Effects of maternal binge alcohol consumption on emotional, cognitive and addictive behaviour in mice

Lídia Cantacorps Centellas

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Hace constar

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Fdo. Dra. Olga Valverde Granados

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"No temas a las dificultades, lo mejor surge de ellas."

Rita Levi-Montalcini

"Tot està per fer i tot és possible."

Miquel Martí i Pol

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ABSTRACT

Maternal alcohol binge drinking during pregnancy can be deleterious for the developing foetus, leading to a wide range of long-lasting morphological and neurobehavioural disabilities known as foetal alcohol spectrum disorders, associated with a higher risk of developing substance use disorders later in life. We sought to assess the effects of prenatal and postnatal alcohol exposure on cognitive, emotional, motor and addictive behaviour in mice and its underlying molecular mechanisms. Pregnant C57BL/6 female mice underwent a procedure to model alcohol binge drinking either during gestation or throughout both the gestation and lactation periods. Then, male offspring were assessed for their behaviour at adulthood. Binge alcohol exposure during early brain development induces cognitive deficits, increased anxiety-like behaviour, motor coordination impairments, and age-dependent locomotor activity alterations. Behavioural effects are associated with an upregulation of pro-inflammatory signalling, gliosis, neuronal death, myelin impairments and epigenetic modifications in the prefrontal cortex and hippocampus. Furthermore, early alcohol exposed mice show alterations in brain network connectivity. Curcumin treatment ameliorates anxiety and cognitive dysfunctions, and rescues alcohol-induced neuroinflammation. In addition, mice exposed to alcohol *in utero* and postnatally show increased susceptibility to later alcohol and cocaine intake compared with their counterparts. Molecular analyses of the prefrontal cortex and striatum of these animals suggest alterations in the glutamatergic excitability within the mesocorticolimbic reward system following cocaine-induced reinstatement. Altogether, our results reveal that maternal binge-like alcohol consumption induces molecular alterations in offspring's brain that may underlie the long-lasting impairments in offspring's behaviour.

RESUM

El consum maternal d'alcohol en afartament durant l'embaràs pot resultar perjudicial per al fetus en desenvolupament, donant lloc a una àmplia gamma de discapacitats físiques i mentals conegudes com a trastorns de l'espectre alcohòlic fetal que persisteixen al llarg de la vida i estan associades a un major risc de desenvolupar trastorns d'ús de substàncies en el futur. En aquesta tesi hem tractat d'avaluar els efectes de l'exposició prenatal i postnatal a l'alcohol en la conducta cognitiva, emocional, motora i addictiva en ratolins i els mecanismes moleculars subjacents a aquests. Les femelles de ratolins C57BL/6 embarassades van ser sotmeses a un procediment per modelar el consum d'alcohol en afartament durant la gestació o bé, al llarg dels períodes de gestació i lactància. A continuació es va avaluar el comportament de la descendència masculina a l'edat adulta. S'ha observat que l'exposició d'alcohol en afartament durant el desenvolupament cerebral indueix dèficits cognitius, augment de l'ansietat, alteracions de coordinació motora i de l'activitat locomotora en funció de l'edat. Els efectes del comportament estan associats a un increment de la senyalització proinflamatòria, gliosi, mort neuronal, deteriorament de la mielina i modificacions epigenètiques en el còrtex prefrontal i l'hipocamp, així com també alteracions en la connectivitat de la xarxa neuronal. El tractament de curcumina alleuja l'ansietat i les disfuncions cognitives, i restableix la neuroinflamació induïda per l'alcohol. A més, els ratolins exposats a l'alcohol durant la gestació i la lactància mostren una major susceptibilitat a la ingesta posterior d'alcohol i cocaïna en comparació amb els seus homòlegs. Els anàlisis moleculars de l'escorça prefrontal i de l'estriat d'aquests animals suggereixen la presència d'alteracions en l'excitabilitat glutamatèrgica en el sistema de recompensa mesocorticolimbic després de la recaiguda induïda per cocaïna. En conjunt, els nostres resultats indiquen que el consum maternal d'alcohol en afartament provoca alteracions moleculars en el cervell de la descendència com a mecanisme subjacent a les alteracions relatives al comportament persistents.

List of abbreviations

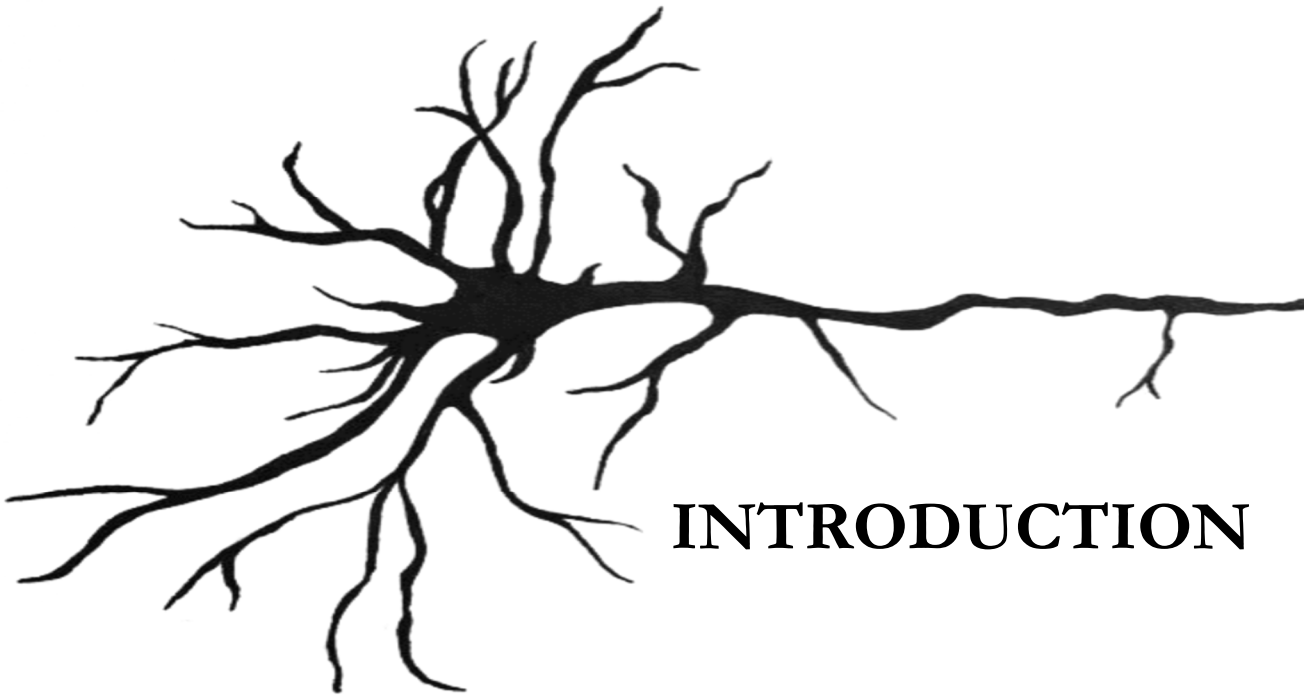
ΔFosB	Delta FosB
5-HT	Serotonin
ADH	Alcohol dehydrogenase
ADHD	Attention-deficit/hyperactivity disorder
ALDH	Aldehyde dehydrogenase
AMPA	Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMPAR	AMPA receptor
ANOVA	Analysis of variance
ARBD	Alcohol-related birth defects
ARND	Alcohol-related neurodevelopmental disabilities
AST	Astaxanthin
BAC	Blood alcohol concentration
BBB	Blood-brain barrier
BCA	Bicinchoninic acid
BDNF	Brain-derived neurotrophic factor
BrdU	5'-bromo-2'-deoxyuridine
BSA	Bovine serum albumin
CA	*Cornu ammonis*
CCO	Cytochrome-c oxidase
CDC	Centers for Disease Control and Prevention
CNS	Central nervous system
CPP	Conditioned place preference
CREB	cAMP-responsive element binding
CYP2E1	Cytochrome P450 2E1
D1R	Dopamine receptor 1
D2R	Dopamine receptor 2
DA	Dopamine
DARPP	Dopamine- and cAMP-regulated phosphoprotein
DAT	Dopamine transporter

DG	Dentate gyrus
DID	Drinking in the dark
DMSO	Dimethyl sulfoxide
DNMT	DNA methyl transferases
DSM	Diagnostic and Statistical Manual of Mental Disorders
dSTR	Dorsal striatum
EGCG	(-)-Epigallocatechin-3-gallate
ELISA	Enzyme-linked immunosorbent assay
EPM	Elevated plus maze
EtG	Ethyl glucuronide
EtS	Ethyl sulfate
FAEE	Fatty acid ethyl esters
FAS	Foetal Alcohol Syndrome
FASD	Foetal Alcohol Spectrum Disorders
FR	Fixed ratio
GABA	Gamma-aminobutyric acid
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GC-FID	Gas chromatography with flame ionization detector
GD	Gestational day
GFAP	Glial fibrillary acidic protein
GluA1	Glutamate ionotropic AMPA receptor subunit 1
GluA2	Glutamate ionotropic AMPA receptor subunit 2
H	Histone
HAT	Histone acetyltransferase
HDAC	Histone deacetylase
HMGB1	High-mobility group box 1
HPA	Hypothalamic-pituitary-adrenal
HPC	Hippocampus
Iba-1	Ionized calcium-binding adapter molecule 1
IGF	Insulin-like growth factor

IL	Interleukin
IOM	Institute of Medicine of the National Academies
LHb	Lateral habenula
LMNA	Lamin A/C
LTP	Long-term potentiation
Lys	Lysine
MAG	Myelin-associated glycoprotein
MBP	Myelin basic protein
miRNA	microRNA
MOG	Myelin oligodendrocyte glycoprotein
mPFC	Medial prefrontal cortex
MYRF	Myelin regulatory factor
NAc	Nucleus accumbens
NAD	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
NCAM	Neural cell adhesion molecule
ND-PAE	Neurodevelopmental Disorder Associated with Prenatal Alcohol Exposure
NeuN	Neuronal nuclei
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NIAAA	National Institute on Alcohol Abuse and Alcoholism
NLR	NOD-like receptor
NLRP3	NOD-like receptor family, pyrin domain containing protein 3
NMDA	N-methyl-D-aspartate
NOD	Nucleotide-binding oligomerization domain
NOR	Novel object recognition
OD	Optical density
OPC	Oligodendrocyte progenitor cell
PAE	Prenatal alcohol exposure
PB	Phosphate buffer

PBS	Phosphate saline buffer
pCREB	phospho-CREB
PD	Postnatal day
PEth	Phosphatidylethanol
PFAS	Partial Foetal Alcohol Syndrome
PFC	Prefrontal cortex
PLAE	Prenatal and lactation alcohol exposure
PLP	Proteolipid protein
PPAR	Peroxisome proliferator-activated receptor
PR	Progressive ratio
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SA	Self-administration
SEM	Standard error of the mean
SGZ	Subgranular zone
STR	Striatum
TBS	Tris-buffered saline
TBS-T	Tris-buffered saline with Tween-20
TLR	Toll-like receptor
TNF-α	Tumour necrosis factor-α
US	United States
VCL	Vinculin
vSTR	Ventral striatum
VTA	Ventral tegmental area

INDEX



INTRODUCTION

1. Alcohol

1.1. General properties

Alcohol (ethanol) is a two-carbon molecule that interacts with other biomolecules via hydrogen bonding and weak hydrophobic interactions (Abrahao et al., 2017). It is a product of vegetal sugar fermentation, colourless in liquid form, volatile, flammable, and with a slight chemical odour. It can be used as a solvent, fuel or antiseptic. In addition, ethanol is the psychoactive substance present in alcoholic beverages, fermented or distilled.

After consumption, alcohol is absorbed into the blood stream through the stomach and intestines, and it readily crosses the blood-brain barrier (BBB). Alcohol is distributed with body water and is found at approximately the same concentration in all tissues, including the nervous system. Most of the metabolism of alcohol occurs in the liver, where alcohol is converted to acetaldehyde by multiple isoenzymes of alcohol dehydrogenase (ADH). This oxidation is accompanied by the reduction of nicotinamide adenine dinucleotide (NAD^+) to NADH, generating a highly reduced cytosolic environment in the hepatocytes (Zakhari, 2006). 90-95% of alcohol is eliminated by oxidation and the rest is excreted through the kidneys, lungs and skin (Deng and Deitrich, 2007). Alcohol's metabolism in the brain is limited, the mitochondrial cytochrome P450 2E1 (CYP2E1), catalase and other pathways might generate acetaldehyde, which then is rapidly metabolized further (Most et al., 2014). The mitochondrial aldehyde dehydrogenase (ALDH) converts acetaldehyde to acetate resulting in the formation of NADH, which is oxidized by the electron transport chain.

Acetaldehyde, the primary metabolite of alcohol, is highly reactive and toxic, and it is responsible for the flushing effect that encompasses face flushing, nausea, vomiting, headache, tachycardia and sweating. In addition, acetaldehyde can cross the BBB and may have direct actions in the brain. Most

of the acetate resulting from alcohol metabolism escapes the liver into the blood. Eventually, it is metabolized to CO_2 by the Krebs cycle generating acetyl-CoA in tissues like the heart, the skeletal muscle and the brain (Zakhari, 2013). Alcohol can also be non-oxidatively metabolized by two minor pathways that end up with production of fatty acid ethyl esters or phosphatidyl ethanol, which may play a role on alcohol-induced tissue damage (Zakhari, 2006).

Alcohol's effects on brain function depend on its concentration in the blood over time and occur across a range from the low millimolar range to 100 mM. Low blood alcohol concentration (BAC) of ~28 mg/dL (~6 mM) can be reliably distinguished in both humans and animal models (Abrahao et al., 2017). Levels of 50-80 mg/dL are associated with anxiolytic and euphoric effects and lead to slowed reaction times, motor incoordination, and mild speech, memory and attention impairments. At concentrations up to 230 mg/dL (50 mM), locomotor disruption, cognitive impairment and sedation escalate. Above this level, strong sedation and respiratory depression can lead to coma or death (Dasgupta, 2017).

In regards to alcohol's pharmacological actions in the brain, it has putative bindings sites with specific neuronal membrane proteins involved in signal transmission, resulting in changes in neural activity, as shown in **Figure 1** (Spanagel, 2009; Vengeliene et al., 2009). Acutely, alcohol acts as an inhibitor of the N-methyl-D-aspartate (NMDA) glutamate receptor and enhances γ-aminobutyric acid (GABA) release, therefore acting as a depressor of the central nervous system (CNS). In addition, alcohol affects other neurotransmitter systems, including opioids, serotonin (5-HT), dopamine (DA) and endocannabinoids. Non-ligand ion channels also constitute a primary target of alcohol. However, these primary inhibitory or facilitatory actions on ion channels and receptors depend on the alcohol concentration and the subunit composition of a particular channel, which might explain the

diversity of alcohol's pharmacological actions (Erdozain and Callado, 2014; Volkow et al., 2017).

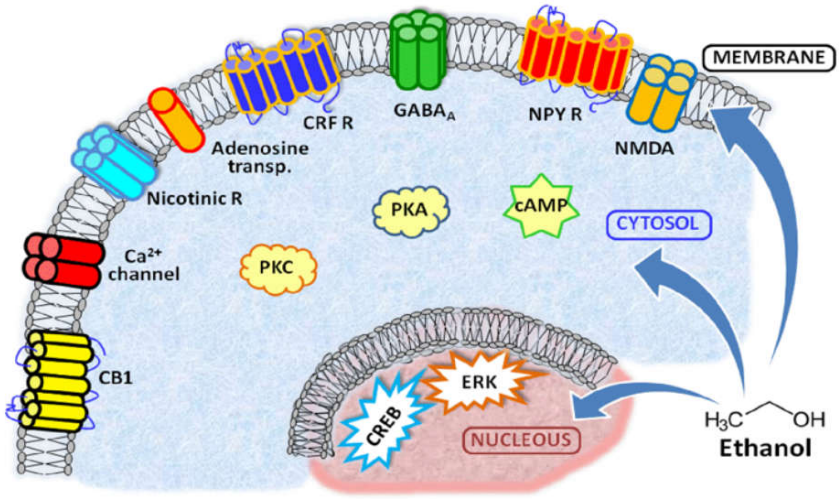


Figure 1. A representation of the great number of cellular components that are modulated by alcohol exposure directly or indirectly: membrane receptors, cytosolic signalling elements, and transcription factors in the nucleus. R, receptor; NPY, neuropeptide Y; GABA, γ-amino-butyric acid; NMDA; N-methyl-D-aspartic acid; CRF, corticotrophin-releasing factor; CB1, Cannabinoid Receptor 1; PKC and PKA, protein kinase C and A; cAMP, cyclic adenosine monophosphate; ERK, extracellular-signal-regulated kinase; CREB, cAMP-responsive element binding protein. Obtained from Erdozain and Callado (2014).

1.2. Epidemiology and patterns of consumption

Alcohol is the psychoactive drug most consumed worldwide. In 2016, 32.5% of the global population were current drinkers. By gender, 25% of women and 39% of men were current drinkers. The prevalence of alcohol consumption varies considerably by geographic location (**Figure 2**). Drinking prevalence is lowest in low-to-middle socio-demographic index locations, where larger differences between men and women are also found. Conversely, in locations with high socio-demographic index, the prevalence of current drinkers

between genders is similar. Alcohol is consumed by more than half of the population in the European region (59.9% of current drinkers), the Region of the Americas (54.1%) and the Western Pacific region (53.8%) (World Health Organization, 2018).

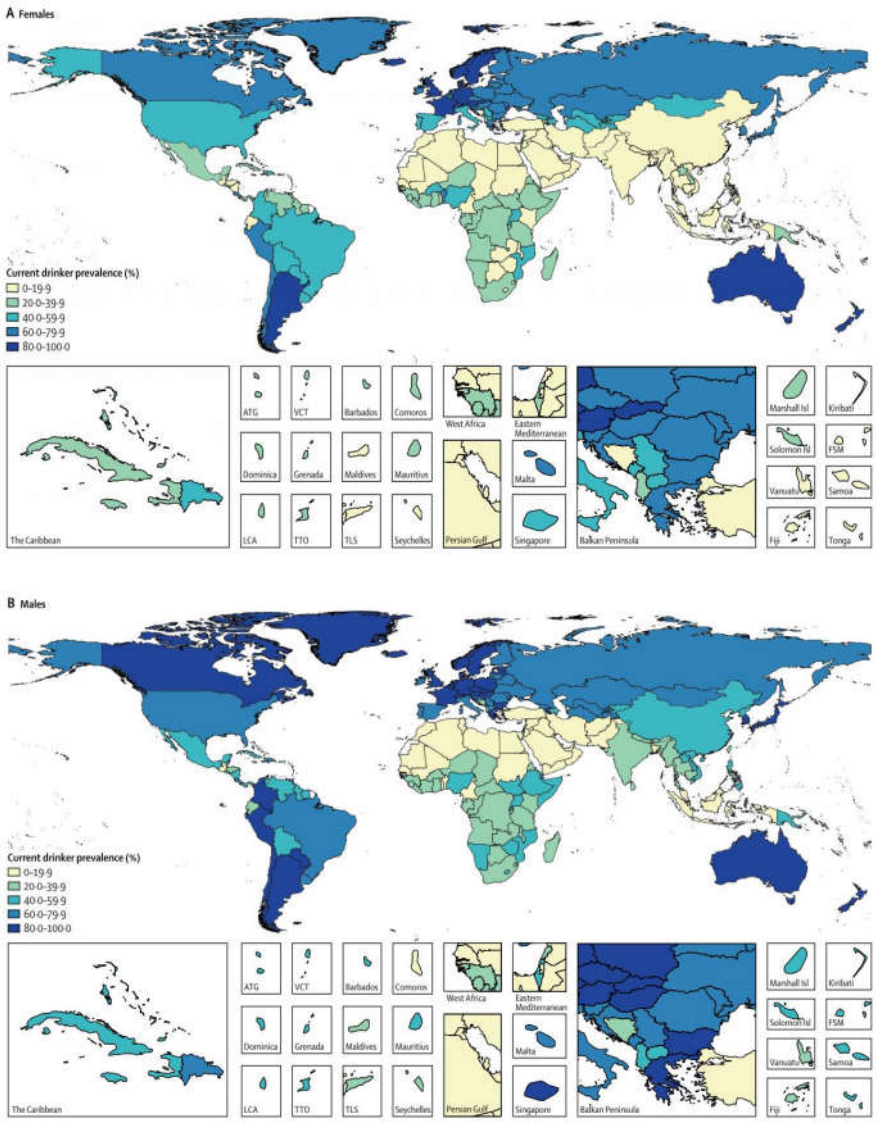


Figure 2. Age-standardised prevalence of current drinking for females (A) and males (B) in 2016, in 195 locations. Current drinkers are defined as individuals who reported having consumed alcohol within the past 12 months. ATG, Antigua and Barbuda;

VCT, Saint Vincent and the Grenadines; Isl, Islands; FSM, Federated States of Micronesia; LCA, Saint Lucia; TTO, Trinidad and Tobago; TLS, Timor-Leste. Obtained from GBD 2016 Alcohol Collaborators (2018).

Alcohol use is a leading risk factor for global disease burden and causes substantial health loss. Around 3 million people die each year as a consequence of alcohol abuse, which represents 5.3% of total deaths (World Health Organization, 2018). According to a recent systematic review and meta-analysis, the risk of all-cause mortality rises with increasing levels of consumption (GBD 2016 Alcohol Collaborators, 2018). Among the population aged 15-49 years, alcohol use is the leading global risk factor for risk-attributable disease burden, accounting for 2.2% of female deaths and 6.8% of male deaths.

In Spain, alcohol is the drug of abuse more consumed among the population aged 15-64 years, with a prevalence of current drinkers of 75.2%. Its consumption is widely socially accepted and the production of alcoholic beverages, such as beer and wine, is an important source of economic activity. Interestingly, the risk perceived by the society associated with alcohol consumption is low, since only 25.6% of the population consider that the consumption of 5-6 alcoholic drinks during the weekend can produce health problems (EDADES, 2018). Among the Spanish adolescent population (aged 14-18 years), 75.6% reported drinking in the last 12 months and 67% during the last 30 days. In addition, the average age of first alcohol use is at 14 years old, being one of the drugs with an earliest age of onset consumption (ESTUDES, 2017).

One of the most popular patterns of high-risk alcohol consumption is binge drinking, which is defined as the consumption of four or more drinks for women and five or more drinks for men in a single occasion (over 2 h or less). The difference between males and females has been attributed to differences in the distribution of body fat and lower gastric levels of the alcohol-

metabolizing enzyme ADH in females (Koob et al., 2014). Specifically, the National Institute on Alcohol Abuse and Alcoholism (NIAAA) considers binge drinking when BAC reaches levels of 80 mg/dL (Wechsler and Nelson, 2001; NIAAA, 2016). But what is a 'drink'? In order to normalize the quantity of alcohol that contains each alcoholic beverage, the concept of 'standard drink unit' is employed. However, different countries define standard drinks differently. In United States (US), a standard drink contains roughly 14 g of pure alcohol (NIAAA, 2019), while in Spain it contains 10 g of pure alcohol (Rodríguez-Martos Dauer et al., 1999).

Binge drinking is the pattern of consumption more common among teenagers and young adult population. Globally, approximately 40% of drinkers aged 15 years or above engage in heavy episodic alcohol drinking, being around 18% among the total population. Also, heavy episodic drinking is particularly prevalent (≥20%) among young people (15-19-year-olds) in Europe and high-income countries such as Australia, Canada, New Zealand and the US (World Health Organization, 2018). In US, binge drinking is most common among younger adults aged 18-34 years (CDC, 2017). In Spain, 31.7% of adolescent population (aged 14-18 years) reported binge drinking in the last month (ESTUDES, 2017).

1.3. Alcohol use among pregnant women

Historically, women have consumed less alcohol than men. Even though, over time, the changing societal role of women has led to a rise in the frequency and levels of alcohol consumption. Young women in Europe are becoming more frequent binge drinkers (Wilsnack et al., 2009; Davoren et al., 2016). According to the Centers for Disease Control and Prevention (CDC) of the US, 46% of women of the general population reported alcohol drinking in the last 30 days, and 12% of women engage in binge drinking 3 times per month (CDC, 2017).

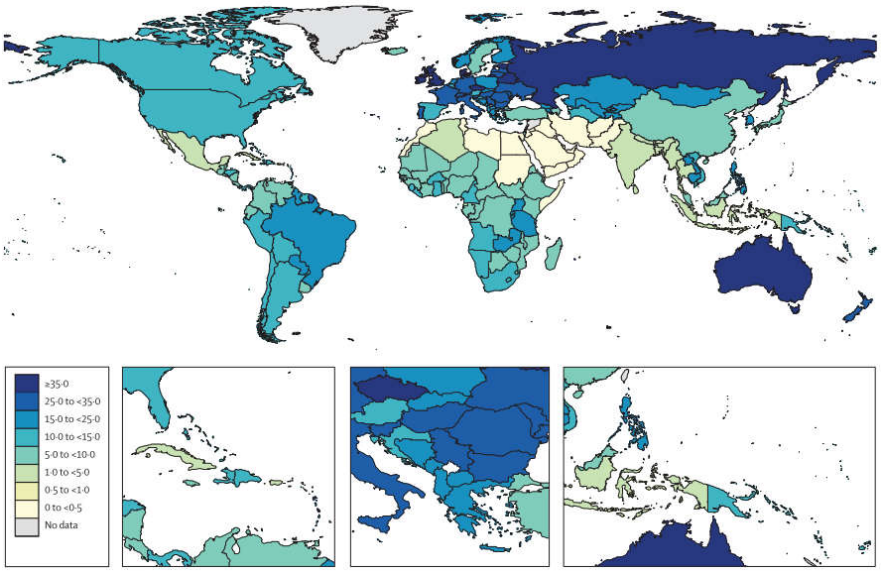


Figure 3. Global prevalence (%) of alcohol use during pregnancy among the general population in 2012. Obtained from Popova et al. (2017a).

The global prevalence of women who consume alcohol while pregnant has been estimated to be 9.8% (**Figure 3**). Alarmingly, a quarter of women in the European region drink alcohol during pregnancy, being the highest prevalence (25.2%), while the lowest prevalence was found in the Eastern-Mediterranean region (0.2%), which includes most of the Muslim populations, a religion associated with very high rates of abstention from alcohol (Popova et al., 2017a, 2018). Ireland is the country with the highest prevalence of alcohol use among pregnant women (60.4%), followed by Belarus (46.6%), Denmark (45.8%), United Kingdom (41.3%) and Russia (36.5%). Furthermore, in Europe, 10.7% of women who drank any amount of alcohol during pregnancy, reported binge drinking. In Canada and US, about 10% and 15% of pregnant women consume alcohol, respectively. Moreover, the prevalence of binge drinking during pregnancy is estimated to be around 3% (Tan et al., 2015; Popova et al., 2017b).

The highest prevalence of binge drinking during pregnancy worldwide is in the African region (3.1%), while the Western Pacific region has the lowest one

(1.8%) (Lange et al., 2017b). Remarkably, among women who engage in binge drinking, pregnant women report a higher frequency of bingeing than non-pregnant women (Tan et al., 2015).

Numerous risk factors are related with maternal alcohol consumption during pregnancy and pre-conceptional binge drinking. These include maternal age, unplanned pregnancy, substance use, marital status, history of physical and emotional abuse, mental health, self-esteem, nutrition, and socioeconomic status (Caetano et al., 2006). For instance, women with a substance use disorder diagnosed reported a higher frequency of binge episodes during pregnancy than non-diagnosed women (Bakhireva et al., 2018).

1.4. Alcohol use during breastfeeding

Alcohol consumed by a mother passes easily into her breast milk at similar concentrations to those found in the bloodstream. It is estimated that a nursing child is exposed to 5-6% of the weight-adjusted maternal alcohol dose ingested, even though newborns metabolize alcohol at approximately half the rate of adults (Koren, 2002). About half of all lactating women in Western countries consume alcohol while breastfeeding, and 80% of the women who drank alcohol during pregnancy continued to do so during lactation (Haastrup et al., 2014), even though the prevalence varies across different countries (May et al., 2016). In France, 6.8% of women reported binge drinking during breastfeeding, and 7.0 and 6.4% of nursing women reported light and moderate drinking, respectively (Dumas et al., 2017).

Hence, the increasing rates of binge alcohol drinking in recent years among young adult population, and especially amongst women of childbearing age, is of special concern since alcohol consumption during pregnancy has been established as a risk factor for adverse birth outcomes, including miscarriage, stillbirth and premature delivery, and can result in a range of lifelong disabilities known as Foetal Alcohol Spectrum Disorders (FASD), as discussed below.

2. Foetal Alcohol Spectrum Disorders

2.1. Historical background

First evidences of the association between maternal alcoholism and birth defects can be found in the literature from centuries ago. In the biblical *Book of Judges* 13:3-4, from the Old Testament, a statement linking drinking alcohol during pregnancy and the well-being of the child is cited: "Thou shalt conceive, and bear a son. Now therefore, beware, I pray thee, and drink not wine nor strong drink, and eat not any unclean thing: For, lo, thou shalt conceive, and bear a son; and no razor shall come on his head; for the child shall be a Nazarite unto God from the womb". Ancient Greek and Roman philosophers/scientists were also concerned about the effects of alcohol intoxication at the time of conception to induce deformities in offspring (Calhoun and Warren, 2007). Aristotle's quote in *Problemata* "foolish, drunken, or haire-brain women most often bring forth children like unto themselves, *morosos et languidos*" reveals his awareness about prenatal alcohol effects (Burton, 1621). Also, there was a Carthaginian custom prohibiting the consumption of alcohol on the wedding night "in order that defective children might not be conceived" (Brown et al., 2019a).

However, it was not until the first half of the 18th century when the Royal College of Physicians in London described children from alcoholic mothers as "weak, feeble, and distempered children, who must be instead of an advantage and strength, a charge to their Country" (Royal College of Physicians of London, 1726). This report was published after the distilling restrictions in England were lifted, which in turn flooded the market with gin, leading to the so-called "gin epidemic". From 1720 to 1750 birth rates dropped and death rates increased, especially among children under 5 years of age. A committee of the Middlesex Sessions reported in 1736, "Unhappy mothers habituate themselves… children are born weak and sickly, and often look shrivelled and old, as though they had numbered many years". In 1751 an act

of Parliament enforcing controls on the manufacture and sale of distilled spirits effectively ended the epidemic (Warner and Rosett, 1975).

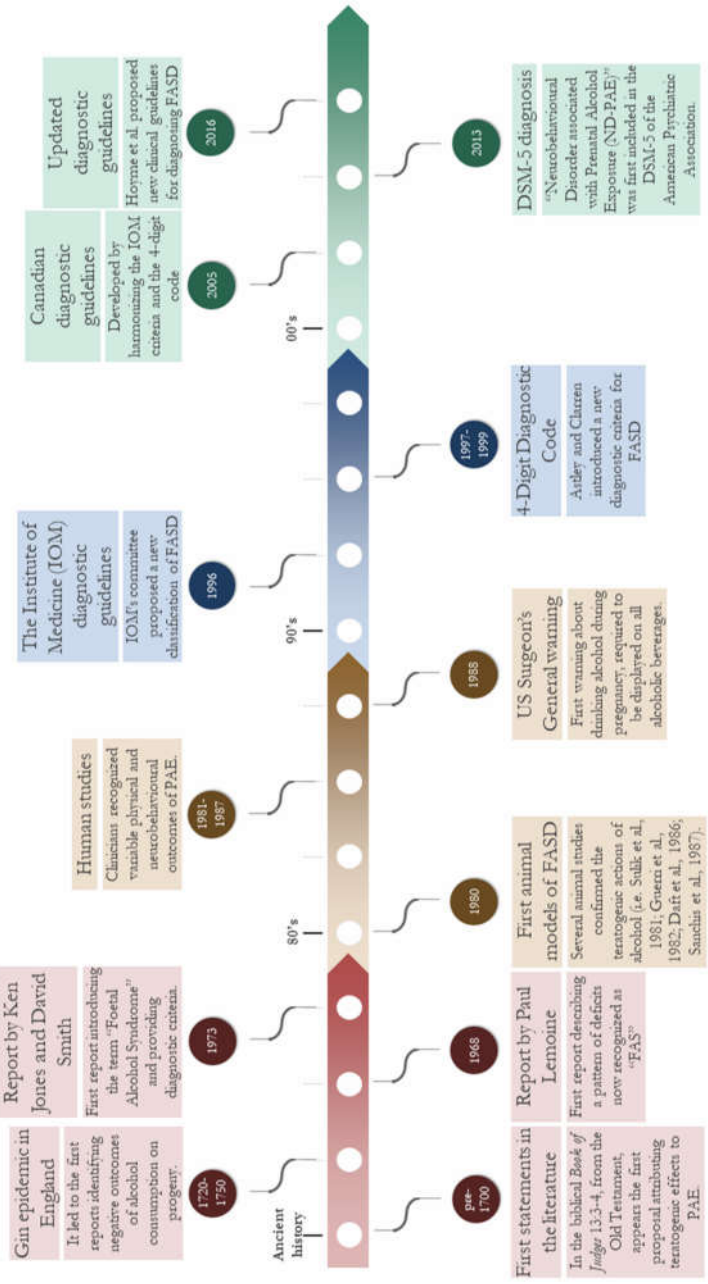


Figure 4. Schematic timeline of FASD history.

Even though, in the US, medical literature on alcohol and its action during pregnancy was not developed until the 19th century. In 1826 the American Temperance Society was founded, leading to the appearance of the temperance movement, which promoted medical research linking prenatal alcohol exposure (PAE) to adverse birth outcomes and pushed for the legal prohibition of alcohol. In the mid-1800s physicians threw themselves into the debate over the inheritance of alcohol effects on offspring. Evidence of damage caused by maternal drinking was being used to encourage total abstinence and throughout the mid-century, several reports and commentaries continued to appear (Warner and Rosett, 1975). By 1899, a physician of the Convict Prison in Liverpool (England) described the offspring of imprisoned alcoholic women, concluding that alcohol had a direct toxic effect on the foetus (Sullivan, 1899). Also, Nicloux (1900) demonstrated that alcohol ingested by the mother was able to cross the placenta and reach the foetus.

At the beginning of the 20th century, the eugenics movement considered alcohol as a "racial poison". Many eugenicists stood up for alcohol prohibition, arguing that alcohol was a dangerous substance which was leading to the decline of civilization and causing the disintegration of the Anglo-Saxon race. Several animal studies were reported demonstrating the adverse effects of alcohol on offspring (Hodge, 1903; Ballantyne, 1904; Nice, 1912, 1917; Stockard and Papanicolaou, 1916; Macdowell and Vicari, 1917). In 1920, the production, importation, transportation and sale of alcoholic beverages was prohibited in the US, Canada and some European countries for thirteen years. During this time, scarce medical articles about alcohol and pregnancy were published, and some scientists rejected the conclusions of their earlier studies due to the controversial nature of the topic. Furthermore, obstetricians took an ambiguous position towards alcohol, since they were using alcohol as an anaesthetic (Brown et al., 2019a). Thus, the scepticism about earlier evidences grew up during the late 1930's and early 1940's. Yet, the scientific and medical communities renewed their interest for the teratogenic effects of alcohol after

observing the disaster consequences of thalidomide and the atomic explosions in Hiroshima in the 1950s-1960s.

The first observation of a pattern of symptoms that we now recognize as FASD in a group of 127 children born to alcoholic mothers was attributed to Paul Lemoine, a French paediatrician (Lemoine et al., 1968). Even though, his article was not internationally recognized. In 1973, two American paediatricians, Ken Jones and David Smith, also described a pattern of deficits in children of women who had consumed alcohol during pregnancy and introduced for the first time the term "Foetal Alcohol Syndrome" (Jones and Smith, 1973), which focused new attention on alcohol use as a serious public health issue. Their findings encouraged a series of case reports and posterior research into the mechanisms of prenatal alcohol exposure (Jones et al., 1973). In 1977, the first public awareness warning was issued by the US Food and Drug Administration *Drug Bulletin* and by the NIAAA in the CDC Mortality and Morbidity Weekly Report.

In the 1980's several studies using animal models of FASD came out, increasing the knowledge about the alcohol's teratogenic effects (i.e. Daft et al., 1986; Guerri and Grisolía, 1982; Guerri and Sanchis, 1986; Sulik et al., 1981). In 1988 the Surgeon General of the US Public Health Service released a federal-level warning against drinking alcohol during pregnancy, which was required to be displayed on all alcoholic beverages. Later, in 1996, the Institute of Medicine of the National Academies (IOM) of the US launched new diagnostic criteria with five categories differentiated under the FASD umbrella: FAS with and without confirmed alcohol exposure, partial FAS (PFAS), alcohol-related neurodevelopmental disabilities (ARND) and alcohol-related birth defects (ARBD) (Stratton et al., 1996). PFAS was used for those individuals with confirmed PAE, evidence of some facial characteristics and either growth, CNS deficits, or a complex pattern of behavioural or cognitive abnormalities. ARND refers to those with CNS deficits or a complex pattern of behavioural or cognitive abnormalities, while ARBD includes individuals

with congenital physical abnormalities. IOM's diagnostic categories were further revised and redefined by Hoyme and colleagues (2005, 2016).

Astley and Clarren introduced the 4-Digit Diagnostic Code (Astley and Clarren, 1997, 2000) which provided standardized ordinal measurement scales that increased the objectivity of diagnosis and reflected the diverse continuum of disabilities found within FASD. However, difficulties in reaching a consensus on categorization could arise from these guidelines. Thus, in 2005 the Canadian diagnostic guidelines were published, which unified the IOM's guidelines with the 4-Digit Diagnostic Code (Chudley et al., 2005). In 2013, the 5th edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) included for the first time the 'Neurodevelopmental Disorder Associated with Prenatal Alcohol Exposure (ND-PAE)', which can be diagnosed regardless of the presence of physical anomalies with confirmation of maternal alcohol exposure.

Nowadays, the recognition of FASD as a clinical disorder is well established, with scientific evidence of CNS damage induced by alcohol exposure *in utero* in all the FASD subtypes which can be recognized using current diagnostic tools.

2.2. Epidemiology of FASD

Estimating the prevalence of FASD is complicated due to the variability between different diagnostic criteria and because of the heterogeneity in terms of methodology of the studies to estimate the prevalence (Farag, 2014). Furthermore, it is suggested that 80% of children with FASD are not diagnosed or misdiagnosed (7%) (May et al., 2018).

Among children and youth in the general population, the global prevalence of FASD is estimated to be 77.3 per 10,000 people (0.77%), and one in 13 pregnant women who consumed any amount of alcohol during pregnancy delivers a child with FASD. In line with the prevalence of alcohol use during

pregnancy, FASD is more prevalent in the European region (198.2 per 10,000 people; 1.9%) while the Eastern-Mediterranean region has the lowest prevalence (1.3 per 10,000 population; 0.1%). Of 187 countries, South Africa is estimated to have the highest prevalence of FASD (11.1% of the population), followed by Croatia (5.3%) and Ireland (4.7%) (Lange et al., 2017a). However, other studies estimate the global prevalence of FASD to be about 2.3% among the general population (Roozen et al., 2016b) and to range from 2% to 5% in a representative Midwestern US community of first-grade students (6-7 years old) (May et al., 2014, 2018). Given that FASD is a life-long disability, it is estimated that more than 11 million individuals between 0 and 18 years of age are affected in the general population worldwide (Popova et al., 2017a).

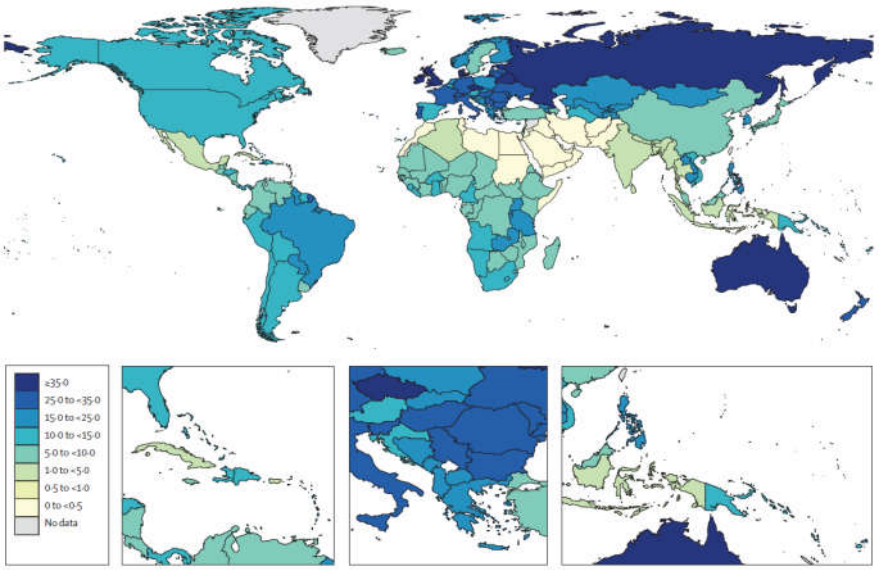


Figure 5. Global prevalence (per 10,000 people) of FAS among the general population in 2012. Obtained from Popova et al. (2017a).

The global prevalence of FAS, the most severe form into the FASD continuum, is estimated to be 14.6 per 10,000 people (0.15%) (**Figure 5**). As for FASD, the highest prevalence of FAS is in the European region (37.4 per

10,000 people; 0.37%) and the lowest in the Eastern-Mediterranean region (0.2 per 10,000 people; 0.002%). Also, South Africa is the country with the highest prevalence of FAS (585.3 per 10,000 people; 5.85%) (Popova et al., 2017a).

In addition, it is estimated that one in every 67 mothers who consume alcohol during pregnancy deliver a child with FAS (Popova et al., 2017a). For the US, the prevalence of FAS exceeds that of other birth defects, such as Down's syndrome, anencephaly or spina bifida, even though the prevalence ratio of FAS to FASD is about two out of ten (Popova et al., 2018), indicating that FAS is only the tip of the iceberg.

Among the general population of US, the pooled prevalence is estimated to be about 2 per 1,000 for FAS and 15 per 1,000 for FASD, while in Canada, the pooled prevalence of FAS and FASD is estimated to be about 1 per 1,000 and 5 per 1,000, respectively (Popova et al., 2017b).

Interestingly, a recent study reported that the pooled prevalence of FASD among special subpopulations (children in care, correctional, special education, specialized clinical and Aboriginal populations) is higher compared with the general population (Popova et al., 2019). In fact, the prevalence of alcohol use during pregnancy in the Aboriginal populations is found to be three to four times higher than in the general population.

FASD is the leading preventable cause of mental impairment and developmental disabilities in the western world. It has been estimated that it has an annual economic burden of $24,308 per adult and $22,810 per child (Greenmyer et al., 2018), which could be minimised by early diagnosis and interventions.

2.3. Diagnosis of FASD

FASD is the umbrella term used to describe the physical, mental and behavioural disabilities that can be present in children exposed prenatally to alcohol (Clarke and Gibbard, 2003; Murawski et al., 2015). Mental alterations can vary from learning deficits and mental retardation to behavioural and psychiatric disorders so there is a wide variability in signs and symptoms among the individuals affected (Jacobson and Jacobson, 2002). For this reason, the assignment of a FASD is a complex medical diagnostic process that requires a multidisciplinary approach (Cook et al., 2016). A lack of accurate diagnosis can lead to inappropriate patient care, increased risk of secondary disabilities, and inaccurate estimates for incidence and prevalence (Benz et al., 2009; Brown et al., 2019b).

