Science 296:678–682.

Wilson DA, Peterson J, Basavaraj BS, Saito M (2011) Local and regional network function in behaviorally relevant cortical circuits of adult mice following postnatal alcohol exposure. Alcohol Clin Exp Res 35:1974–1984.

Wu CS, Hew CP, Lu HC (2011) Lasting impacts of prenatal cannabinoid exposure and the role of endogenous cannabinoids in the developing brain. Future Neurol 6:459–480.

Wu CS, Morgan D, Hew CP, Andrews MJ, Leishman E, Spencer CM, Czyzyk T, Bradshaw H, Mackie K, Lu HC (2014) Long-term consequences of perinatal fatty acid amino hydrolase inhibition. Br J Pharmacol 171:1420–1434.

Ying SW, Futter M, Rosenblum K, Webber MJ, Hunt SP, Bliss TV, Bramham CR (2002) Brain-derived neurotrophic factor induces long-term potentiation in intact adult hippocampus: requirement for ERK activation coupled to CREB and upregulation of Arc synthesis. J Neurosci 22:1532–1540.

Young C, Straiko MM, Johnson SA, Creeley C, Olney JW (2008) Ethanol causes and lithium prevents neuroapoptosis and suppression of pERK in the infant mouse brain. Neurobiol Dis 31:355–360.