Assessment of maternal prenatal alcohol intake is an essential part of the diagnostic process, which is measured by quantity of alcohol consumed (standard drinks per day), frequency and timing during gestation. Then, the presence or absence of the characteristic structural features of FASD must be evaluated, as well as, the three cardinal facial characteristics (short palpebral fissures, smooth philtrum and thin vermilion border of the upper lip). In addition, a comprehensive neurodevelopmental evaluation is crucial. Once the prenatal exposure history, dysmorphology assessment and neuropsychological testing have been obtained, genetic testing is used if other teratogenic conditions with similar facial phenotype are being considered for a differential diagnosis (Sokol et al., 2003; Hoyme et al., 2016). Early identification of prenatally exposed infants is critical to withhold the long-term behavioural problems. The median age of diagnosis is at 3.3 years, 6.5% are diagnosed at birth while 63% of FASD children are diagnosed at 5 years of age (Elliott et al., 2008).

Thus, the first step is the identification of prenatal exposure to alcohol. Documented PAE is defined as the following (one or more conditions must be met):

- Consumption of ≥6 drinks/week for ≥2 weeks during pregnancy
- Consumption of ≥3 drinks per occasion on ≥2 occasions during pregnancy
- Documentation of alcohol-related social or legal problems in proximity to (before or during) the index pregnancy
- Positive testing with established alcohol-exposure biomarkers during pregnancy or at birth
- Increased prenatal risk associated with drinking during pregnancy as assessed by a validated screening tool

There are some current available screening questionnaires for pregnant women, such as the AUDIT (Alcohol Use Disorders Identification Test), TWEAK (Tolerance, Worry about drinking, Eye-opener, Amnesia and Cut-down) and the T-ACE (Tolerance, Annoyed, Cut-down, and Eye-Opener) tests, aimed at differentiating at-risk from non-risk drinkers. Still, diagnosis often relies on maternal self-report and some pregnant and nursing mothers might underreport their drinking behaviour because of the stigma and fear of punishment. For this reason, it is necessary to use objective diagnostic tools to detect prenatal exposure to alcohol.

The examination and measurement of known biomarkers in various bio-specimens is the most reliably measure currently available (Joya et al., 2012; Chabenne et al., 2014; Bager et al., 2017). Residual products of alcohol metabolism that are stored in various tissues, such as fatty acid ethyl esters (FAEEs), ethyl glucuronide (EtG), ethyl sulfate (EtS) and phosphatidylethanol (PEth), are direct biomarkers derived from alcohol. In addition, indirect biomarkers, which are generated due to the toxic effect of alcohol on organ systems, include the hepatic membrane glycoprotein enzyme gamma-glutamyl

transpeptidase, the hepatic enzymes alanine aminotransferase and aspartate aminotransferase, the glycoprotein carbohydrate-deficient transferrin, the mean corpuscular volume of the erythrocytes, and whole-blood-associated aldehyde. Recently, the use of insulin-like growth factors (IGF-I and IGF-II) has also been proposed as surrogate biomarkers of alcohol damage during foetal development (Andreu-Fernández et al., 2019).

Biological matrices in which these biomarkers can be assessed include maternal and neonatal blood samples, which reflect recent alcohol exposure, as substances are constantly eliminated from the bloodstream. Urine also reflects recent exposure and allows for the detection of EtG and EtS. Hair is useful to reflect exposure over time since substances are stored as the hair grows, such as FAEEs and EtG. The meconium allows the detection of alcohol exposure during the last months of gestation, since FAEEs, EtG and EtS do not cross the human placenta, causing meconium to serve as a reservoir of foetal chemical exposures. Thus, the presence of FAEE in the meconium reflects foetal alcohol metabolism. Actually, the prevalence of PAE measured by meconium testing was 4 times higher than the prevalence measured by maternal self-reports (Lange et al., 2014). FAEEs, EtG and EtS can also be detected in the placenta, which reflects months of exposure. Recently, the detection of the placental growth factor has been proposed as a reliably marker of foetal brain defects after *in utero* alcohol exposure, since its expression correlates with placental vascular impairments (Lecuyer et al., 2017).

However, currently available screening questionnaires to diagnose and confirm FASD are still unreliable, and biomarkers are insensitive for pregnant women. Hence, there is a dire need to discover novel biomarkers with better sensitivity and specificity for detecting even low-to-moderate drinking during pregnancy (Chabenne et al., 2014).

According to the updated clinical guidelines for diagnosis (Hoyme et al., 2016), FASD includes FAS, PFAS, ARND and ARBD. A summary of the diagnostic criteria for the four categories is presented in **Table 1**.

Categories	Criteria for diagnosis
FAS	Requires all features, A-D (with or without documented PAE): A. Pattern of minor facial anomalies B. Prenatal and/or postnatal growth deficiency C. Deficient brain growth, abnormal morphogenesis or abnormal neurophysiology D. Neurobehavioural impairment
PFAS	For children with documented PAE, requires features A and B: A. Pattern of minor facial anomalies B. Neurobehavioural impairment For children without documented PAE, requires all features, A-C: A. Pattern of minor facial anomalies B. Growth deficiency or deficient brain growth, abnormal morphogenesis, or abnormal neurophysiology C. Neurobehavioural impairment
ARND	Requires features A and B (this diagnosis cannot be made in children <3 years of age): A. Documented PAE B. Neurobehavioural impairment
ARBD	Requires features A and B: A. Documented PAE B. One or more specific major malformations demonstrated in animal models and human studies to be the result of PAE

Table 1. Updated criteria for diagnosis of FASD [adapted from Hoyme et al. (2016)].

However, with the introduction of ND-PAE into the DSM-5, there has been some controversy about the necessity of retaining both ARND and ND-PAE

as diagnostic entities. ND-PAE was proposed to facilitate the identification of those individuals affected by PAE (with confirmed alcohol history) with behavioural and mental health problems (Doyle and Mattson, 2015; Kable and Mukherjee, 2017). ND-PAE can be diagnosed either in the presence or absence of the physical effects of PAE (requires confirmation of minimal PAE), and neurobehavioural problems must cause clinically significant impairments in social, academic, occupational, or other areas of functioning.

PAE is associated with a broad spectrum of neurobehavioural impairments, which were designated into three domains: neurocognition, self-regulation and adaptive functioning. The neurocognitive domain includes five potential symptoms, such as impairments in global intellectual functioning, executive functioning, learning, memory, or visual-spatial reasoning. The self-regulation domain includes three possible symptoms: mood or behavioural dysregulation, attention deficits and impairments in impulse control. The adaptive domain includes impairments in communication, social, daily living skills or motor functioning (Mattson et al., 2013; Kable and Mukherjee, 2017).

ND-PAE requires ≥1 deficits in neurocognition and in self-regulation plus ≥2 deficits in adaptive functioning, while ARND can be diagnosed based on global cognitive deficits alone or with evidence of ≥2 deficits in behavioural domains in the absence of cognitive deficits (Kable et al., 2016).

Even though the morphological alterations can be already assessed in infants and small children, the cognitive and neurobehavioural phenotypes might evolve over time (Subramoney et al., 2018), as described in **Table 2**.

Among the birth defects and malformations, deficits in body weight and height, small head circumference, volume reductions of the whole brain, structural alterations of specific brain regions (corpus callosum, cerebellar vermis, basal ganglia or hippocampus (HPC)), or abnormalities of grey and white matter are the most commonly observed in children with FASD (Toga et al., 2006; Sowell et al., 2008; Guerri et al., 2009; Jones et al., 2010; Lebel et

al., 2011; Dörrie et al., 2014; Gautam et al., 2015). In addition, functional deficits in the SNC have been documented by neuroimaging techniques in FASD children (Fagerlund et al., 2006; Norman et al., 2009; Moore et al., 2014; Donald et al., 2015).

Newborn period and early infancy (0-2 years)	**Early childhood (3-5 years)**	**Middle childhood (6-12 years)**	**Adolescence (13-21 years)** ***(in addition to the symptoms occurring in childhood)***	**Adulthood (>21 years)** ***(in addition to childhood and adolescence symptoms)***
Growth deficits	Growth deficits	Growth deficits	Poor judgement	Reduced daily living skills
Feeding difficulties, irritability	Talkative, intrusive, friendly, short-tempered	Inattention, impulsivity, poor understanding of social interactions	Poor understanding of abstract concepts	Impaired peer relationships, susceptibility to victimization
Sleep disturbances	Hyperactivity	Hyperactivity	Conduct disorder	Precarious employment
Congenital anomalies, delayed cognitive development	Language development disorder, poor fine and/or gross motor skills	Impaired functional communication skills, impaired coordination and postural control	Deficits in language processing, inappropriate sexual behaviours	Psychiatric disorders: *ADHD, panic or mood disorders, substance use disorders*
Epilepsy or seizures	Comorbid attachment disorder	Deficits in executive functioning, memory deficits in learning	Dropping out of school or training programs, delinquency	

Table 2. Developmental aspects of FASD [adapted from Dörrie et al. (2014)].

The pathognomonic pattern of facial dysmorphology includes short palpebral fissures, thin upper lip and smooth philtrum (Del Campo and Jones, 2017), among other features (**Figure 6**).

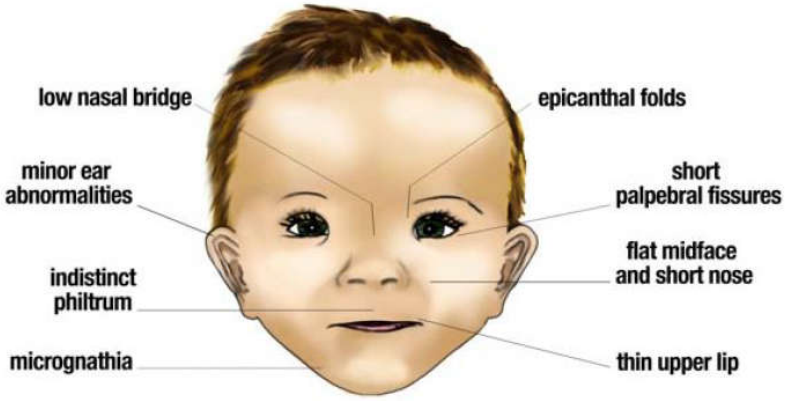


Figure 6. Facial characteristics associated with FAS (NIAAA, 2011).

The facial phenotype of FAS, although most commonly associated with PAE, can also be observed in other genetic and teratogenic conditions, known as phenocopies. The conditions to be considered in the differential diagnosis of FASD include Cornelia de Lange, Velocardiofacial, 15q Duplication, Noonan, Williams, Foetal Valproate Syndrome, Maternal Phenylketonuria effects or Toluene embryopathy (Manning and Hoyme, 2007; Hoyme et al., 2016).

2.4. Factors affecting the extent of alcohol-induced teratogenicity

The wide variability of phenotypes among individuals affected by PAE suggests that alcohol's teratogenic effects can be moderated or exacerbated by the influence of some biological and environmental factors (Nguyen et al., 2010; Alfonso-Loeches and Guerri, 2011; May and Gossage, 2011).

The quantity of alcohol ingested highly correlates with outcome severity; a higher level of alcohol consumption, along with longer duration of exposure, will generally lead to more adverse effects. However, a linear relationship between dosage and severity may not always be expected (Nguyen et al., 2010); as even low-to-moderate levels of alcohol can produce significant neurobehavioural consequences (Mamluk et al., 2017; Sarman, 2018).

The pattern of alcohol consumption can also modulate the severity of resultant deficits; binge-like drinking resulting in more severe neuropathology and behavioural alterations than chronic exposure (Flak et al., 2014). Thus, a high peak of BAC appears to be a significant risk factor for prenatal damage, correlating with increased child dysmorphology and negative cognitive outcomes (May et al., 2013).

The developmental timing of alcohol exposure also accounts for phenotypic differences. As different organ systems develop at different rates and times during gestation, alcohol exposure during critical periods of development can strongly influence the ontogeny of specific systems (Guerri et al., 2009; Sadrian et al., 2014). The CNS is developing throughout the gestation period and continues to do so postnatally, with some neurodevelopmental processes occurring until adulthood (Thompson and Nelson, 2001; Andersen, 2003; de Graaf-Peters and Hadders-Algra, 2006; Semple et al., 2013). However, each brain region has different developmental trajectories and therefore might be vulnerable to the adverse effects of alcohol at different time points, as shown in **Figure 7** (Jacobson, 1997; Chen et al., 2003). For instance, craniofacial dysmorphology appears to arise only when high levels of alcohol exposure occur during the embryonic stage of gastrulation (corresponding to the third week of human gestation), altering the neural crest development (Sulik, 2005; Lipinski et al., 2012; Smith et al., 2014).

Typically, events that occur in the first trimester have a greater impact on outcome than events occurring in the third trimester, including structural embryogenesis, neurogenesis and cell migration. Thus, neural tube defects, microcephaly and migration defects can cause severe neurodevelopmental impairments, while disruptions of third-trimester developmental processes may have a significant impact on neural circuit construction, which may not become obvious for years but can account for later risk to psychopathology (Georgieff et al., 2018).

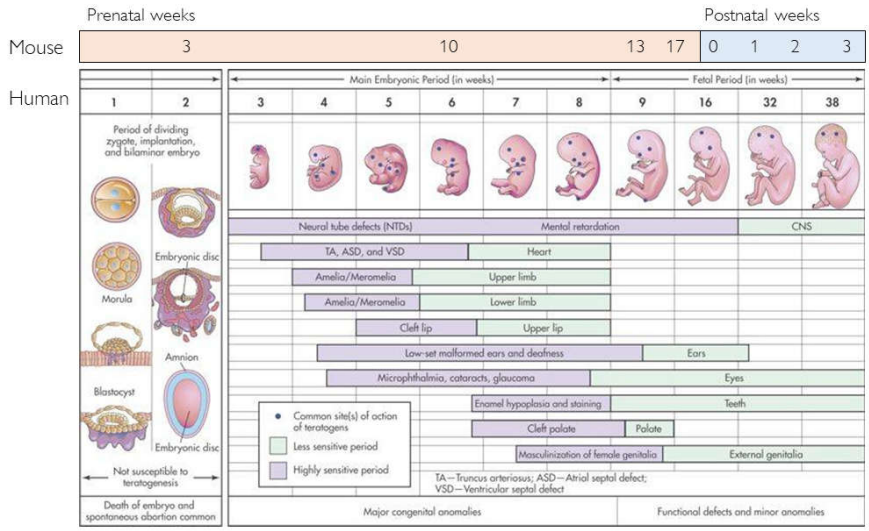


Figure 7. Sensitive periods of prenatal development to the exposure of teratogens in humans and rodents. Adapted from Moore and Persaud, (1993).

Another critical period is the neuroepithelial cell proliferation and migration stage, which occurs from week 7 to 20 of human gestation. Most of the brain areas begin to differentiate at this stage, except the cerebellum. Alcohol exposure during this period impairs neuronal migration, cell generation timing and reduces the number of neurons and glial cells in the neocortex, the HPC and the sensory nucleus (Rubert et al., 2006). Perturbation of neuroglial proliferation and migration can lead to long-term abnormalities in the cerebral cortex and brain size, as noted in FAS and FASD individuals (Shenoda, 2017; Delatour et al., 2019). Furthermore, around the 7th week of human gestation, the corpus callosum and the glial midline begin to develop. Thus, alcohol exposure at this time point might disrupt the development of the callosal formation, leading to agenesis, hypoplasia or other abnormalities.

Finally, alcohol also interferes with the brain growth spurt, a period characterized by dendritic arborisation and development of glial cells, which occurs during the third trimester of the human gestation and extends until two years of life. Also, the cerebellum undergoes its most rapid period of development during this stage. Thus, alcohol exposure at this period might

produce microcephaly, neuronal cell loss and synaptogenesis and glial derangements (Dikranian et al., 2005). The caudate nucleus, and the frontal and parietal cortices are the most vulnerable regions at this time (Cortese et al., 2006). Studies in rodents demonstrated that postnatal alcohol exposure reduced the number of cortical pyramidal neurons and altered cortical and striatal interneuron distribution and morphology, since these periods are most similar to late gestational development in humans (Ross et al., 2015).

Thus, alcohol interferes with distinct ontogenetic stages of neural development. Consequently, the pattern of structural and functional abnormalities depends on the developmental timing of alcohol exposure.

In addition, the highest risk of giving birth to a child with FASD is reported in mothers drinking during all trimesters of gestation, whereas the risk decreases with earlier abstention from drinking during pregnancy (Delano et al., 2019). Also, drinking during the first trimester increased 12 times the likelihood of developing FASD, first and second trimester drinking increased FASD outcomes 61 times, while drinking throughout the pregnancy, 65 times (May et al., 2013).

The genetic background of both the mother and foetus influences the effect of alcohol on the developing foetus. Alcohol's metabolism and organism's functional sensitivity to alcohol is affected by genetic polymorphisms. Specific alleles of ADH (*ADH1B*2* and *ADH1B*3*) encode for isoenzymes that allow faster alcohol metabolism rates, thereby affording protection for FASD outcomes. In addition, *ALDH2*2*, which encodes for a low activity variant of the mitochondrial ALDH, confers protection against the development of alcohol dependence, since it results in elevated levels of acetaldehyde following alcohol consumption, causing an unpleasant physiological state. This polymorphism has been found among 30% of the Asian population; although no studies exploring the protective effect of the presence of this allele for alcohol-induced teratogenesis have been conducted yet (Warren and Li, 2005).

Experimental animal studies have also demonstrated differential vulnerability to motor deficits in rat lines selectively bred for high and low alcohol sensitivity (Thomas et al., 2003).

Environmental factors related to prenatal care and nutrition are also important risk modifiers in FASD. A complex interaction exists between nutrition and alcohol, since food affects the rate of alcohol absorption and metabolism, but alcohol often alters the requirement for and absorption of nutrients. Alcohol exposure in combination with low nutrient levels increases the risk for FASD. Riboflavin, calcium, omega-3 fatty acids, zinc and B vitamins deficiencies are the major nutritional deficiencies related with FASD risk. Other risk factors for FASD include polysubstance abuse, maternal age, ethnicity, and socioeconomic status (May and Gossage, 2011).

An age-related increase in BAC despite exposure to the same dose of alcohol has been documented (Church et al., 1990). Thus, it is speculated that alcohol absorption and metabolism are altered by age. Also, gravidity (number of previous pregnancies) and parity (number of previous births) are maternal risk factors; therefore, the risk of having a most severally affected child with FASD is higher for those mothers who had one child with FASD previously (Jones, 2011).

2.5. Animal models of FASD

Preclinical studies have investigated the effects of foetal alcohol exposure on the developing brain and the subsequent consequences to the adult brain. Animal models, ranging from *Caenorhabditis elegans*, *Drosophila*, zebrafish and *Xenopus* to rodents (mouse, rat or guinea pig) and non-human primates, have been shown to mimic some aspects of the human condition (Patten et al., 2014b; Barron et al., 2016).

Rodent models are useful for exploring basic biological questions that cannot yet be explored in higher-order animals. Indeed, rodents are the most

commonly used animal species in FASD research as their physiological responses to alcohol are similar to that of humans and the neurobehavioural outcomes of prenatal exposure to alcohol have been fairly consistent with human studies (Gil-Mohapel et al., 2010).

Rodent models of FASD emerged rapidly after the first clinical publication describing FAS. Various exposure paradigms have been used with rodents and these vary in terms of timing of alcohol exposure, dose and pattern of alcohol exposure, and route of administration of alcohol, which makes that the findings from these models are not always consistent. Nevertheless, significant face validity in terms of modelling many of the behavioural effects associated with FASD in the clinical population has been found in a variety of animal models (Petrelli et al., 2018).

The exposure window in which alcohol is administered can have a different impact depending on the developmental timing of exposure, as mentioned above. However, the gestation period of rodents is much shorter than that of human beings, and brain development still occurs following birth in these species. In rats and mice it is estimated that the first trimester equivalent to human gestation is from gestational day (GD) 1-10, the second trimester equivalent corresponds to GD 10-20 and the third trimester equivalent occurs following birth, from postnatal day (PD) 1-10 (Patten et al., 2014b).

In addition to the timing of exposure, the route of administration is a critical factor to consider when choosing a model of FASD, since the BAC curve varies whether alcohol is consumed voluntarily or if it is administered in a single bolus by oral intubation. For instance, data from rodent models have shown that binge-type exposure induced by intragastric intubation of alcohol produces higher peak BACs than a more continuous exposure with a higher daily dose but lower peak BACs (Barron et al., 2016). In invertebrates and simple vertebrates (*C. elegans, Xenopus,* zebrafish) alcohol exposure is usually by bath application, while in rodents and non-human primates there are three

major methods of alcohol administration: ingestion (through liquid, solid diet, or intragastric intubation), injection, or inhalation (**Figure 8**).

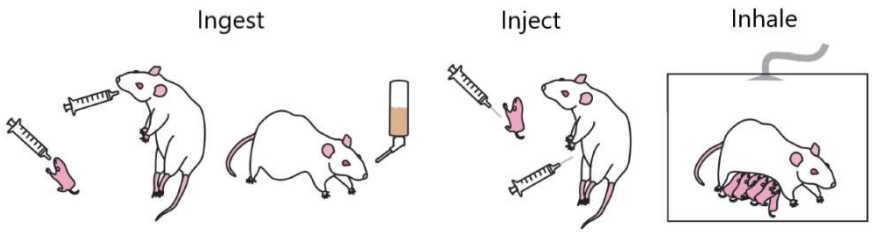


Figure 8. Common alcohol administration techniques in rodents used to examine the effects of PAE in offspring. Alcohol can be ingested by the animal via gavage administration (during gestation or during the early postnatal period) or orally ingested as a liquid diet. Alcohol injections can be administered to pregnant dams or pups. Also, dams with their litter can be placed in vapor chambers and be exposed to inhaled gaseous alcohol. Adapted from Patten et al. (2014b).

Voluntary drinking models involve less handling of the animals and therefore, less stress associated with the procedure. However, this method does not allow for the precise control over dosage or timing of exposure, which can lead to increased variability in the BACs achieved. A major advantage of intragastric intubation is the precise control over the dose administered, even though this is a much more invasive procedure. Alcohol administration by injection is a useful method to evaluate the acute effects of alcohol with limited handling-induced stress. Nevertheless, this method does not accurately resemble what occurs following alcohol consumption in humans. The inhalation mode of administration is the less commonly used, it does not mimic the route of intake of humans and moreover, it can cause irritation to the upper respiratory tract.

Most studies using mouse models employ chronic exposure paradigms, but intermittent exposure is also common, particularly in studies where critical periods of vulnerability are being examined. Even though the third trimester equivalent occurs following birth in mice, they are still used, resembling many of the features of FASD observed in humans (Patten et al., 2014b).

2.6. Mechanisms of alcohol-induced teratogenicity

The molecular mechanisms underlying the effects of developmental alcohol exposure on the CNS are still not well understood, even though available experimental and clinical data provide evidence that alcohol interferes with several molecular and cellular events that play a role in the correct formation of the developing CNS (Guerri, 2002; Alfonso-Loeches and Guerri, 2011).

Alcohol may have an impact on molecules that regulate key developmental processes, interfering with cell proliferation, migration, growth and differentiation, or even cell survival, depending on the developmental timing and levels of alcohol exposure. During the first trimester equivalent to human gestation, alcohol exposure can alter the development of the neural tube and crest, leading to microcephaly, ocular malformations and facial dysmorphology, which characterizes FAS. During the second trimester, alcohol can affect the proliferation of neuronal precursors and the formation of radial glia cells, leading to abnormalities in migration. Finally, alcohol can induce cell death, synaptogenesis disruptions and persistent deficits in neuronal plasticity during the period equivalent to the third trimester (Brocardo et al., 2016). Furthermore, many neurotransmitters, adhesive molecules, transcription factors, and trophic factors can be modulated by foetal alcohol exposure (Patten et al., 2014b; Ross et al., 2015). Here, some of the molecular mechanisms that could be underpinning alcohol-induced teratogenic effects will be highlighted.

2.6.1. Neuroinflammation

Neuroinflammation is the term used to describe the broad range of immune responses that occur in the CNS, primarily concerning microglia and astrocytes. It is considered a double-edged sword for the CNS, since acute inflammatory response tends to be beneficial, while chronic inflammation is most often detrimental and damaging to nervous tissue (Streit et al., 2004).

Microglial cells, generally considered the main resident macrophage-like innate immune cells of the brain, play an immune surveillance function in the healthy CNS parenchyma, maintaining intact tissue homeostasis. Their cell bodies remain stationary, but their processes are continuously elongating and retracting to explore the surrounding extracellular space and communicate directly with neurons, astrocytes, and blood vessels. Upon recognition of damage or immunological stimuli, microglia switches from a ramified, "resting" state to an activated, amoeboid phenotype as it takes on a phagocytic role. Activated microglia can serve diverse functions essential to neuron survival, including cellular maintenance and innate immunity. During development, microglial cells are involved in programmed elimination of neural cells and neuronal survival, releasing trophic and anti-inflammatory factors. In addition to clearance of dead cells, microglial phagocytic activity is crucial for synaptic homeostasis, participating in the process of neuronal pruning during development (Paolicelli et al., 2011; Bilbo et al., 2012; Nayak et al., 2014; Cowan and Petri, 2018). In the mature brain, microglia facilitate the migration of stem cells to the site of injury (Tay et al., 2017). The transition from the resting but surveying microglial phenotype to an activated one is tightly regulated by several intrinsic and extrinsic factors (Kierdorf and Prinz, 2013). However, microglia can become overactivated after adverse environmental stimuli, neuronal death or damage cues. Excessive microglia activation can induce detrimental neurotoxic effects by the excess production of cytotoxic factors such as reactive oxygen species (ROS) and tumour necrosis factor-α (TNF-α). Microglia can also cause excitotoxic neuronal death directly by inducing the release of glutamate (Block et al., 2007; Hanisch and Kettenmann, 2007; Hickman et al., 2018). Hence, even though microglial activation is crucial for host defence and neuron survival, its overactivation results in deleterious and neurotoxic effects (Czeh et al., 2011).

Astrocytes comprise the most numerous cell type of the glia family, which give structural and metabolic support to surrounding neurons while maintaining

homeostasis of the extracellular milieu through clearance of potassium and neurotransmitters (Bélanger and Magistretti, 2009). Astrocytes can also regulate excitability through the clearance of glutamate and glycine as well as by the release of gliotransmitters (Adermark and Bowers, 2016). Furthermore, they play a key role in regulating the formation, maturation, stability and maintenance of synapses. Astrocytes are immunocompetent cells as well, and like microglia, can become activated. Activated astrocytes contribute to neuroinflammation releasing a wide array of immune mediators, such as cytokines, chemokines and growth factors, and can modulate microglial activation state (Colombo and Farina, 2016). In addition, activated microglia can induce the activation of a subtype of reactive astrocytes which show neurotoxic effects (Liddelow et al., 2017). Hence, both microglia and astrocytes play a dual role in CNS immune response (Norden et al., 2016; Jha et al., 2019). It is known that a dynamic crosstalk exists between glia, neurons and BBB endothelial cells; thus, it is expected that the neuroinflammatory response from one type of cell will directly influence another (Holm et al., 2012; Tian et al., 2012; Jha et al., 2019).

Glial cell activation is triggered upon recognition of invading pathogens or tissue damage by a plethora of subsets of immune receptors, such as Toll-like receptors (TLRs), nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), scavenger receptors and numerous cytokine and chemokine receptors, which are expressed in both microglia and astrocyte cells. TLRs recognize pathogen-associated molecular patterns and danger-associated molecular patterns and their activation (with the exception of TLR3) recruits MyD88-dependent pathway, which triggers the transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signalling with subsequent production of pro-inflammatory cytokines, including TNF-α, interleukin (IL)-1β and IL-6 (Moresco et al., 2011; Lim and Staudt, 2013; Takeda and Akira, 2015). Whereas TLRs are membrane-spanning receptors, NLRs are cytoplasmic sensors that oligomerize to form a platform for the

inflammasome, a multisubunit complex that processes pro-IL-1β into its mature form via caspase-1 action (Franchi et al., 2009; Hanamsagar et al., 2012; Guo et al., 2015). Of all the NLR genes identified, NLRP3 is the best characterized inflammasome, which is capable to react to multiple distinct stimuli and can be activated by a wide range of endogenous and environmental signals (Tschopp and Schroder, 2010; Liu et al., 2013; Baroja-Mazo et al., 2014). TLR agonists induce its expression in a NF-κB-dependent manner (Bauernfeind et al., 2009; Qiao et al., 2012). Microglia and astrocytes express the complete repertoire of identified TLRs and NLRs, however inflammasome activity in astrocytes remains unclear (Shastri et al., 2013). There is also evidence that both oligodendrocytes and neurons can express TLRs, but their role in innate immune responses are less well defined (Carpentier et al., 2008).

The ubiquitously expressed nuclear protein, high-mobility group box 1 (HMGB1) is an endogenous cytokine-like molecule expressed in all cell types that upon release can directly activate TLRs. Preclinical models reveal that alcohol increases the expression of HMGB1 and TLRs in the brain (Zou and Crews, 2014; Wang et al., 2015b; Crews et al., 2017). Thus, alcohol exposure may trigger the release of the endogenous TLR4 ligand HMGB1 from neurons contributing to neuroimmune signalling through TLR4 receptor activation (Vetreno and Crews, 2012; Crews et al., 2013; Wang et al., 2015b; Crews and Vetreno, 2016). In addition, a second mechanism has been described to be involved in alcohol-induced activation of the neuroimmune system. Consumed alcohol makes the gut become permeable or "leaky" and release HMGB1, which activates gut TLR4 receptors that allows bacterial endotoxins such as lipopolysaccharide to enter the bloodstream, stimulating proinflammatory cytokine production in the liver. Then, proinflammatory cytokines in the blood can cross the BBB and increase neuroimmune gene expression (Crews and Vetreno, 2014; Leclercq et al., 2014; de Timary et al., 2017).

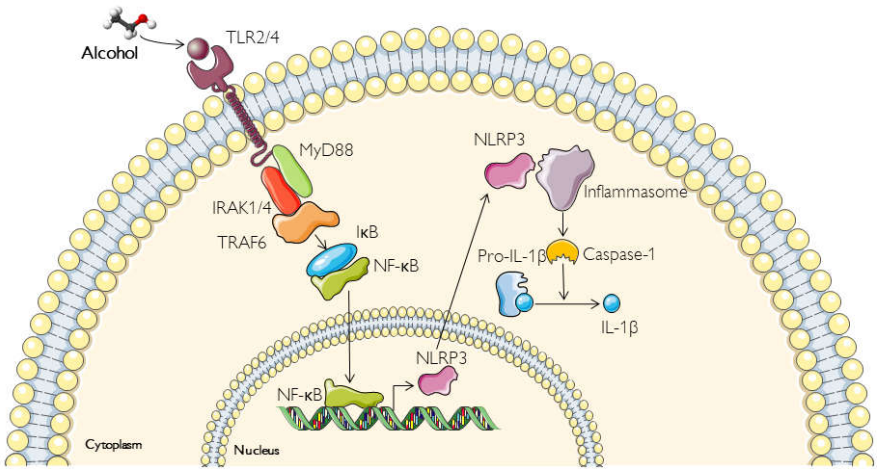


Figure 9. Alcohol-induced neuroimmune signalling in the CNS.

In the brain, alcohol activates and recruits TLR4 within the lipid rafts of glial cells, triggering both microglia and astroglia activation (Fernandez-Lizarbe et al., 2009; Alfonso-Loeches et al., 2010). Alcohol also up-regulates TLR2 expression in microglial cells and potentiates TLR4/TLR2 recruitment, triggering the cascade to endorse microglia activation (**Figure 9**) (Fernandez-Lizarbe et al., 2013). Evidences from *in vitro* and *in vivo* studies indicate that chronic alcohol treatment induces the activation of glial cells, upregulating the production of inflammatory mediators in the brain, involving TLR4- and TLR2-mediated signalling with the induction of NF-κB (Vallés et al., 2004; Blanco et al., 2005; Oak et al., 2006; Zou and Crews, 2010). Indeed, mice lacking TLR4 or TLR2 were protected against alcohol-induced neuroinflammation and induced cell death and behavioural associated effects (Pascual et al., 2011, 2014a; Alfonso-Loeches et al., 2014). Similarly, inhibition of TLR4-MyD88 pathway reduced alcohol-induced behavioural effects (Wu et al., 2012); thus, supporting the role of alcohol in promoting deleterious adaptations in neuroimmune signalling (Coller and Hutchinson, 2012; Mayfield et al., 2013; Leclercq et al., 2014; Robinson et al., 2014; Montesinos et al., 2016a; Ciafrè et al., 2018; Erickson et al., 2019).

Accordingly, prolonged alcohol consumption induces an activated state of microglia, which is accompanied by an increase in microglia cell number (Pascual et al., 2014a; Pradier et al., 2018) and increased levels of pro-inflammatory cytokines, such as TNF-α and IL-1β, in the cerebral cortex and cerebellum (Qin et al., 2008; Alfonso-Loeches et al., 2010; Lippai et al., 2013b). However, the chronic activation of glial cells may result in neuronal cell death, since sustained exposure of neurons to pro-inflammatory mediators can cause neuronal cell death (**Figure 10**) (Guerri and Pascual, 2010; Lehnardt, 2010; Heneka et al., 2014; Yang et al., 2014). Multiple mechanisms might be involved in alcohol-induced neurodegeneration. For instance, activation of the microglial complement-phagosome pathway has been shown to lead to dopaminergic neuron loss in a mouse model (Bodea et al., 2014) and increased levels of advanced glycation end-product-albumin from activated microglial cells have also been demonstrated to play an important role in promoting alcohol-induced neurodegeneration in both rats and humans (Byun et al., 2014). Also, TLR4 signalling has been shown to increase synaptic NMDA receptors, neuronal excitability and subsequent neurotoxicity through activation of kinase cascades (Balosso et al., 2014). Another mechanism of alcohol-induced hyperexcitability is through inhibition of glial glutamate transporters (Zou and Crews, 2005).

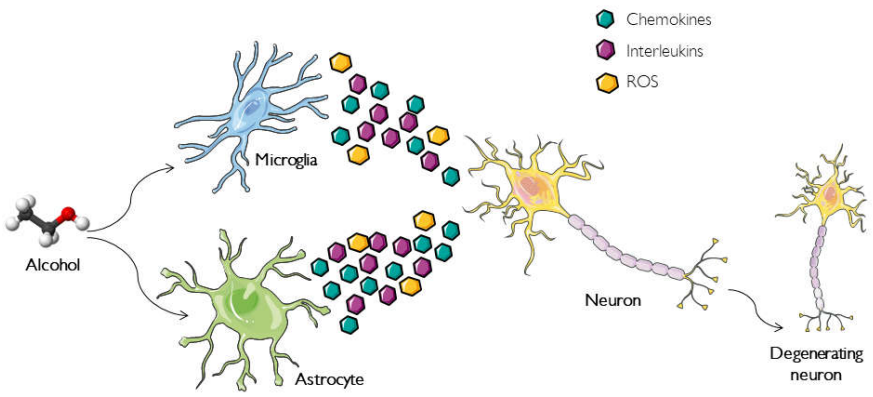


Figure 10. Gliosis-induced neuron cell death triggered by alcohol exposure.

In accordance, binge alcohol exposure induced the activation of neuroinflammatory pathways in rodent models, associated with increased oxidative stress and neuronal degeneration (Collins and Neafsey, 2012; Tajuddin et al., 2014). Therefore, excessive alcohol intake results in neurodegeneration, which has been linked to a variety of cognitive deficits, and neuroinflammation is thought to be a factor in alcohol-induced cell death. Studies in alcoholic humans find cortical thinning in multiple brain areas which overlap with the findings from rodent models of binge alcohol drinking (Crews and Vetreno, 2014).

The activation of glial cells and the upregulation of pro-inflammatory mediators in the brain have been reported in rodents exposed to alcohol during the third trimester equivalent period of human gestation (Drew and Kane, 2014; Topper et al., 2015; Boschen et al., 2016; Terasaki and Schwarz, 2016), indicating an increase of the neuroinflammatory response. Also, *in utero* alcohol exposure promoted microglia activation, produced abnormalities in neocortical development (Komada et al., 2017) and evoked an astroglial response (Brolese et al., 2014), with increases in glial fibrillary acidic protein (GFAP) and its mRNA levels (Guerri and Renau-Piqueras, 1997). Thus, alcohol-induced neuroinflammation has been established as a key factor in the neurotoxicity observed in FASD models (Chastain and Sarkar, 2014; Wilhelm and Guizzetti, 2015; Saito et al., 2016). The finding that alcohol induces neuroinflammatory processes in FASD models is important, as even transient neuroinflammation or alterations in glial cell function during the CNS development can affect neural development and survival, impairing the proper brain architecture and connectivity, which could be an underlying cause of cognitive and psychiatric disorders in adults (Bodnar and Weinberg, 2013; Guizzetti et al., 2014; Kane and Drew, 2016; Raineki et al., 2017). Furthermore, alcohol not only affects signalling pathways within a cell type but also strongly alters the ability of glial cells to send information to neurons and *vice versa*, disrupting the series of events that lead to the correct

development of the brain and the proper formation of the brain circuit architecture. It has been hypothesized that PAE leads to a long-term sensitization of microglia resulting in persistent inflammatory signalling in the brain following insult (Chastain and Sarkar, 2014; Wilhelm and Guizzetti, 2015). This priming hypothesis would be consistent with the studies that report increased inflammation in the adult brain of PAE animals.

2.6.2. Oxidative stress

Evidence from animal models of PAE involve oxidative stress and dysregulation of the endogenous antioxidant system in the mechanisms of action of alcohol that may contribute to FASD pathology (Brocardo et al., 2011). Alcohol metabolism induces the generation of ROS and reactive nitrogen species (RNS) by activating the mitochondrial respiratory chain with the subsequent formation of superoxide ($O2^{-\bullet}$), hydroxyl radical ($OH^{\bullet}$), hydrogen peroxide (H_2O_2), or nitric oxide ($^{\bullet}NO$), or via its oxidation by enzymes such as CYP2E1, which generate hydroxyethyl radicals, leading to an imbalance in the intracellular redox state (see **Figure 11**). Most part of alcohol metabolism occurs in the liver, so its effects on the intracellular redox state in the CNS most likely result from a direct dysregulation of mitochondrial bioenergetics.

At physiological conditions, ROS participate in the signal transduction of intracellular pathways by acting as messenger molecules affecting processes such as the activity of enzymes involved in synaptic plasticity and modulating learning and memory (Massaad and Klann, 2011). However, cell damage occurs when ROS and/or RNS levels accumulate and self-perpetuate over time, interacting with carbohydrates, proteins, lipids and nucleic acids. The developing brain is particularly vulnerable to oxidative stress due to its high concentrations of unsaturated fatty acids, high oxygen consumption rate and high content of metals catalysing free radical formation. Furthermore, alcohol exposure *in utero* increases oxidative stress in the foetus, which compromises

numerous intracellular signalling pathways, DNA integrity and causes genetic alterations, which can ultimately culminate in cell death (Hansen, 2006; Bhatia et al., 2019). The ability of the foetus to metabolize alcohol is limited, since the activity of the metabolizing enzymes (ADH, CYP2E1) gradually increases with gestational age, even though non-oxidative pathways (FAEE synthases) are present in foetuses early in gestation. Moreover, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzyme activity was found to be increased in alcohol-exposed embryos (Dong et al., 2010), which is the major source of ROS in neurons, glia, and cerebral blood vessels (Infanger et al., 2006; Sorce and Krause, 2009).

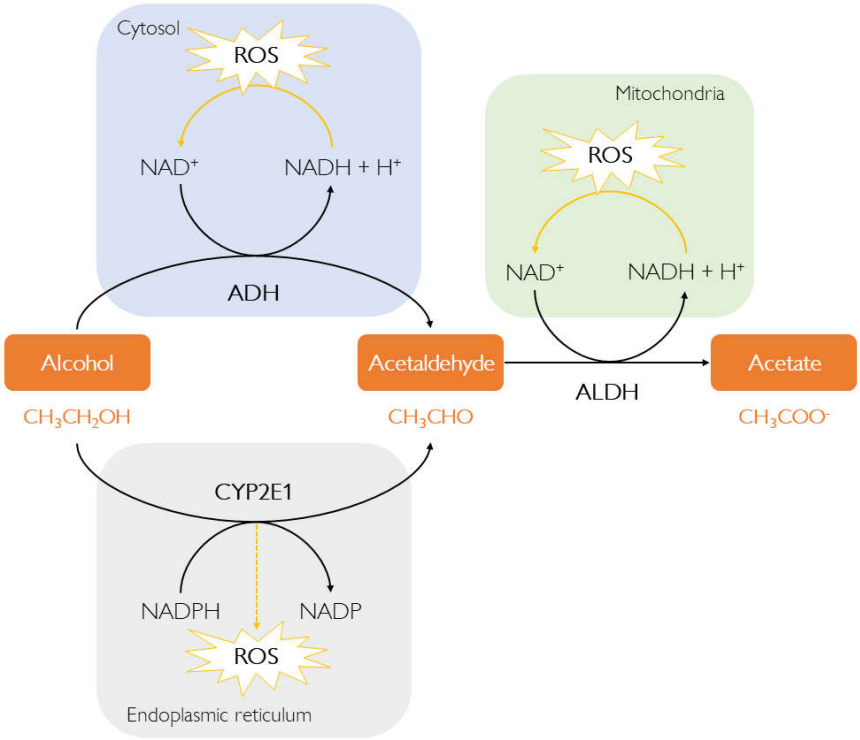


Figure 11. Production of ROS by alcohol metabolism. The enzymes ADH, CYP2E1 and catalase (not shown) contribute to oxidative metabolism of alcohol. ADH, present in the cytosol, converts alcohol to acetaldehyde. This reaction involves an intermediate carrier of electrons, NAD^+, which is reduced by two electrons to form NADH. At high levels of alcohol consumption, CYP2E1 present in the endoplasmic reticulum

becomes involved in metabolizing alcohol to acetaldehyde. Acetaldehyde is further metabolized mainly by ALDH2 in the mitochondria to form acetate and NADH. Both alcohol metabolism by CYP2E1 and the re-oxidation of NADH via the electron transport chain in the mitochondria results in the formation of ROS.

In order to prevent cell damage, the endogenous antioxidant system can inhibit the formation of ROS or remove free radicals. Non-enzymatic antioxidants include thiols and glutathione, while enzymatic antioxidants include superoxide dismutase, catalase and the glutathione peroxidase system. However, during prenatal and early postnatal development, levels of antioxidants are much lower than in mature cells, being the developing neurons much more susceptible to oxidative stress. Furthermore, several studies have shown that the content and the activity of antioxidant enzymes is reduced in the CNS of animals exposed to alcohol (Brocardo et al., 2011; Fontaine et al., 2016). In addition, alcohol-induced dysregulations in redox status during development can have long-lasting consequences in the adult brain (Brocardo et al., 2016). For instance, PAE adult rats prenatally exposed to alcohol presented higher levels of lipid peroxides and protein carbonyls in the brain, as well as, decreased levels of glutathione (Dembele et al., 2006). Hence, animals exposed to alcohol during the period of brain development show increased lipid peroxidation, protein peroxidation and DNA damage, suggesting that oxidative stress, and ROS in particular, might play a major role in the neuropathology and the development of some of the behavioural symptoms associated with FASD (Ramachandran et al., 2001; Heaton et al., 2003; Smith et al., 2005; Perez et al., 2006; Patten et al., 2014a).

2.6.3. Dysregulation of cell survival signalling

Several studies on experimental animal models of FASD have provided evidence of the neurotoxic effects of alcohol, causing reductions in brain mass and cell loss (Jaatinen and Rintala, 2008; Luo, 2012; Petrelli et al., 2018). Alcohol promotes the activation of signalling pathways associated with cell

death (Pascual et al., 2003; Young et al., 2003; Dong et al., 2010), leading to neuronal cell death.

Increased activation of caspase-3, an executioner protease which triggers apoptosis, was found in the brain after developmental alcohol exposure, inducing the death of post-mitotic neurons in the cerebral cortex and cerebellum (Olney et al., 2002a; Young et al., 2005). A single alcohol exposure during the synaptogenesis period (first two postnatal weeks in rodents) induced the neurodegeneration of Purkinje cells and other neurons in the cerebellum and brainstem nuclei (Dikranian et al., 2005). Also, adult mice exposed to a binge alcohol episode at PD7 showed reduced number of frontal cortical parvalbumin interneurons and pyramidal neurons (Coleman et al., 2012) and increased caspase-3 immunoreactivity in brain regions comprising the extended hippocampal circuit (Wozniak et al., 2004). Furthermore, brain and cerebral cortex volumes were reduced in adult mice after alcohol treatment at PD7. In particular, calretinin and parvalbumin subtypes of GABAergic neurons showed a striking reduction (Smiley et al., 2015). Neuronal death induced by acute alcohol exposure at PD7 seems to be mediated by the mitochondrial pathway of apoptosis, involving Bax activation, cytochrome c release and caspase-3 activation (Mooney and Miller, 2001; Saito et al., 2016), since alcohol-induced microglial activation associated with apoptotic neuronal death was abolished in the knockout mouse for the proapoptotic *bax* gene (Ahlers et al., 2015). In addition, the levels of ROS following alcohol exposure were mitigated in $Bax^{-/-}$ mice, conferring neuroprotection (Heaton et al., 2006).

The up-regulation of caspase-3 in the brain has been associated with the alteration of some neurotransmitter receptors. It has been postulated that alcohol exerts its apoptogenic action by a dual mechanism: blockade of NMDA glutamate receptors and hyperactivation of $GABA_A$ receptors (Olney et al., 2002b). Hence, through its GABAmimetic and NMDA antagonist effects, alcohol impairs the function of these receptors during brain ontogeny,

which can lead to apoptotic neurodegeneration. These alterations could also underlie some of the learning and memory deficits observed following foetal alcohol exposure (Ikonomidou et al., 2000).

The NMDA receptor plays an important role in neuronal plasticity during development and later in life during learning. However, its overactivation leads to increases in intracellular calcium (Ca^{2+}) and consequent cell death, a process named excitotoxicity (Lau and Tymianski, 2010; Collins and Neafsey, 2016). Alcohol acutely blocks the NMDA receptor, but chronic exposure may lead to a compensatory increase in either receptor number or glutamate release, actions that may contribute to the excitotoxic cell death (Crews et al., 2004). This process may also occur in the developing brain. Actually, an impairment in the glutamatergic system after maternal alcohol exposure during gestation and lactation has been described, associated with disruption of Ca^{2+} homeostasis, energy deficits and oxidative damage in the immature HPC of rat's offspring (Cesconetto et al., 2016). Further evidences of alcohol-induced excitotoxic mechanisms were demonstrated in the perinatal brain, since treatment with NMDA receptor antagonists (MK-801 or memantine) in rat pups exposed to alcohol attenuated alcohol-induced behavioural impairments (Thomas et al., 2002; Idrus et al., 2014).

Alcohol-induced neurotoxicity can also be related to oxidative stress and pro-inflammatory factors. Alcohol increases DNA binding of NF-κB transcription factor, known for its ubiquitous roles in inflammatory and immune responses (O'Neill and Kaltschmidt, 1997; Mémet, 2006). In the CNS, NF-κB is expressed in neurons and glia and is activated by stimuli of oxidative stress, cytokines and glutamate. NF-κB activation induces a pro-inflammatory cascade leading to the production of cytokines, which has been reported to play a role in alcohol-induced neurotoxicity (Vallés et al., 2004; Crews et al., 2006b; Crews and Nixon, 2009; Qin and Crews, 2012). For instance, TNF-α

can directly potentiate glutamate neurotoxicity by inhibiting glutamate uptake through NF-κB mechanisms (McCoy and Tansey, 2008).

A number of studies have indicated that alcohol promotes the signalling pathways leading to cell death, but at the same time interferes with those pathways involved in cell survival such as those associated with neurotrophic factors action (Alfonso-Loeches and Guerri, 2011).

Alcohol inhibits cyclic adenosine monophosphate (cAMP) production, a second messenger derived from ATP, probably either by reducing the activity of adenylyl cyclase, which converts ATP to cAMP, or stimulating phosphodiesterase activity, which catalyses the degradation of cAMP (Liu et al., 2014). Consequently, alcohol interferes with the transcription factor cAMP responsive element-binding protein (CREB) pathway. CREB family transcription factors are activated by phosphorylation and promote neuronal survival, protecting neurons from excitotoxicity and apoptosis through regulating the transcription of pro-survival factors (Lonze and Ginty, 2002; Mantamadiotis et al., 2002). CREB activation stimulates the expression of brain-derived neurotrophic factor (BDNF), which is a crucial neurotrophin that activates cell signalling pathways involved in cell survival, differentiation, synaptic strength and plasticity, and dendritic out-growth (Barco et al., 2002; Bramham and Messaoudi, 2005; Kowiański et al., 2018). Accordingly, neuronal cells showed decreased levels of cAMP and BDNF following alcohol treatment, increasing cellular oxidative status and apoptotic cell death (Boyadjieva and Sarkar, 2013). It has also been shown that binge alcohol treatment decreases phospho-CREB (pCREB) immunoreactivity in the brain and reduces levels of BDNF, coinciding with the peak of neurodegeneration (Crews and Nixon, 2009). Thus, reduced CREB transcription contributes to alcohol-induced neurotoxicity (Zou and Crews, 2006).

Neurotrophin signalling plays an important role in neuronal survival, proliferation, migration and differentiation in the developing brain (Park and

Poo, 2013). It has been reported that alcohol exposure during the foetal development diminishes the levels of neurotrophic factors, such as BDNF, impairing BDNF-mediated activation of survival pathways and leading to neuronal cell death (Climent et al., 2002; Boschen and Klintsova, 2017). Other neurotrophic factors, including IGF, nerve growth factor, and glial-derived neurotrophic factor, might also be reduced in the presence of alcohol. In addition, when immature neurons are exposed to alcohol, the newly developed cells may respond abnormally to certain guidance or trophic factors (Lindsley et al. 2003). Therefore, alteration by alcohol to either of these mechanisms leads to a pathological cascade of events, which ultimately may contribute to FASD. Indeed, alcohol exposure during the equivalent period to the third trimester of human gestation inhibited CREB phosphorylation in neonatal and adult mice, which was accompanied by impaired cognitive function (Subbanna et al., 2014b).

Alcohol-induced loss of trophic signals combined with the induction of oxidative stress and pro-inflammatory signals is one of the key mechanisms underlying the neurodegeneration induced by alcohol. The mechanisms leading to cell death can also be responsible for maladaptive neuroplastic rearrangement of surviving neurons leading to functional deficits in the CNS, representing one of the main pathogenetic mechanisms of alcohol-induced damage to the developing brain (Granato and Dering, 2018).

2.6.4. Interference with neural progenitors' proliferation

The process of proliferation of pluripotent neural stem cells to give rise to progenitor cells that differentiate into distinct lineages (neuronal, astroglial or oligodendroglial) is known as neurogenesis/gliogenesis. After cell differentiation, cells migrate and become fully functional neurons or glia that are integrated into the brain circuitry. Neurogenesis primarily occurs during embryonic development, although it also occurs in at least two brain regions of the adult brain, the subgranular zone (SGZ) of the hippocampal dentate

gyrus (DG) (Danglot et al., 2006; Spalding et al., 2013) and in the subventricular zone of the anterior lateral ventricles, where olfactory bulb neurons are generated (Alvarez-Buylla and Garcia-Verdugo, 2002). Specifically, the pyramidal neurons that reside in the *Cornu Ammonis* areas CA1 and CA3 are generated during embryonic development, while the granule cell population of the DG is initially produced during embryonic development and continues to do so postnatally. Even though in the rodent brain a proliferative SGZ is well established to be maintained into adulthood, in adult humans appears to be a decline of neurogenesis (Boldrini et al., 2018; Paredes et al., 2018; Sorrells et al., 2018; Moreno-Jiménez et al., 2019).

Another mechanism of alcohol's actions on the foetus is the disruption of neurogenesis, which can lead to neuronal cell loss (Crews and Nixon, 2003; Nixon, 2006; Geil et al., 2014). Binge alcohol exposure in adolescent and adult rats decreased neural progenitor cell proliferation and survival (Nixon and Crews, 2002; Crews et al., 2006a; Morris et al., 2010); as well, chronic alcohol exposure reduced hippocampal neurogenesis and dendritic growth of newborn neurons (He et al., 2005). Studies in non-human primates revealed long-lasting reductions in neurogenesis due to alcohol consumption during adolescence (Taffe et al., 2010). Furthermore, a reduction of neurogenesis in the HPC of human alcoholics has been recently reported (Dhanabalan et al., 2018; Le Maître et al., 2018), consistent with animal studies.

Foetal exposure to alcohol has been shown to impact neurogenesis during brain development and maturation in animal models. Thus, alcohol-induced inflammation during development may also contribute to the detrimental effects for hippocampal neurogenesis (Green and Nolan, 2014). Preclinical studies demonstrated that postnatal alcohol administration reduced the population of progenitor cells in the HPC (Redila et al., 2006; Ieraci and Herrera, 2007; Klintsova et al., 2007; Uban et al., 2010; Hamilton et al., 2011; Olateju et al., 2018). Even though, other studies reported no disruption of hippocampal neurogenesis by PAE (Choi et al., 2005; Helfer et al., 2009; Gil-

Mohapel et al., 2014; Hamilton et al., 2016) or even an increase (Gil-Mohapel et al., 2011; Coleman et al., 2012). Presumably, variations in alcohol dosage, route of administration, developmental timing of exposure and age at the time of assessment might account for this diverse range of findings observed among different studies (Gil-Mohapel et al., 2010).

2.6.5. Myelin impairments

Recent evidence suggests that deficits in white matter integrity found in humans with FASD could be explained by myelin fibre disruptions. Myelin is composed of dense layers of lipid membranes wrapped around the axons to provide electrical insulation and can profoundly affect neural circuit connectivity. The main constituents of myelin are glycolipids (70%) and proteins (30%) (Baumann and Pham-Dinh, 2001). Bundles of myelinated axons give rise to the appearance of the white matter, although many axons in the grey matter that contain neuronal cell bodies and dendrites are also myelinated (Nickel and Gu, 2018). Oligodendrocytes provide myelination and trophic support for the maintenance of axonal integrity in the CNS (Aggarwal et al., 2011). During embryonic development, oligodendrocyte progenitor cells (OPCs) populate the forebrain from distinct telencephalic germinal regions. At birth, the majority of forebrain OPCs and mature oligodendrocytes are derived from the ventral telencephalic ventricular zone. However, this population is almost entirely replaced by OPCs and oligodendrocytes originated at more dorsal regions during the early postnatal period. Postnatally, OPCs continue to populate the forebrain, where they contribute to neural plasticity and repair. Upon differentiation, oligodendrocytes start to express the myelinating genes, a process driven by promyelinating transcription factors such as the myelin regulatory factor (MYRF), which directly regulates the expression of genes underlying myelination and maintenance of mature oligodendrocytes and myelin structure (Emery et al., 2009; Bujalka et al., 2013; Mitew et al., 2014; Li and Richardson, 2016). In humans, the third trimester of gestation represents a period of rapid oligodendrogenesis and myelination,

which continues during the first few decades of life; whereas in mice, oligodendrogenesis and myelination mostly occur during the first and second weeks after birth. Notwithstanding, continued myelin remodelling throughout lifetime has been reported in both animal models and humans, contributing to plasticity in the brain (Purger et al., 2016).

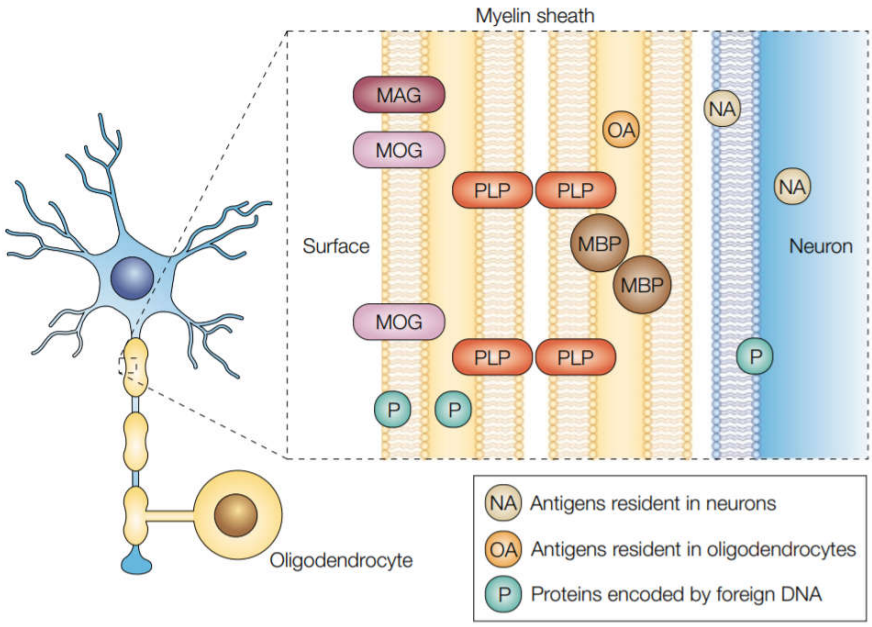


Figure 12. Representation of the myelin sheath structure (Hemmer et al., 2002).

The impact of alcohol on myelin has been established in various animal models. Acute exposure to alcohol during adolescence resulted in damaged myelin in rats (Vargas et al., 2014). Also, adolescent rodents treated intermittently with alcohol showed structural alterations of myelin (Pascual et al., 2014b; Montesinos et al., 2015). Alcohol can affect myelination at multiple stages of oligodendrocyte development through different potential pathways (Rice and Gu, 2019). Alcohol can disrupt OPC differentiation and survival, since OPCs that have yet to differentiate into oligodendrocytes are more vulnerable to the excitotoxic damage induced by alcohol. Moreover, alcohol can interfere with cell signalling pathways involved in early life brain

development, such as Wnt or BDNF signalling, which play a key role in myelination (Gaesser and Fyffe-Maricich, 2016). Mature myelinating oligodendrocytes or myelin itself can also be affected by alcohol. The proteins that constitute the actual myelin sheath (**Figure 12**), including myelin basic protein (MBP), proteolipid protein (PLP), myelin-associated glycoprotein (MAG), and myelin oligodendrocyte glycoprotein (MOG) have been evaluated in preclinical studies as readout of alcohol-induced myelin damage. Accordingly, reduced expression of myelin proteins was reported in the human alcoholic brain (Lewohl et al., 2005).

Imaging studies revealed white matter abnormalities in children with FASDs, associated with neurobehavioural dysfunctions (Sowell et al., 2008; Norman et al., 2009; Lebel et al., 2011; Gautam et al., 2015; Fan et al., 2016). Gestational alcohol exposure also reduced the expression of MBP in the brain of mouse embryos (Ozer et al., 2000). In addition, alterations in oligodendrocyte morphology, maturation, differentiation and survival have been reported in third trimester-equivalent preclinical models of FASD, when oligodendrocytes begin maturation and myelination. A reduced number of mature oligodendrocytes and OPCs within the corpus callosum was found in alcohol-exposed mice at PD16, with persistent abnormalities, such as reduced MBP expression, until adulthood (Newville et al., 2017). Similarly, *in utero* exposure of the foetal non-human primate brain to alcohol triggered oligodendrocyte cell loss (Creeley et al., 2013). Altogether, these studies indicate that early-life alcohol exposure affects oligodendrocyte development and survival and myelination, which may have a great impact on axonal size and ability to effectively transmit action potentials (Guizzetti et al., 2014).

2.6.6. Epigenetic modifications

The role of epigenetics in alcohol-induced damage has been highlighted in recent years (Shukla et al., 2008; Mahnke et al., 2017). Epigenetics refers to changes in gene expression that do not involve DNA coding sequence

modifications. Epigenetic marks can be transitory but can also be transgenerationally transmitted. However, the mechanism by which an epigenetic signature becomes heritable is still not well understood. Epigenetic mechanisms include DNA methylation, post-translational histone modifications (acetylation, methylation, phosphorylation, ubiquitinylation, ADP-rybosylation and sumoylation), and modifications in non-coding RNAs (including microRNAs, long non-coding RNAs, and small interfering RNAs). Modifications in DNA mostly occur by methylation at C5 position of cytosine associated with CpG dinucleotides in the promoter regions of constitutively active genes. DNA is methylated by DNA methyl transferases (DNMT) and this process is reversed by demethylases.

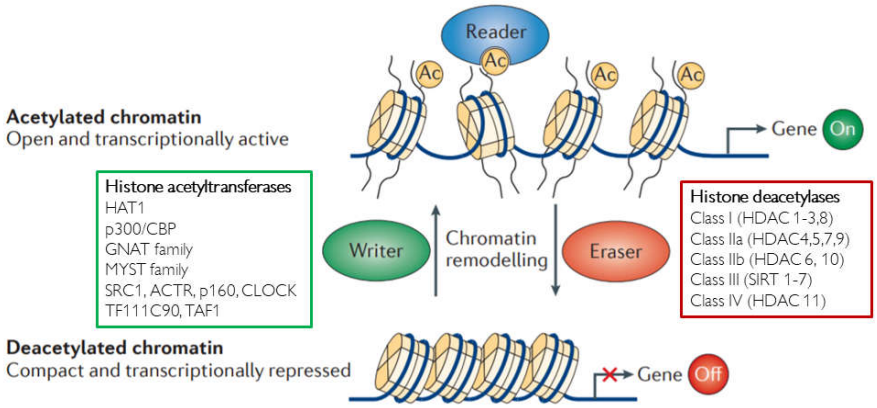


Figure 13. Histone acetylation, chromatin condensation and gene expression. Acetylation targets Lys residues in the amino-terminal tails of core histone proteins, which inhibits the folding of nucleosome arrays into secondary and tertiary chromatin structures. Thus, histone tail acetylation results in chromatin decondensation, thereby allowing access to transcription factors and other transcription co-activators. Adapted from (Verdin and Ott, 2015).

The N-terminal tails of histone proteins can be modified by acetylation at lysine (Lys) or methylation at Lys and arginine residues. Histones are acetylated by histone acetyltransferases (HATs) and deacetylated by histone deacetylases (HDACs), whilst histone methylations are usually mediated by histone

methyltransferases (**Figure 13**). Histone acetylation almost always correlates with chromatin accessibility and transcriptional activity, while Lys methylation can have different effects depending on which residue is modified. Furthermore, distinct histone modifications can influence each other and may also interact with DNA methylation (Bernstein et al., 2007; Latham and Dent, 2007).

Alcohol and/or its metabolites, acetaldehyde and acetate, may induce histone acetylation and methylation changes by altering the activity of the enzymes that mediate these processes, such as HDACs and HATs (Zakhari, 2013). The activity of these enzymes is regulated, in part, by the concentrations of their required substrates and cofactors (Legube and Trouche, 2003). Thus, the cell's metabolic state is tightly related with the epigenetic regulation. The ratio of NADH to NAD^+ fluctuates in response to changes in metabolism. Alcohol metabolism produces a significant increase in the NADH/NAD^+ ratio, resulting in alterations of the carbohydrate and glucose metabolism. As NADH accumulates, NAD^+ becomes depleted and the oxidation of acetyl-CoA by the Krebs cycle is inhibited because of a lack of oxidized coenzymes. Then, the acetyl-CoA is used by HATs to acetylate histones as a substrate from which to transfer the acetyl group (Chater-Diehl et al., 2017). In accordance, alcohol was shown to increase HAT activity (Park et al., 2005). Histone modifications by alcohol have been related with an upregulation of gene transcription, such as acetylation of histone H3 at Lys 9 (H3K9), whereas H3K9 methylation is repressive toward transcription. Increases in H3K9 acetylation, HAT activity, and p300 HAT protein were associated with chronic alcohol feeding (Bardag-Gorce et al., 2007). In addition, acute alcohol decreased HDAC activity and increased acetylation of histones H3 and H4, an effect that was reversed during alcohol withdrawal, associated with anxiety-like behaviours (Pandey et al., 2008; Berkel and Pandey, 2017). Increased acetylation levels of histone 4 at Lys 5 and Lys 12 (H4K5 and H4K12, respectively) have been reported in the prefrontal cortex (PFC) of adolescent

mice after intermittent alcohol treatment (Pascual et al., 2009) and after binge alcohol drinking (Montesinos et al., 2016b). Furthermore, chronic alcohol abuse in humans may result in global increases in trimethylation levels of histone 3 at Lys 4 (H3K4me3), which is a promoter-enriched chromatin mark of actively transcribed genes (Ponomarev, 2013; Krishnan et al., 2014).

Early life experiences can modify epigenetic regulatory factors affecting gene expression (Kundakovic and Champagne, 2015; McGowan and Roth, 2015), such as alcohol exposure during development. Gestation and lactation periods are significantly vulnerable to epigenetic alterations, as active changes occur in the epigenome as part of neurodevelopmental processes (MacDonald and Roskams, 2009; Keverne et al., 2015; Banik et al., 2017). First evidence of alcohol-induced epigenetic modifications during embryogenesis was reported by Garro et al. (1991), who described alterations in DNA methylation in foetuses of alcohol-treated mice. Since then, numerous animal studies have shown epigenetic alterations after *in utero* alcohol exposure. Alcohol enhanced the specific acetylation of Lys 14 on histone 3 (H3K14) at G9a exon1 (Lys dimethyltransferase) in the mouse brain during synaptogenesis leading to neurodegeneration (Subbanna et al., 2014a), effects that were prevented with the pre-administration of a G9a/GLP inhibitor (Subbanna and Basavarajappa, 2014). In addition, alcohol increased the acetylation of histone 4 at Lys 8 (H4K8) at cannabinoid type 1 receptor exon1, upregulating its function and causing neurobehavioural dysfunctions (Subbanna et al., 2014b). Elevated mRNA levels of histone modifying genes (HDAC2, HDAC4 and G9a) were also reported in the foetal brain of alcohol-exposed rats (Gangisetty et al., 2015). A global decrease in histone H3 and H4 acetylation was reported in the cerebellum of perinatally alcohol-exposed rats, which has been proposed to account for the motor deficits associated with FASDs (Guo et al., 2011). In another study, decreased histone activation marks (histone 3 Lys 4 trimethylation (H3K4me3) and H3K9 acetylation) and increased repressive marks (H3K9 dimethylation; H3K9me2) were found in the rat hypothalamus

after foetal alcohol exposure (Bekdash et al., 2013). Moreover, mice receiving acute alcohol exposure on GD7 exhibited alterations in H3K9me2 and acetylation, which strongly correlated with the development of craniofacial anomalies (Veazey et al., 2015).

Genome-wide analysis of DNA methylation patterns in the adult mouse brain revealed widespread differences between alcohol-exposed mice and their counterparts, indicating long-lasting epigenetic alterations following prenatal alcohol exposure (Chater-Diehl et al., 2016). Decreased global DNA methylation levels and suppressed DNMT expression were found in a mouse model of FASD (Abbott et al., 2018), although other studies reported increases in DNMT activity in the HPC of rodents perinatally exposed to alcohol (Perkins et al., 2013). Also, distinct whole-genome DNA methylation patterns were identified in children with FASD, associated with the long-term adverse effects of PAE (Frey et al., 2018; Cobben et al., 2019).

In addition, gestational alcohol exposure altered the expression of microRNAs (miRNAs) and target gene expression in foetal mouse brains (Wang et al., 2009). *In vivo* and *in vitro* models of FASD have identified numerous miRNAs that are impacted by developmental alcohol exposure, including miR-9, miR-20a, miR-21, miR-30, miR-103, miR-151, miR-153, miR-335, and miR-140-3p (Boschen et al., 2018). It also has been shown that binge prenatal alcohol exposure induced changes in the expression of genes associated with apoptosis, cellular redox and inflammation as a short-term effect, while differential expression in brain miRNAs was maintained into adulthood (Mantha et al., 2014). Plasma miRNAs in alcohol drinking pregnant women have been assessed to predict infant outcomes, since their dysregulation during development play a significant role in the aetiology of FASDs (Balaraman et al., 2013, 2016).

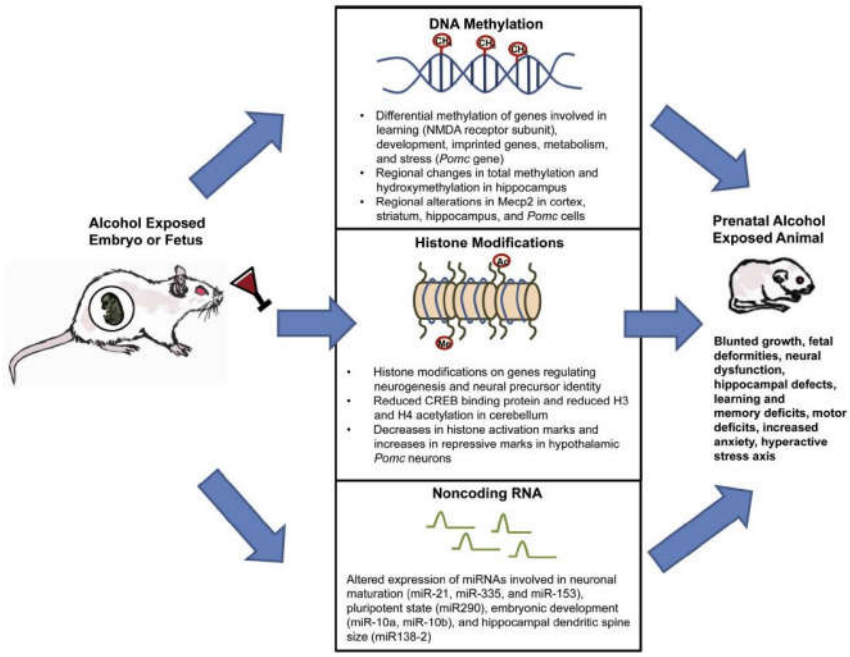


Figure 14. Role of epigenetic processes in the development of FASD. *In vivo* and *in vitro* studies have shown evidence that early developmental alcohol exposure alters DNA methylation, histone modifications and miRNAs. These epigenetic modifications are involved in mediating the development of FASD symptoms. Obtained from Chastain and Sarkar (2017).

Collectively, these results indicate that alcohol exposure during development may have an impact on the epigenetic regulation of genes involved in several key developmental processes, underlying one of the mechanisms of alcohol-induced teratogenesis in FASD (**Figure 14**) (Haycock, 2009; Basavarajappa and Subbanna, 2016; Liyanage et al., 2017; Lussier et al., 2017; Mandal et al., 2017a; Boschen et al., 2018). Indeed, early alcohol exposure has been shown to alter the epigenetic regulation of genes involved in imprinting, neural and glial development, cell cycle regulation and nervous system growth (Ramsay, 2010; Kleiber et al., 2014; Resendiz et al., 2014). Furthermore, alterations in DNA methylation profiles have been found in alcohol-exposed rodent offspring, associated with changes in the expression of several genes involved in multiple biological functions and have been proposed as biomarkers of

prenatal alcohol exposure (Liu et al., 2009; Ungerer et al., 2013; Marjonen et al., 2015; Laufer et al., 2017). Recently, the first report describing a global decrease in DNA methylation, histone methylation and a global increase in histone acetylation in the human brain associated with *in utero* alcohol exposure has been published (Jarmasz et al., 2019).

2.6.7. Other mechanisms

Long-term alterations to brain anatomy, including atypical developmental cortical thinning and abnormal subcortical development as a result of intrauterine alcohol exposure have been broadly described in FASD models (Abbott et al., 2016, 2018), which could be explained by some of the mechanisms described above. Other mechanisms mediating alcohol's adverse effects on neurobiological and neurobehavioural outcomes include neuroendocrine dysfunction, alterations in gene expression, cell morphology, migration and neurotransmission.

The hypothalamic-pituitary-adrenal (HPA) axis is a complex neuroendocrine system involved in adaptation to stressful situations (Armario, 2010). Early-life stress is associated with alterations of the HPA axis functioning (Ros-Simó and Valverde, 2012; Maccari et al., 2014; Spanagel et al., 2014). In that sense, foetal alcohol exposure could be considered a stressor. Alcohol consumption during pregnancy can induce changes in maternal endocrine function, disrupting the hormonal interactions between maternal and foetal systems. Moreover, because alcohol can readily cross the placenta, maternal alcohol consumption can result in direct alteration of foetal neuroendocrine function (Weinberg et al., 2008; Workman et al., 2015).

Clinical studies indicated that neuroendocrine disruptions may occur in children with FASD (Keiver et al., 2015; McLachlan et al., 2016). Animal models of PAE strongly support these findings, demonstrating impaired HPA axis responsivity in adulthood (Zhang et al., 2005; Hellemans et al., 2010; Comeau et al., 2014), associated with molecular and behavioural changes.

Acute alcohol administration at GD7 in mice caused HPA axis dysfunction, showing elevated corticosterone and adrenocorticotropic hormone levels following a restraint stress exposure (Wieczorek et al., 2015), promoting the release of glucocorticoids. A decrease in proopiomelanocortin gene expression -induced by hypermethylation of the promoter- and a decrement in the levels of its derived peptide β-endorphin were reported in adult offspring after foetal alcohol exposure, resulting in hyperresponsiveness to stress in adulthood (Bekdash et al., 2014; Gangisetty et al., 2014). Thus, HPA axis hyperactivity in PAE animals is expected to increase the likelihood to develop psychopathologies after adolescent stress exposure (Raineki et al., 2014, 2016).

Preclinical research has demonstrated that early alcohol exposure can also induce alterations in gene expression (Eberhart and Parnell, 2016). Studies utilizing DNA microarray analyses have shown dysregulations of genes involved in neurodevelopment, apoptosis and energy metabolism (Kleiber et al., 2012; El Shawa et al., 2013; Mantha et al., 2014; Lussier et al., 2015; Mandal et al., 2015) in different brain areas of PAE animals. Further, developmental timing-dependent gene expression alterations have been found in adult PAE mice (Kleiber et al., 2013). Recently, a study using high-throughput RNA-sequencing revealed a range of transcriptome-wide effects on the developing HPC after chronic binge alcohol exposure during gestation, involving key global pathways for healthy foetal development (Lunde-Young et al., 2019).

In addition, alcohol affects neuronal migration along radial glia fascicles (Guerri, 1998; Thompson and Nelson, 2001; Suzuki, 2007). During embryogenesis, radial glial cells provide physical support and chemical guidance for the migration of neuroblasts, the precursors of neurons. As observed in autopsies of children with FAS and in animal models of FASD, foetal alcohol exposure induces alterations in radial glia guiding fibres leading to disruptions in neuronal migration and subsequent abnormalities in the morphology of the cerebral cortex (Zhou et al., 2001; Guerri, 2002; Rubert et

al., 2006; Skorput et al., 2015; Louth et al., 2018; Delatour et al., 2019). Accordingly, alterations in radial glial cell populations delayed neuronal migration in rats exposed to alcohol during gestation (Aronne et al., 2011).

Neuronal migration along the radial glial scaffold is regulated by complex molecular interactions between neuronal and glial cells. Various molecules play a role in these cell-cell interactions, such as glycoproteins, membrane lipids, and both GABA and glutamate acting as chemical attractants (de Graaf-Peters and Hadders-Algra, 2006). In line with this, *in utero* alcohol exposure promoted premature migration by potentiating GABA signalling (Shenoda, 2017). Alcohol may also interfere with cell-to-cell contact by its ability to specifically inhibit cellular adhesive properties (Chen et al., 2003). Animal research demonstrated that alcohol exposure *in utero* alters the expression of cell adhesion molecules, affecting neuron-glia interactions and subsequently, migration, synaptogenesis and plasticity (Bearer, 2001; Miñana et al., 2002).

Alterations in cell morphology, synaptic plasticity and neurotransmission have also been reported in animal models of FASD (An et al., 2013; An and Zhang, 2015). Moderate levels of alcohol during gestation affected the dendritic length, branching and spine density of neurons in the nucleus accumbens (NAc) and dorsal striatum (dSTR) of adult rats (Rice et al., 2012), as well chronic alcohol exposure decreased dendritic spine density of newborn neurons, interfering with synaptic connectivity (Golub et al., 2015). Morphometric changes in hippocampal neurons were also found after a binge-type alcohol exposure during the prenatal period (Jakubowska-Doğru et al., 2017). These morphological changes could be explained by disruption of the cytoskeleton homeostasis (Reis et al., 2015) or by inhibition of astrocyte-mediated neurite outgrowth by alcohol during development (Guizzetti et al., 2014). Furthermore, early alcohol exposure can induce changes in synaptic plasticity by modulating the receptor subunit expression and activity of receptors at the synapse (Fontaine et al., 2016).

Thus, structural alterations at the synapse level could be underlying the functional changes found in PAE models (Berman and Hannigan, 2000; Wang et al., 2006; Krawczyk et al., 2016; Ma, 2019). For instance, animal studies demonstrated that PAE attenuated GABAergic inhibition, leading to neuronal hyperexcitability during development (Galindo et al., 2005; Toso et al., 2006; Zhou et al., 2010; Nirgudkar et al., 2016), altered glutamatergic (Toso et al., 2005; Samudio-Ruiz et al., 2009, 2010; Brady et al., 2013; Brolese et al., 2015) and dopaminergic neurotransmission (Schneider et al., 2005; Sobrian et al., 2005; Fabio et al., 2015b). Dysfunctions in other neurotransmitter systems were also reported (Lee et al., 2008; Ohta et al., 2010; Olateju et al., 2017; Seleverstov et al., 2017). Further, alterations in neuronal Ca^{2+} dynamics were found in a mouse model of FASD, which could explain the hyperexcitability of Purkinje cells (Servais et al., 2007).

2.7. Treatments and interventions for FASD

No effective treatment is currently available for FASD, since alcohol-induced damage seems to be irreversible. The most effective strategy to prevent FASD is abstinence from alcohol during pregnancy and breastfeeding periods, and some preventive interventions including education and media campaigns aimed at increasing knowledge of FASD and the harmful effects of alcohol consumption during pregnancy have been developed (Roozen et al., 2016a; Symons et al., 2018). Also, interventions aimed at reducing alcohol intake in women at risk of PAE have been conducted. However, researchers have been working for years to develop some therapeutic strategies in order to counteract or ameliorate the neurobehavioural outcome and improve the quality of life of individuals affected by FASD.

Parent education and training are essential to promote a nurturing home environment, which has been proved to be protective against secondary conditions associated with FASD. In addition, a number of intervention programs have been developed to address cognitive, attention and self-

regulation deficits that usually accompany children with FASD (Paley and O'Connor, 2011; Petrenko and Alto, 2017).

One pharmaceutical approach would be the use of stimulant medications, which have been successful in treating attention-deficit/hyperactivity disorder (ADHD). These drugs may reduce hyperactivity and impulsivity, as assessed in preclinical studies (Juárez and Guerrero-Álvarez, 2015). Atomoxetine (Strattera), a noradrenaline reuptake inhibitor clinically prescribed for ADHD, has now been tested in a phase III clinical trial for children with FASD and ADHD (*ClinicalTrials.gov Identifier: NCT00418262*).

NMDA receptor antagonists, such as MK-801 and memantine, have been demonstrated to attenuate alcohol-induced hyperactivity and motor coordination deficits, with concomitant neuroprotective effects when administered during the withdrawal phase (Idrus and Thomas, 2011). Moreover, administration of a histamine H3 receptor antagonist, ABT-239, improved the retention of acquired information in PAE animals (Savage et al., 2010).

Preclinical models of FASD have also used neuroprotective peptides to mitigate neuropathologies and behavioural impairments resulting from developmental alcohol exposure. The neuroactive peptides NAP and SAL, which are derived from activity-dependent neuroprotective protein and activity-dependent neurotrophic factor, respectively, administered in pregnant dams prevented alcohol-induced damage in the offspring (Incerti et al., 2010). NAP might protect against alcohol's teratogenesis by antagonizing alcohol's inhibition of L1 cell adhesion molecule, which is implicated in cell-to-cell contact (Idrus and Thomas, 2011). Administration of neuroactive peptides in adolescents exposed to alcohol prenatally can reduce behavioural deficits in memory and learning tasks. Obestatin, a newly discovered peptide with anti-inflammatory and antioxidant activities, which is encoded by the ghrelin gene and released from the gut, has been demonstrated to improve spatial memory

deficits in a rat model of FASD (Toosi et al., 2019). Similarly, neurotrophic and growth factor administration might reduce alcohol-induced adverse effects on insulin-dependent signalling pathways and apoptosis (Gupta et al., 2016).

In addition, pharmacological modulation of nuclear receptors has been proposed as potential targets, since they simultaneously regulate the expression of several genes. The activation of the alpha isoforms of the peroxisome proliferator-activated receptor (PPAR-α) by fenofibrate administration prevented the expression of hyperactivity in a rat model of early alcohol exposure (Marche et al., 2011).

Since neuroinflammation contributes to alcohol-induced neurodegeneration, it has been suggested that anti-inflammatory agents could be effective in the treatment of FASD. PPAR-γ agonists, including pioglitazone, inhibit microglia activation and the production of pro-inflammatory mediators. In a mouse model of FASD, PPAR-γ agonists prevented alcohol-induced neuron cell death and blocked microglia activation (Kane et al., 2011).

The pharmaceutical vinpocetine, a vasodilator and anti-inflammatory agent, inhibits the enzyme phosphodiesterase type I, an action that stimulates cAMP signalling pathway and prolongs CREB protein activation and thereby strengthens synaptic connections, facilitating long-term potentiation (LTP). Animal studies showed that vinpocetine attenuates alcohol-related impairments in cortical plasticity and reduces learning and memory deficits associated with developmental alcohol exposure (Medina et al., 2006; Krahe et al., 2009; Nunes et al., 2011). However, clinical studies with this drug remain to be evaluated (Murawski et al., 2015).

Deficiencies in micronutrients contribute to abnormal foetal development and exacerbate the damaging effects of alcohol on the developing foetus (Sebastiani et al., 2018). Hence, nutritional supplementation during pregnancy may attenuate alcohol's teratogenic effects.

Some nutritional interventions target oxidative stress. Administration of nutrients with high antioxidant properties (vitamin C, vitamin E, omega-3 fatty acids) in pregnant rodent females reduced the alcohol-induced oxidative stress, cell loss and behavioural impairments in offspring (Tiwari et al., 2012). Even though, a clinical trial using high doses of vitamins C and E in women with alcohol-exposed pregnancies was terminated because of safety concerns (Goh et al., 2007). In preclinical studies, antioxidant treatment also had a positive impact at the neuroanatomical level (Joya et al., 2015). Astaxanthin (AST) is a carotenoid pigment with anti-inflammatory and antioxidant properties. Preclinical research indicates that AST might mitigate embryonic growth retardation induced by PAE, as well alcohol-induced oxidative stress and inflammation (Zheng et al., 2014; Zhang et al., 2018). Anthocyanins, a class of flavonoids present in various foods, counteracted the inhibition of glutamatergic neurotransmission, synaptic dysfunction, $GABA_{B1}R$ activation and neuronal apoptosis induced by developmental alcohol exposure in the rat brain (Shah et al., 2015). Another antioxidant with natural origin is curcumin, the main curcuminoid found in turmeric (*Curcuma longa*). It has been shown that curcumin presents protective effects against alcohol-induced apoptosis, in addition to its antioxidant and anti-inflammatory effects in preclinical models of FASD (Tiwari and Chopra, 2012; Muralidharan et al., 2017). Also, the flavonoid (-)-Epigallocatechin-3-gallate (EGCG) is a powerful antioxidant extracted from green tea which has been shown to ameliorate growth retardation in embryos caused by alcohol (Long et al., 2010). Nowadays, clinical studies are using EGCG as a promising tool for the improvement of cognitive performance in FAS patients, as it has previously shown efficacy for Down's syndrome (*ClinicalTrials.gov Identifier: NCT02558933*).

Other nutrients such as choline, betaine, folic acid, methionine, and zinc can influence the epigenetic profiles and potentially attenuate alcohol-induced changes to the epigenome. Preclinical research demonstrated that supplementation with β-carotene (provitamin A), nicotinamide (the amide of

vitamin B3) and zinc all may reduce alcohol's effects on foetal development (Summers et al., 2008). Folic acid deficiency during pregnancy induces neural tube defects; thus, folate supplementation can help to protect against these congenital malformations. Prenatal folic acid administration mitigated alcohol's teratogenic effects, including growth retardation, physical anomalies and neuronal loss (Shrestha and Singh, 2013). Choline is the precursor for the synthesis of acetylcholine and acts as an essential amino acid that plays an important role in CNS development (Zeisel, 2011; Barron et al., 2016). The feasibility and acceptability of maternal choline supplementation in heavy drinking pregnant women was evaluated in a clinical trial, since findings from animal studies suggested that choline can mitigate the alcohol-induced adverse effects on growth and neurocognitive function (Jacobson et al., 2018).

In addition, subjects with FASD ingest inadequate levels of certain nutrients, such as omega-3 fatty acids, vitamin D, and choline (Werts et al., 2014). Therefore, they may benefit from nutrient supplementation (Bastons-Compta et al., 2018). Postnatal omega-3 supplementation restored glutathione levels and reduced lipid peroxidation in PAE animals, reducing oxidative stress in the brain (Patten et al., 2013). Also, acute treatment with omega-3 after a binge alcohol neurotoxic exposure in neonates reduced hyperlocomotion and anxiety-like behaviours (Balaszczuk et al., 2019). It has also been demonstrated that postnatal choline administration can attenuate alcohol's adverse effects on working memory deficits in adolescent rats (Schneider and Thomas, 2016; Waddell and Mooney, 2017). Current clinical studies are examining the effectiveness of choline supplementation as a neurodevelopmental intervention in children with FASD (Wozniak et al., 2015).

Melatonin is also a highly effective antioxidant which is secreted by the pineal gland under physiological conditions. It has been reported that melatonin treatment prevents oxidative damage induced by maternal alcohol administration and reduces homocysteine levels, providing neuroprotective effects in the cerebellum of animal models (Bagheri et al., 2015). Moreover,

lithium could be considered a neuroprotectant against foetal alcohol toxicity, since lithium treatment in mice exposed to a postnatal binge alcohol episode reduced a broad range of anatomical, physiological and behavioural effects induced by alcohol during development (Sadrian et al., 2012).

Other studies suggest that exercise interventions may enhance learning and memory in rodents prenatally exposed to alcohol (Klintsova et al., 2012). The beneficial effects of physical activity on cognitive function are associated with increasing levels of growth factors, such as BDNF, and neuronal plasticity enhancements. Moreover, environmental enrichment has been shown to improve behavioural outcomes associated with PAE in animal models (Hamilton et al., 2014). Clinical studies are developing motor training and exercise interventions to investigate their efficacy in individuals with FASD (Murawski et al., 2015).

Regenerative medicines, such as the use of stem cells, are novel strategies starting to be explored in FASD. In that sense, transplantation of neural stem cells in rodent models attenuated the alcohol-induced impairments in cognitive function and social interaction behaviour (Shirasaka et al., 2012; Poulos et al., 2014).

Furthermore, the pharmacological inhibition of the Lys dimethyltransferase G9a, which mediates the H3K9me2 mark, prior to alcohol exposure in neonatal mice prevented alcohol-induced deficits in memory and social recognition in adult mice, indicating an epigenetic origin of alcohol's effects (Subbanna and Basavarajappa, 2014).

Overall, multiple interventions strategies (such as nutritional and exercise interventions) as well as more traditional interventions (educational, occupational and/or physical therapies) may mitigate a wider range of cognitive impairments when translated to clinical cases of FASD (Murawski et al., 2015; Wilhoit et al., 2017).

3. Drug addiction

Individuals with FASD display higher vulnerability to develop secondary neuropsychiatric disabilities later in life, such as substance use disorders (Popova et al., 2016). Previous clinical studies have suggested that *in utero* alcohol exposure may increase the risk for later alcohol abuse and other drug dependencies (Baer et al., 2003; Alati et al., 2006). Preclinical research also shows that animals prenatally exposed to drugs of abuse are more responsive to such drugs and other psychotropic substances. The neurobiological substrates involved in the vulnerability to drug abuse attributable to early-life alcohol exposure are still not fully understood, although the main mechanism presumably involved is the dysfunction of the mesocorticolimbic neurocircuitry, the brain reward system (Koob and Le Moal, 2001).

Drug addiction is a chronically relapsing disorder that has been characterized by i) compulsive drug-seeking and drug-taking behaviour, ii) loss of control over limiting drug intake, and iii) emergence of a negative emotional state (dysphoria, anxiety or irritability) reflecting a motivational withdrawal syndrome when access to the drug is prevented (Koob and Volkow, 2010). It has been further conceptualized as a progression from impulsive to compulsive behaviour, ending in chronic, relapsing drug taking (Koob and Le Moal, 2005). The transition from occasional, controlled drug use to the loss of control over drug-seeking and drug-taking and to chronic relapse even after protracted abstinence is a hallmark of addiction, which involves neuroplasticity, and may begin with initial drug use in vulnerable individuals or individuals at particularly vulnerable developmental periods.

Approximately 5.5% of the adult population (271 million people) has engaged in nonmedical or illicit drug use at least once in the previous year, with approximately 13% among the estimated past-year users (35 million) going on to substance dependence on illicit drugs. This corresponds to a prevalence of

drug use disorders of 0.71% globally among the population aged 15-64 (UNODC, 2019).

After cannabis, the most commonly used illicit drugs are psychostimulants (amphetamine, cocaine, LSD, "ecstasy" and other hallucinogens). Globally, it is estimated that 18 million people are past-year users of cocaine, corresponding to 0.4% of the global population (UNODC, 2019). In Spain, cocaine is the second most consumed illicit drug; 10% of the population (aged 15-64 years) reported cocaine consumption at least once in their lives, and around 2% have consumed cocaine in the last 12 months (EDADES, 2018).

3.1. Neurobiological substrates of drug addiction

The brain reward system (**Figure 15**) is originated in the ventral tegmental area (VTA), a DA-rich nucleus located in the ventral midbrain which provides dopaminergic innervation to limbic structures (that is, mesolimbic pathway, such as the amygdala, ventral pallidum, HPC and NAc) and cortical areas (that is, mesocortical pathway, including the PFC, the orbitofrontal cortex and the anterior cingulate) (Feltenstein and See, 2008).

The primary reward circuit includes dopaminergic projections from the VTA to the NAc, which release DA in response to reward-related stimuli (and in some cases, aversion-related stimuli). However, dopaminergic transmission does not fully account for the acute reinforcing effects of addictive drugs, other neurotransmitter systems including glutamate, GABA, opioid peptides, cannabinoids and 5-HT are also involved. In this sense, the NAc receives glutamatergic projections from the medial PFC (mPFC), HPC and amygdala, as well as other regions. The VTA receives such inputs from the lateral dorsal tegmentum, the lateral habenula (LHb) and the hypothalamus, as well as both GABAergic and glutamatergic innervations from the extended amygdala. There are also GABAergic projections from the NAc to the VTA; projections through the direct pathway directly innervate the VTA, whereas projections

through the indirect pathway innervate the VTA via intervening GABAergic neurons in the ventral pallidum (Russo and Nestler, 2014).

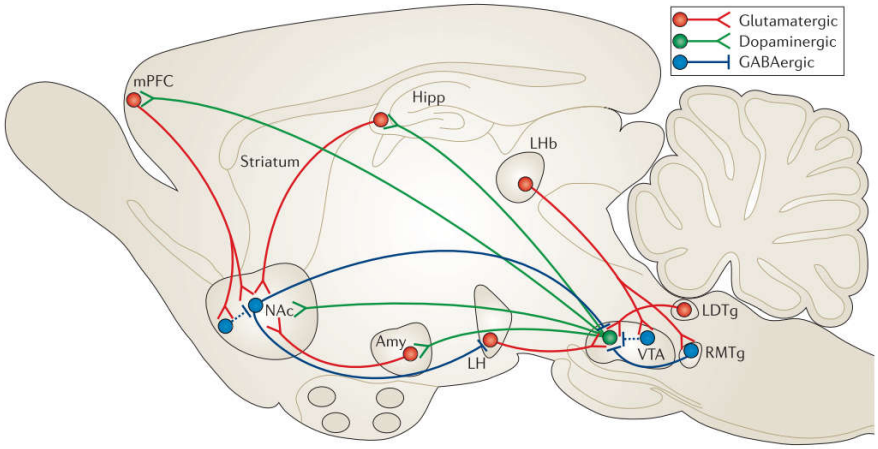


Figure 15. Sagittal section of a representative rodent brain illustrating the major dopaminergic, glutamatergic and GABAergic pathways to and from the VTA and NAc involved in the acute reinforcing actions of drugs of abuse. The dashed lines indicate internal inhibitory projections. mPFC, medial prefrontal cortex; Hipp, hippocampus; LHb, lateral habenula; NAc, nucleus accumbens; Amy, amygdala; LH, lateral hypothalamus; LDTg, lateral dorsal tegmentum; RMTg, rostromedial tegmentum; VTA, ventral tegmental area. Obtained from Russo and Nestler (2014).

The NAc and the ventral pallidum appear to be involved in the primary reinforcing effects of drugs of abuse, the amygdala and HPC play an important role in conditioning learning, while the cortical areas regulate emotional responses, cognitive control and executive function, with repeated drug exposure leading to cellular adaptations in the prefrontal-NAc glutamatergic pathway, which mediate persistent addictive behaviours. In other words, the mesolimbic pathway is involved in the acute reinforcing effects of drugs and various conditioned responses related to craving and relapse, whereas changes in the mesocortical pathway mediate the conscious drug experience, drug craving and a loss of behavioural inhibition related to compulsive drug-seeking and drug-taking behaviours.

The transition from controlled to compulsive drug-taking behaviour has been associated with a shift at the neural level from prefrontal cortical to striatal control, as well as with a progression from ventral (including the NAc) to dorsal subregions of the striatum (STR), which is associated with habit formation (Gerdeman et al., 2003; Everitt and Robbins, 2005, 2013).

All drugs of abuse, despite their initial effects on distinct molecular targets, exert a series of common functional effects on the reward circuitry to impair its function, causing an individual to lose control over drug consumption (Nestler, 2005). Within the population of VTA DA neurons, those projecting to the medial NAc shell appear to be the primary target of addictive drugs, producing an enhancement of dopaminergic signalling from the midbrain VTA to the NAc in ventral STR (vSTR).

In terms of their neurochemical actions, psychostimulants increase the synaptic availability of several monoamines, including 5-HT, DA and norepinephrine, either indirectly by inhibiting their reuptake (for example, cocaine) or directly by enhancing their release from presynaptic terminals (for example, amphetamines). The acute reinforcing effects of cocaine appear to be mediated primarily by increasing synaptic levels of DA in the NAc through blockade of the presynaptic DA transporter (DAT) (Ikegami and Duvauchelle, 2004; Dong and Nestler, 2014). In contrast, alcohol activates $GABA_A$ receptors or GABA release in the VTA, NAc, and amygdala by either direct actions at the $GABA_A$ receptor or through indirect release of GABA. However, alcohol might also facilitate the release of opioid peptides in the VTA, NAc and central nucleus of the amygdala, and the release of DA in the NAc through a direct action either in the VTA or the NAc (**Figure 16**).

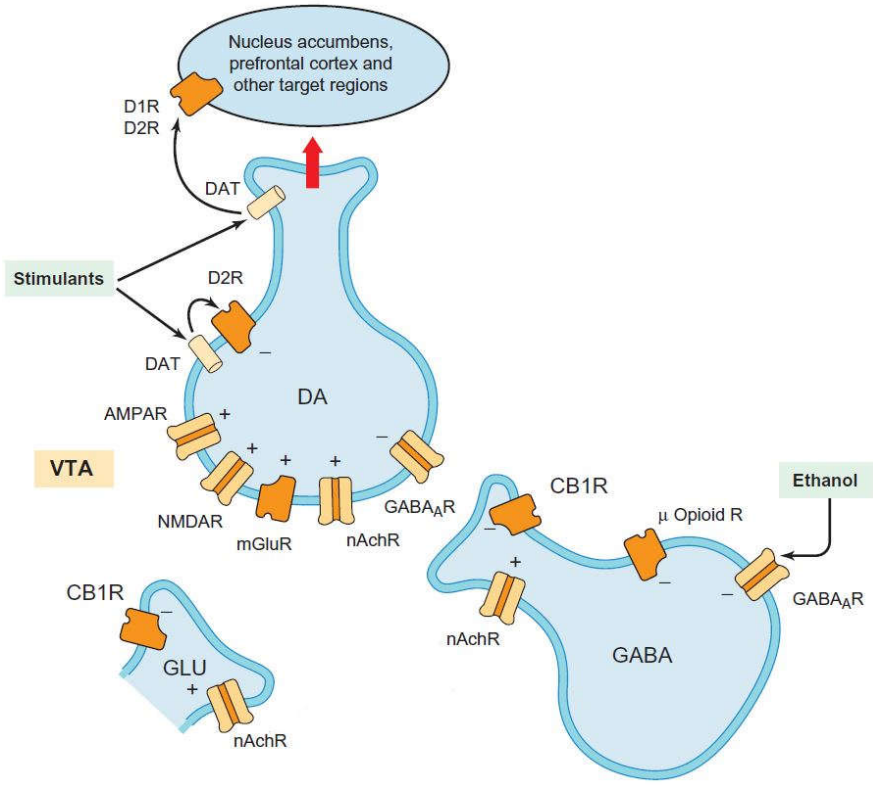


Figure 16. All reinforcing drugs increase DA transmission in the mesocorticolimbic DA system, but they use different mechanisms. Psychostimulants, such as cocaine, interact with the DAT to elevate extracellular DA levels. Opiates, alcohol and cannabinoids are believed to decrease GABA transmission in the VTA, thereby disinhibiting DA neurons. However, alcohol can also directly excite DA neurons via voltage-gated ion channels. Dopamine-independent mechanisms also may contribute significantly to the reinforcing effects of drugs of abuse. Adapted from Wolf (2012).

Natural rewards, including food, sex or interpersonal relationships can activate this system, although drugs of abuse promote this activation with higher intensity than natural rewards (Wolf, 2002). Consequently, the overactivation of the reward system by drugs of abuse induces neuroadaptations that alter the normal function of this system modifying the natural rewarding properties in drug abusers (Koob and Le Moal, 2008).

Chronic exposure to drugs of abuse induces adaptive changes in different components of the dopaminergic system, such as alterations in DA receptors. G protein-coupled DA receptors are divided into two subclasses: the D_1-type and D_2-type receptors. D_1-type receptors (D1 and D5) are primarily coupled to $G\alpha_s$ and induce the production of cAMP. D_2-type receptors (D2, D3 and D4) are $G\alpha_{i/o}$-coupled and inhibit the production of cAMP. In the CNS, D_1-like receptors display a post-synaptic anatomical distribution, while D_2-type receptors are both pre- and post-synaptic located. Both are expressed in brain regions that receive dopaminergic inputs, such as dSTR and NAc, and in a lesser extent, in the HPC, neocortex, hypothalamus and thalamus (Vallone et al., 2000; Beaulieu and Gainetdinov, 2011).

Furthermore, long-term drug consumption can result in strengthening or weakening of synaptic connectivity, affecting monoamine and glutamatergic neurotransmitter signalling. Synaptic strength is controlled by the insertion or removal of glutamate receptors, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and NMDA. As for their subunit composition, a shift in the AMPA receptors (AMPAR) subunit composition, from calcium-impermeable GluA2-containing to calcium-permeable GluA2-lacking AMPARs, has been reported to facilitate AMPAR currents, contributing to LTP in animal studies of addiction (Woodward Hopf and Mangieri, 2018). Changes in GluA1/GluA2 ratio may be associated with addictive-like conduct, such as the incubation of cocaine-seeking behaviour (Wolf, 2016). The up-regulation of AMPA/NMDA ratio increases the responsiveness of the NAc to glutamate, which is released by cortical and limbic terminals when exposed to drugs or drug cues (Conrad et al., 2008).

In addition, several drugs of abuse upregulate the cAMP pathway and cause CREB protein activation in the NAc, although such changes are generally short. In contrast, ΔFosB isoforms are highly stable products of the *fosB* gene that accumulate following chronic drug exposure and remain high after a long period, regulating complex behaviours related to the addiction process

(McClung and Nestler, 2003; Nestler, 2008). Changes in gene expression initiated by these transcription factors contribute to 'sensitizing' adaptations that promote drug-taking as well as 'compensatory' adaptations that oppose drug-taking.

These neuroadaptations caused by chronic drug exposure induce tolerance to the rewarding effects, resulting in the need to consume higher doses of the drug to produce the same euphoric feelings. Therefore, as drug use duration increases, the positive reinforcing effects are diminished resulting in dysphoria states, contributing to the development of addictive behaviour.

Even though, it is estimated that only 10-20% of people who use drugs ultimately become addicted (Warner, 1995). Many genetic, environmental, and social factors contribute to the determination of a person's unique susceptibility to using drugs initially, sustaining drug use, and undergoing the progressive changes in the brain that characterize addiction.

Factors that increase vulnerability to addiction include family history, early exposure to drug use (adolescence is among the periods of greatest vulnerability to addiction, but also foetal development), exposure to high-risk environments, and certain mental illnesses (such as mood disorders, ADHD, psychoses, and anxiety disorders) (Volkow et al., 2016).

3.2. FASD as a risk factor for drug addiction

As mentioned above, it has been suggested that alcohol exposure during development can account for an increased risk to develop substance use disorders later in life. Even though, there is still scarce research on the neurobiological mechanisms underlying the association between prenatal substance exposure and subsequent drug abuse in exposed offspring (Behnke and Smith, 2013). In this thesis, we focused on the effects of FASD on later alcohol and cocaine abuse vulnerability.

3.2.1. Alcohol

Clinical data support an increased risk of alcohol abuse later in life following prenatal exposure (Yates et al., 1998; Baer et al., 2003; Streissguth et al., 2004; Alati et al., 2006; Pfinder et al., 2014; Parolin et al., 2016), since alcohol intake by offspring is predicted more effectively by prenatal exposure to alcohol than by family history. This epidemiological evidence has also been suggested in preclinical studies.

Studies with rodents during the third-trimester equivalent period to human gestation demonstrated that prenatal exposure to alcohol increased the response to alcohol's flavour and odour, attenuating the sensitivity to its aversive effects, which was confirmed by tests with human neonates of mothers reporting moderate alcohol intake during pregnancy (Spear and Molina, 2005; Youngentob and Glendinning, 2009; Gore-Langton and Spear, 2019). In addition, early alcohol exposure can yield later enhancement of alcohol intake in rodents (Chotro and Arias, 2006; Chotro et al., 2007; Fabio et al., 2013; Parker et al., 2016; Brancato et al., 2018).

Enhanced alcohol intake is not the only consequence of early alcohol exposure that could increase risk for abuse among adolescents or adults. Tolerance to alcohol, and physiological responses to alcohol such as hypothermia, alcohol intake associated with stress or social circumstances, or susceptibility to alcohol reinforcement are factors that could promote later abuse of alcohol. Some preclinical studies have shown altered temperature regulation after prenatal exposure to alcohol, as well as, heightened responsivity to stress due to HPA axis hyperactivity (Spear and Molina, 2005). Also, long-lasting alterations in neurotransmitter systems, such as GABA and glutamate systems, can account for postnatal differences in the reinforcement effects of alcohol. Thus, foetal alcohol exposure could sensitize the brain regions and the developmental processes involved in drug addiction, causing long-lasting alterations on neurobehavioural functions and increasing propensity to later

abuse, as it is hypothesized to occur when drinking during adolescence (Guerri and Pascual, 2010).

3.2.2. Cocaine and other psychostimulants

Preclinical research indicates that PAE animals as adults are more responsive to alcohol as well as to other psychoactive drugs. Intrauterine drug exposure might predispose animals to increased reward-driven behaviours and increased self-administration of drugs (Glantz and Chambers, 2006).

Previous studies have shown that psychostimulants can restore the alterations of neuronal activity in the VTA in rodent models of FASD (Xu and Shen, 2001; Choong and Shen, 2004a), thus suggesting that animals exposed prenatally to alcohol may respond differently to the reinforcing effects of psychostimulant drugs. Accordingly, an increased sensitivity to cocaine reinforcing effects in the conditioned place preference (CPP) and augmented cocaine consumption in a free two-bottle choice paradigm (Barbier et al., 2008), in addition to enhanced locomotor sensitization to cocaine and amphetamine (Barbier et al., 2009), were reported in adult male offspring rats exposed to alcohol. Recently, increased amphetamine-induced CPP and amphetamine intravenous self-administration (SA) have been reported in a rat model of FASD (Wang et al., 2019b). Moreover, rats receiving alcohol during gestation self-administered more amphetamine and exerted greater effort to obtain the drug under a progressive ratio (PR) schedule (Hausknecht et al., 2015; Wang et al., 2018), suggesting increased psychostimulant addiction risk in individuals with FASD later in life. However, the molecular mechanisms underlying the behavioural alterations caused by psychostimulants in PAE animals remain to be elucidated.

HYPOTHESIS AND OBJECTIVES

1. Hypothesis

Considering the theoretical frame exposed in the *Introduction*, we **hypothesized** that binge alcohol drinking during critical periods for brain development, such as gestation and lactation, produces behavioural and molecular alterations in the brain increasing the vulnerability to develop secondary neuropsychiatric disorders later in adolescence or adulthood, such as drug addiction.

2. Objectives

In accordance with our hypothesis, the **main aim** of this thesis was to investigate the long-term consequences of maternal binge alcohol consumption during critical periods for brain development on cognitive, motor, emotional and addictive behaviour in adult male mice. Behavioural studies were combined with biochemical analysis in order to achieve our main objective.

The **specific objectives** of the present work were the following:

Objective 1. To assess the effects of binge-like alcohol consumption during gestation and lactation on cognitive, motor and anxiety-like behaviour in adult offspring mice.

- **a)** To establish a model of maternal binge-like alcohol drinking during prenatal period or both prenatal and lactation periods using the Drinking-in-the-Dark test.
- **b)** To evaluate the spontaneous maternal care behaviour along the first postnatal week and the consequences on body weight and litter size in our experimental conditions.
- **c)** To assess the spontaneous locomotor activity and locomotor coordination using the rotarod in offspring mice.

d) To determine the effects of maternal binge-like alcohol consumption on cognitive performance in adult offspring. Specifically, evaluation of working memory, object recognition memory and left-right discrimination reversal learning.

e) To study the long-term consequences of early alcohol exposure on anxiety-related behaviour using the elevated plus maze.

Objective 2. To study the underlying molecular mechanisms to the behavioural alterations observed. We were particularly interested in neuroinflammation, neuronal cell death, myelin damage, alterations in neurogenesis, epigenetic modifications and brain network connectivity alterations.

a) To analyse the pro-inflammatory response in the brain due to early alcohol exposure. To study the involvement of the TLRs, inflammasome, and pro-inflammatory mediators, such as IL-1β.

b) To determine whether prenatal and lactational alcohol exposure induces alterations in adult hippocampal neurogenesis using the BrdU assay.

c) To evaluate the integrity of the myelin sheath by analysing the expression of myelin proteins in adult offspring mice.

d) To study the effects of alcohol exposure on epigenetic post-translational modifications in the brain by analysing the activity of the enzymes involved in this process and histone acetylation marks.

e) To assess the brain network connectivity in adult mice exposed to alcohol during development using the cytochrome-c oxidase (CCO) labelling.

Objective 3. To evaluate the effects of curcumin as a therapeutic strategy to counteract the behavioural and molecular alterations in offspring mice exposed to alcohol during development.

a) To assess the putative therapeutic effects of a curcumin treatment during the adolescence period on cognitive performance in adult offspring. Evaluation of spatial working memory, object recognition memory and left-right discrimination reversal learning.

b) To evaluate the effects of curcumin on anxiety-related behaviour using the elevated plus maze.

c) To examine the potential effects of curcumin on counteracting the molecular alterations found in early alcohol exposed mice, focusing on epigenetic modifications and neuroinflammatory processes. Evaluation of HAT enzyme activity, as well as, glial cell activation (microglia and astrocytes) and production of pro-inflammatory mediators.

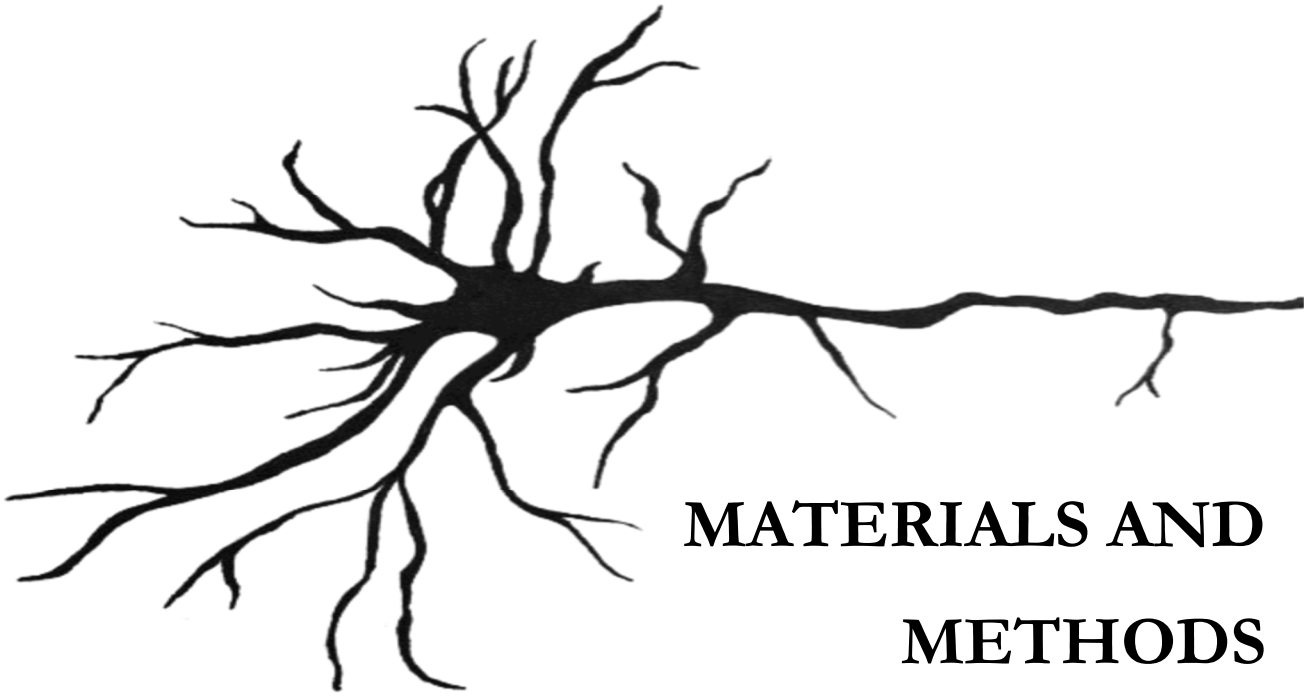
Objective 4. To investigate the increased risk to develop addictive behaviours after developmental alcohol exposure later in life and its underlying molecular mechanisms. Specifically, we were interested in the vulnerability to alcohol and cocaine addiction.

a) To evaluate the rewarding properties of alcohol in adult offspring mice using the conditioned place preference.

b) To assess the rewarding properties of cocaine in adult offspring mice using the conditioned place preference.

c) To examine the effects of alcohol exposure during gestation and lactation periods on cocaine-induced locomotor sensitization.

d) To study the reinforcing effects of cocaine using the operant self-administration paradigm during acquisition, extinction and reinstatement phases. To analyse the neuroadaptations in the dopaminergic and glutamatergic systems in our experimental conditions.



MATERIALS AND METHODS

1. Experimental procedure

To reproduce an episodic pattern of binge alcohol drinking during pregnancy and lactation periods, we exposed pregnant and nursing C57BL/6 female mice to an alcohol solution using the drinking-in-the-dark (DID) test paradigm, which has been proposed as a useful binge drinking model of FASD (Boehm et al., 2008). Subsequently, male offspring were assessed for their cognitive, emotional and motor function, as well as, addictive-like behaviours at adulthood. Furthermore, neurochemical alterations in the brain of alcohol-exposed offspring mice were investigated, as explained below (see **Figure 17**).

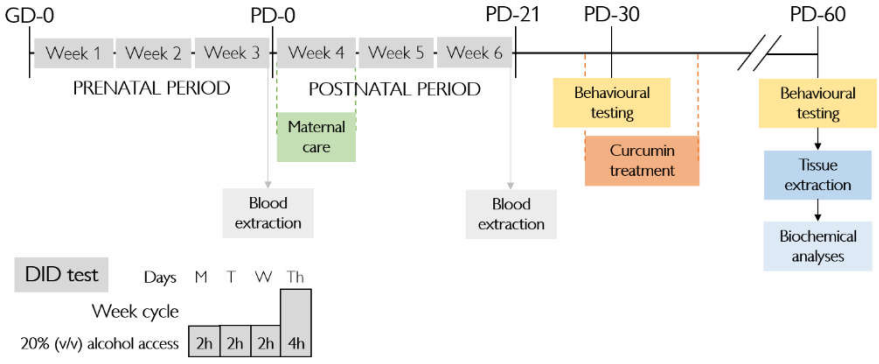


Figure 17. Schematic representation of experimental design.

2. Animals

Twelve-week-old male and female C57BL/6 inbred mice were purchased from Charles River (Barcelona, Spain) and shipped to our animal facility (UBIOMEX, PRBB) to be used as breeders. Upon arrival, they were housed in standard cages at constant temperature (21 ± 1ºC) and humidity (55 ± 10%), under a reversed light-dark cycle (white lights on 20:00-08:00 h). After one week of acclimatization, breeding pairs were mated, and pregnant females were observed daily for parturition. For each litter, the date of birth was designated as postnatal day (PD) 0. Pups remained with their mothers for 21 days and were then weaned (PD21). After weaning, male offspring were housed in groups of 4. Food and water were available *ad libitum* except during

the DID procedure, as described below. Every effort was made to minimize the number of animals used and their suffering. All animal care and experimental procedures were approved by the local ethics committee (CEEA-PRBB) and conducted in accordance with the European Union Directive 2010/63/EU guidelines on the protection of animals used for scientific purposes.

3. Drugs

Ethyl alcohol was purchased from Merck (Darmstadt, Germany) and diluted in tap water in order to obtain a 20% (v/v) alcohol solution for the Drinking-in-the-dark test. For the conditioned place preference, absolute alcohol was dissolved in sterile 0.9% NaCl (physiological saline, pH=7.4) in order to obtain 1 or 2 g/kg body weight solutions. Cocaine hydrochloride was purchased in Alcaliber S.A. (Madrid, Spain) with the authorization of the "Agencia Española del Medicamento y Productos Sanitarios" (Ministerio de Sanidad, Consumo y Bienestar Social) and prepared in 0.9% NaCl immediately before administration. Curcumin (Turmeric) (Sigma-Aldrich, Madrid, Spain) was prepared in 20% dimethyl sulfoxide (DMSO) and 80% sterile saline solution in order to obtain a 100 mg/kg body weight solution.

4. Behavioural tests

4.1. Drinking-in-the-dark (DID) test

This procedure was conducted as previously reported (Rhodes et al., 2005; Esteve-Arenys et al., 2017), commencing two days after mating. Pregnant females were randomly assigned to two groups: alcohol and water-exposed (control). Briefly, water bottles were replaced with 10-ml graduated cylinders fitted with sipper tubes containing either 20% (v/v) alcohol in tap water or only tap water 3h after the lights were turned off. Following a 2h-access period, individual intake was recorded, and the original water drinking bottles were returned to the home cage. During this period, female mice were

individually housed, and each corresponding male breeding pair was removed from the home cage for the DID procedure. This procedure was repeated on days 2 and 3 and fresh fluids were provided each day (from Monday to Wednesday). On day 4 (Thursday), alcohol or water cylinders were left for 4h and fluid intakes were recorded. Food was removed during the alcohol exposure periods and then, returned to the home cage. Two empty control cages (water and alcohol) were placed in the rack to measure general liquid loss (leakage/evaporation) and drip values were subtracted from the drinking values. Fluid intakes (g/kg body weight) were calculated on the basis of average 2-day body weight values, as dams were weighed at 2-day intervals (Mondays and Wednesdays). The procedure was maintained throughout the 3-week gestation and/or 3-week lactation periods. Food and water consumption during *ad libitum* periods were measured in both pregnant and nursing female mice.

4.2. Neonatal offspring assessment

Litters were left undisturbed until PD5, when litter size was determined. The weights of the entire litter were collected on PD5 and PD21 and average pup weight was determined by dividing the total litter weight by the number of pups present in each litter, as previously described (Brady et al., 2012).

4.3. Maternal care behaviour

Spontaneous maternal behaviour was recorded 3 times per day (08:00, 13:00 and 17:00) from PD1 to PD7 as previously reported, with minor modifications (Fodor et al., 2012; Gracia-Rubio et al., 2016b). In short, the behaviour of each mother (n=6 per group) was scored 20 times at 3-minute intervals (20 observations x 3 periods per day x 7 days = 420 observations/mother). The following behaviours were scored as present or absent and quantified in a checklist: 1) mother licking and grooming offspring; 2) mother nursing offspring in an "arched-back" posture with rigid limbs; 3) mother nursing in a

"blanket" posture; 4) mother nursing in a "passive" posture; 5) mother "out of the nest" (no maternal contact); and 6) "nest-building".

4.4. Spontaneous locomotor activity

Offspring mice were evaluated for their basal locomotor activity during adolescence (PD30) and adulthood (PD60). Animals were placed in locomotor activity boxes (24x24x24 cm) (LE8811 IR, Panlab s.l.u., Barcelona, Spain) and horizontal (deambulations) and vertical (rearings) movements were automatically recorded for 45 min as previously described (Gracia-Rubio et al., 2016b).

4.5. Rotarod

Late adolescent mice (PD48) were trained at a fixed speed (4 rpm) on the accelerating rotarod apparatus (five-line accelerating rotarod; LE 8200, Panlab s.l.u.) as previously reported (Bilkei-Gorzo et al., 2005; Moscoso-Castro et al., 2016). The rotarod accelerated from 4 to 40 rpm over 2 min. Motor coordination was measured in a session comprising ten consecutive trials interleaved by 30-s breaks. The mean of rpm achieved prior to falling in the last four trials was averaged as a measure of motor coordination.

4.6. Elevated plus maze

Elevated plus maze (EPM) was performed to evaluate anxiety-like behaviour in the offspring at PD30 and PD60 as previously reported (Gracia-Rubio et al., 2016b). The apparatus (Panlab s.l.u.) consisted of a black maze with four arms (16×5 cm) set in the form of a cross from a neutral central square (5×5 cm). Two arms were closed up by vertical walls (closed arms) while the other two perpendicular arms had open edges (open arms). The maze was elevated 30 cm above the floor in dim lighting conditions (30 lux). At the outset of the 5-min observation session, each mouse was placed in the central area, facing an open arm. The total number of entries (placing all four paws into the arm)

and the time spent in the open and closed arms (as a percentage of the total test time) were recorded by automated tracking software (Smart, Panlab s.l.u.).

4.7. Spontaneous alternation Y-maze test

Adult mice (PD60) were assessed for spatial working memory as previously reported (Arai et al., 2001). Briefly, mice were placed in the centre of a Y-shaped maze with two equal arms, each 395 mm long and separated by 120º angles, and were allowed to freely explore for 8 min. Arm choices were manually recorded. Three consecutive choices of three different arms were counted as an alternation. The score was obtained following this formula:

$$\text{Score (\%)} = \frac{\text{Number of alternations x 3}}{\text{Total number of choices - 2}} \text{ x 100}$$

4.8. Novel object recognition (NOR) test

Recognition memory, a hippocampal-dependent task, was evaluated in PLAE mice and their counterparts (PD60) as previously reported (Maccarrone et al., 2002), with minor modifications. The test was performed in a white Plexiglas box (50 x 50 x 30 cm) with white vertical walls under dim light intensity (30 lux) in the middle of the field. The objects to be discriminated were a black plastic object (Object A) and a red wooden object (Object B). Firstly, mice were individually habituated to the box for 15 min. After 24 h, the acquisition trial took place and mice were allowed to explore the box for 10 min in the presence of either object A or object B alone (counterbalanced). A 10-min retention trial occurred 24 h later with the presence of objects A and B in the open-field. The time spent by the animal exploring object A (tA) and object B (tB) was recorded using Smart software (Panlab s.l.u.). The recognition index (%) was defined as [tB/(tA + tB)] x 100 for the animals exploring object A in the acquisition trial or [tA/(tA + tB)] x 100 for the mice exploring object B during the acquisition trial, being 't' the time each subject spent exploring an object.

To evaluate the effects of curcumin treatment on recognition memory in adult offspring mice (PD60), NOR test was conducted as previously described (Moscoso-Castro et al., 2016; Luján et al., 2019), with minor modifications. First, mice were placed in the centre of an empty black open field (27 x 31 x 25 cm) with moderate luminosity (100 lux) and allowed to freely explore it for 15 min. Next day, they were presented with two identical objects for 8 min and exploration time was recorded. The retention trial took place 72 h later, in which one of the familiar objects was replaced by a novel object and animals were allowed to explore for 8 min. Exploration time was recorded and discrimination index (%) was defined as $[(t_{\text{novel object}} - t_{\text{familiar object}})/(t_{\text{novel object}} + t_{\text{familiar object}})]$.

4.9. T-maze alternation test

Adult offspring male mice (PD60) were assessed for left-right discrimination learning using a T-maze (length of stem 30 cm, length of arms 30 cm, width 12 cm, wall height 16 cm) filled with water (23 ± 2ºC) as previously described (Filali and Lalonde, 2009). A platform (11 x 11 cm) was submerged at the end of each arm 1 cm beneath the surface. Turning preference was tested during the first two trials. The platform was then placed on the least preferred arm, counterbalancing the number of mice reinforced on either side. Mice were placed in the stem of the T-maze and swam either to the left or to the right until finding the submerged platform for a maximum of 60 s. After reaching the platform, mice were removed from the maze to allow them to recover and then placed back in the maze for up to a maximum of 25 trials. Acquisition criterion was achieved when a mouse reached the platform in 5 consecutive trials. Once the acquisition criterion was acquired, animals did not perform any other trial. After 48 h, the reversal learning phase was conducted, in which the escape platform was placed on the opposite arm. Latency to reach the platform and number of trials to reach criterion were recorded.

4.10. Conditioned place preference (CPP)

Adult offspring mice (PD60) were tested for the rewarding properties of food, alcohol or cocaine using an unbiased CPP paradigm. Different sets of animals were used for each experiment. The apparatus consisted of two equally sized compartments (30x29x35 cm), differing in terms of visual and tactile cues, connected by a corridor (14x29x35 cm) (Cibertec S.A., Madrid, Spain). One compartment had white-painted walls with prismatic textured flooring while the other had black walls with a smooth floor. Both compartments were equipped with infrared emitter/detector pairs which allowed us to record the position of the animal and its crossings between compartments.

4.10.1. Food-induced CPP

For the food-induced CPP, mice were first food-deprived to 80-85% of their baseline body weight with limited-access to standard chow pellet (2-3 g/day per animal) for 4 days prior to commencing preconditioning and throughout the procedure, as previously described with some minor modifications (Valverde et al., 2004). The CPP procedure consisted of three different phases: preconditioning (one session), conditioning (six sessions) and testing day (one session). During preconditioning, mice could freely explore both compartments for 20 min. Mice showing strong unconditioned aversion (<33% of the session time) or preference (>67%) for either compartment were excluded from the study. Conditioning training began on the following day. Food-paired mice were confined, for 30 min, to one compartment, in which they had access to palatable food (1g; Cheerios, Nestlé®) on days 2, 4 and 6. On alternate days (3, 5 and 7), mice were confined to the other chamber with no food available. Food-unpaired mice were alternatively placed in each compartment without receiving any food. During conditioning, the central area was blocked by guillotine doors. Finally, the testing session (day 8) was conducted under the same conditions as in the preconditioning phase.

Time spent in each compartment was recorded during the preconditioning and testing sessions. The CPP score for each subject was calculated as the difference between the time spent in the drug-paired compartment during the test and the pre-conditioning phase.

4.10.2. Alcohol-induced CPP

The same procedure was followed for alcohol-induced CPP, but in this case mice were not food-deprived, as previously described with minor modifications (Roger-Sánchez et al., 2012). The conditioning phase consisted of four pairings: mice received an i.p. injection of 1 or 2 g/kg alcohol immediately prior to being confined to the drug-paired compartment for 5 min on days 2, 4, 6 and 8. On the alternate days (3, 5, 7 and 9), mice received physiological saline before confinement to the vehicle-paired compartment for 5 min. Treatments were counterbalanced between compartments. Non-drug paired mice were administered saline prior to confinement to one of the two compartments every day. The test (day 10) was conducted in the same conditions applied in the pre-test session.

4.10.3. Cocaine-induced CPP

A similar procedure was followed for cocaine-induced CPP, with no food-deprivation, as previously described (Luján et al., 2018). The conditioning phase consisted of four pairings: mice received an i.p. injection of 5 or 10 mg/kg cocaine immediately prior to confinement to the drug-paired compartment for 30 min on days 2, 4, 6 and 8, while on alternate days (3, 5, 7 and 9) mice received physiological saline before being confined to the vehicle-paired compartment for 30 min. Treatments were counterbalanced between compartments. Non-drug paired mice were administered saline prior to confinement to one of the two compartments every day. The testing session (20 min) took place on day 10.

4.11. Drug-induced behavioural sensitization

The sensitization to hyperlocomotor responses elicited by cocaine was assessed in adult offspring mice (PD60) as previously described with minor modifications (Gracia-Rubio et al., 2016a). The sensitization procedure consisted of three phases: habituation, acquisition and challenge. In the habituation phase, mice were placed individually into locomotor activity boxes (24 x 24 x 24 cm) (LE881 IR, Panlab, s.l.u.) for 30 min immediately after receiving an i.p. saline injection. On the following five days (acquisition phase), mice were treated daily with cocaine (7.5 or 10 mg/kg; i.p.) or saline immediately prior to confinement in the locomotor activity boxes for 30 min. Following a drug-free period of 8 days after the last cocaine treatment, mice were injected with cocaine (7.5 or 10 mg/kg; i.p.) and the locomotor activity was recorded for 30 min (challenge phase).

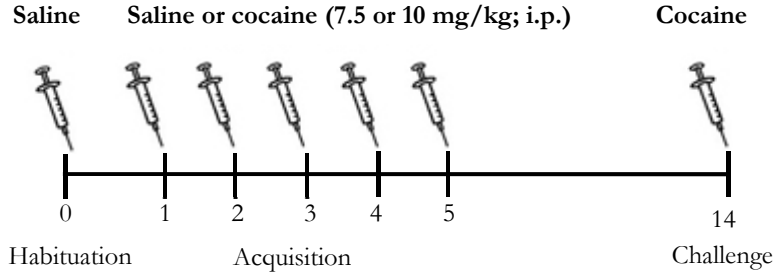


Figure 18. Schematic representation of drug-induced sensitization procedure.

4.12. Operant self-administration paradigm

Catheter implantation surgery

An indwelling Silastic catheter was surgically implanted into the right jugular vein under isoflurane anaesthesia (1.5-2.0%). During surgery, mice were treated with an analgesic (Meloxicam; Metacam, Boehringer Ingelheim, Barcelona, Spain; 0.5 mg/kg injected in a volume of 0.1 mL/10 g, s.c.) and an antibiotic solution (Enrofloxacin; Baytril, Bayer, Barcelona, Spain; 7.5 mg/kg

injected in a volume of 0.03 mL/10 g, i.p.). Following surgery, mice were individually housed and allowed to recover for at least 3 days prior to commencing SA training. During recovery, mice were treated daily with the analgesic and the antibiotic solutions. The home cages were placed upon thermal blankets to avoid post-anaesthesia hypothermia.

Acquisition of self-administration

The SA experiment was carried out in mouse operant chambers (Model ENV-307A-CT, Med Associates, Inc. Cibertec S.A., Madrid, Spain), as previously described with some minor modifications (Soria et al., 2008; López-Arnau et al., 2017; Luján et al., 2018). The chambers were housed in sound- and light-attenuated boxes and contained two holes, one of which was defined as active and the other as inactive. Nose-poking into the active hole produced a cocaine infusion (reinforcement) that was paired with a stimulus light placed above the active hole. Nose-poking into the inactive hole had no consequences. The side on which the active/inactive hole was situated was counterbalanced.

Mice were trained for 2 h per day to nose-poke in order to receive a cocaine infusion (0.75 mg/kg) under a fixed ratio 1 (FR1) schedule of reinforcement for 10 consecutive days (acquisition phase). When mice responded on the active hole, cocaine was delivered in a 20 μL infusion over 2 s via a syringe mounted on a microinfusion pump (PHM-100A, Med-Associates, Georgia, VT, USA) connected via Tygon tubing (0.96 mm outer diameter, Portex Fine Bore Polythene Tubing, Portex Limited, UK) to a single-channel liquid swivel (375/25, Instech Laboratories, Plymouth Meeting, PA, USA) and to the intravenous catheter implanted in the mice. Each infusion was followed by a 15-s time-out period during which nose-poking on the active hole had no consequences. Each acquisition-phase session began with a priming infusion of the drug, with the box light turned on for 3 s and then deactivated for the rest of the session.

Acquisition criteria were met when: 1) mice performed at least 5 cocaine infusions per session, 2) ≥ 65% of responses were received at the active hole, and 3) the number of reinforcements deviated less than 20% from the mean number of reinforcements in two consecutive days. After 10 days of training, mice achieving the acquisition criteria were moved to a PR session. In the PR session (2 h), the response requirement to earn an injection escalated throughout the following series: 1–2–3–5–12–18–27–40–60–90–135–200–300–450–675–1000.

The number of cocaine infusions received on the last day of the acquisition phase was used as the baseline to determine extinction criteria in each mouse. Non-acquiring animals were excluded from the study.

Extinction and reinstatement

Once acquisition criteria were met, the cocaine was removed, and mice were tested for latency to extinguish nose-poking behaviour over successive once-daily 2 h sessions. During the extinction phase, nose-pokes into the active hole produced neither cocaine infusion nor stimulus light presentation. Extinction criteria were met when response levels decreased to ≤40% of the acquisition baseline levels in two consecutive days for each mouse. Twenty-four hours after achieving the extinction criteria, mice underwent a cocaine-primed reinstatement session. Mice were confined to the operant chambers for 2h immediately after being administered cocaine (10 mg/kg; i.p.). Nose-poking had no consequences in any of the holes.

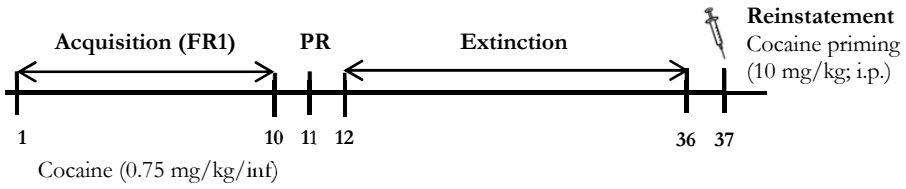


Figure 19. Schematic representation of cocaine self-administration procedure.

5. Biochemical assays

5.1. BAC analysis

Blood was collected from the tail vein of the female mice immediately after the final alcohol intake session of gestation or lactation (4th day of third week). Samples were placed on ice for slow coagulation and were centrifuged at 10,000 xg for 40 min at 4ºC to obtain cell-free plasma, which was stored at -80ºC until the alcohol quantification analysis was performed. 5 µl aliquots of each sample were transferred to sample microvials, combined with 5 µl of internal standard solution (0.2 g/l isopropanol) and placed in a water bath at 50ºC for 5 min. Then, 1 ml of the headspace gas was injected manually with a Hamilton syringe into the gas chromatograph (Agilent 7890A GC-system) equipped with a flame ionization detector (GC-FID). Alcohol and internal standard were separated on a capillary column (Supelcowax-10®, 30m x 250µm x 0.25µm) using helium as a carrier gas at a constant flow rate of 2.0ml/min (split ratio 20:1). The column oven temperature was isothermal at 55ºC and the injector and the FID system were set at 150 and 200ºC, respectively. Alcohol concentrations were quantified from linear standard curves (10 – 200 mg/dl alcohol) using the peak area ratios of alcohol to the internal standard using Agilent Chemstation software.

5.2. Extraction and preparation of tissue samples

To evaluate the expression of pro-inflammatory mediators, myelin proteins and epigenetic marks, as reported in Article 1 and Article 3 of this thesis, total protein extracts were isolated from PFC and HPC brain areas (250 mg tissue/0.5 ml lysis buffer) extracted from adult mice (PD70) after behavioural testing in the Y-maze and the object recognition test. The tissue was first homogenized in cold lysis buffer (1% Nonidet P-40, 20 mM Tris-HCl pH8, 130 mM NaCl, 10 mM NaF, 10 µg/ml aprotinin, 10 µg/ml leupeptin, 10 mM DTT, 1 mM Na3VO4 and 1 mM PMSF). Homogenates were kept on ice for

30 min, centrifuged at 15,000 xg for 15 min, and the supernatant was collected to determine protein concentration. Also, to determine nuclear proteins, the cytoplasmic and nuclear extracts were isolated using the Epiquik Nuclear Extraction kit, according to manufacturer's instructions (Epigentek, Farmingdale, NY). The lysate protein concentration was measured by the Pierce BCA (bicinchoninic acid) assay kit (Thermo Fisher Scientific, Spain).

To evaluate the effects of curcumin on PLAE mice, mice were sacrificed by cervical dislocation after behavioural testing (Y-maze, NOR test and EPM) and PFC and HPC structures were obtained, as reported in Article 4 of this thesis. For the analysis of brain samples from animals in the cocaine SA experiment, mice were sacrificed 30 min after the cocaine-primed reinstatement session, and PFC, STR and HPC were extracted, as reported in Article 5 of this thesis. After extraction, brain areas were quickly frozen in dry ice and stored at -80ºC until used, as previously described (Moscoso-Castro et al., 2017). Tissue was homogenized in 30 µL of lysis buffer [0.15 NaCl, 1% TX-100, 10% glycerol, 1mM EDTA, 50 mM TRIS pH=7.4 and a phosphatase and protease inhibitor cocktail (complete ULTRA Protease Inhibitor Cocktail Tablets and PhosSTOP Inhibitor Cocktail Tablets, respectively; Roche, Basel, Switzerland)] per mg (wet weight). Homogenates were centrifuged at 15,000 xg for 15 min at 4ºC and the resulting supernatants were collected for protein quantification. The lysate protein concentration was determined using a stock solution of 5 mg/mL bovine serum albumin (BSA) as a protein standard.

5.3. Western Blot

As reported in Article 1 and Article 3 of this thesis, brain lysates were separated by SDS-PAGE gels and immunoblotting was performed as previously described (Alfonso-Loeches et al., 2010). Membranes were incubated overnight at 4ºC with primary antibodies (see **Table 3**). After washing with Tris-buffered saline (TBS) (100 mmol/L NaCl, 10 mmol/L Tris, pH=7.4) and 0.1% Tween-20 (TBS-T), blots were incubated with their respective HRP-

conjugated antibodies: anti-rat (1/1000, Santa Cruz Biotechnology), anti-mouse (1/5000, Sigma-Aldrich) or anti-rabbit (1/20000, Sigma-Aldrich). Blots were developed using the ECL system (ECL Plus, Amersham Biosciences). Some membranes were stripped for 1 h at 60°C in SDS solution (2% SDS, 0.85% 2-ME, and 65 mM Tris-HCl (pH 6.8), washed and incubated with anti-GAPDH (1/3000, Chemicon), anti-Lamin A/C (LMNA; 1/1000, Cell Signalling) or anti-Vinculin (1:10000, Abcam) for 2 h as loading controls. A densitometry analysis was assessed with Alpha-Ease FC, version Alpha Imager 2200 (Alpha Innotech Corporation). For comparative purposes, control values were normalized to 100% and the respective protein expression values were adjusted according to the normalization factor.

Antibody	Description	Host	Dilution	Company
Caspase-1	*Caspase-1/Interleukin-1 converting enzyme (ICE)*	Rabbit	1:100	Santa Cruz Biotechnology
Caspase-3	*Caspase-3 p17 subunit (active)*	Rabbit	1:1000	Cell Signaling
GAPDH	*Glyceraldehyde 3-phosphate dehydrogenase*	Mouse	1:3000	Chemicon
GFAP	*Glial fibrillary acidic protein*	Rabbit	1:500	Sigma-Aldrich
H3K9ac	*Histone H3 Acetyl-K9*	Rabbit	1:1000	Cell signalling
H4K5ac	*Histone H4 Acetyl-K5*	Rabbit	1:10000	Abcam
H4K12ac	*Histone H4 Acetyl-K12*	Rabbit	1:1000	Cell signalling
H3K4me3	*Histone H3 (tri-methyl k4)*	Rabbit	1:1000	Abcam
K-Ac	*Acetyl Lysine*	Rabbit	1:1000	Abcam
LMNA	*Lamin A/C*	Mouse	1:1000	Cell Signalling
MAG	*Myelin-associated glycoprotein*	Rabbit	1:5000	Abcam

MBP	*Myelin Basic Protein*	Rat	1:1000	Abcam
MYRF	*Myelin Regulatory Factor*	Rabbit	1:1000	Millipore
NeuN	*Neuronal Nuclei*	Mouse	1:100	Chemicon
NF-κB p65	*Nuclear factor NF-Kappa-B p65 subunit*	Rabbit	1:50	Santa Cruz Biotechnology
NLRP3	*Nod-like receptor family, Pyrin Domain Containing protein 3*	Goat	1:1000	Abcam
PLP	*Myelin proteolipid protein*	Rabbit	1:1000	Abcam
TLR2	*Toll-like receptor 2*	Rabbit	1:200	Santa Cruz Biotechnology
TLR4	*Toll-like receptor 4*	Mouse	1:200	Santa Cruz Biotechnology
VCL	*Vinculin*	Rabbit	1:10000	Abcam

Table 3. Primary antibodies used in chemiluminescent immunoblotting.

As reported in Article 4 and Article 5 of this thesis, fluorescent Western Blot technique was used to determine protein expression in tissue homogenates. Equal amounts of protein (16 μg) for each sample were mixed with loading buffer (153 mM TRIS pH=6.8, 7.5% SDS, 40% glycerol, 5 mM EDTA, 12.5% 2-β-mercaptoethanol and 0.025% bromophenol blue) and loaded onto 10% polyacrylamide gels, and transferred to PVDF sheets (Immobilion-P, Merck, Burlington, USA). Membranes were blocked with 5% BSA in TBS-T for 1 h and then immunoblotted using the primary antibodies listed in **Table 4** overnight at 4 ºC. Finally, membranes were incubated for 1 h with their respective secondary fluorescent antibodies: anti-mouse (1:2500, IRDye 800, Rockland) and anti-rabbit (1:2500, IRDye 680, Rockland). Protein expression was quantified using a LI-COR Odyssey scanner and software (LI-COR Biosciences, Lincoln, USA). Protein densities were normalized to the

detection of the housekeeping control gene in the same samples and expressed as a fold-change of the control group.

Antibody	Description	Host	Dilution	Company
β-tubulin	*beta-tubulin*	Mouse	1:5000	BD Pharmingen
β-tubulin	*class III beta-tubulin*	Rabbit	1:1000	Abcam
ΔFosB	*delta FosB*	Mouse	1:250	Abcam
CREB	*cAMP response element-binding protein*	Rabbit	1:500	MERCK
D1R	*dopamine receptor 1*	Rabbit	1:500	Abcam
D2R	*dopamine receptor 2*	Rabbit	1:500	MERCK
DARPP-32	*dopamine- and cAMP–regulated phosphoprotein*	Rabbit	1:1000	Cell Signaling
GAPDH	*glyceraldehyde-3-phosphate dehydrogenase*	Mouse	1:2500	Santa Cruz Biotechnology
GluA1	*AMPA receptor subunit 1*	Rabbit	1:1000	MERCK
GluA2	*AMPA receptor subunit 2*	Rabbit	1:1000	MERCK
IL-6	*Interleukin-6*	Mouse	1:500	Santa Cruz Biotechnology
NFκB/p65	*Nuclear factor NF-Kappa-B p65 subunit*	Mouse	1:100	Santa Cruz Biotechnology
pCREB	*phosphorylated CREB*	Rabbit	1:1000	MERCK
pDARPP-32 (Thr34)	*phosphorylated DARPP-32(Thr34)*	Rabbit	1:1000	MERCK
TNF-α	*Tumor necrosis factor alpha*	Rabbit	1:500	Abcam

Table 4. Primary antibodies used in fluorescent immunoblotting.

5.4. Interleukin-1β enzyme-linked immunosorbent assay (ELISA)

Brain tissue was homogenized in cold lysis buffer (1% Nonidet P-40, 20 mM Tris-HCl pH8, 130 mM NaCl, 10 mM NaF, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 10 mM DTT, 1 mM Na3VO4 and 1 mM PMSF) (250 mg tissue/0.5 ml). Lysis samples were kept on ice for 30 min and centrifuged at maximum speed (13,000 rpm) for 15 min. The supernatant was collected for protein and cytokine determination. Protein was determined by using the Pierce BCA assay kit (Thermo Fisher Scientific, Spain). The cytokine levels of IL-1β were measured using the ELISA kit (Bender MedSystems GmbH, Austria), following the manufacturer´s instructions, as previously reported (Alfonso-Loeches et al., 2016). IL-1β levels were expressed as pg/mg of protein.

5.5. Measurement of HDAC and HAT enzymatic activities

The activity of HAT and HDAC was measured in the nuclear fraction using the EpiQuik™ HAT Activity/Inhibitor Assay Kit (Epigentek, Madrid, Spain), and the Epigenase HDAC Activity/Inhibition Direct Assay Kit (Epigentek), respectively. In these kits, the microplates contain the respective substrates stably coated onto the wells. The nuclear extract samples of PFC and HPC were added to catalyse the intended reaction. The products were then detected in ELISA-like reactions using specific antibodies, as indicated in the manufacturer´s instructions. Each sample´s optical density (OD) was measured at 450 nm and the results were expressed as OD/h/mg protein.

5.6. Cytochrome c oxidase histochemistry

Adult mice (PD60) were sacrificed and their brains were quickly removed, frozen in isopentane (Sigma–Aldrich) at −70°C and stored at −40°C to preserve brain tissue and enzyme activity. Next, 30 μm-thick coronal brain sections were obtained using a cryostat microtome (Microm International

GmbH, model HM 505-E, Heidelberg, Germany). The sections were mounted on slides and stored at −40°C prior to processing with quantitative cytochrome-c oxidase (CCO) histochemistry.

A modified version of the quantitative CCO histochemical method developed by Gonzalez-Lima and Jones (1994) was used. Staining variability across different staining baths was controlled by including sets of tissue standards. These standards were obtained from sections of brain homogenates of previously known CCO activity determined spectrophotometrically. Following the previously described protocol by Conejo et al. (2013), sets of standards cut at different thicknesses (10, 30, 50 and 70 μm) were included with each batch of slides. Each set of slides was fixed for 5 min with a 0.5% glutaraldehyde solution, rinsed three times in phosphate buffer (PB) and preincubated during 5 min in a solution containing 0.05 M Tris buffer at pH 7.6 with 275 mg/l cobalt chloride, 10% (w/v) sucrose, and 5 ml dimethylsulfoxide. The sections were then rinsed in PB (pH 7.6; 0.1 M) and were incubated at 37°C for 1 h in the dark and with continuous stirring, in a solution containing 50 mg 3,3'-diaminobenzidine, 15 mg cytochrome c (Sigma-Aldrich) and 4 g sucrose per 100 ml PB (pH 7.4; 0.1 M). The reaction was stopped by fixing the tissue in buffered formalin (10% w/v sucrose and 4% formaline) for 30 min at room temperature. The slides were then dehydrated, cleared with xylene and coverslipped with Entellan (Merck, Darmstadt, Germany).

Evaluation of regional brain cytochrome c oxidase activity

CCO histochemical staining intensity was measured by densitometric analysis using a computer-assisted image analysis workstation (MCID, InterFocus Imaging Ltd., Linton, England), which includes specific image analysis software. Four measurements of relative optical density in three consecutive sections were obtained for each region, making up a total of twelve measures per region. To establish comparisons and consider possible staining variations

across brain sections from different staining baths, measurements were also obtained from CCO-stained brain homogenate standards. Regression curves between section thickness (10, 30, 50, 70 micrometer-thick sections) and CCO activity, previously measured by spectrophotometric assay in each set of standards were calculated. Finally, average relative optical density measured in each brain region was converted into CCO activity units (1 unit: 1 μmoL of cytochrome c oxidized/min/g tissue wet weight at 23°C) using the previously calculated regression curve in each homogenate standard. The average measure per region was carried out for each section and animal. These included cingulate, prelimbic, and infralimbic areas of the medial prefrontal cortex and primary motor cortex. In addition, the following regions were also analysed: STR, NAc, lateral septal nucleus, bed nucleus of the stria terminalis, ventral pallidum (substantia innominata), anterodorsal and anteroventral thalamic nuclei; hippocampal subfields including CA1, CA3 and DG of the dorsal and ventral HPC; lateral and medial habenula; medial, basal, lateral and central amygdaloid nuclei; medial, medial lateral part and lateral nuclei of the mammillary bodies; pars reticularis and pars compacta of the substantia nigra and ventral tegmental area. Likewise, granular and molecular layers of the central (lobules II, III), culmen (lobules IV-V) and uvula (lobules IX) of the cerebellum were taken into account. The selected brain regions were defined according to an interactive online atlas, the Allen Mouse Brain Atlas (version 2, 2011; http://atlas.brain-map.org/).

5.7. BrdU immunofluorescence labelling

On PD60, neurogenesis was evaluated in the DG of the HPC. Animals were injected three times at 2h intervals with a thymidine analogue, 5'-bromo-2'-deoxyuridine (BrdU, Sigma-Aldrich; 100 mg/kg; i.p.) as previously described with minor modifications (Chen et al., 2012; Johansson et al., 2015). Brain tissue was collected 3 or 30 days after the last BrdU injection to determine neural cell proliferation and survival, respectively. Mice were anesthetized and transcardially perfused with 0.1M PB followed by ice-cold 4%

paraformaldehyde PB solution. Brains were removed, postfixed in 4% paraformaldehyde PB solution overnight at 4 ºC and stored in 30% v/v sucrose in 0.1M PB at 4 ºC. After freezing in dry ice, 30 μm-thick coronal tissue sections were obtained using a microtome. Subsequently, floating brain sections were washed three times in 0.1M phosphate saline buffer (PBS), treated with 2N HCl for 30min at 37ºC and neutralized in tap water, followed by immunofluorescent staining. After extensive washes in PBS, sections were blocked with a PBS solution containing 0.25% Tween 20 and 5% normal goat serum for 1h at room temperature. The following primary antibodies were then incubated in the same solution for 48h at 4ºC: rat anti-BrdU (1:300; Abcam, Cambridge, UK), mouse anti-neuronal specific nuclear protein (NeuN) (1:1000; Merck). After three washes in PBS, floating sections were incubated with fluorescent secondary antibodies for 2h at room temperature without light: goat anti-rat IgG Alexa Fluor 488 (1:500; Invitrogen, Barcelona, Spain), goat anti-mouse IgG Alexa Fluor 555 (1:500; Invitrogen). Finally, sections were mounted on slides using Fluoroshield (Sigma-Aldrich) and a coverslip was placed on top for microscopy imaging.

Image analysis

Six brain sections containing the DG of the HPC of each subject were analysed bilaterally. Samples were visualized and digitized under a 20x objective using a laser scanning confocal microscope (Leica TCS SP5; Mannheim, Germany). Z-plane image stacks (1,024x1,024 pixels) were taken at 2 μm. BrdU+ cells with NeuN colabelling in the DG of the HPC were quantified using ImageJ software (NIH, Bethesda, MD, USA) and the average of each sample was calculated.

5.8. Iba-1 and GFAP immunofluorescence staining

Microglia and astrocyte activation in the DG of the HPC was evaluated using ionized calcium-binding adapter molecule 1 (Iba-1) and GFAP markers. The

immunodetection of Iba-1+ (microglia) and GFAP+ (astrocytes) cells was followed as previously described (Gracia-Rubio et al., 2016b). Mice were anesthetized and transcardially perfused with 0.1M PB followed by ice-cold 4% paraformaldehyde (Merck) PB solution. Brains were quickly removed, postfixed in 4% paraformaldehyde PB solution overnight at 4 ºC and stored in 30% v/v sucrose in 0.1M PB at 4 ºC. After freezing in dry ice, 30 μm-thick coronal tissue sections were obtained using a microtome. Then, free-floating brain sections were washed three times in 0.1M PBS and then were blocked with a PBS solution containing 0.3% Triton X-100 (Sigma-Aldrich) and 3% normal goat serum (Vector Laboratories Inc., Burlingame, CA) for 2h at room temperature. The following primary antibodies were then incubated in the same solution overnight at 4ºC: rabbit polyclonal anti-Iba1 (1:500; Wako Pure Chemical Industries, Japan), rabbit polyclonal anti-GFAP (1:500; Dako, Glostrup, Denmark). After three washes in PBS, floating sections were incubated with fluorescent secondary antibodies for 2h at room temperature without light: goat anti-rabbit IgG Alexa Fluor 488 (1:500; Invitrogen, Barcelona, Spain), goat anti-rabbit IgG Alexa Fluor 555 (1:500; Invitrogen). Then, slides were washed three times for 10 min and incubated with Hoechst 33258 (1:10.000; Invitrogen) for 5 min to visualize cell nuclei. Finally, sections were mounted on slides using FluorSave (Calbiochem, Darmstadt, Germany) and coverslipped for microscopy.

Image analysis

A minimum of three images were taken for each subject bilaterally. Sample areas were visualized under a 20x objective in a Leica DMR microscope, and digitized using a digital camera Leica DFC 300 FX (Vashaw Scientific Inc, Atlanta, GA). Immunoreactive cells were quantified using ImageJ software (NIH, Bethesda, MD). The background was subtracted by adjusting the detection threshold density, and we only considered the signal density above the threshold. The investigator was blind to the groups analysed. The number

and percentage of stained pixels per area was measured and the average of each sample was calculated.

6. Statistical analysis

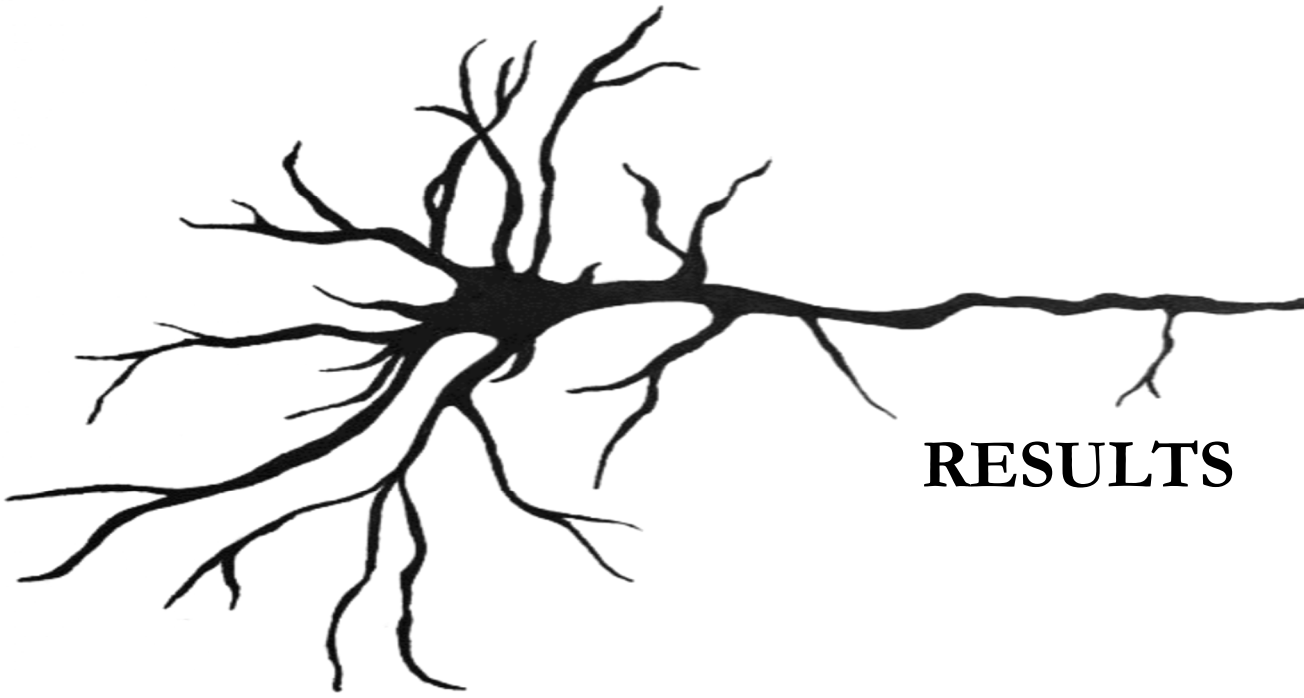
Data obtained from the DID test were analysed using two-way analysis of variance (ANOVA) with *group* (alcohol vs. water) as a between-subject factor and *day* as a within-subject factor, followed by Bonferroni post-hoc comparisons when required. One-way ANOVA with repeated measures was used to analyse alcohol intake during the DID test. Correlations between maternal alcohol intake levels and behavioural and neurochemical results were performed by Pearson's correlation analyses. Maternal care data were analysed using two-way ANOVA with repeated measures. Subsequent Bonferroni post-hoc comparisons were calculated when required. Data from Y-maze, object recognition, rotarod test and EPM were analysed using unpaired or paired (as corresponding) two-tailed Student's t-test. Locomotor activity results were analysed using two-way ANOVA with group and time as factors of variation or by unpaired two-tailed Student's t-test to compare total deambulations and rearings between groups. Results obtained in the BrdU assay and immunoblotting were analysed using unpaired two-tailed Student's t-test. Iba-1 and GFAP quantification and protein expression of pro-inflammatory mediators in curcumin experiment were analysed by two-way ANOVA with *group* and *treatment* as factors of variation.

Data from CPP were evaluated using two-way ANOVA with *group* and *treatment* as factors of variation. We calculated a three-way ANOVA with repeated measures for the locomotor sensitization with *group* and *treatment* as between-subject factors and *day* of test as a within-subject factor. To analyse the acquisition, extinction and reinstatement of the SA, a three-way ANOVA with repeated measures was performed with *group* as a between-subject factor and *day of training* and *nose-poke* (active vs. inactive holes) as within-subject factors. When required, ANOVA analyses were followed by Bonferroni post-

hoc tests. The unpaired two-tailed Student t-test was used to analyse the differences between groups in the total of active nose-pokes, total of cocaine consumption, day of acquisition, PR test and day of extinction. The area under the curve was calculated for extinction curve and compared between groups using the Student t-test.

Group differences in mean brain CCO activity measured in each brain region were analysed by Student's t-tests. In order to evaluate possible changes in functional relationship between regional brain activity caused by the DID test, regional CCO activity data were analysed in terms of pair-wise correlations within each experimental group (Puga et al., 2007). Pearson product–moment correlations were calculated for interregional correlation analysis, including pair-wise comparisons of each region showing a mean between-group difference as revealed by Student's *t*-tests. CCO activity values were normalized by dividing the measured activity of each brain region by the average CCO activity value of the hippocampal areas measured for each animal. This was done in order to reduce possible differences in the intensity of CCO staining not resulting from experimental manipulations. To ensure the reliability of correlations, a 'jackknife' procedure (Shao and Tu, 1995) was performed based on the calculation of all possible pair-wise correlations resulting from the removal of one subject each time and only considering the correlations that remained significant ($p<0.01$) across all possible combinations. This is a conservative method, sensitive to outliers, which avoids magnified type-1 errors caused by the large number of interregional correlations computed in relation to the sample sizes. This statistical analysis was performed using the statistics module featured in SigmaPlot 12 software (Systat Software Inc., Germany).

Statistical analyses were performed using SPSS Statistics v23. Data were expressed as mean ± standard error of the mean (SEM). The α-level of statistical significance was set at $p<0.05$.



RESULTS

ARTICLE 1

Maternal alcohol binge drinking induces persistent neuroinflammation associated with myelin damage and behavioural dysfunctions in offspring mice.

Cantacorps L, Alfonso-Loeches S, Moscoso-Castro M, Cuitavi J, Gracia-Rubio I, López-Arnau R, Escubedo E, Guerri C, Valverde O.

Neuropharmacology. 2017 Sep 1; 123:368-384. Epub 2017 Jun 29.

doi: 10.1016/j.neuropharm.2017.05.034.

ARTICLE 2

Altered brain functional connectivity and behaviour in a mouse model of maternal alcohol binge-drinking.

Cantacorps L, González-Pardo H, Arias JL, Valverde O, Conejo NM.

Prog Neuropsychopharmacol Biol Psychiatry. 2018 Jun 8; 84(Pt A):237-249. Epub 2018 Mar 8.

doi: 10.1016/j.pnpbp.2018.03.006

ARTICLE 3

Long-term epigenetic changes in offspring mice exposed to alcohol during gestation and lactation.

Cantacorps L, Alfonso-Loeches S, Guerri C, Valverde O.

J Psychopharmacol. 2019 Epub Jun 18.

doi: 10.1177/0269881119856001

ARTICLE 4

Curcumin treatment attenuates alcohol-induced alterations in a mouse model of foetal alcohol spectrum disorders.

Cantacorps L, Montagud-Romero S, Valverde O.

Prog Neuro-Psychopharmacology Biol Psychiatry. 2020 Jun 8;100

doi: 10.1016/j.pnpbp.2020.109899

ARTICLE 5

Prenatal and postnatal alcohol exposure increases vulnerability to cocaine addiction in adult mice.

Cantacorps L, Montagud-Romero S, Luján MÁ, Valverde O.

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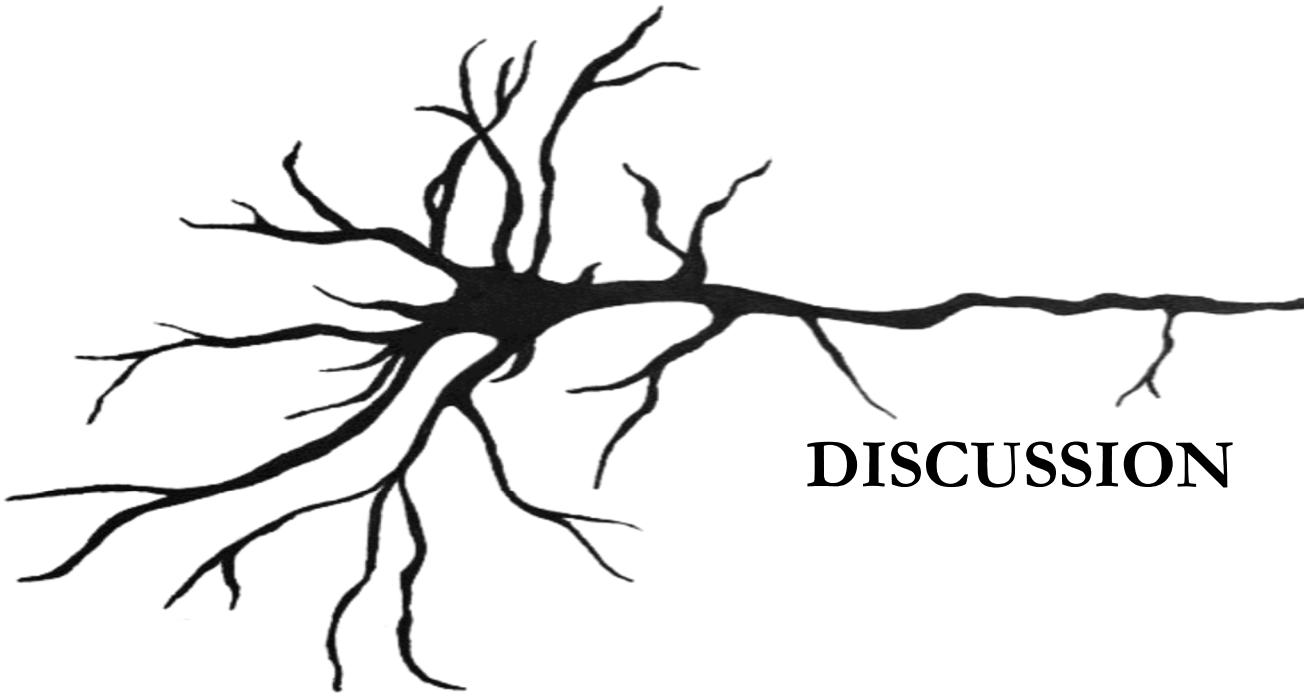


DISCUSSION

Alcohol is one of the most detrimental neurotoxic compounds to affect the developing foetus. Of the various organ systems affected, the brain is the most severely impacted (Caputo et al., 2016). Alcohol drinking during pregnancy, even at low-to-moderate doses, can have deleterious effects on the early development of the foetus and continued growth (Valenzuela et al., 2012; Subramoney et al., 2018). Exposure to alcohol during foetal development can impair brain ontogeny, leading to a wide range of long-lasting physical, neurocognitive and behavioural dysfunctions collectively known as FASD (Sokol et al., 2003; Dörrie et al., 2014). The CNS is particularly vulnerable to the teratogenic effects of alcohol owing to its extended developmental period. Therefore, alterations related with the CNS are among the most common and severe manifestations of FASD, impairing a wide range of functions, such as learning, memory attention, fine motor coordination, judgement, social interaction and emotional behaviour (Riley et al., 2011). Even though, the subsequent behavioural outcome is markedly influenced by several factors including dosage, pattern and developmental timing of alcohol exposure, as reviewed in the *Introduction*. Binge alcohol drinking has recently emerged as a popular pattern of consumption among teenagers and young adult population, including pregnant women (Popova et al., 2017b), which is of great concern.

To our knowledge, the effects of prenatal and lactational binge alcohol exposure using an animal model of voluntary alcohol consumption have been scarcely addressed, since most of the previous studies used a parenteral route of alcohol administration to reproduce a binge episode (Mantha et al., 2014; Wagner et al., 2014; Jakubowska-Doğru et al., 2017). Furthermore, most of them focused on different stages of gestation or postnatal period but did not cover for the full length of gestation and lactation periods.

The molecular mechanisms underlying alcohol-induced neuroteratogenesis are complex. Therefore, the long-lasting consequences of maternal alcohol binge drinking on the adult offspring brain are still not fully understood.

Furthermore, binge alcohol drinking during pregnancy has been identified as a risk factor for the development of mental disorders later in life, such as substance abuse (Barr et al., 2006). However, the increased vulnerability to drug addiction in binge alcohol-exposed offspring and the neurobiological mechanisms underlying the heightened risk have been scarcely addressed.

Hence, this thesis has been focused on *evaluating the long-term consequences of maternal binge alcohol consumption during critical periods for brain development -prenatal and lactational periods- on cognitive, motor, emotional and addictive behaviour in adult male mice and its underlying mechanisms.*

1. Behavioural characterization of our FASD model

There are no current animal models that completely recapitulate all the characteristic features of FASD present in humans (Gil-Mohapel et al., 2010). Thus, different models are continuously being developed in animal studies with variations in the developmental timing of alcohol exposure, the duration and the mode of administration. In this thesis, we chose a voluntary consumption strategy for alcohol exposure instead of intraperitoneal injections or intragastric intubation in order to produce the least amount of maternal stress. The oral route of administration has the advantage that it better resembles the human situation, in which alcohol is being consumed orally, and therefore enters the blood stream through the same mechanisms by which it occurs in humans. Furthermore, there are a limited number of rodent studies in which alcohol is administered both pre- and postnatally as a model of alcohol exposure during the period equivalent to all three trimesters.

To reproduce an episodic pattern of excessive alcohol drinking during pregnancy and lactation periods, we exposed pregnant and nursing C57BL/6 female mice to an alcohol solution using the DID paradigm (Rhodes et al., 2005; Thiele and Navarro, 2014; Thiele et al., 2014). In addition, we chose a dose of 20% (v/v) alcohol in accordance with previous studies from our

laboratory showing that higher levels of alcohol intake were found with this dose compared with 30% (v/v), and mice exposed to 20% (v/v) achieved BACs higher than 80 mg/dL (Esteve-Arenys et al., 2017). Few studies have previously used the DID procedure as a model of prenatal and postnatal binge-like alcohol exposure. Boehm et al. (2008) employed the DID test paradigm throughout the gestational period to model binge drinking in C57BL/6 mice and proposed it as a useful model of FASD. Our study differs from this in that we extended maternal alcohol consumption until weaning (PD21), given that a significant phase of brain development occurs after birth in rodents. Furthermore, our procedure better mimics the human condition in which some alcoholic mothers continue to drink throughout the breastfeeding period (Breslow et al., 2007; Haastrup et al., 2014; May et al., 2016). When a lactating woman consumes alcohol, it is transferred into the milk, equalling the levels found in maternal blood (Guerri and Sanchis, 1986). Thus, alcohol can be transferred through the breast milk to the pups, but scarce research data is available regarding the long-term effects of binge-like alcohol drinking during lactation (Mennella, 2001; Haastrup et al., 2014).

Few rodent strains voluntarily consume alcohol to reach similar levels of BACs as that achieved by humans, but C57BL/6 mice are known for its drinking preference for alcohol over water and DID has been proved to mimic binge drinking in C57BL/6 mice and allowing them to reach high BACs (Rhodes et al., 2007; Crabbe et al., 2011). In our study, C57BL/6 females consumed higher amounts of alcohol during the binge drinking sessions (4h-access) compared with the three previous days (2h-access) of each DID weekly cycle. During gestation, females were exposed to three binge episodes, while females exposed during gestation and lactation periods underwent a total of six binge episodes, reaching BACs of 80 mg/dL approximately after the binge drinking sessions, which are defined as binge intoxication levels (NIAAA, 2016). In addition, we found a positive correlation between alcohol intake levels, volume consumed and BACs, indicating that females drinking higher amounts of

alcohol may result in more defects on their progeny as alcohol swiftly crosses the placenta and can be detected in the foetus almost immediately after a peak rise in maternal BAC (Burd et al., 2012). Interestingly, we found an increase of water consumption in the control group during the lactational DID procedure compared with the gestation period, but this effect was also observed in alcohol-exposed females during *ad libitum* access periods. Therefore, we attributed this to an increased physiological demand of fluid intake to support milk production after parturition (Bentley, 1998).

The levels of alcohol consumption achieved by females in this study were consistent with values for C57BL/6 mice in previous studies (Allan et al., 2003; Boehm et al., 2008; Brady et al., 2012). Even though the levels of BAC were not determined in all the experiments of this study, the amount of alcohol intake by females was comparable to that assessed from females producing BACs of 80 mg/dL.

Food intake and maternal body weight gain were not affected by alcohol consumption as no differences were observed between females exposed to water or alcohol during the DID test. Similarly, offspring of alcohol-drinking dams did not differ in weight, litter size or pup mortality compared with their counterparts, yet later behavioural assessment yielded to long-lasting cognitive deficits, motor coordination impairments, and anxiety-related behaviour. The overall pattern of behavioural alterations was consistent and reproducible across the different experiments, indicating that although alcohol exposure by voluntary maternal consumption may be variable from litter to litter, this model produces subtle, reproducible, and long-lasting effects in the adult brain. Further, it is likely that BACs reached by dams are pharmacologically relevant given the behavioural alterations observed in their offspring.

In agreement with our findings, preclinical studies reported no differences in litter size or pup weight in rodent models of alcohol exposure *in utero* (Savage

et al., 2002; Allan et al., 2003; Choi et al., 2005; Barbier et al., 2009; Kleiber et al., 2011; Brady et al., 2012; Cullen et al., 2014). In contrast, other reports have shown reductions in brain and body weight and reduced litter size in alcohol-exposed offspring (Chappell et al., 2007; Dasgupta et al., 2007; El Shawa et al., 2013; Comeau et al., 2014; Helfer et al., 2014; An and Zhang, 2015; Abbott et al., 2016), consistent with human findings in neuroimaging studies revealing global brain volume reductions in FASD patients (Donald et al., 2015). Interestingly, Boehm et al. (2008) reported reduced body weight in adolescent alcohol-exposed offspring mice, but not in adult mice, suggesting that this deficit was reversed by compensatory mechanisms. Such discrepancies in the literature might be explained by differences in alcohol dosage and timing of exposure.

In addition, we evaluated the maternal care behaviour, since there are evidences that maternal care-taking behaviour can be disrupted in alcoholic mothers (Jacobson, 1997; Christensen and Bilenberg, 2000). Mother-pup interaction plays an important role during the first period of postnatal life, as variations in maternal behaviour can induce alterations in endocrine, emotional and cognitive responsivity to stress in offspring. Indeed, animals experiencing non-nurturing maternal care show increased anxiety-like behaviour and enhanced stress responses in addition to epigenetic alterations (Weaver et al., 2004; Meaney and Szyf, 2005; McGowan et al., 2011). Observations of rodents during the postpartum period indicate that offspring receiving greater levels of maternal care, evaluated by 'pup licking and grooming' and 'arched-back nursing' behaviours, show more modest HPA stress responses, a phenomenon involving epigenetic rearrangements (Kundakovic and Champagne, 2015). The quality of maternal care during early postnatal period can be modulated by a variety of environmental manipulations, such as social isolation or environmental enrichment. Notably, our results do not show robust alterations in maternal care behaviour due to maternal binge alcohol drinking during gestation and lactation periods.

Furthermore, there is no compromised nurturing effect on pup's body growth, indicating that maternal care is appropriate in our experimental model. In agreement with our findings, Raineki et al. (2017) reported that maternal licking and grooming and self-directed behaviour were not affected by alcohol consumption during pregnancy in mice. Similarly, no differences in pup retrieval and time spent on the nest were found between alcohol-exposed and control mice (Brady et al., 2012). Thus, behavioural alterations observed in PAE offspring (Weinberg et al., 2008; Hellemans et al., 2010; Schneider et al., 2011) might be primarily due to the direct effects of alcohol exposure and not to altered maternal behaviour. However, a previous study showed alterations in nursing behaviour and increased negative maternal care in female rats exposed to alcohol during gestation (Workman et al., 2015); even though, the frequency of licking and grooming was not affected. In addition, low-levels of alcohol administered via gavage during late gestation increased arched-back nursing and affected pup retrieval in Sprague-Dawley but not in Long Evans rats, indicating a strain-specific effect (Popoola et al., 2015). Also, maternal behaviour was disrupted after first-experience with alcohol intoxication in lactating rats, but repeated exposures to alcohol led to maternal tolerance and decreased the behavioural reactivity of infants to alcohol (Pepino et al., 2002). Hence, variations in alcohol dosage, route and pattern of administration, and strain differences can have a different impact on maternal behaviour.

Behavioural assessment of PAE and PLAE offspring revealed a significant long-lasting impact of maternal binge-like alcohol drinking during prenatal and/or postnatal periods on the motor domain, cognitive function and emotional behaviour in offspring mice.

A subtle range of cognitive defects has been described in children with PAE, including disabilities in learning and memory, social interaction and attention deficits (Clarke and Gibbard, 2003; Zuccolo et al., 2013; Murray et al., 2016). Thus, we evaluated spatial working memory in PAE and PLAE mice using the

Y-maze spontaneous alternation test, which is mainly dependent on PFC function (Lalonde, 2002; Yoon et al., 2008). Even though we did not find differences between PAE mice and their counterparts in the cognitive performance, a reduced score in PLAE mice was found, indicating a deficit in working memory as assessed with the Y-maze task. Deficits in spatial learning and memory have also been reported in other preclinical models of PAE (Berman and Hannigan, 2000; Marquardt and Brigman, 2016). Rats prenatally exposed to alcohol showed spatial working and reference memory impairments that became more evident as task difficulty increased (Popović et al., 2006). Also, executive function impairments assessed by reversal learning in the Y-maze were found in PAE adolescent and adult mice (Allan et al., 2014; Marquardt et al., 2014), supporting our findings. However, prenatal chronic moderate alcohol exposure (6% v/v alcohol) did not impair the performance in the Y-maze task neither in adult or aged rats (Cullen et al., 2014). Such discrepancies with our results might be attributed to the lower concentration of alcohol used, the lower maternal BACs achieved (~30 mg/dL) and the different ages at which offspring were tested. Indeed, the amount of maternal alcohol intake appears to be related with the behavioural outcome observed in the offspring. Accordingly, the score obtained in the Y-maze task by PLAE mice in our study correlated with each corresponding maternal BAC, whereas no significant correlation was found in PAE mice, which did not show any impairment.

We also evaluated PAE and PLAE mice in the NOR test, a hippocampal-dependent task (Barker and Warburton, 2011). No evidences of object recognition memory impairment were found 24h after the acquisition trial as assessed in *Article 1*, even though we found a reduced discrimination index in PLAE mice 72h following the first exposure in the NOR test as described in *Article 4*. In *Article 1*, NOR test was performed in a white open field arena and only one object was displayed during the acquisition trial, introducing a novel object in the retention trial 24h later. In contrast, in *Article 4* the NOR test was

performed in a black open field and two identical objects were presented on the acquisition trial, and one of the familiar objects was replaced with a novel object on the retention trial, that took place 72h later. Thus, it is plausible that differences in methodology and timing of assessment may account for such discrepancies. Interestingly, Allan et al. (2003) reported increased novelty exploratory behaviour in a mouse model of PAE using a voluntary drinking paradigm, whereas reduced exploration of the novel object was reported in rats exposed to alcohol during prenatal and preweaning periods (Popović et al., 2006; Marche et al., 2011).

Another study of our group evaluated spatial and working memory using the radial arm maze, showing a higher number of errors in PLAE mice than in the control group, in addition to corroborating the findings in the NOR test (Montagud-Romero et al., 2019). Also, spatial working memory impairments in the radial arm maze were found in rats exposed to alcohol during the second quarter of prenatal life (Salami et al., 2004). Other studies reported deficits in spatial memory tasks, such as the Barnes maze or the delay-non-match-to-place test (Kleiber et al., 2011; Brady et al., 2012; Houlé et al., 2017), in C57BL/6 mice.

We also reported subtle alterations in spatial working memory and cognitive flexibility in PLAE mice as assessed by the left-right discrimination task, in which different brain areas are involved, including the PFC and the HPC (Deacon and Rawlins, 2006). The number of trials needed to reach learning criterion in the acquisition phase was higher in PLAE mice, revealing impairments in the acquisition of learning. Also, an effect of PLAE in the latency to reach the platform was found in both acquisition and reversal phases, indicating mild cognitive deficits in PLAE adult offspring mice.

Deficits in fine motor control tasks and postural balance have been reported in FASD patients (Connor et al., 2006; Lucas et al., 2014). Consistent with

these findings, we found motor coordination impairments in both PAE and PLAE mice at late adolescence as assessed by a reduced latency to fall in the accelerating rotarod test, which involves a whole network of motor skill learning-associated brain areas, including cerebellum (Shiotsuki et al., 2010; Scholz et al., 2015). Accordingly, motor deficits were found in rats exposed chronically to 24% v/v alcohol during gestation (Tong et al., 2013) and in mice prenatally exposed to 18% v/v alcohol (Cebolla et al., 2009). In addition, mouse pups receiving alcohol injections from PD4-9 showed deficits in rotarod and balance beam performance (Bonthius et al., 2015). Also, adolescent rats exposed daily to 4g/kg alcohol from GD6 to PD21 showed reduced motoric capacity in the rotarod (Bagheri et al., 2015). Nevertheless, neither adolescent nor adult offspring mice exposed to alcohol prenatally showed alterations in the rotarod performance (Boehm et al., 2008). Likewise, chronic intragastric administration of 6 g alcohol/kg/day throughout gestation did not produce rotarod impairments in adult rat offspring (Dursun et al., 2006), albeit higher maternal BACs were recorded in these studies. Hence, in that case, a linear correlation between the levels of alcohol ingested and the outcome severity should not be expected.

ADHD is often diagnosed as a comorbid condition in FASD-affected individuals (Popova et al., 2016). Therefore, we evaluated spontaneous locomotor activity in PLAE mice at different time points, adolescence (PD30) and adulthood (PD60). We found age-dependent alterations in locomotor activity, with adolescent PLAE mice showing hyperactivity while adult PLAE mice showed diminished locomotor activity compared with control group. Noteworthy, such differences were only significant when data were analysed by collapsing the whole 45-min period. As indicated by some studies, it is during adolescence that hyperactivity is more often diagnosed in FASD patients and the alteration may become less pronounced during adulthood (Kleiber et al., 2011). In accordance with our findings, previous studies have reported locomotor activity to be increased by alcohol dosage and reduced

with age in PAE rats (Kim et al., 2013; Muñoz-Villegas et al., 2017). Similarly, hyperactivity in juvenile but hypoactivity in adult rats as a consequence of early exposure to alcohol has been previously reported (Marche et al., 2011). The differences obtained between pre- and post-pubertal stages could most probably be explained by the major brain maturation processes that occur during adolescence (Sisk and Foster, 2004; Fuhrmann et al., 2015). Also, diminished locomotor activity was appreciated when assessed at adulthood in other rat studies (Dursun et al., 2006; Ohta et al., 2010). Hypoactivity at adulthood may be a result of damage to the developing frontal cortices, affecting the frontostriatal circuitry, like others pointed out (Alberry and Singh, 2016; Schambra et al., 2016). However, other studies using a lowest dose of alcohol and shorter periods of alcohol exposure reported no differences in basal locomotor activity after developmental alcohol exposure in mice (Brady et al., 2012; Alberry and Singh, 2016) or even a decrease in locomotion in adolescent rats (Abate et al., 2017), suggesting that a higher dose of alcohol and longer periods of exposure are required to induce changes in locomotor activity.

Finally, anxiety-like behaviour was assessed using the EPM. PLAE mice showed a reduced percentage of the time spent in the open arms of the maze at adolescence (PD30) and adulthood (PD60), indicating a long-lasting anxiogenic effect of binge-like developmental alcohol exposure. Furthermore, these findings were replicated in subsequent studies (Montagud-Romero et al., 2019). Consistently, an increase in anxiety-like behaviour following foetal alcohol exposure has been reported in mouse (Kleiber et al., 2011; El Shawa et al., 2013; Abbott et al., 2016; Schambra et al., 2016) and rat (Dursun et al., 2006; Cullen et al., 2013; Baculis et al., 2015) studies. However, other studies showed no effect on anxiety-related responses in mice (Boehm et al., 2008; Sanchez Vega et al., 2013; Alberry and Singh, 2016) and rats (Barbier et al., 2008; Muñoz-Villegas et al., 2017) or even an anxiolytic effect in rats (Ohta et al., 2010). Such differences might be attributed to the pattern and route of

alcohol administration, the period of exposure, the species and the strain used. Notwithstanding, higher rates of anxiety have been found among FASD patients (Hellemans et al., 2008).

Overall, greater behavioural alterations in the motor and cognitive domains were found in PLAE mice than in PAE group, possibly owing to a cumulative effect of alcohol exposure or the developmental timing of alcohol exposure during early postnatal development. Indeed, some brain areas including the HPC, are perinatally developed in both rodents and humans (Semple et al., 2013).

2. Molecular alterations in the brain induced by developmental binge-like alcohol exposure

Early alcohol exposure can elicit a neuroimmune response in the developing brain involving changes in intracellular pathways, which could be one of the mechanisms underlying the behavioural and cognitive alterations observed. In accordance, we found enhanced expression of pro-inflammatory factors in the PFC and the HPC of PAE and PLAE adult mice, indicating that maternal binge alcohol drinking induces persistent neuroimmune alterations that persist until adulthood. TLR4, NF-κB/p65, NLRP3, caspase-1 and IL-1β levels increased mainly in the PFC after foetal alcohol exposure, while pre- and postnatal alcohol exposure led to an increase in all the aforementioned markers, in addition to TLR2, in the PFC and HPC. Furthermore, a positive correlation was found between the levels of expression of the neuroimmune markers and the maternal BACs, indicating that higher amounts of alcohol intake would lead to greater activation of the pro-inflammatory pathways. In addition, we reported an increase in GFAP expression in the PFC of PAE mice, and in both PFC and HPC of PLAE mice, suggesting an activation of astrocytes that persists until adulthood. Also, a diminished expression of the neuronal marker NeuN associated with an increase of caspase-3/p17, an

apoptotic biomarker, was found in the PFC of PLAE mice. Thus, our results demonstrate that maternal binge-like alcohol drinking induces a persistent deregulation of the neuroimmune response in the offspring's brain that culminate with neuronal apoptotic cell death. These alterations would lead to long-term alcohol-related impairments in learning, memory and behavioural dysfunction, such as the deficits in executive functioning.

Indeed, developmental alcohol-induced neuroinflammation can contribute to neuron cell loss and subsequent impairment of brain functioning (Glass et al., 2010; Chastain and Sarkar, 2014; Guizzetti et al., 2014; Heneka et al., 2014; Wilhelm and Guizzetti, 2015; Saito et al., 2016). The sustained activation of microglia and astrocytes increases the release of pro-inflammatory and neurotoxic factors, including cytokines, chemokines and ROS, which may result in neurodegeneration by promoting apoptotic cell death (Simi et al., 2007; Lehnardt, 2010). Consistently, previous studies reported widespread apoptotic-induced neurodegeneration in the rodent brain after alcohol exposure during the brain growth spurt period (Ikonomidou et al., 2000; Olney et al., 2002a, 2002b). Furthermore, increased levels of pro-inflammatory factors in the maternal serum and offspring's brain associated with a long-lasting increase of microglia markers and reduction of synaptic markers were reported in a mouse model of FASD, effects that were prevented in TLR4 $^{-/-}$ mice (Pascual et al., 2017). These results corroborate that the innate immune response through TLR4 activation might play an important role in the alcohol-induced neurodevelopmental defects.

In addition, since the immune system plays a key role during CNS development, deregulation or overactivation of immune functions during early brain development may cause long-term effects on both neuronal function and cognitive impairment. For instance, IL-1β within the HPC seems to be required for the maintenance of LTP, even though elevated levels of this cytokine might impair learning and memory processes (Bilbo et al., 2012).

Recently, it has been reported that moderate PAE in rats upregulates TLR4 signalling enhancing TNF-α and IL-1β expression but suppresses the TLR4-mediated innate immune response to lipopolysaccharide in the HPC (Wang et al., 2019a). Presumably, PAE challenges the immune system during development, which may result in attenuated innate immune response to later pro-inflammatory stimuli in the brain.

Abnormalities in white matter structure associated with poorer myelination have been reported in children with FASD (Sowell et al., 2008). Accordingly, in our study we found reduced expression of protein components of the myelin sheath in the PFC and the HPC of alcohol-exposed offspring mice. MAG and PLP proteins were reduced in the PFC and HPC of PAE mice, while MBP levels were not modified by *in utero* alcohol exposure alone. However, gestational and lactational alcohol exposure decreased the levels of MAG, MBP and PLP in both brain areas assessed, probably because it is during the third trimester-equivalent period that myelination mostly occurs in rodents (Semple et al., 2013). In addition, the expression of one of the promyelinating transcription factors, MYRF, was reduced in the PFC of PAE mice and in both PFC and HPC of PLAE mice. Remarkably, the protein expression levels correlated negatively with the levels of maternal alcohol intake, which suggests that the effects of alcohol on myelin disruptions might be dose dependent. Thus, combined pre- and postnatal binge alcohol exposure leads to persistent myelination deficits, which can affect the correct neuronal transmission and cognitive function. Alcohol-induced myelin disruptions may also impair the neurotransmission in corticocerebellar projections having an impact on motor skill learning (McKenzie et al., 2014), as observed in our mouse model of FASD at late adolescence. In fact, impaired rotarod learning has been associated with hypomyelination (Kuhn et al., 1995).

In addition, the involvement of pro-inflammatory signalling in myelination defects has been reported in rodent models of maternal immune activation,

which show myelin and axonal abnormalities in the SNC (Makinodan et al., 2008; Farrelly et al., 2015). Indeed, we have found negative correlations between the expression of inflammatory markers and myelin proteins analysed, indicating that an enhanced expression of pro-inflammatory factors is associated with a reduction in the expression of constitutive myelin proteins. The involvement of alcohol-induced TLR4 pro-inflammatory signalling in myelin derangements has already been observed in the brain of adolescent and adult mice after alcohol binge drinking (Alfonso-Loeches et al., 2012; Pascual et al., 2014b; Montesinos et al., 2015), revealing a role for TLR4-mediated immune response in the myelin alterations induced by alcohol. Similarly, it has been shown that inhibition of TLR4-MyD88 signalling pathway reduces acute alcohol-induced motor impairments in mice (Wu et al., 2012). In addition, it has been recently shown that myelin disruptions are prevented in mice deficient in the TLR4 function exposed to alcohol during gestation and lactation periods (Pascual et al., 2017). Therefore, these results suggest that alcohol creates a pro-inflammatory microenvironment in the developing brain, which affects the myelinization process, leading to behavioural dysfunctions, including motor coordination alterations.

Furthermore, we provide evidence of persistent epigenetic alterations in the brain of our mouse model of FASD including post-translational histone modifications and changes in the activity of HAT and HDAC enzymes. Increased acetylation levels of H4K5 and H4K12 and total K acetylation were found in the PFC of PLAE mice, while in the HPC just H4K5 acetylation levels were increased. Moreover, the levels of H4K5 acetylation in the PFC correlated positively with maternal alcohol intake levels, indicating a dose-dependent effect of alcohol exposure on the acetylation of H4K5. Histone acetylation has been generally associated with an open chromatin structure. Hence, our results would indicate that pre- and postnatal binge-like alcohol exposure induces brain region-specific epigenetic modifications associated with an active state of gene transcription in the PFC of adult offspring mice.

Accordingly, increased levels of H4K5 and H4K12 acetylation have been reported after binge alcohol drinking in adolescent rodents (Pascual et al., 2009; Montesinos et al., 2016b).

As a result of alcohol metabolism, the levels of acetyl-CoA may increase, which in turn can promote histone acetylation, as acetyl-CoA acts as a substrate from which to transfer the acetyl group and as a limiting factor for the activity of the HAT enzyme, which mediates histone acetylation (Legube and Trouche, 2003). Also, alcohol metabolization to acetaldehyde consumes NAD+ and produces NADH, attenuating the activity of NAD+-dependent enzymes. The sirtuin family of HDACs is NAD+-dependent; thus, alcohol could inhibit HDAC activity. In order to elucidate the mechanism underlying such epigenetic changes, we measured the activity of HAT and HDAC enzymes. PLAE mice showed enhanced HAT activity in the PFC, indicating that a deregulation of such chromatin-modifying enzyme might be involved in the epigenetic alterations found in the PFC of PLAE mice. Thus, alcohol exposure during foetal development may increase the levels of acetyl-CoA leading to increased HAT activity and subsequent histone acetylation (Chater-Diehl et al., 2017). Still, the mechanism involved in the augmented H4K5 acetylation in the HPC remains to be elucidated.

Consistent with our findings, preclinical research has shown increased acetylation levels of H3K14 and H4K8 in mice after early postnatal alcohol exposure (Subbanna et al., 2014a, 2014b) and increased acetylation of H3K9 in mouse foetuses encountering an acute alcohol exposure on GD7 (Veazey et al., 2015). Similarly, increased levels of H3K9 and H3K14 acetylation have been found in the cardiac tissue of FASD rodent models (Zhang et al., 2014; Peng et al., 2015), associated with an upregulated HAT activity without affecting HDAC. However, a global decrease in histone H3 and H4 acetylation in the cerebellum has been reported after perinatal alcohol vapour exposure (Guo et al., 2011) and decreased H3K9 acetylation levels were found in the

hypothalamus of rats exposed to alcohol during gestation (Bekdash et al., 2013). Furthermore, increased HDAC2 and HDAC4 expression has been reported in female offspring rats after foetal alcohol exposure (Gangisetty et al., 2015). Nevertheless, in our study no changes in H3K9 acetylation and H3K4me3 were found in the brain areas assessed. Such contrasting findings suggest that the epigenetic modifications induced by *in utero* alcohol exposure might be organ- or cell type-specific (Liyanage et al., 2017; Mandal et al., 2017b).

Recent evidence indicates that the adverse consequences of early alcohol exposure in cognition and behaviour might be associated with persistent histone modifications. Epigenetic regulatory mechanisms play a key role in memory and learning processes (Nelson and Monteggia, 2011; Sen, 2015). Acetylation of H4K5 and H4K12 is considered a key mark of active transcriptional activity of genes associated with long-term memory formation and synaptic plasticity (Peleg et al., 2010; Park et al., 2013; Peixoto and Abel, 2013). In addition, supporting the idea that histone acetylation acts as a molecular memory aid, it has been demonstrated that promoting histone acetylation through HDAC inhibition improves memory formation in rodents (Guan et al., 2009; Gräff and Tsai, 2013; Penney and Tsai, 2014; Volmar and Wahlestedt, 2015; Villain et al., 2016). The specific class IIa HDAC4 is required for learning and memory in mice (Kim et al., 2012). In accordance, we have recently reported that increased HDAC4 activity is associated with the cognitive impairments present in PLAE mice, which were reversed by trichostatin A treatment, an inhibitor of HDACs (Montagud-Romero et al., 2019). Even though we have shown significant elevated HAT activity associated with increased histone acetylation in the brain of PLAE mice, HDAC inhibition, which prevents histone deacetylation, ameliorated the cognitive performance in PLAE mice; therefore, a broader complexity of mechanisms might be involved in the cognitive deficits induced by developmental binge alcohol exposure.

Furthermore, epigenetic mechanisms have been shown to regulate neuroinflammation (Garden, 2013). For instance, the modulation of neuroinflammatory responses through pharmacological blockade of HDAC activity has demonstrated an important role for HDACs in the regulation of gene expression related with CNS inflammation (Suh et al., 2010; Licciardi et al., 2013).

Changes in histone acetylation and HAT activity were found in adult mice drinking alcohol, in addition to cognitive and anxiety-related behavioural impairments. However, these changes were not observed in mice lacking TLR4, demonstrating that TLR4 signalling via alterations of chromatin structure can promote the inflammatory damage observed after chronic alcohol exposure (Alfonso-Loeches et al., 2010; Pascual et al., 2011). Likewise, the epigenetic changes induced by intermittent alcohol treatment were abrogated in adolescent TLR4$^{-/-}$ mice (Montesinos et al., 2016b). Therefore, it is plausible that the long-lasting epigenetic changes induced by pre- and postnatal binge alcohol exposure in the brain might be contributing to the neuroimmune alterations found in PLAE mice. Recently, it has been shown that neonatal alcohol exposure induces epigenetic modifications related with microglia priming (Chastain et al., 2019). Alcohol-exposed adult rats showed enduring increases in H3K9 acetylation enrichment at TNF-α and IL-6 promoter regions, suggesting a possible epigenetic mechanism for the long-term immune disruption associated with microglial priming, which could lead to exaggerated immune response to stress in adulthood.

Moreover, the alterations in myelin proteins reported in our FASD model could be related to epigenetic changes. Oligodendrocyte development and myelination in the CNS are tightly regulated by epigenetic mechanisms, including DNA methylation, histone modifications and miRNAs (Emery, 2010; Liu and Casaccia, 2010; Liu et al., 2016; Koreman et al., 2018). Indeed, intermittent alcohol treatment during adolescence in mice led to reductions of

myelin-related gene expression and altered chromatin modifying genes involved in histone modifications in the PFC (Wolstenholme et al., 2017). In addition, it has been shown that HDAC inhibition prevents white matter injury in a murine model of traumatic brain injury by promoting microglia polarization toward the beneficial M2 phenotype (Wang et al., 2015a). Hence, binge alcohol exposure during early brain development could induce derangements in the myelin sheath through epigenetic mechanisms that promote neuroinflammation and in turn, affect myelin formation.

All together, these alterations in myelin integrity, neuroinflammation, neuron cell loss and epigenetic modifications induced by early alcohol exposure may lead to aberrant functionality in the brain. Indeed, we have found cumulative changes in the baseline metabolic capacity of different brain regions, as assessed by CCO immunoreactivity quantification. The mitochondrial enzyme CCO is used as a metabolic marker of neuronal activity since it is linked with ATP generation within the mitochondria of neurons (Wong-Riley, 1989). Therefore, quantitative CCO histochemistry is a reliable method for measuring neuronal oxidative metabolic capacity of brain regions (Gonzalez-Lima and Cada, 1998; Riha et al., 2011). Furthermore, CCO activity may be sensitive to the oxidative stress induced by *in utero* alcohol exposure, as it can produce mitochondrial dysfunction leading to apoptotic neuron cell death (Ramachandran et al., 2001; Reddy et al., 2013). To our knowledge, this is the first study reporting changes in regional brain CCO activity in adult offspring mice exposed to alcohol during gestation and lactation periods.

Accordingly, increased CCO activity has been reported in the DG of the HPC, the central nucleus of the amygdala, the medial lateral nucleus of the mammillary bodies and the granular and molecular layers of the central lobules of the cerebellum, whilst a diminished CCO immunoreactivity was found in the VTA and the LHb of PLAE mice compared with their counterparts. Some of these brain areas are associated with emotional and reward processing, such

as the central nucleus of the amygdala which processes emotionally relevant sensory information and its dysfunction has been associated with anxiety and alcohol dependence (Gilpin et al., 2015). In fact, PAE has been reported to attenuate GABAergic inhibition in the basolateral amygdala, which projects to the central amygdala and other brain regions, leading to neuronal hyperexcitability and anxiety-like behaviour in rats (Zhou et al., 2010). This finding agrees well with the increased anxiety-like behaviour observed in PLAE mice at both adolescence and adulthood.

The mammillary bodies have been related with anxiety as well, as they constitute a critical brain region to the effects of anxiolytic drugs (Kataoka et al., 1982; González-Pardo et al., 2006). Increased CCO activity in the medial lateral nucleus of the mammillary bodies has been found following chronic alcohol exposure in rats (Rubio et al., 1996) and consistent with our findings, increased brain glucose utilization in the mammillary bodies, the central amygdala and the HPC has been reported in rats after a history of alcohol consumption (Williams-Hemby et al., 1996). Therefore, increased CCO activity in these limbic structures may be associated with increased emotional and anxiety-like behaviour in PLAE mice.

The cerebellum is involved in motor learning and coordination, and its dysfunction produces alterations in movement coordination. Thus, we postulated that the increased CCO activity found in the cerebellum could be related with the impairment in the rotarod performance reported in PLAE mice. Furthermore, a role for the cerebellum in non-motor functions, such as emotion regulation and social cognition has been recently recognised (Shakiba, 2014; Strata, 2015; Adamaszek et al., 2017), which lead us to hypothesize the involvement of cerebellar dysfunction in the anxiety effects of developmental alcohol exposure.

The DG neural circuitry mediates a variety of mnemonic processes (Kesner, 2018). Therefore, alterations in the DG activity may account for memory impairments. However, as the subgranular zone of the DG is the major brain region where new neurons are generated at adulthood (Chambers et al., 2015), we speculated that the increase in hippocampal CCO activity observed in PLAE mice could be the result of an increase in neurogenesis. Accordingly, we found a higher number of BrdU+ cells in the DG of adult PLAE mice compared with control group, suggesting an increase in proliferation and survival of newly born neurons in the adult HPC following pre- and postnatal alcohol exposure. However, this issue has yielded some discrepancy in the literature, since previous studies have reported a reduction in hippocampal neurogenesis, no alterations or even an increase following developmental alcohol exposure (Boehme et al., 2011; Hamilton et al., 2011; Gil-Mohapel et al., 2014), as mentioned in the *Introduction*. Actually, some authors pointed out that moderate alcohol exposure during gestation has no effect on the generation of new neurons in the adult DG when mice are housed under standard conditions, but leads to marked impairment in the ability to mount a neurogenic response to enriched environments (Choi et al., 2005; Kajimoto et al., 2013). This effect might be related with the impaired ability to integrate newborn neurons into the hippocampal network. Interestingly, the quantitative analysis of doublecortin immunoreactivity in the DG led to a reduction of newly differentiated and migrating neuroblasts in PLAE mice, associated with diminished levels of BDNF (Montagud-Romero et al., 2019), according with preclinical studies demonstrating that alcohol exposure inhibits neurogenesis (Morris et al., 2010; Anderson et al., 2012; Campbell et al., 2014; Golub et al., 2015). Thus, our results may be interpreted as a compensatory mechanism to the neuroadaptations caused by early alcohol exposure.

The analysis of interregional within-group correlations in CCO activity showed alterations in functional brain connectivity in PLAE mice when compared to control mice. Three discrete and parallel closed reciprocal

functional brain networks were detected involving the amygdala complex, the cerebellum and the ventral HPC together with brainstem regions. Conversely, a more complex single brain network was detected in the control group, with numerous cross-correlations between anatomically related brain regions comprising the prelimbic cortex, dorsal and ventral hippocampal regions, the amygdala complex, the mammillary body, brainstem regions (including the VTA) and cerebellar regions. The only negative cross-correlation was found between the prelimbic region and the lateral septum in PLAE mice. Since the prelimbic cortex indirectly projects to the lateral septum via the infralimbic region (Vertes, 2004), the negative correlation found in CCO activity could be related with an abnormal modulation of the lateral septum affecting motivation and anxiety-like behaviour (Jonsson et al., 2017).

Such results indicate that PLAE mice display disrupted functional brain network connectivity as compared with control mice. In accordance, altered fronto-cerebellar and cortico-limbic connectivity has been reported in human alcoholics (Herting et al., 2011; Rogers et al., 2012) and in rats exposed intermittently to alcohol during adolescence (Broadwater et al., 2018). In addition, disrupted functional brain connectivity has also been reported in humans (Donald et al., 2015; Infante et al., 2017) and rodents (El Shawa et al., 2013; Louth et al., 2016; Rodriguez et al., 2016) with PAE using neuroimaging techniques, leading to reduced coordinated activity between spatially distinct brain areas. Therefore, functional connectivity derangements could be underlying some of the neurobehavioural alterations, including cognitive, emotional, and motor deficits described in PLAE mice, as well in human studies of FASD (Moore et al., 2014).

3. The therapeutic use of curcumin for FASD

Curcumin is a yellow-pigmented spice derived from the rhizomes of *Curcuma longa* that has been widely used in food for flavour and colour and as an herbal

medicine in ancient China and India (Begum et al., 2008; Jurenka, 2009). Numerous pharmacological activities have been attributed to curcumin, including antioxidant, antimicrobial, anti-inflammatory and antineoplastic properties. Research has shown curcumin to be a highly pleiotropic molecule capable of interacting with numerous molecular targets (Deguchi, 2015). Furthermore, curcumin's molecular structure and its ability to cross the BBB provide a promising avenue for neuroprotection (Sarker et al., 2015). Indeed, the neuroprotective role of curcumin against alcohol-induced brain damage has been described (Rajakrishnan et al., 1999), as has its effectiveness in preventing cognitive deficits and reducing the oxidative stress and neuroinflammation associated with chronic alcohol exposure (Tiwari and Chopra, 2013; Yu et al., 2013a; Ikram et al., 2019). There is evidence that curcumin diminishes alcohol-induced inflammation through the inhibition of NF-κB (Singh and Aggarwal, 1995; Nanji et al., 2003; Cho et al., 2007), attenuating the expression of pro-inflammatory molecules, such as cytokines and chemokines (Chen et al., 2018; Kahkhaie et al., 2019), and thus, preventing microglia activation (Karlstetter et al., 2011; Ghasemi et al., 2019). Moreover, curcumin can modulate epigenetic mechanisms by inhibiting the activity of the p300/CBP HAT enzyme, preventing histone hyperacetylation (Marcu et al., 2006; Hassan et al., 2019).

Several clinical and preclinical studies have shown beneficial effects of dietary curcumin in neurodegenerative diseases, including Alzheimer's and Parkinson's diseases, cancer, inflammatory gastrointestinal conditions and mood disorders (Chandra et al., 2001; Lim et al., 2001; Ishrat et al., 2009; Xu et al., 2009; Chimakurthy and Talasila, 2010; Hoppe et al., 2013; Agrawal et al., 2015; Zhang et al., 2015; Montagud-Romero et al., 2016; Bassani et al., 2017; Panahi et al., 2018).

Based on these considerations, we tested curcumin for its ability to inhibit inflammation and oxidative damage, as well for its epigenetic effects on HAT,

in our mouse model of FASD. Our results show that curcumin treatment during the peri-adolescence period (PD28-35) can ameliorate the cognitive deficits present in adult PLAE mice (PD60), including spatial working memory, object recognition and left-right discrimination reversal learning. Furthermore, curcumin had an anxiolytic effect, as assessed in the EPM. Regarding the molecular alterations, we found that the alcohol-induced glia activation (assessed by GFAP and Iba-1 expression) and subsequent elevation of pro-inflammatory factors (NF-κB and IL-6) in the brain was rescued by curcumin treatment in PLAE mice; but it was not able to reverse the increased HAT activity in PLAE mice.

Inhibition of histone acetylation by curcumin has been shown to rescue histone acetylation levels in the mouse foetal heart (Zhang et al., 2014) and reduce alcohol-induced foetal cardiac apoptosis (Yan et al., 2017), indicating that curcumin can be protective against the detrimental effects of foetal alcohol exposure. However, scarce experimental studies investigating the effects of curcumin in FASD models are available. In rats exposed to alcohol postnatally, chronic curcumin treatment was found to attenuate oxidative stress, neuroinflammation, and apoptosis, mitigating the behavioural deficits induced by alcohol (Tiwari and Chopra, 2012). Similarly, curcumin rescued alcohol-induced developmental defects in a zebrafish model of FASD (Muralidharan et al., 2017).

In our study, although curcumin could ameliorate the cognitive deficits induced by alcohol exposure during gestation and lactation periods in adult mice, its ineffectiveness in reversing the HAT activity could be attributed to the dosage and duration of the treatment. Notwithstanding its action through epigenetic mechanisms cannot be discarded, since no post-translational histone modifications were evaluated following curcumin treatment.

Therefore, our results suggest that curcumin's ability to protect against the effects of developmental alcohol exposure is mainly based on its anti-inflammatory and neuroprotective properties. Thus, the polyphenol curcumin could be used as a therapeutic agent to ameliorate the cognitive deficits and attenuate the pro-inflammatory response induced by alcohol exposure in FASD-affected individuals.

4. Vulnerability to alcohol and cocaine consumption and its neurobiological substrates in PLAE mice

Alcohol exposure *in utero* produces persistent alterations in brain areas controlling motivational and reward circuits. Indeed, alcohol exposure during the prenatal period seems to be predictive of adolescent and adult alcohol use and misuse (Baer et al., 2003; Alati et al., 2006). Premature exposure to alcohol in rodents promotes a heightened affinity for the drug later in life (Spear and Molina, 2005; Chotro et al., 2007). However, the neurobiological mechanisms underlying such susceptibility are still not well understood.

To date, few studies have assessed the effects of foetal alcohol exposure on the rewarding properties of alcohol in adult offspring using the CPP paradigm. Our results show an attenuation of the conditioned response to the alcohol-paired compartment in adult PLAE mice. This effect is more pronounced at the highest dose of alcohol administered (2 g/kg), although both doses used in the present study are effective doses for control mice. Such results led us to hypothesize that pre- and postnatal alcohol exposure reduces the sensitivity to the rewarding effects of alcohol in adult mice, which may need higher doses of alcohol to obtain the same rewarding effect as control mice.

Barbier et al. (2008) reported no differences in the conditioned response to 1 g/kg alcohol in the CPP paradigm in 2-month old rats exposed to alcohol throughout gestation and lactation. However, a significant increase in the time spent in the alcohol-paired compartment (at both 2 and 3 g/kg alcohol doses)

in early alcohol-exposed rats was reported, while the control group did not show any conditioned response (Barbier et al., 2009). Such contrasting findings could be explained by variations in the alcohol exposure pattern, alcohol dosing, and the rodent strain used.

In addition, voluntary alcohol intake, as assessed in the two-bottle choice and oral SA paradigms, was found to be increased in adolescent PLAE mice (Montagud-Romero et al., *under review*). Such data are in accordance with previous studies showing an increase in alcohol consumption. Preclinical findings showed that a single binge-like alcohol exposure, during a period equivalent to third-trimester of neural development, would seem to be enough to exacerbate dependence-like alcohol consumption in mice (Bosse et al., 2019). Similarly, rats with PAE showed exhibited increased alcohol consumption and preference in a two-bottle choice paradigm during adolescence (Fabio et al., 2013, 2015a; Chang et al., 2015). Also, when adult rats were submitted to a forced 10% alcohol solution exposure for 3 weeks, early life alcohol-exposed rats displayed an increase in alcohol consumption. Similar results were obtained in rats exposed to a free choice paradigm (Barbier et al., 2009). Furthermore, early-alcohol exposed rats were more resistant to the hypnotic effects of alcohol, as assessed by the loss of the righting reflex. Hence, the susceptibility to ulterior alcohol intake in rodents exposed to alcohol during developmental stages seems to be associated with a decreased sensitivity to the aversive effects of alcohol, such as sedation and hypothermia (Abel et al., 1981; Arias et al., 2008).

The acute pleasant effects of alcohol result from activation of dopaminergic pathways in the mesocorticolimbic system. The VTA plays a central role in reward processing, with its dopaminergic projections to the NAc, the amygdala and the PFC. In this study we found decreased neuronal activity in the VTA in PLAE mice, as assessed by CCO activity, which could be related to the reduced sensitivity to the rewarding effects of alcohol. Indeed, it has been previously reported that PAE induces alterations in the

mesocorticolimbic system in rodents, including the reduced electrical activity of DA neurons located in the substantia nigra and the VTA (Choong and Shen, 2004b; Shen and Choong, 2006) caused by an overexcitation that blocks depolarization, contributing to the reduced dopaminergic function. Such mechanism is also observable following repeated exposure to drugs of abuse after prolonged withdrawal, as a transient increase first occurs in neuronal activity. Accordingly, our results support the hypothesis that the mesocorticolimbic system is functionally impaired in PLAE mice, which might explain the increased drug-seeking and drug-taking behaviour in subjects exposed to alcohol during early development.

We have also reported a reduced neuronal activity in the LHb of PLAE mice, an epithalamic structure connecting forebrain to midbrain regions with a crucial role in the regulation of midbrain monoaminergic systems (Lecca et al., 2014). Descending projections from the LHb inhibit a proportion of VTA dopaminergic neurons through a selective GABAergic innervation to the VTA (Ji and Shepard, 2007; Omelchenko et al., 2009). Although VTA dopaminergic neurons reciprocally project to the LHb, inhibiting the LHb to promote reward (Stamatakis et al., 2013). Indeed, habenula neurons are usually inhibited by reward-predicting cues and excited by the absence of reward (Matsumoto and Hikosaka, 2007). Dysfunctions of the LHb have been implicated in depression and drug addiction, particularly in alcohol use and withdrawal (Velasquez et al., 2014; Shah et al., 2017). Lesions of the LHb increase voluntary alcohol intake in rats (Haack et al., 2014). Therefore, the attenuated conditioned response to alcohol in the CPP paradigm of PLAE mice could be related to a diminished activity of the LHb as assessed by CCO immunoreactivity.

Besides, epigenetic mechanisms may contribute to the increased vulnerability to later alcohol abuse in FASD patients. Epigenetic modifications play an important role in neurodevelopment, and changes induced by alcohol exposure during development in key brain regions can lead to adult

psychopathology, such as increased risk for substance use disorders (Kyzar et al., 2016). In recent years, alterations in the enzymes responsible for chromatin remodelling have been identified as molecular mechanisms for alcohol-related disorders (Pandey et al., 2008; Krishnan et al., 2014). HDAC activity has been reported to be inhibited following acute alcohol exposure in the amygdala, associated with increased global histone acetylation (Moonat et al., 2013). Following withdrawal from chronic alcohol exposure, animals display increased anxiety-like behaviour and increased HDAC activity. Blocking HDAC activity prevents the development of alcohol withdrawal-related anxiety in rats (Palmisano and Pandey, 2017). Hence, the alterations that we reported in HDAC and HAT enzymatic activity might underlie the enhanced risk to alcohol abuse in PLAE mice. Furthermore, epigenetic modifications induced by PAE have been reported to alter the proopiomelanocortin gene expression and other genes related with HPA axis function, altering HPA reactivity and consequently, contributing to the risk for stress-related alcohol addiction (Ngai et al., 2015; Pandey et al., 2017).

In addition, preclinical studies have shed light on the increased vulnerability to psychostimulant drug addiction in individuals with FASD (Glantz and Chambers, 2006). In this study, we evaluated the long-term effects of pre- and postnatal binge alcohol exposure on the reinforcing effects of cocaine in adult offspring mice. To our knowledge, this is the first study to demonstrate increased cocaine-taking behaviour in a mouse model of FASD.

An increased conditioned response to the cocaine-paired compartment in the CPP paradigm was found in PLAE mice when compared to their counterparts, based on the lowest dosage of cocaine used (5 mg/kg), whilst no differences were found in the 10 mg/kg cocaine-induced CPP between PLAE and control group. This effect is specific for cocaine's rewarding effects, as developmental alcohol exposure does not alter the CPP to food, which is a natural reward. In addition, such results would indicate that PLAE mice do not show anhedonia-like behaviour, as, like their counterparts, they show an enhanced motivation

towards the food-paired compartment. Furthermore, we evaluated behavioural sensitization to repeated administration of cocaine in PLAE and control adult mice. An attenuated locomotor sensitization to the lowest dose of cocaine used (7.5 mg/kg) was observed in mice exposed to alcohol during early development, while no differences between groups were observed in mice sensitized to a repeated treatment of 10 mg/kg cocaine. The findings obtained in the CPP paradigm suggest that pre- and postnatal binge alcohol exposure increases the sensitivity to the conditioned rewarding effects of cocaine.

Subsequently, we evaluated PLAE mice in an operant SA paradigm, which allows us to study drug-seeking and drug-taking behaviour, hallmarks of the addictive behaviour in humans. PLAE mice show an increased drug-seeking and drug-taking behaviour during the acquisition phase of the SA procedure receiving cocaine infusions of 0.75 mg/kg. However, no differences are found in the PR session between PLAE and control mice, which is an indicator of the effort level each subject is willing to exert in order to gain another drug infusion. PLAE animals extinguished drug-seeking behaviour and relapsed after a 10 mg/kg cocaine priming similarly to the control group. Nevertheless, the number of active nose-pokes on the first day of extinction and at reinstatement is lower in PLAE mice than in control group.

Our results illustrate that maternal binge alcohol drinking during gestation and lactation periods increases the reinforcing effects of cocaine in adult offspring and diminishes cocaine-induced locomotor sensitization. Given that different neural substrates are involved in drug-induced behavioural sensitization and in the associative learning processes that lead to CPP or drug SA, our findings are conceivable (Runegaard et al., 2018). In agreement with our results, previous research has shown increased cocaine intake and cocaine-induced CPP in adult offspring rats exposed to alcohol during gestation and lactation periods (Barbier et al., 2008). Likewise, increased sensitivity in the CPP induced by amphetamine, another psychostimulant drug, was observed in

PAE rats upon reaching adulthood (Wang et al., 2019b). In addition, they self-administered more amphetamine under a PR schedule of reinforcement (Hausknecht et al., 2015; Wang et al., 2018). In contrast to our findings, increased locomotor sensitization to psychostimulants, such as cocaine and amphetamine, was described in adult rats exposed to alcohol during gestation and lactation periods (Barbier et al., 2009). Interestingly, Macht et al. (2017) reported sex-specific effects of developmental alcohol exposure on cocaine-induced CPP in adulthood, since male offspring rats showed a reduced sensitivity, whilst their female counterparts displayed increased sensitivity to cocaine cues.

In order to elucidate the neural mechanisms underlying the behavioural alterations found in response to cocaine in PLAE mice, molecular changes in the PFC and the STR were evaluated after cocaine-induced reinstatement in the SA procedure. Our findings show alterations in the GluA1/GluA2 ratio in these brain areas in PLAE mice compared with their counterparts following cocaine-primed reinstatement. Although PLAE mice show an increase in the GluA1/GluA2 ratio in the PFC, connotative of enhanced glutamatergic excitability, a reduction is found in the STR, revealing diminished neuronal excitability therein. It has been reported that drug exposure evokes glutamatergic synaptic plasticity in the brain's reward circuitry (Thomas et al., 2008; Lüscher, 2016; Cooper et al., 2017). Indeed, neuroadaptations in the PFC to enhance excitatory output have been associated with drug-seeking behaviour (Van den Oever et al., 2010). Glutamatergic projections from the mPFC to the NAc have been reported to mediate cocaine-induced behavioural neuroplasticity (Kalivas, 2004; Zhang et al., 2019) and specifically, cocaine-primed reinstatement of drug-seeking behaviour (McFarland et al., 2003). Cocaine SA in mice has been shown to reduce excitatory glutamatergic signalling in the NAc shell (Schramm-Sapyta et al., 2006). In addition, GluA2-lacking AMPARs in the NAc regulate the incubation of cocaine craving after prolonged withdrawal (Conrad et al., 2008).

In a rodent model of FASD, Hausknecht et al. (2015) reported a persistent augmentation of calcium-permeable AMPAR expression in VTA dopaminergic neurons, which could lead to an enhanced excitatory synaptic strength. Moreover, binge alcohol exposure during the third trimester-equivalent period increased the frequency of spontaneous excitatory glutamatergic postsynaptic currents in the basolateral amygdala (Baculis et al., 2015).

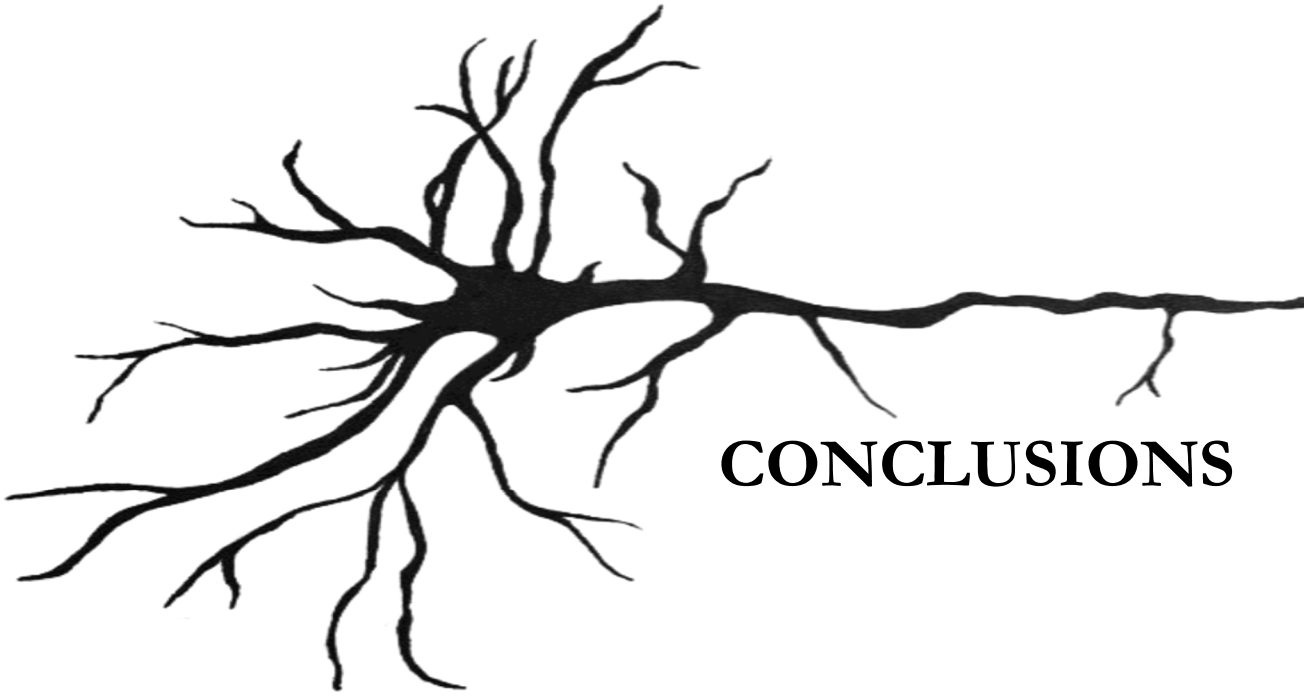
In our study, no changes in the phosphorylation of CREB were found in PLAE mice, but surprisingly, a decreased protein expression of ΔFosB in the STR was observed. Drugs of abuse induce a short-term CREB activation, whereas chronic drug treatment induces an accumulation of ΔFosB in the NAc that persists after weeks of withdrawal (McClung and Nestler, 2003). We expected that alcohol exposure during development would induce the accumulation of ΔFosB in the NAc, thus resulting in greater cocaine-induced reward and intake. However, we found a decreased ΔFosB expression in the STR of PLAE mice. Notwithstanding, we should consider that brain areas were extracted after cocaine-induced reinstatement, which was lower in PLAE mice. Therefore, we cannot discard the role of ΔFosB in the heightened sensitivity to cocaine reinforcing effects in PLAE mice, even though other mechanisms might be involved.

Addictive drugs stimulate striatal dopaminergic receptors inducing changes in intracellular pathways that may underlie functional and structural neuroplasticity (Philibin et al., 2011). However, no significant differences were found in our study regarding the protein expression of D1R and D2R between PLAE and control mice. Similarly, no changes were observed in the phosphorylation of the dopamine- and cyclic AMP-regulated phosphoprotein of 32 kDa (DARPP-32), which plays a key role in the integration of dopaminergic and glutamatergic signalling in the STR (Gould and Manji, 2005; Zachariou et al., 2006).

As mentioned earlier, previous studies have shown that PAE causes a persistent reduction in the spontaneous electrical activity of midbrain dopaminergic neurons (Choong and Shen, 2004b) and a lower frequency of evoked action potentials, leading to decreased excitability of the VTA (Wang et al., 2006). Furthermore, an imbalance between D1R and D2R has been found in the dSTR of PAE rats, resulting in alterations of corticostriatal synaptic plasticity (Zhou et al., 2012). However, inconsistent findings have been described regarding D1R and D2R expression in FASD studies. Boggan et al. (1996) reported an elevation of D1R binding in adolescent PAE mice, but not in adulthood, while D2R binding was not affected. In contrast, decreased D1R binding in the STR and increased D2R mRNA levels were observed in adult PLAE rats (Barbier et al., 2008). Furthermore, a report in rhesus monkeys showed that moderate alcohol exposure during early gestation reduced the striatal D2R binding in adulthood, whereas middle-to-late alcohol gestation exposure heightened dopaminergic function, suggesting a time-dependent effect (Schneider et al., 2005).

An increased expression of DAT in the PFC and the STR of PAE mice has also been reported (Kim et al., 2013), whilst a decrease in DAT binding in the STR was found in rats with foetal alcohol exposure (Barbier et al., 2009). Interestingly, higher DA levels in the NAc were found in PAE rats when compared to controls, presumably as a result of a down-regulation of post-synaptic receptors or desensitization of presynaptic receptors (Muñoz-Villegas et al., 2017). Adolescent PAE rats displayed greater dopaminergic activity in the VTA after receiving a postnatal challenge of alcohol than controls (Fabio et al., 2015b). However, a lower baseline level of striatal dopaminergic activity has been reported in other models of FASD (Carneiro et al., 2005). So, as Fabio et al. (2015) proposed, animals exposed to alcohol *in utero* might have similar or lower dopaminergic function compared to controls and, when re-exposed to the drug, they exhibit heightened DA activity.

In short, our findings demonstrate that binge-like alcohol exposure during pregnancy and breastfeeding in a mouse model alters the sensitivity to the reinforcing effects of alcohol and cocaine in adulthood, leading to increased drug-taking behaviour. Neuroadaptations affecting the glutamatergic excitability within the mesocorticolimbic reward system could be mediating these behavioural alterations.



CONCLUSIONS

The main **conclusions** of this Doctoral Thesis can be summarized as follows:

1. Drinking-in-the-dark test is a useful procedure to mimic binge-like alcohol consumption during pregnancy and breastfeeding periods in a mouse model of FASD.
2. Maternal binge-like alcohol consumption during gestation and lactation periods induces cognitive deficits, increased anxiety-like behaviour, age-dependent alterations in locomotor activity and motor coordination impairments in adult offspring mice.
3. Maternal care behaviour, body weight and litter size are not affected by binge alcohol exposure during prenatal and lactation periods.
4. Prenatal and postnatal binge alcohol exposure induces persistent upregulation of TLR4-dependent pro-inflammatory factors and glial cells activation, leading to neuronal cell death in the PFC and HPC of adult offspring mice.
5. Reduced expression of myelin proteins is found in the PFC and HPC of adult mice exposed to binge alcohol during prenatal and lactational periods.
6. Mice with pre- and postnatal binge alcohol exposure show epigenetic modifications at adulthood, including increased histone acetylation and increased HAT activity in the PFC.
7. Long-lasting effects on neuronal oxidative metabolism in some brain areas in addition to alterations in functional brain networks were found in adult mice following maternal binge-like alcohol drinking during prenatal and lactational periods.
8. Curcumin treatment during the periadolescence ameliorates the cognitive deficits and reverses the pro-inflammatory effects induced by prenatal and postnatal alcohol exposure in our mouse model.
9. Prenatal and lactational binge-like alcohol exposure alters the sensitivity to the rewarding effects of alcohol later in life, leading to increased alcohol intake in adult offspring mice.

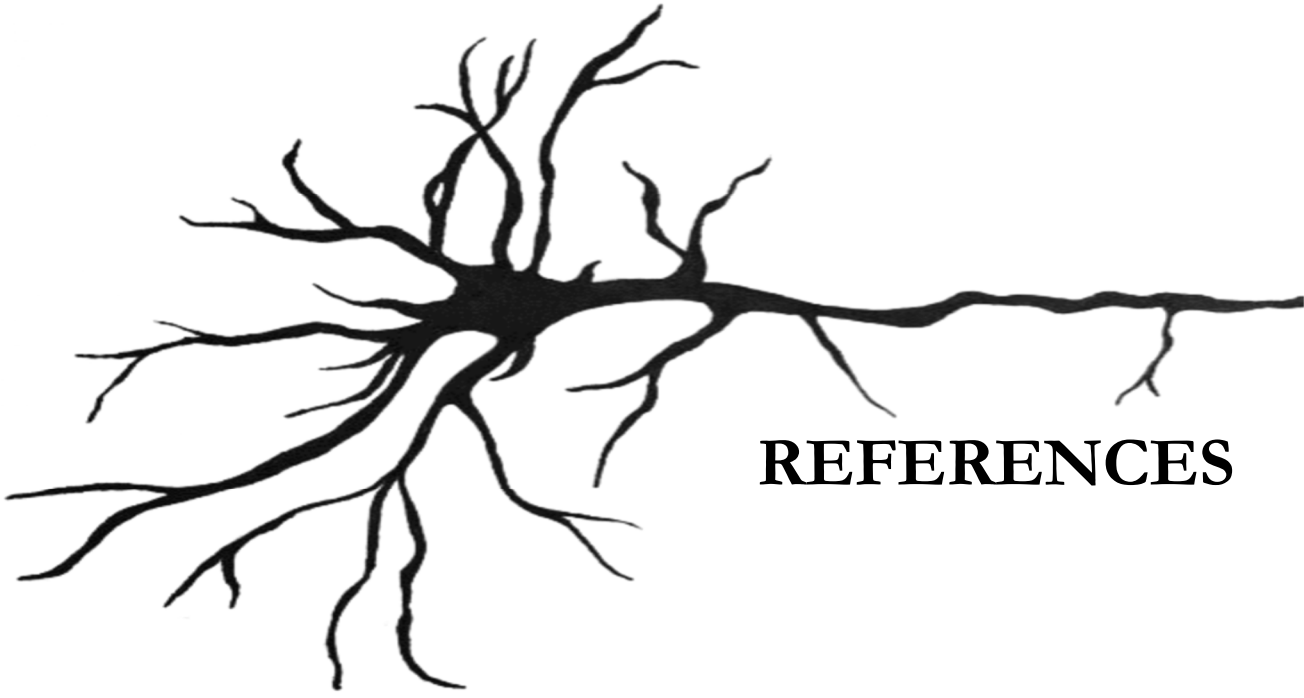
10. Mice with pre- and postnatal binge alcohol exposure show increased conditioned response to the rewarding effects of cocaine but diminished behavioural sensitization following repeated cocaine treatment.
11. Increased cocaine-seeking and cocaine-taking behaviour in the operant SA paradigm was found in mice exposed to binge-like alcohol exposure during gestation and lactation periods.
12. Alterations in AMPAR subunits GluA1/GluA2 ratio in the PFC and STR of adult PLAE mice following cocaine-induced reinstatement were found, suggesting that glutamatergic neuroadaptations in the mesocorticolimbic system might underpin the increased vulnerability to cocaine abuse.

Les principals **conclusions** d'aquesta Tesi Doctoral es poden resumir a continuació:

1. El procediment de *Drinking-in-the-dark* és útil per a modelar un consum d'alcohol d'afartament durant l'embaràs i la lactància en un model murí de trastorn de l'espectre alcohòlic fetal.
2. El consum maternal d'alcohol en forma d'afartament durant els períodes de gestació i lactància provoca dèficits cognitius, augment de l'ansietat, alteracions de l'activitat locomotora en funció de l'edat i alteracions de la coordinació motora en ratolins descendents adults.
3. El comportament maternal, el pes corporal i la mida de les ventrades no es veuen afectats per l'exposició a l'alcohol durant el període prenatal i la lactància.
4. L'exposició prenatal i post-natal d'afartament a l'alcohol indueix una regulació a l'alça de factors proinflamatoris dependents del TLR4 i l'activació de les cèl·lules glials, que condueixen a una mort neuronal a l'escorça prefrontal i a l'hipocamp en ratolins descendents adults.
5. Els ratolins exposats a l'alcohol seguint un patró d'afartament durant el període prenatal i el de lactància presenten una disminució en l'expressió de proteïnes de la mielina a l'escorça prefrontal i a l'hipocamp.
6. Els ratolins exposats a l'alcohol en forma d'afartament durant el període prenatal i el de lactància presenten modificacions epigenètiques a l'etapa adulta, incloent un augment en l'acetilació d'histones i en l'activitat enzimàtica de la HAT a l'escorça prefrontal.
7. S'han trobat canvis persistents al llarg del temps en el metabolisme neuronal oxidatiu en algunes àrees del cervell, així com alteracions en les xarxes funcionals del cervell en ratolins adults exposats a l'alcohol durant el període prenatal i el de lactància.
8. El tractament amb curcumina durant l'adolescència millora els dèficits cognitius i, al mateix temps, contraresta els efectes proinflamatoris

induïts per l'exposició prenatal i postnatal a l'alcohol en el nostre model murí.

9. L'exposició prenatal i postnatal a l'alcohol en forma d'afartament altera la sensitivitat als efectes reforçants de l'alcohol, donant lloc a un augment de la ingesta d'alcohol en la descendència a l'etapa adulta.
10. Els ratolins exposats a l'alcohol sota un patró d'afartament durant el període prenatal i el de lactància presenten un major condicionament als efectes reforçants de la cocaïna i una disminució de la sensibilització locomotora en resposta a un tractament repetit de cocaïna.
11. Els ratolins exposats a l'alcohol en forma d'afartament durant el període prenatal i el de lactància presenten un increment del comportament de cerca i consum de cocaïna en el paradigma operant de l'autoadministració.
12. Els ratolins exposats a l'alcohol en forma d'afartament durant el període prenatal i el de lactància presenten alteracions en la proporció de les subunitats GluA1/GluA2 del receptor AMPA a l'escorça prefrontal i a l'estriat després de la recaiguda induïda per cocaïna, la qual cosa suggereix que canvis neuroadaptatius en l'excitabilitat glutamatèrgica en el sistema mesocorticolímbic podrien estar explicant la major vulnerabilitat a l'abús de cocaïna.



REFERENCES

Abate, P., Reyes-Guzmán, A.C., Hernández-Fonseca, K., and Méndez, M. (2017). Prenatal ethanol exposure modifies locomotor activity and induces selective changes in Met-enk expression in adolescent rats. Neuropeptides *62*: 45–56.

Abbott, C.W., Kozanian, O.O., Kanaan, J., Wendel, K.M., and Huffman, K.J. (2016). The Impact of Prenatal Ethanol Exposure on Neuroanatomical and Behavioral Development in Mice. Alcohol. Clin. Exp. Res. *40*: 122–133.

Abbott, C.W., Rohac, D.J., Bottom, R.T., Patadia, S., and Huffman, K.J. (2018). Prenatal Ethanol Exposure and Neocortical Development: A Transgenerational Model of FASD. Cereb. Cortex *28*: 2908–2921.

Abel, E., Bush, R., and Dintcheff, B. (1981). Exposure of rats to alcohol in utero alters drug sensitivity in adulthood. Science (80-.). *212*: 1531–1533.

Abrahao, K.P., Salinas, A.G., and Lovinger, D.M. (2017). Alcohol and the Brain: Neuronal Molecular Targets, Synapses, and Circuits. Neuron *96*: 1223–1238.

Adamaszek, M., D'Agata, F., Ferrucci, R., Habas, C., Keulen, S., Kirkby, K.C., et al. (2017). Consensus Paper: Cerebellum and Emotion. Cerebellum *16*: 552–576.

Adermark, L., and Bowers, M.S. (2016). Disentangling the Role of Astrocytes in Alcohol Use Disorder. Alcohol. Clin. Exp. Res. *40*: 1802–1816.

Aggarwal, S., Yurlova, L., and Simons, M. (2011). Central nervous system myelin: structure, synthesis and assembly. Trends Cell Biol. *21*: 585–93.

Agrawal, R., Sandhu, S.K., Sharma, I., and Kaur, I.P. (2015). Development and evaluation of curcumin-loaded elastic vesicles as an effective topical anti-inflammatory formulation. AAPS PharmSciTech *16*: 364–74.

Ahlers, K.E., Karaçay, B., Fuller, L., Bonthius, D.J., and Dailey, M.E. (2015). Transient activation of microglia following acute alcohol exposure in developing mouse neocortex is primarily driven by BAX-dependent neurodegeneration. Glia *63*: 1694–1713.

Alati, R., Mamun, A. Al, Williams, G.M., O'Callaghan, M., Najman, J.M., Bor, W., et al. (2006). In utero alcohol exposure and prediction of alcohol disorders in early adulthood: A birth cohort study. Arch. Gen. Psychiatry *63*: 1009–1016.

Alberry, B., and Singh, S.M. (2016). Developmental and behavioral consequences of early life maternal separation stress in a mouse model of fetal alcohol spectrum disorder. Behav. Brain Res. *308*: 94–103.

Alfonso-Loeches, S., and Guerri, C. (2011). Molecular and behavioral aspects of the actions of alcohol on the adult and developing brain. Crit. Rev. Clin. Lab. Sci. *48*: 19–47.

Alfonso-Loeches, S., Pascual-Lucas, M., Blanco, A.M., Sanchez-Vera, I., and Guerri, C. (2010). Pivotal role of TLR4 receptors in alcohol-induced neuroinflammation and brain damage. J. Neurosci. *30*: 8285–95.

Alfonso-Loeches, S., Pascual, M., Gómez-Pinedo, U., Pascual-Lucas, M., Renau-Piqueras, J., and Guerri, C. (2012). Toll-like receptor 4 participates in the myelin disruptions associated with chronic alcohol abuse. Glia *60*: 948–964.

Alfonso-Loeches, S., Ureña-Peralta, J., Morillo-Bargues, M.J., Gómez-Pinedo, U., and Guerri, C. (2016). Ethanol-Induced TLR4/NLRP3 Neuroinflammatory Response in Microglial Cells Promotes Leukocyte Infiltration Across the BBB. Neurochem. Res. *41*: 193–209.

Alfonso-Loeches, S., Ureña-Peralta, J.R., Morillo-Bargues, M.J., Oliver-De La Cruz, J., and Guerri, C. (2014). Role of mitochondria ROS generation in ethanol-induced NLRP3 inflammasome activation and cell death in astroglial cells. Front. Cell. Neurosci. *8*: 216.

Allan, A.M., Chynoweth, J., Tyler, L. a, and Caldwell, K.K. (2003). A mouse model of prenatal ethanol exposure using a voluntary drinking paradigm. Alcohol. Clin. Exp. Res. *27*: 2009–16.

Allan, A.M., Goggin, S.L., and Caldwell, K.K. (2014). Prenatal alcohol exposure modifies glucocorticoid receptor subcellular distribution in the medial prefrontal cortex and impairs frontal cortex-dependent learning. PLoS One *9*: e96200.

Alvarez-Buylla, A., and Garcia-Verdugo, J.M. (2002). Neurogenesis in adult subventricular zone. J. Neurosci. *22*: 629–34.

An, L., Yang, Z., and Zhang, T. (2013). Imbalanced Synaptic Plasticity Induced Spatial Cognition Impairment in Male Offspring Rats Treated with Chronic Prenatal Ethanol Exposure. Alcohol. Clin. Exp. Res. *37*: 763–770.

An, L., and Zhang, T. (2015). Prenatal ethanol exposure impairs spatial cognition and synaptic plasticity in female rats. Alcohol *49*: 581–8.

Andersen, S.L. (2003). Trajectories of brain development: point of vulnerability or window of opportunity? Neurosci. Biobehav. Rev. *27*: 3–18.

Anderson, M.L., Nokia, M.S., Govindaraju, K.P., and Shors, T.J. (2012). Moderate drinking? Alcohol consumption significantly decreases neurogenesis in the adult hippocampus. Neuroscience *224*: 202–9.

Andreu-Fernández, V., Bastons-Compta, A., Navarro-Tapia, E., Sailer, S., and Garcia-Algar, O. (2019). Serum concentrations of IGF-I/IGF-II as biomarkers of alcohol damage during foetal development and diagnostic markers of Foetal Alcohol Syndrome. Sci. Rep. *9*: 1–10.

Arai, K., Matsuki, N., Ikegaya, Y., and Nishiyama, N. (2001). Deterioration of spatial learning performances in lipopolysaccharide-treated mice. Jpn. J. Pharmacol. *87*: 195–201.

Arias, C., Molina, J.C., Mlewski, E.C., Pautassi, R.M., and Spear, N. (2008). Acute sensitivity and acute tolerance to ethanol in preweanling rats with or without prenatal experience with the drug. Pharmacol. Biochem. Behav. *89*: 608–622.

Armario, A. (2010). Activation of the hypothalamic-pituitary-adrenal axis by addictive drugs: different pathways, common outcome. Trends Pharmacol. Sci. *31*: 318–25.

Aronne, M.P., Guadagnoli, T., Fontanet, P., Evrard, S.G., and Brusco, A. (2011). Effects of prenatal ethanol exposure on rat brain radial glia and neuroblast migration. Exp. Neurol. *229*: 364–71.

Astley, S., and Clarren, S. (1997). Diagnostic guide for fetal alcohol syndrome and related conditions: The 4-digit diagnostic code, 1st edn. (Seattle: University of Washington).

Astley, S., and Clarren, S. (2000). Diagnosing the full spectrum of fetal alcohol-exposed individuals: introducing the 4-digit diagnostic code. Alcohol Alcohol *35*: 400–10.

Baculis, B.C., Diaz, M.R., and Fernando Valenzuela, C. (2015). Third trimester-equivalent ethanol exposure increases anxiety-like behavior and glutamatergic transmission in the basolateral amygdala. Pharmacol. Biochem. Behav. *137*: 78–85.

Baer, J.S., Sampson, P.D., Barr, H.M., Connor, P.D., and Streissguth, A.P. (2003). A 21-year longitudinal analysis of the effects of prenatal alcohol exposure on young adult drinking. Arch. Gen. Psychiatry *60*: 377–385.

Bager, H., Christensen, L.P., Husby, S., and Bjerregaard, L. (2017). Biomarkers for the Detection of Prenatal Alcohol Exposure: A Review. Alcohol. Clin. Exp. Res. *41*: 251–261.

Bagheri, F., Goudarzi, I., Lashkarbolouki, T., and Elahdadi Salmani, M. (2015). Melatonin prevents oxidative damage induced by maternal ethanol administration and reduces homocysteine in the cerebellum of rat pups. Behav. Brain Res. *287*: 215–25.

Bakhireva, L.N., Shrestha, S., Garrison, L., Leeman, L., Rayburn, W.F., and Stephen, J.M. (2018). Prevalence of alcohol use in pregnant women with substance use disorder. Drug Alcohol Depend. *187*: 305–310.

Balaraman, S., Schafer, J.J., Tseng, A.M., Wertelecki, W., Yevtushok, L., Zymak-Zakutnya, N., et al. (2016). Plasma miRNA profiles in pregnant women predict infant outcomes following prenatal alcohol exposure. PLoS One *11*: 1–20.

Balaraman, S., Tingling, J.D., Tsai, P.-C., and Miranda, R.C. (2013). Dysregulation of

microRNA expression and function contributes to the etiology of fetal alcohol spectrum disorders. Alcohol Res. *35*: 18–24.

Balaszczuk, V., Salguero, J.A., Villarreal, R.N., Scaramuzza, R.G., Mendez, S., and Abate, P. (2019). Hyperlocomotion and anxiety- like behavior induced by binge ethanol exposure in rat neonates. Possible ameliorative effects of Omega 3. Behav. Brain Res. *372*: 112022.

Ballantyne, J. (1904). A manual of antenatal pathology and hygiene of the embryo. (Edinburg (UK): William Green and Sons).

Balosso, S., Liu, J., Bianchi, M.E., and Vezzani, A. (2014). Disulfide-Containing High Mobility Group Box-1 Promotes N-Methyl-d-Aspartate Receptor Function and Excitotoxicity by Activating Toll-Like Receptor 4-Dependent Signaling in Hippocampal Neurons. Antioxid. Redox Signal. *21*: 1726–1740.

Banik, A., Kandilya, D., Ramya, S., Stünkel, W., Chong, Y., and Dheen, S. (2017). Maternal Factors that Induce Epigenetic Changes Contribute to Neurological Disorders in Offspring. Genes (Basel). *8*: 150.

Barbier, E., Houchi, H., Warnault, V., Pierrefiche, O., Daoust, M., and Naassila, M. (2009). Effects of prenatal and postnatal maternal ethanol on offspring response to alcohol and psychostimulants in long evans rats. Neuroscience *161*: 427–440.

Barbier, E., Pierrefiche, O., Vaudry, D., Vaudry, H., Daoust, M., and Naassila, M. (2008). Long-term alterations in vulnerability to addiction to drugs of abuse and in brain gene expression after early life ethanol exposure. Neuropharmacology *55*: 1199–1211.

Barco, A., Alarcon, J.M., and Kandel, E.R. (2002). Expression of constitutively active CREB protein facilitates the late phase of long-term potentiation by enhancing synaptic capture. Cell *108*: 689–703.

Bardag-Gorce, F., French, B.A., Joyce, M., Baires, M., Montgomery, R.O., Li, J., et al. (2007). Histone acetyltransferase p300 modulates gene expression in an epigenetic manner at high blood alcohol levels. Exp. Mol. Pathol. *82*: 197–202.

Barker, G.R.I., and Warburton, E.C. (2011). When Is the Hippocampus Involved in Recognition Memory? J. Neurosci. *31*: 10721–10731.

Baroja-Mazo, A., Martín-Sánchez, F., Gomez, A.I., Martínez, C.M., Amores-Iniesta, J., Compan, V., et al. (2014). The NLRP3 inflammasome is released as a particulate danger signal that amplifies the inflammatory response. Nat. Immunol. *15*: 738–48.

Barr, H.M., Bookstein, F.L., O'Malley, K.D., Connor, P.D., Huggins, J.E., and Streissguth, A.P. (2006). Binge drinking during pregnancy as a predictor of psychiatric disorders on the Structured Clinical Interview for DSM-IV in Young Adult Offspring. Am. J. Psychiatry *163*: 1061–1065.

Barron, S., Hawkey, A., Fields, L., and Littleton, J.M. (2016). Animal Models for Medication Development and Application to Treat Fetal Alcohol Effects. Int. Rev. Neurobiol. *126*: 423–40.

Basavarajappa, B., and Subbanna, S. (2016). Epigenetic Mechanisms in Developmental Alcohol-Induced Neurobehavioral Deficits. Brain Sci. *6*: 12.

Bassani, T.B., Turnes, J.M., Moura, E.L.R., Bonato, J.M., Cóppola-Segovia, V., Zanata, S.M., et al. (2017). Effects of curcumin on short-term spatial and recognition memory, adult neurogenesis and neuroinflammation in a streptozotocin-induced rat model of dementia of Alzheimer's type. Behav. Brain Res. *335*: 41–54.

Bastons-Compta, A., Astals, M., Andreu-Fernandez, V., Navarro-Tapia, E., and Garcia-Algar, O. (2018). Postnatal nutritional treatment of neurocognitive deficits in fetal alcohol spectrum disorder. Biochem. Cell Biol. *96*: 213–221.

Bauernfeind, F.G., Horvath, G., Stutz, A., Alnemri, E.S., MacDonald, K., Speert, D., et al. (2009). Cutting edge: NF-kappaB activating pattern recognition and cytokine receptors license NLRP3 inflammasome activation by regulating NLRP3 expression. J. Immunol. *183*: 787–91.

Baumann, N., and Pham-Dinh, D. (2001). Biology of Oligodendrocyte and Myelin in the Mammalian Central Nervous System. Physiol. Rev. *81*: 871–927.

Bearer, C.F. (2001). L1 Cell Adhesion Molecule Signal Cascades: Targets for Ethanol Developmental Neurotoxicity. Neurotoxicology *22*: 625–633.

Beaulieu, J.-M., and Gainetdinov, R.R. (2011). The Physiology, Signaling, and Pharmacology of Dopamine Receptors. Pharmacol. Rev. *63*: 182–217.

Begum, A.N., Jones, M.R., Lim, G.P., Morihara, T., Kim, P., Heath, D.D., et al. (2008). Curcumin structure-function, bioavailability, and efficacy in models of neuroinflammation and Alzheimer's disease. J. Pharmacol. Exp. Ther. *326*: 196–208.

Behnke, M., and Smith, V.C. (2013). Prenatal Substance Abuse: Short- and Long-term Effects on the Exposed Fetus. Pediatrics *131*: e1009–e1024.

Bekdash, R., Zhang, C., and Sarkar, D. (2014). Fetal alcohol programming of hypothalamic proopiomelanocortin system by epigenetic mechanisms and later life vulnerability to stress. Alcohol. Clin. Exp. Res. *38*: 2323–2330.

Bekdash, R.A., Zhang, C., and Sarkar, D.K. (2013). Gestational choline supplementation normalized fetal alcohol-induced alterations in histone modifications, DNA methylation, and proopiomelanocortin (POMC) gene expression in β-endorphin-producing POMC neurons of the hypothalamus. Alcohol. Clin. Exp. Res. *37*: 1133–42.

Bélanger, M., and Magistretti, P.J. (2009). The role of astroglia in neuroprotection.

Dialogues Clin. Neurosci. *11*: 281–95.

Bentley, G.R. (1998). Hydration as a limiting factor in lactation. Am. J. Hum. Biol. *10*: 151–161.

Benz, J., Rasmussen, C., and Andrew, G. (2009). Diagnosing fetal alcohol spectrum disorder: History challenges and future directions. Paediatr. Child Health (Oxford). *14*: 231–237.

Berkel, T.D.M., and Pandey, S.C. (2017). Emerging Role of Epigenetic Mechanisms in Alcohol Addiction. Alcohol. Clin. Exp. Res. *41*: 666–680.

Berman, R.F., and Hannigan, J.H. (2000). Effects of prenatal alcohol exposure on the hippocampus: spatial behavior, electrophysiology, and neuroanatomy. Hippocampus *10*: 94–110.

Bernstein, B.E., Meissner, A., and Lander, E.S. (2007). The Mammalian Epigenome. Cell *128*: 669–681.

Bhatia, S., Drake, D.M., Miller, L., and Wells, P.G. (2019). Oxidative stress and DNA damage in the mechanism of fetal alcohol spectrum disorders. Birth Defects Res. bdr2.1509.

Bilbo, S.D., Smith, S.H., and Schwarz, J.M. (2012). A lifespan approach to neuroinflammatory and cognitive disorders: A critical role for glia. J. Neuroimmune Pharmacol. *7*: 24–41.

Bilkei-Gorzo, A., Racz, I., Valverde, O., Otto, M., Michel, K., Sastre, M., et al. (2005). Early age-related cognitive impairment in mice lacking cannabinoid CB1 receptors. Proc. Natl. Acad. Sci. U. S. A. *102*: 15670–5.

Biswas, S.K., McClure, D., Jimenez, L.A., Megson, I.L., and Rahman, I. (2005). Curcumin Induces Glutathione Biosynthesis and Inhibits NF-κB Activation and Interleukin-8 Release in Alveolar Epithelial Cells: Mechanism of Free Radical Scavenging Activity. Antioxid. Redox Signal. *7*: 32–41.

Blanco, A.M., Valles, S.L., Pascual, M., and Guerri, C. (2005). Involvement of TLR4/Type I IL-1 Receptor Signaling in the Induction of Inflammatory Mediators and Cell Death Induced by Ethanol in Cultured Astrocytes. J. Immunol. *175*: 6893–6899.

Block, M.L., Zecca, L., and Hong, J.-S. (2007). Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. Nat. Rev. Neurosci. *8*: 57–69.

Bodea, L.-G., Wang, Y., Linnartz-Gerlach, B., Kopatz, J., Sinkkonen, L., Musgrove, R., et al. (2014). Neurodegeneration by activation of the microglial complement-phagosome pathway. J. Neurosci. *34*: 8546–56.

Bodnar, T., and Weinberg, J. (2013). Prenatal Alcohol Exposure: Impact on Neuroendocrine–Neuroimmune Networks. In Neural-Immune Interactions in Brain Function and Alcohol Related Disorders, (Boston, MA: Springer US), pp 307–357.

Boehm, S.L., Moore, E.M., Walsh, C.D., Gross, C.D., Cavelli, A.M., Gigante, E., et al. (2008). Using drinking in the dark to model prenatal binge-like exposure to ethanol in C57BL/6J mice. Dev. Psychobiol. *50*: 566–78.

Boehme, F., Gil-Mohapel, J., Cox, A., Patten, A., Giles, E., Brocardo, P.S., et al. (2011). Voluntary exercise induces adult hippocampal neurogenesis and BDNF expression in a rodent model of fetal alcohol spectrum disorders. Eur. J. Neurosci. *33*: 1799–811.

Boggan, W.O., Xu, W., Shepherd, C.L., and Middaugh, L.D. (1996). Effects of prenatal ethanol exposure on dopamine systems in C57BL/6J mice. Neurotoxicol. Teratol. *18*: 41–8.

Boldrini, M., Fulmore, C.A., Tartt, A.N., Simeon, L.R., Pavlova, I., Poposka, V., et al. (2018). Human Hippocampal Neurogenesis Persists throughout Aging. Cell Stem Cell *22*: 589-599.e5.

Bonthius, D.J., Winters, Z., Karacay, B., Bousquet, S.L., and Bonthius, D.J. (2015). Importance of genetics in fetal alcohol effects: Null mutation of the nNOS gene worsens alcohol-induced cerebellar neuronal losses and behavioral deficits. Neurotoxicology *46*: 60–72.

Boschen, K.E., Keller, S.M., Roth, T.L., and Klintsova, A.Y. (2018). Epigenetic mechanisms in alcohol- and adversity-induced developmental origins of neurobehavioral functioning. Neurotoxicol. Teratol. *66*: 63–79.

Boschen, K.E., and Klintsova, A.Y. (2017). Neurotrophins in the Brain: Interaction With Alcohol Exposure During Development. Vitam. Horm. *104*: 197–242.

Boschen, K.E., Ruggiero, M.J., and Klintsova, A.Y. (2016). Neonatal binge alcohol exposure increases microglial activation in the developing rat hippocampus. Neuroscience *324*: 355–66.

Bosse, K.E., Chiu, V.M., Lloyd, S.C., and Conti, A.C. (2019). Neonatal alcohol exposure augments voluntary ethanol intake in the absence of potentiated anxiety-like behavior induced by chronic intermittent ethanol vapor exposure. Alcohol *79*: 17–24.

Boyadjieva, N.I., and Sarkar, D.K. (2013). Cyclic adenosine monophosphate and brain-derived neurotrophic factor decreased oxidative stress and apoptosis in developing hypothalamic neuronal cells: role of microglia. Alcohol. Clin. Exp. Res. *37*: 1370–9.

Brady, M.L., Allan, A.M., and Caldwell, K.K. (2012). A limited access mouse model of prenatal alcohol exposure that produces long-lasting deficits in hippocampal-

dependent learning and memory. Alcohol. Clin. Exp. Res. *36*: 457–66.

Brady, M.L., Diaz, M.R., Iuso, A., Everett, J.C., Valenzuela, C.F., and Caldwell, K.K. (2013). Moderate Prenatal Alcohol Exposure Reduces Plasticity and Alters NMDA Receptor Subunit Composition in the Dentate Gyrus. J. Neurosci. *33*: 1062–1067.

Bramham, C.R., and Messaoudi, E. (2005). BDNF function in adult synaptic plasticity: The synaptic consolidation hypothesis. Prog. Neurobiol. *76*: 99–125.

Brancato, A., Castelli, V., Cavallaro, A., Lavanco, G., Plescia, F., and Cannizzaro, C. (2018). Pre-conceptional and peri-gestational maternal Binge Alcohol Drinking produces inheritance of mood disturbances and alcohol vulnerability in the adolescent offspring. Front. Psychiatry *9*: 1–13.

Breslow, R. a, Falk, D.E., Fein, S.B., and Grummer-Strawn, L.M. (2007). Alcohol consumption among breastfeeding women. Breastfeed. Med. *2*: 152–7.

Broadwater, M.A., Lee, S.-H., Yu, Y., Zhu, H., Crews, F.T., Robinson, D.L., et al. (2018). Adolescent alcohol exposure decreases frontostriatal resting-state functional connectivity in adulthood. Addict. Biol. *23*: 810–823.

Brocardo, P.S., Gil-Mohapel, J., and Christie, B.R. (2011). The role of oxidative stress in fetal alcohol spectrum disorders. Brain Res. Rev. *67*: 209–225.

Brocardo, P.S., Gil-Mohapel, J., Wortman, R., Noonan, A., Mcginnis, E., Patten, A.R., et al. (2016). The Effects of Ethanol Exposure During Distinct Periods of Brain Development on Oxidative Stress in the Adult Rat Brain. Alcohol. Clin. Exp. Res. *41*: 26–37.

Brolese, G., Lunardi, P., Broetto, N., Engelke, D.S., L??rio, F., Batassini, C., et al. (2014). Moderate prenatal alcohol exposure alters behavior and neuroglial parameters in adolescent rats. Behav. Brain Res. *269*: 175–184.

Brolese, G., Lunardi, P., Souza, D.F. de, Lopes, F.M., Leite, M.C., and Gonçalves, C.-A. (2015). Pre- and postnatal exposure to moderate levels of ethanol can have long-lasting effects on hippocampal glutamate uptake in adolescent offspring. PLoS One *10*: e0127845.

Brown, J.M., Bland, R., Jonsson, E., and Greenshaw, A.J. (2019a). A Brief History of Awareness of the Link Between Alcohol and Fetal Alcohol Spectrum Disorder. Can. J. Psychiatry *64*: 164–168.

Brown, J.M., Bland, R., Jonsson, E., and Greenshaw, A.J. (2019b). The Standardization of Diagnostic Criteria for Fetal Alcohol Spectrum Disorder (FASD): Implications for Research, Clinical Practice and Population Health. Can. J. Psychiatry *64*: 169–176.

Bujalka, H., Koenning, M., Jackson, S., Perreau, V.M., Pope, B., Hay, C.M., et al.

(2013). MYRF is a membrane-associated transcription factor that autoproteolytically cleaves to directly activate myelin genes. PLoS Biol. *11*: e1001625.

Burd, L., Blair, J., and Dropps, K. (2012). Prenatal alcohol exposure, blood alcohol concentrations and alcohol elimination rates for the mother, fetus and newborn. J. Perinatol. *32*: 652–659.

Burton, R. (1621). The anatomy of melancholy (Weinheim (Germany): Clarendon Press).

Byun, K., Bayarsaikhan, D., Bayarsaikhan, E., Son, M., Oh, S., Lee, J., et al. (2014). Microglial AGE-albumin is critical in promoting alcohol-induced neurodegeneration in rats and humans. PLoS One *9*: e104699.

Caetano, R., Ramisetty-Mikler, S., Floyd, L.R., and McGrath, C. (2006). The epidemiology of drinking among women of child-bearing age. Alcohol. Clin. Exp. Res. *30*: 1023–30.

Calhoun, F., and Warren, K. (2007). Fetal alcohol syndrome: Historical perspectives. Neurosci. Biobehav. Rev. *31*: 168–171.

Campbell, J.C., Stipcevic, T., Flores, R.E., Perry, C., and Kippin, T.E. (2014). Alcohol exposure inhibits adult neural stem cell proliferation. Exp. Brain Res. *232*: 2775–2784.

Campo, M. Del, and Jones, K.L. (2017). A review of the physical features of the fetal alcohol spectrum disorders. Eur. J. Med. Genet. *60*: 55–64.

Cantacorps, L., Alfonso-Loeches, S., Guerri, C., and Valverde, O. (2019). Long-term epigenetic changes in offspring mice exposed to alcohol during gestation and lactation. J. Psychopharmacol. 026988111985600.

Cantacorps, L., Alfonso-Loeches, S., Moscoso-Castro, M., Cuitavi, J., Gracia-Rubio, I., López-Arnau, R., et al. (2017). Maternal alcohol binge drinking induces persistent neuroinflammation associated with myelin damage and behavioural dysfunctions in offspring mice. Neuropharmacology *123*: 368–384.

Cantacorps, L., González-Pardo, H., Arias, J.L., Valverde, O., and Conejo, N.M. (2018). Altered brain functional connectivity and behaviour in a mouse model of maternal alcohol binge-drinking. Prog. Neuropsychopharmacol. Biol. Psychiatry *84*: 237–249.

Caputo, C., Wood, E., and Jabbour, L. (2016). Impact of fetal alcohol exposure on body systems: A systematic review. Birth Defects Res. Part C - Embryo Today Rev. *108*: 174–180.

Carneiro, L.M. V, Diógenes, J.P.L., Vasconcelos, S.M.M., Aragão, G.F., Noronha, E.C., Gomes, P.B., et al. (2005). Behavioral and neurochemical effects on rat offspring after prenatal exposure to ethanol. Neurotoxicol. Teratol. *27*: 585–92.

Carpentier, P.A., Duncan, D.S., and Miller, S.D. (2008). Glial toll-like receptor signaling in central nervous system infection and autoimmunity. Brain. Behav. Immun. *22*: 140–147.

Carr, G.D., Phillips, A.G., and Fibiger, H.C. (1988). Independence of amphetamine reward from locomotor stimulation demonstrated by conditioned place preference. Psychopharmacology (Berl). *94*: 221–6.

CDC (2017). Data & Statistics | FASD | NCBDDD | CDC.

Cebolla, A.M., Cheron, G., Hourez, R., Bearzatto, B., Dan, B., and Servais, L. (2009). Effects of maternal alcohol consumption during breastfeeding on motor and cerebellar Purkinje cells behavior in mice. Neurosci. Lett. *455*: 4–7.

Cesconetto, P.A., Andrade, C.M., Cattani, D., Domingues, J.T., Parisotto, E.B., Filho, D.W., et al. (2016). Maternal Exposure to Ethanol During Pregnancy and Lactation Affects Glutamatergic System and Induces Oxidative Stress in Offspring Hippocampus. Alcohol. Clin. Exp. Res. *40*: 52–61.

Chabenne, A., Moon, C., Ojo, C., Khogali, A., Nepal, B., and Sharma, S. (2014). Biomarkers in fetal alcohol syndrome. Biomarkers Genomic Med. *6*: 12–22.

Chambers, A.L., Maqbool, A., Fowler, M., and Wigmore, P. (2015). Adult hippocampal neurogenesis: Role in cognition and disease. In Dentate Gyrus: Structure, Role in Disease, and Potential Health Implications, Zackery Lowes, ed. pp 1–46.

Chandra, V., Pandav, R., Dodge, H.H., Johnston, J.M., Belle, S.H., DeKosky, S.T., et al. (2001). Incidence of Alzheimer's disease in a rural community in India: The Indo-US Study. Neurology *57*: 985–989.

Chang, G.-Q., Karatayev, O., and Leibowitz, S.F. (2015). Prenatal exposure to ethanol stimulates hypothalamic CCR2 chemokine receptor system: Possible relation to increased density of orexigenic peptide neurons and ethanol drinking in adolescent offspring. Neuroscience *310*: 163–175.

Chappell, T.D., Margret, C.P., Li, C.X., and Waters, R.S. (2007). Long-term effects of prenatal alcohol exposure on the size of the whisker representation in juvenile and adult rat barrel cortex. Alcohol *41*: 239–51.

Chastain, L.G., Franklin, T., Gangisetty, O., Cabrera, M.A., Mukherjee, S., Shrivastava, P., et al. (2019). Early life alcohol exposure primes hypothalamic microglia to later-life hypersensitivity to immune stress: possible epigenetic mechanism. Neuropsychopharmacology *44*: 1579–1588.

Chastain, L.G., and Sarkar, D.K. (2014). Role of microglia in regulation of ethanol neurotoxic action. Int. Rev. Neurobiol. *118*: 81–103.

Chastain, L.G., and Sarkar, D.K. (2017). Alcohol effects on the epigenome in the germline: Role in the inheritance of alcohol-related pathology. Alcohol *60*: 53–66.

Chater-Diehl, E.J., Laufer, B.I., Castellani, C.A., Alberry, B.L., and Singh, S.M. (2016). Alteration of Gene Expression, DNA Methylation, and Histone Methylation in Free Radical Scavenging Networks in Adult Mouse Hippocampus following Fetal Alcohol Exposure. PLoS One *11*: e0154836.

Chater-Diehl, E.J., Laufer, B.I., and Singh, S.M. (2017). Changes to histone modifications following prenatal alcohol exposure: An emerging picture. Alcohol *60*: 41–52.

Chen, G., Liu, S., Pan, R., Li, G., Tang, H., Jiang, M., et al. (2018). Curcumin Attenuates gp120-Induced Microglial Inflammation by Inhibiting Autophagy via the PI3K Pathway. Cell. Mol. Neurobiol. *38*: 1465–1477.

Chen, Q., Kogan, J.H., Gross, A.K., Zhou, Y., Walton, N.M., Shin, R., et al. (2012). SREB2/GPR85, a schizophrenia risk factor, negatively regulates hippocampal adult neurogenesis and neurogenesis-dependent learning and memory. Eur. J. Neurosci. *36*: 2597–2608.

Chen, W.-J. a, Maier, S.E., Parnell, S.E., and West, J.R. (2003). Alcohol and the developing brain: neuroanatomical studies. Alcohol Res. Health *27*: 174–180.

Chimakurthy, J., and Talasila, M. (2010). Effects of curcumin on pentylenetetrazole-induced anxiety-like behaviors and associated changes in cognition and monoamine levels. Psychol. Neurosci. *3*: 239–244.

Cho, J.-W., Lee, K.-S., and Kim, C.-W. (2007). Curcumin attenuates the expression of IL-1β, IL-6, and TNF-α as well as cyclin E in TNF-α-treated HaCaT cells; NF-κB and MAPKs as potential upstream targets. Int. J. Mol. Med. *19*: 469–474.

Choi, I.Y., Allan, A.M., and Cunningham, L.A. (2005). Moderate Fetal Alcohol Exposure Impairs the Neurogenic Response to an Enriched Environment in Adult Mice. Alcohol. Clin. Exp. Res. *29*: 2053–2062.

Choong, K., and Shen, R. (2004a). Methylphenidate restores ventral tegmental area dopamine neuron activity in prenatal ethanol-exposed rats by augmenting dopamine neurotransmission. J. Pharmacol. Exp. Ther. *309*: 444–51.

Choong, K., and Shen, R. (2004b). Prenatal ethanol exposure alters the postnatal development of the spontaneous electrical activity of dopamine neurons in the ventral tegmental area. Neuroscience *126*: 1083–1091.

Chotro, M.G., and Arias, C. (2006). Exposure to low and moderate doses of alcohol on late gestation modifies infantile response to and preference for alcohol in rats. Ann. Ist. Super. Sanita *42*: 22–30.

Chotro, M.G., Arias, C., and Laviola, G. (2007). Increased ethanol intake after prenatal ethanol exposure: studies with animals. Neurosci. Biobehav. Rev. *31*: 181–91.

Christensen, H.B., and Bilenberg, N. (2000). Behavioural and emotional problems in children of alcoholic mothers and fathers. Eur. Child Adolesc. Psychiatry *9*: 219–226.

Chudley, A., Conry, J., Cook, J., Loock, C., Rosales, T., and LeBlanc, N. (2005). Fetal alcohol spectrum disorder: Canadian guidelines for diagnosis. Can. Med. Assoc. J. *172*: S1–S21.

Church, M.W., Abel, E.L., Dintcheff, B.A., and Matyjasik, C. (1990). Maternal age and blood alcohol concentration in the pregnant Long-Evans rat. J. Pharmacol. Exp. Ther. *253*: 192–9.

Ciafrè, S., Carito, V., Tirassa, P., Ferraguti, G., Attilia, M.L., Ciolli, P., et al. (2018). Ethanol Consumption and Innate Neuroimmunity. Biomed. Rev. *28*: 49.

Clarke, M.E., and Gibbard, W.B. (2003). Overview of fetal alcohol spectrum disorders for mental health professionals. Can. Child Adolesc. Psychiatr. Rev. *12*: 57–63.

Climent, E., Pascual, M., Renau-Piqueras, J., and Guerri, C. (2002). Ethanol exposure enhances cell death in the developing cerebral cortex: role of brain-derived neurotrophic factor and its signaling pathways. J. Neurosci. Res. *68*: 213–25.

Cobben, J.M., Krzyzewska, I.M., Venema, A., Mul, A.N., Polstra, A., Postma, A. V, et al. (2019). DNA methylation abundantly associates with fetal alcohol spectrum disorder and its subphenotypes. Epigenomics *11*: 767–785.

Coleman, L.G., Oguz, I., Lee, J., Styner, M., and Crews, F.T. (2012). Postnatal day 7 ethanol treatment causes persistent reductions in adult mouse brain volume and cortical neurons with sex specific effects on neurogenesis. Alcohol *46*: 603–12.

Coller, J.K., and Hutchinson, M.R. (2012). Implications of central immune signaling caused by drugs of abuse: mechanisms, mediators and new therapeutic approaches for prediction and treatment of drug dependence. Pharmacol. Ther. *134*: 219–45.

Collins, M.A., and Neafsey, E.J. (2012). Neuroinflammatory pathways in binge alcohol-induced neuronal degeneration: oxidative stress cascade involving aquaporin, brain edema, and phospholipase A2 activation. Neurotox. Res. *21*: 70–8.

Collins, M.A., and Neafsey, E.J. (2016). Alcohol, Excitotoxicity and Adult Brain Damage: An Experimentally Unproven Chain-of-Events. Front. Mol. Neurosci. *9*: 8–11.

Colombo, E., and Farina, C. (2016). Astrocytes: Key Regulators of Neuroinflammation. Trends Immunol. *37*: 608–620.

Comeau, W.L., Winstanley, C.A., and Weinberg, J. (2014). Prenatal alcohol exposure

and adolescent stress - unmasking persistent attentional deficits in rats. Eur. J. Neurosci. *40*: 3078–3095.

Conejo, N.M., Cimadevilla, J.M., González-Pardo, H., Méndez-Couz, M., and Arias, J.L. (2013). Hippocampal inactivation with TTX impairs long-term spatial memory retrieval and modifies brain metabolic activity. PLoS One *8*: e64749.

Connor, P.D., Sampson, P.D., Streissguth, A.P., Bookstein, F.L., and Barr, H.M. (2006). Effects of prenatal alcohol exposure on fine motor coordination and balance: A study of two adult samples. Neuropsychologia *44*: 744–751.

Conrad, K.L., Tseng, K.Y., Uejima, J.L., Reimers, J.M., Heng, L.-J., Shaham, Y., et al. (2008). Formation of accumbens GluR2-lacking AMPA receptors mediates incubation of cocaine craving. Nature *454*: 118–21.

Cook, J.L., Green, C.R., Lilley, C.M., Anderson, S.M., Baldwin, M.E., Chudley, A.E., et al. (2016). Fetal alcohol spectrum disorder: a guideline for diagnosis across the lifespan. Can. Med. Assoc. J. *188*: 191–197.

Cooper, S., Robison, A.J., and Mazei-Robison, M.S. (2017). Reward Circuitry in Addiction. Neurotherapeutics *14*: 687–697.

Cortese, B.M., Moore, G.J., Bailey, B.A., Jacobson, S.W., Delaney-Black, V., and Hannigan, J.H. (2006). Magnetic resonance and spectroscopic imaging in prenatal alcohol-exposed children: Preliminary findings in the caudate nucleus. Neurotoxicol. Teratol. *28*: 597–606.

Cowan, M., and Petri, W.A. (2018). Microglia: Immune Regulators of Neurodevelopment. Front. Immunol. *9*: 2576.

Crabbe, J.C., Harris, R.A., and Koob, G.F. (2011). Preclinical studies of alcohol binge drinking. Ann. N. Y. Acad. Sci. *1216*: 24–40.

Creeley, C.E., Dikranian, K.T., Johnson, S.A., Farber, N.B., and Olney, J.W. (2013). Alcohol-induced apoptosis of oligodendrocytes in the fetal macaque brain. Acta Neuropathol. Commun. *1*: 23.

Crews, F.T., Collins, M. a., Dlugos, C., Littleton, J., Wilkins, L., Neafsey, E.J., et al. (2004). Alcohol-induced neurodegeneration: when, where and why? Alcohol. Clin. Exp. Res. *28*: 350–64.

Crews, F.T., Mdzinarishvili, A., Kim, D., He, J., and Nixon, K. (2006a). Neurogenesis in adolescent brain is potently inhibited by ethanol. Neuroscience *137*: 437–445.

Crews, F.T., and Nixon, K. (2003). Alcohol, neural stem cells, and adult neurogenesis. Alcohol Res. Health *27*: 197–204.

Crews, F.T., and Nixon, K. (2009). Mechanisms of neurodegeneration and

regeneration in alcoholism. Alcohol Alcohol *44*: 115–27.

Crews, F.T., Nixon, K., Kim, D., Joseph, J., Shukitt-Hale, B., Qin, L., et al. (2006b). BHT blocks NF-kB activation and ethanol-induced brain damage. Alcohol. Clin. Exp. Res. *30*: 1938–1949.

Crews, F.T., Qin, L., Sheedy, D., Vetreno, R.P., and Zou, J. (2013). High mobility group box 1/Toll-like receptor danger signaling increases brain neuroimmune activation in alcohol dependence. Biol. Psychiatry *73*: 602–12.

Crews, F.T., and Vetreno, R.P. (2014). Neuroimmune basis of alcoholic brain damage. Int. Rev. Neurobiol. *118*: 315–57.

Crews, F.T., and Vetreno, R.P. (2016). Mechanisms of neuroimmune gene induction in alcoholism. Psychopharmacology (Berl). *233*: 1543–57.

Crews, F.T., Walter, T.J., Coleman, L.G., and Vetreno, R.P. (2017). Toll-like receptor signaling and stages of addiction. Psychopharmacology (Berl). *234*: 1483–1498.

Cullen, C.L., Burne, T.H., Lavidis, N. a, and Moritz, K.M. (2014). Low dose prenatal alcohol exposure does not impair spatial learning and memory in two tests in adult and aged rats. PLoS One *9*: e101482.

Cullen, C.L., Burne, T.H.J., Lavidis, N. a., and Moritz, K.M. (2013). Low Dose Prenatal Ethanol Exposure Induces Anxiety-Like Behaviour and Alters Dendritic Morphology in the Basolateral Amygdala of Rat Offspring. PLoS One *8*: 1–12.

Curtis, M.J., Alexander, S., Cirino, G., Docherty, J.R., George, C.H., Giembycz, M.A., et al. (2018). Experimental design and analysis and their reporting II: updated and simplified guidance for authors and peer reviewers. Br. J. Pharmacol. *175*: 987–993.

Czeh, M., Gressens, P., and Kaindl, A.M. (2011). The yin and yang of microglia. Dev. Neurosci. *33*: 199–209.

Daft, P.A., Johnston, M.C., and Sulik, K.K. (1986). Abnormal heart and great vessel development following acute ethanol exposure in mice. Teratology *33*: 93–104.

Danglot, L., Triller, A., and Marty, S. (2006). The development of hippocampal interneurons in rodents. Hippocampus *16*: 1032–1060.

Dasgupta, A. (2017). Alcohol a double-edged sword. In Alcohol, Drugs, Genes and the Clinical Laboratory, (Elsevier), pp 1–21.

Dasgupta, S., Adams, J. a, and Hogan, E.L. (2007). Maternal alcohol consumption increases sphingosine levels in the brains of progeny mice. Neurochem. Res. *32*: 2217–24.

Davoren, M.P., Demant, J., Shiely, F., and Perry, I.J. (2016). Alcohol consumption among university students in Ireland and the United Kingdom from 2002 to 2014: A

systematic review. BMC Public Health *16*:.

Deacon, R.M.J., and Rawlins, J.N.P. (2006). T-maze alternation in the rodent. Nat. Protoc. *1*: 7–12.

Deguchi, A. (2015). Curcumin Targets in Inflammation and Cancer. Endocrine, Metab. Immune Disord. Targets *15*: 88–96.

Delano, K., Koren, G., Zack, M., and Kapur, B.M. (2019). Prevalence of Fetal Alcohol Exposure by Analysis of Meconium Fatty Acid Ethyl Esters; A National Canadian Study. Sci. Rep. *9*: 1–6.

Delatour, L.C., Yeh, P.W., and Yeh, H.H. (2019). Ethanol Exposure In Utero Disrupts Radial Migration and Pyramidal Cell Development in the Somatosensory Cortex. Cereb. Cortex *29*: 2125–2139.

Dembele, K., Yao, X.-H., Chen, L., and Nyomba, B.L.G. (2006). Intrauterine ethanol exposure results in hypothalamic oxidative stress and neuroendocrine alterations in adult rat offspring. Am. J. Physiol. Regul. Integr. Comp. Physiol. *291*: R796-802.

Deng, X.-S., and Deitrich, R. a (2007). Ethanol metabolism and effects: nitric oxide and its interaction. Curr. Clin. Pharmacol. *2*: 145–53.

Denny, L., Coles, S., and Blitz, R. (2017). Fetal Alcohol Syndrome and Fetal Alcohol Spectrum Disorders. Am. Fam. Physician *96*: 515–522.

Dhanabalan, G., Maître, T.W. Le, Bogdanovic, N., Alkass, K., and Druid, H. (2018). Hippocampal granule cell loss in human chronic alcohol abusers. Neurobiol. Dis. *120*: 63–75.

Dikranian, K., Qin, Y.Q., Labruyere, J., Nemmers, B., and Olney, J.W. (2005). Ethanol-induced neuroapoptosis in the developing rodent cerebellum and related brain stem structures. Dev. Brain Res. *155*: 1–13.

Donald, K.A., Eastman, E., Howells, F.M., Adnams, C., Riley, E.P., Woods, R.P., et al. (2015). Neuroimaging effects of prenatal alcohol exposure on the developing human brain: a magnetic resonance imaging review. Acta Neuropsychiatr. *27*: 251–269.

Dong, J., Sulik, K.K., and Chen, S. (2010). The role of NOX enzymes in ethanol-induced oxidative stress and apoptosis in mouse embryos. Toxicol. Lett. *193*: 94–100.

Dong, S., Zeng, Q., Mitchell, E.S., Xiu, J., Duan, Y., Li, C., et al. (2012). Curcumin enhances neurogenesis and cognition in aged rats: implications for transcriptional interactions related to growth and synaptic plasticity. PLoS One *7*: e31211.

Dong, Y., and Nestler, E.J. (2014). The neural rejuvenation hypothesis of cocaine addiction. Trends Pharmacol. Sci. *35*: 374–83.

Dörrie, N., Föcker, M., Freunscht, I., and Hebebrand, J. (2014). Fetal alcohol spectrum disorders. Eur. Child Adolesc. Psychiatry *23*: 863–75.

Doyle, L.R., and Mattson, S.N. (2015). Neurobehavioral Disorder Associated with Prenatal Alcohol Exposure (ND-PAE): Review of Evidence and Guidelines for Assessment. Curr. Dev. Disord. Reports *2*: 175–186.

Drew, P.D., and Kane, C.J.M. (2014). Fetal alcohol spectrum disorders and neuroimmune changes. Int. Rev. Neurobiol. *118*: 41–80.

Dumas, A., Toutain, S., and Simmat-Durand, L. (2017). Alcohol Use During Pregnancy or Breastfeeding: A National Survey in France. J. Womens. Health (Larchmt). *26*: 798–805.

Dursun, I., Jakubowska-Doğru, E., and Uzbay, T. (2006). Effects of prenatal exposure to alcohol on activity, anxiety, motor coordination, and memory in young adult Wistar rats. Pharmacol. Biochem. Behav. *85*: 345–55.

Eberhart, J.K., and Parnell, S.E. (2016). The Genetics of Fetal Alcohol Spectrum Disorders. Alcohol. Clin. Exp. Res. *40*: 1154–65.

EDADES (2018). Observatorio Español de las Drogas y las Adicciones.

Elliott, E.J., Payne, J., Morris, A., Haan, E., and Bower, C. (2008). Fetal alcohol syndrome: a prospective national surveillance study. Arch. Dis. Child. *93*: 732–7.

Emery, B. (2010). Regulation of Oligodendrocyte Differentiation and Myelination. Science (80-.). *330*: 779–782.

Emery, B., Agalliu, D., Cahoy, J.D., Watkins, T.A., Dugas, J.C., Mulinyawe, S.B., et al. (2009). Myelin Gene Regulatory Factor Is a Critical Transcriptional Regulator Required for CNS Myelination. Cell *138*: 172–185.

Engblom, D., Bilbao, A., Sanchis-Segura, C., Dahan, L., Perreau-Lenz, S., Balland, B., et al. (2008). Glutamate Receptors on Dopamine Neurons Control the Persistence of Cocaine Seeking. Neuron *59*: 497–508.

Erdozain, A.M., and Callado, L.F. (2014). Neurobiological alterations in alcohol addiction: a review. Adicciones *26*: 360–70.

Erickson, E.K., Grantham, E.K., Warden, A.S., and Harris, R.A. (2019). Neuroimmune signaling in alcohol use disorder. Pharmacol. Biochem. Behav. *177*: 34–60.

Esteve-Arenys, A., Gracia-Rubio, I., Cantacorps, L., Pozo, O.J., Marcos, J., Rodríguez-Árias, M., et al. (2017). Binge ethanol drinking during adolescence modifies cocaine responses in mice. J. Psychopharmacol. *31*: 86–95.

ESTUDES (2017). Observatorio Español de las Drogas y las Adicciones.

Everitt, B.J., and Robbins, T.W. (2005). Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. Nat. Neurosci. *8*: 1481–9.

Everitt, B.J., and Robbins, T.W. (2013). From the ventral to the dorsal striatum: Devolving views of their roles in drug addiction. Neurosci. Biobehav. Rev. *37*: 1946–1954.

Fabio, M.C., Macchione, A.F., Nizhnikov, M.E., and Pautassi, R.M. (2015a). Prenatal ethanol increases ethanol intake throughout adolescence, alters ethanol-mediated aversive learning, and affects μ but not δ or κ opioid receptor mRNA expression. Eur. J. Neurosci. *41*: 1569–79.

Fabio, M.C., March, S.M., Molina, J.C., Nizhnikov, M.E., Spear, N.E., and Pautassi, R.M. (2013). Prenatal ethanol exposure increases ethanol intake and reduces C-fos expression in infralimbic cortex of adolescent rats. Pharmacol. Biochem. Behav. *103*: 842–852.

Fabio, M.C., Vivas, L.M., and Pautassi, R.M. (2015b). Prenatal ethanol exposure alters ethanol-induced Fos immunoreactivity and dopaminergic activity in the mesocorticolimbic pathway of the adolescent brain. Neuroscience *301*: 221–234.

Fagerlund, A., Heikkinen, S., Autti-Rämö, I., Korkman, M., Timonen, M., Kuusi, T., et al. (2006). Brain metabolic alterations in adolescents and young adults with fetal alcohol spectrum disorders. Alcohol. Clin. Exp. Res. *30*: 2097–104.

Fan, J., Jacobson, S.W., Taylor, P.A., Molteno, C.D., Dodge, N.C., Stanton, M.E., et al. (2016). White matter deficits mediate effects of prenatal alcohol exposure on cognitive development in childhood. Hum. Brain Mapp. *37*: 2943–2958.

Farag, M. (2014). Diagnostic issues affecting the epidemiology of fetal alcohol spectrum disorders. J. Popul. Ther. Clin. Pharmacol. *21*: e153-8.

Farrelly, L., Föcking, M., Piontkewitz, Y., Dicker, P., English, J., Wynne, K., et al. (2015). Maternal immune activation induces changes in myelin and metabolic proteins, some of which can be prevented with risperidone in adolescence. Dev. Neurosci. *37*: 43–55.

Feizolahi, F., Azarbayjani, M.-A., Nasehi, M., Peeri, M., and Zarrindast, M.-R. (2019). The combination of swimming and curcumin consumption may improve spatial memory recovery after binge ethanol drinking. Physiol. Behav. *207*: 139–150.

Feltenstein, M.W., and See, R.E. (2008). The neurocircuitry of addiction: an overview. Br. J. Pharmacol. *154*: 261–74.

Fernandez-Lizarbe, S., Montesinos, J., and Guerri, C. (2013). Ethanol induces TLR4/TLR2 association, triggering an inflammatory response in microglial cells. J. Neurochem. *126*: 261–73.

Fernandez-Lizarbe, S., Pascual, M., and Guerri, C. (2009). Critical role of TLR4 response in the activation of microglia induced by ethanol. J. Immunol. *183*: 4733–44.

Filali, M., and Lalonde, R. (2009). Age-related cognitive decline and nesting behavior in an APPswe/PS1 bigenic model of Alzheimer's disease. Brain Res. *1292*: 93–9.

Flak, A.L., Su, S., Bertrand, J., Denny, C.H., Kesmodel, U.S., and Cogswell, M.E. (2014). The Association of Mild, Moderate, and Binge Prenatal Alcohol Exposure and Child Neuropsychological Outcomes: A Meta-Analysis. Alcohol. Clin. Exp. Res. *38*: 214–226.

Fodor, A., Klausz, B., Pintér, O., Daviu, N., Rabasa, C., Rotllant, D., et al. (2012). Maternal neglect with reduced depressive-like behavior and blunted c-fos activation in Brattleboro mothers, the role of central vasopressin. Horm. Behav. *62*: 539–51.

Fontaine, C.J., Patten, A.R., Sickmann, H.M., Helfer, J.L., and Christie, B.R. (2016). Effects of pre-natal alcohol exposure on hippocampal synaptic plasticity: Sex, age and methodological considerations. Neurosci. Biobehav. Rev. *64*: 12–34.

Franchi, L., Eigenbrod, T., Muñoz-Planillo, R., and Nuñez, G. (2009). The inflammasome: a caspase-1-activation platform that regulates immune responses and disease pathogenesis. Nat. Immunol. *10*: 241–7.

Frey, S., Eichler, A., Stonawski, V., Kriebel, J., Wahl, S., Gallati, S., et al. (2018). Prenatal Alcohol Exposure Is Associated With Adverse Cognitive Effects and Distinct Whole-Genome DNA Methylation Patterns in Primary School Children. Front. Behav. Neurosci. *12*: 1–13.

Fuhrmann, D., Knoll, L.J., and Blakemore, S.J. (2015). Adolescence as a Sensitive Period of Brain Development. Trends Cogn. Sci. *19*: 558–566.

Gaesser, J.M., and Fyffe-Maricich, S.L. (2016). Intracellular signaling pathway regulation of myelination and remyelination in the CNS. Exp. Neurol. *283*: 501–511.

Galindo, R., Zamudio, P.A., and Valenzuela, C.F. (2005). Alcohol is a potent stimulant of immature neuronal networks: Implications for fetal alcohol spectrum disorder. J. Neurochem. *94*: 1500–1511.

Gangisetty, O., Bekdash, R., Maglakelidze, G., and Sarkar, D.K. (2014). Fetal Alcohol Exposure Alters Proopiomelanocortin Gene Expression and Hypothalamic-Pituitary-Adrenal Axis Function via Increasing MeCP2 Expression in the Hypothalamus. PLoS One *9*: e113228.

Gangisetty, O., Wynne, O., Jabbar, S., Nasello, C., and Sarkar, D.K. (2015). Fetal Alcohol Exposure Reduces Dopamine Receptor D2 and Increases Pituitary Weight and Prolactin Production via Epigenetic Mechanisms. PLoS One *10*: e0140699.

Garden, G.A. (2013). Epigenetics and the Modulation of Neuroinflammation.

Neurotherapeutics *10*: 782–788.

Garro, A.J., McBeth, D.L., Lima, V., and Lieber, C.S. (1991). Ethanol consumption inhibits fetal DNA methylation in mice: implications for the fetal alcohol syndrome. Alcohol. Clin. Exp. Res. *15*: 395–8.

Gautam, P., Lebel, C., Narr, K.L., Mattson, S.N., May, P.A., Adnams, C.M., et al. (2015). Volume changes and brain-behavior relationships in white matter and subcortical gray matter in children with prenatal alcohol exposure. Hum. Brain Mapp. *36*: 2318–29.

GBD 2016 Alcohol Collaborators (2018). Alcohol use and burden for 195 countries and territories, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet (London, England) *392*: 1015–1035.

Geil, C.R., Hayes, D.M., McClain, J.A., Liput, D.J., Marshall, S.A., Chen, K.Y., et al. (2014). Alcohol and adult hippocampal neurogenesis: promiscuous drug, wanton effects. Prog. Neuropsychopharmacol. Biol. Psychiatry *54*: 103–13.

Georgieff, M.K., Tran, P. V., and Carlson, E.S. (2018). Atypical fetal development: Fetal alcohol syndrome, nutritional deprivation, teratogens, and risk for neurodevelopmental disorders and psychopathology. Dev. Psychopathol. *30*: 1063–1086.

Gerdeman, G.L., Partridge, J.G., Lupica, C.R., and Lovinger, D.M. (2003). It could be habit forming: drugs of abuse and striatal synaptic plasticity. Trends Neurosci. *26*: 184–92.

Ghasemi, F., Bagheri, H., Barreto, G.E., Read, M.I., and Sahebkar, A. (2019). Effects of Curcumin on Microglial Cells. Neurotox. Res. *36*: 12–26.

Gil-Mohapel, J., Boehme, F., Kainer, L., and Christie, B.R. (2010). Hippocampal cell loss and neurogenesis after fetal alcohol exposure: Insights from different rodent models. Brain Res. Rev. *64*: 283–303.

Gil-Mohapel, J., Boehme, F., Patten, A., Cox, A., Kainer, L., Giles, E., et al. (2011). Altered adult hippocampal neuronal maturation in a rat model of fetal alcohol syndrome. Brain Res. *1384*: 29–41.

Gil-Mohapel, J., Titterness, a. K., Patten, a. R., Taylor, S., Ratzlaff, A., Ratzlaff, T., et al. (2014). Prenatal ethanol exposure differentially affects hippocampal neurogenesis in the adolescent and aged brain. Neuroscience *273*: 174–188.

Gilpin, N.W., Herman, M.A., and Roberto, M. (2015). The Central Amygdala as an Integrative Hub for Anxiety and Alcohol Use Disorders. Biol. Psychiatry *77*: 859–869.

Glantz, M.D., and Chambers, J.C. (2006). Prenatal drug exposure effects on subsequent vulnerability to drug abuse. Dev. Psychopathol. *18*: 893–922.

Glass, C.K., Saijo, K., Winner, B., Marchetto, M.C., and Gage, F.H. (2010). Mechanisms Underlying Inflammation in Neurodegeneration. Cell *140*: 918–934.

Goh, Y.I., Ungar, W., Rovet, J., and Koren, G. (2007). Mega-dose vitamin C and E in preventing FASD: The decision to terminate the study prematurely. J. FAS Int. *5*: 1–3.

Golub, H.M., Zhou, Q.-G., Zucker, H., McMullen, M.R., Kokiko-Cochran, O.N., Ro, E.J., et al. (2015). Chronic Alcohol Exposure is Associated with Decreased Neurogenesis, Aberrant Integration of Newborn Neurons, and Cognitive Dysfunction in Female Mice. Alcohol. Clin. Exp. Res. *39*: 1967–1977.

Gonzales-Lima, F., and Jones, D. (1994). Quantitative mapping of cytovhrome oxidase activity in the central auditory system of the gerbil: a study with calibrated activity standards and metal-intensified histochemistry. Brain Res. *660*: 34–49.

Gonzalez-Lima, F., and Cada, A. (1998). Quantitative Histochemistry of Cytochrome Oxidase Activity. In Cytochrome Oxidase in Neuronal Metabolism and Alzheimer's Disease, (Boston, MA: Springer US), pp 55–90.

González-Pardo, H., Conejo, N.M., and Arias, J.L. (2006). Oxidative metabolism of limbic structures after acute administration of diazepam, alprazolam and zolpidem. Prog. Neuro-Psychopharmacology Biol. Psychiatry *30*: 1020–1026.

Gore-Langton, J.K., and Spear, L.P. (2019). Prenatal ethanol exposure attenuates sensitivity to the aversive effects of ethanol in adolescence and increases adult preference for a 5% ethanol solution in males, but not females. Alcohol *79*: 59–69.

Gould, T.D., and Manji, H.K. (2005). DARPP-32: A molecular switch at the nexus of reward pathway plasticity. Proc. Natl. Acad. Sci. *102*: 253–254.

Graaf-Peters, V.B. de, and Hadders-Algra, M. (2006). Ontogeny of the human central nervous system: What is happening when? Early Hum. Dev. *82*: 257–266.

Gracia-Rubio, I., Martinez-Laorden, E., Moscoso-Castro, M., Milanés, M.V., Laorden, M.L., and Valverde, O. (2016a). Maternal Separation Impairs Cocaine-Induced Behavioural Sensitization in Adolescent Mice. PLoS One *11*: e0167483.

Gracia-Rubio, I., Moscoso-Castro, M., Pozo, O.J., Marcos, J., Nadal, R., and Valverde, O. (2016b). Maternal separation induces neuroinflammation and long-lasting emotional alterations in mice. Prog. Neuro-Psychopharmacology Biol. Psychiatry *65*: 104–117.

Gräff, J., and Tsai, L.-H. (2013). The Potential of HDAC Inhibitors as Cognitive Enhancers. Annu. Rev. Pharmacol. Toxicol. *53*: 311–330.

Granato, A., and Dering, B. (2018). Alcohol and the Developing Brain: Why Neurons Die and How Survivors Change. Int. J. Mol. Sci. *19*: 2992.

Green, H.F., and Nolan, Y.M. (2014). Inflammation and the developing brain: consequences for hippocampal neurogenesis and behavior. Neurosci. Biobehav. Rev. *40*: 20–34.

Greenmyer, J.R., Klug, M.G., Kambeitz, C., Popova, S., and Burd, L. (2018). A Multicountry Updated Assessment of the Economic Impact of Fetal Alcohol Spectrum Disorder. J. Addict. Med. *12*: 466–473.

Guan, J.S., Haggarty, S.J., Giacometti, E., Dannenberg, J.H., Joseph, N., Gao, J., et al. (2009). HDAC2 negatively regulates memory formation and synaptic plasticity. Nature *459*: 55–60.

Guerri, C. (1998). Neuroanatomical and neurophysiological mechanisms involved in central nervous system dysfunctions induced by prenatal alcohol exposure. Alcohol. Clin. Exp. Res. *22*: 304–312.

Guerri, C. (2002). Mechanisms involved in central nervous system dysfunctions induced by prenatal ethanol exposure. Neurotox. Res. *4*: 327–35.

Guerri, C., Bazinet, A., and Riley, E.P. (2009). Foetal Alcohol Spectrum Disorders and alterations in brain and behaviour. Alcohol Alcohol *44*: 108–14.

Guerri, C., and Grisolía, S. (1982). Effects of prenatal and postnatal exposure of rats to alcohol: changes in (Na+-K+) ATPase. Pharmacol. Biochem. Behav. *17*: 927–32.

Guerri, C., and Pascual, M. (2010). Mechanisms involved in the neurotoxic, cognitive, and neurobehavioral effects of alcohol consumption during adolescence. Alcohol *44*: 15–26.

Guerri, C., and Renau-Piqueras, J. (1997). Alcohol, astroglia, and brain development. Mol. Neurobiol. *15*: 65–81.

Guerri, C., and Sanchis, R. (1986). Alcohol and acetaldehyde in rat's milk following ethanol administration. Life Sci. *38*: 1543–56.

Guizzetti, M., Zhang, X., Goeke, C., and Gavin, D.P. (2014). Glia and neurodevelopment: focus on fetal alcohol spectrum disorders. Front. Pediatr. *2*: 123.

Guo, H., Callaway, J.B., and Ting, J.P.-Y. (2015). Inflammasomes: mechanism of action, role in disease, and therapeutics. Nat. Med. *21*: 677–687.

Guo, W., Crossey, E.L., Zhang, L., Zucca, S., George, O.L., Valenzuela, C.F., et al. (2011). Alcohol Exposure Decreases CREB Binding Protein Expression and Histone Acetylation in the Developing Cerebellum. PLoS One *6*: e19351.

Gupta, K.K., Gupta, V.K., and Shirasaka, T. (2016). An Update on Fetal Alcohol Syndrome-Pathogenesis, Risks, and Treatment. Alcohol. Clin. Exp. Res. *40*: 1594–602.

Haack, A.K., Sheth, C., Schwager, A.L., Sinclair, M.S., Tandon, S., and Taha, S.A. (2014). Lesions of the Lateral Habenula Increase Voluntary Ethanol Consumption and Operant Self-Administration, Block Yohimbine-Induced Reinstatement of Ethanol Seeking, and Attenuate Ethanol-Induced Conditioned Taste Aversion. PLoS One *9*: e92701.

Haastrup, M.B., Pottegård, A., and Damkier, P. (2014). Alcohol and Breastfeeding. Basic Clin. Pharmacol. Toxicol. *114*: 168–173.

Hamilton, G.F., Bucko, P.J., Miller, D.S., DeAngelis, R.S., Krebs, C.P., and Rhodes, J.S. (2016). Behavioral deficits induced by third-trimester equivalent alcohol exposure in male C57BL/6J mice are not associated with reduced adult hippocampal neurogenesis but are still rescued with voluntary exercise. Behav. Brain Res. *314*: 96–105.

Hamilton, G.F., Jablonski, S.A., Schiffino, F.L., Cyr, S.A. St., Stanton, M.E., and Klintsova, A.Y. (2014). Exercise and environment as an intervention for neonatal alcohol effects on hippocampal adult neurogenesis and learning. Neuroscience *265*: 274–290.

Hamilton, G.F., Murawski, N.J., St.cyr, S.A., Jablonski, S.A., Schiffino, F.L., Stanton, M.E., et al. (2011). Neonatal alcohol exposure disrupts hippocampal neurogenesis and contextual fear conditioning in adult rats. Brain Res. *1412*: 88–101.

Hanamsagar, R., Hanke, M.L., and Kielian, T. (2012). Toll-like receptor (TLR) and inflammasome actions in the central nervous system. Trends Immunol. *33*: 333–342.

Hanisch, U.-K., and Kettenmann, H. (2007). Microglia: active sensor and versatile effector cells in the normal and pathologic brain. Nat. Neurosci. *10*: 1387–94.

Hansen, J.M. (2006). Oxidative stress as a mechanism of teratogenesis. Birth Defects Res. Part C Embryo Today Rev. *78*: 293–307.

Harper, C. (2009). The neuropathology of alcohol-related brain damage. Alcohol Alcohol *44*: 136–40.

Hassan, F.-U., Rehman, M.S.-U., Khan, M.S., Ali, M.A., Javed, A., Nawaz, A., et al. (2019). Curcumin as an Alternative Epigenetic Modulator: Mechanism of Action and Potential Effects. Front. Genet. *10*: 514.

Hausknecht, K., Haj-Dahmane, S., Shen, Y.-L., Vezina, P., Dlugos, C., and Shen, R.-Y. (2015). Excitatory synaptic function and plasticity is persistently altered in ventral tegmental area dopamine neurons after prenatal ethanol exposure. Neuropsychopharmacol. *40*: 893–905.

Haycock, P.C. (2009). Fetal alcohol spectrum disorders: the epigenetic perspective. Biol. Reprod. *81*: 607–17.

He, J., Nixon, K., Shetty, A.K., and Crews, F.T. (2005). Chronic alcohol exposure reduces hippocampal neurogenesis and dendritic growth of newborn neurons. Eur. J. Neurosci. *21*: 2711–2720.

He, Y., Yue, Y., Zheng, X., Zhang, K., Chen, S., and Du, Z. (2015). Curcumin, inflammation, and chronic diseases: how are they linked? Molecules *20*: 9183–213.

Heaton, M.B., Paiva, M., Madorsky, I., Mayer, J., and Moore, D.B. (2003). Effects of ethanol on neurotrophic factors, apoptosis-related proteins, endogenous antioxidants, and reactive oxygen species in neonatal striatum: relationship to periods of vulnerability. Brain Res. Dev. Brain Res. *140*: 237–52.

Heaton, M.B., Paiva, M., Madorsky, I., Siler-Marsiglio, K., and Shaw, G. (2006). Effect of bax deletion on ethanol sensitivity in the neonatal rat cerebellum. J. Neurobiol. *66*: 95–101.

Helfer, J.L., Goodlett, C.R., Greenough, W.T., and Klintsova, A.Y. (2009). The effects of exercise on adolescent hippocampal neurogenesis in a rat model of binge alcohol exposure during the brain growth spurt. Brain Res. *1294*: 1–11.

Helfer, J.L., White, E.R., and Christie, B.R. (2014). Prenatal Ethanol (EtOH) Exposure Alters the Sensitivity of the Adult Dentate Gyrus to Acute EtOH Exposure. Alcohol. Clin. Exp. Res. *38*: 135–143.

Hellemans, K.G.C., Sliwowska, J.H., Verma, P., and Weinberg, J. (2010). Prenatal alcohol exposure: fetal programming and later life vulnerability to stress, depression and anxiety disorders. Neurosci. Biobehav. Rev. *34*: 791–807.

Hellemans, K.G.C., Verma, P., Yoon, E., Yu, W., and Weinberg, J. (2008). Prenatal alcohol exposure increases vulnerability to stress and anxiety-like disorders in adulthood. Ann. N. Y. Acad. Sci. *1144*: 154–175.

Hemmer, B., Archelos, J.J., and Hartung, H.-P. (2002). New concepts in the immunopathogenesis of multiple sclerosis. Nat. Rev. Neurosci. *3*: 291–301.

Heneka, M.T., Kummer, M.P., and Latz, E. (2014). Innate immune activation in neurodegenerative disease. Nat. Rev. Immunol. *14*: 463–477.

Herting, M.M., Fair, D., and Nagel, B.J. (2011). Altered fronto-cerebellar connectivity in alcohol-naïve youth with a family history of alcoholism. Neuroimage *54*: 2582–2589.

Hickman, S., Izzy, S., Sen, P., Morsett, L., and Khoury, J. El (2018). Microglia in neurodegeneration. Nat. Neurosci. *21*: 1359–1369.

Hodge, C. (1903). The influence of alcohol on growth and development. In Physiological Aspects of the Liquor Problem., W.O. Atwater, J.S. Billings, H.P. Bowditch, R.H. Chittenden, and W.H. Welch, eds. (Boston, MA: Houghton Mifflin),

pp 359–375.

Holm, T.H., Draeby, D., and Owens, T. (2012). Microglia are required for astroglial toll-like receptor 4 response and for optimal TLR2 and TLR3 response. Glia *60*: 630–638.

Hoppe, J.B., Coradini, K., Frozza, R.L., Oliveira, C.M., Meneghetti, A.B., Bernardi, A., et al. (2013). Free and nanoencapsulated curcumin suppress β-amyloid-induced cognitive impairments in rats: Involvement of BDNF and Akt/GSK-3β signaling pathway. Neurobiol. Learn. Mem. *106*: 134–144.

Houlé, K., Abdi, M., and Clabough, E.B.D. (2017). Acute ethanol exposure during late mouse neurodevelopment results in long-term deficits in memory retrieval, but not in social responsiveness. Brain Behav. *7*: e00636.

Hoyme, H.E., Kalberg, W.O., Elliott, A.J., Blankenship, J., Buckley, D., Marais, A.-S., et al. (2016). Updated Clinical Guidelines for Diagnosing Fetal Alcohol Spectrum Disorders. Pediatrics *138*: e20154256–e20154256.

Hoyme, H.E., May, P.A., Kalberg, W.O., Kodituwakku, P., Gossage, J.P., Trujillo, P.M., et al. (2005). A Practical Clinical Approach to Diagnosis of Fetal Alcohol Spectrum Disorders: Clarification of the 1996 Institute of Medicine Criteria. Pediatrics *115*: 39–47.

Idrus, N.M., McGough, N.N.H., Riley, E.P., and Thomas, J.D. (2014). Administration of Memantine During Withdrawal Mitigates Overactivity and Spatial Learning Impairments Associated with Neonatal Alcohol Exposure in Rats. Alcohol. Clin. Exp. Res. *38*: 529–537.

Idrus, N.M., and Thomas, J.D. (2011). Fetal alcohol spectrum disorders: experimental treatments and strategies for intervention. Alcohol Res. Health *34*: 76–85.

Ieraci, A., and Herrera, D.G. (2007). Single alcohol exposure in early life damages hippocampal stem/progenitor cells and reduces adult neurogenesis. Neurobiol. Dis. *26*: 597–605.

Ikegami, A., and Duvauchelle, C.L. (2004). Dopamine Mechanisms and Cocaine Reward. Int. Rev. Neurobiol. *62*: 45–94.

Ikonomidou, C., Price, M.T., Stefovska, V., and Ho, F. (2000). Ethanol-Induced Apoptotic Neurodegeneration and Fetal Alcohol Syndrome. Science (80-.). *287*: 1056–1060.

Ikram, M., Saeed, K., Khan, A., Muhammad, T., Khan, M.S., Jo, M.G., et al. (2019). Natural Dietary Supplementation of Curcumin Protects Mice Brains against Ethanol-Induced Oxidative Stress-Mediated Neurodegeneration and Memory Impairment via Nrf2/TLR4/RAGE Signaling. Nutrients *11*: 1082.

Incerti, M., Vink, J., Roberson, R., Wood, L., Abebe, D., and Spong, C.Y. (2010). Reversal of alcohol-induced learning deficits in the young adult in a model of fetal alcohol syndrome. Obstet. Gynecol. *115*: 350–356.

Infanger, D.W., Sharma, R. V., and Davisson, R.L. (2006). NADPH Oxidases of the Brain: Distribution, Regulation, and Function. Antioxid. Redox Signal. *8*: 1583–1596.

Infante, M.A., Moore, E.M., Bischoff-Grethe, A., Tapert, S.F., Mattson, S.N., and Riley, E.P. (2017). Altered functional connectivity during spatial working memory in children with heavy prenatal alcohol exposure. Alcohol *64*: 11–21.

Ishrat, T., Hoda, M.N., Khan, M.B., Yousuf, S., Ahmad, M., Khan, M.M., et al. (2009). Amelioration of cognitive deficits and neurodegeneration by curcumin in rat model of sporadic dementia of Alzheimer's type (SDAT). Eur. Neuropsychopharmacol. *19*: 636–47.

Jaatinen, P., and Rintala, J. (2008). Mechanisms of ethanol-induced degeneration in the developing, mature, and aging cerebellum. The Cerebellum *7*: 332–347.

Jacobson, J.L., and Jacobson, S.W. (2002). Effects of prenatal alcohol exposure on child development. Alcohol Res. Health *26*: 282–6.

Jacobson, S.W. (1997). Assessing the impact of maternal drinking during and after pregnancy. Alcohol Health Res. World *21*: 199–203.

Jacobson, S.W., Carter, R.C., Molteno, C.D., Meintjes, E.M., Senekal, M.S., Lindinger, N.M., et al. (2018). Feasibility and Acceptability of Maternal Choline Supplementation in Heavy Drinking Pregnant Women: A Randomized, Double-Blind, Placebo-Controlled Clinical Trial. Alcohol. Clin. Exp. Res. *42*: 1315–1326.

Jagetia, G., Rajanikant, G., Jagetia, G.C., and Rajanikant, G.K. (2015). Curcumin Stimulates the Antioxidant Mechanisms in Mouse Skin Exposed to Fractionated γ-Irradiation. Antioxidants *4*: 25–41.

Jakubowska-Doğru, E., Elibol, B., Dursun, I., and Yürüker, S. (2017). Effects of prenatal binge-like ethanol exposure and maternal stress on postnatal morphological development of hippocampal neurons in rats. Int. J. Dev. Neurosci. *61*: 40–50.

Jarmasz, J.S., Stirton, H., Basalah, D., Davie, J.R., Clarren, S.K., Astley, S.J., et al. (2019). Global DNA Methylation and Histone Posttranslational Modifications in Human and Nonhuman Primate Brain in Association with Prenatal Alcohol Exposure. Alcohol. Clin. Exp. Res. *43*: 1145–1162.

Jha, M.K., Jo, M., Kim, J.-H., and Suk, K. (2019). Microglia-Astrocyte Crosstalk: An Intimate Molecular Conversation. Neuroscientist *25*: 227–240.

Ji, H., and Shepard, P.D. (2007). Lateral Habenula Stimulation Inhibits Rat Midbrain Dopamine Neurons through a GABAA Receptor-Mediated Mechanism. J. Neurosci.

27: 6923–6930.

Johansson, E.M., García-Gutiérrez, M.S., Moscoso-Castro, M., Manzanares, J., and Valverde, O. (2015). Reduced Contextual Discrimination following Alcohol Consumption or MDMA Administration in Mice. PLoS One *10*: e0142978.

Jones, K.L. (2011). The effects of alcohol on fetal development. Birth Defects Res. C. Embryo Today *93*: 3–11.

Jones, K.L., Hoyme, H.E., Robinson, L.K., Campo, M. Del, Manning, M.A., Prewitt, L.M., et al. (2010). Fetal alcohol spectrum disorders: Extending the range of structural defects. Am. J. Med. Genet. Part A *152*: 2731–2735.

Jones, K.L., and Smith, D.W. (1973). Recognition of the fetal alcohol syndrome in early infancy. Lancet (London, England) *302*: 999–1001.

Jones, K.L., Smith, D.W., Ulleland, C.N., Streissguth, P., and Streissguth, A.P. (1973). Pattern of malformation in offspring of chronic alcoholic mothers. Lancet *301*: 1267–1271.

Jonsson, S., Morud, J., Stomberg, R., Ericson, M., and Söderpalm, B. (2017). Involvement of lateral septum in alcohol's dopamine-elevating effect in the rat. Addict. Biol. *22*: 93–102.

Joya, X., Friguls, B., Ortigosa, S., Papaseit, E., Martínez, S.E., Manich, A., et al. (2012). Determination of maternal-fetal biomarkers of prenatal exposure to ethanol: a review. J. Pharm. Biomed. Anal. *69*: 209–22.

Joya, X., Garcia-Algar, O., Salat-Batlle, J., Pujades, C., and Vall, O. (2015). Advances in the development of novel antioxidant therapies as an approach for fetal alcohol syndrome prevention. Birth Defects Res. Part A Clin. Mol. Teratol. *103*: 163–177.

Juárez, J., and Guerrero-Álvarez, Á. (2015). Effects of methylphenidate and atomoxetine on impulsivity and motor activity in preadolescent rats prenatally-treated with alcohol. Behav. Neurosci. *129*: 756–64.

Jurenka, J.S. (2009). Anti-inflammatory properties of curcumin, a major constituent of Curcuma longa: A review of preclinical and clinical research. Altern. Med. Rev. *14*: 141–153.

Kable, J.A., and Mukherjee, R.A.S. (2017). Neurodevelopmental disorder associated with prenatal exposure to alcohol (ND-PAE): A proposed diagnostic method of capturing the neurocognitive phenotype of FASD. Eur. J. Med. Genet. *60*: 49–54.

Kable, J.A., O'Connor, M.J., Olson, H.C., Paley, B., Mattson, S.N., Anderson, S.M., et al. (2016). Neurobehavioral Disorder Associated with Prenatal Alcohol Exposure (ND-PAE): Proposed DSM-5 Diagnosis. Child Psychiatry Hum. Dev. *47*: 335–46.

Kahkhaie, K.R., Mirhosseini, A., Aliabadi, A., Mohammadi, A., Mousavi, M.J., Haftcheshmeh, S.M., et al. (2019). Curcumin: a modulator of inflammatory signaling pathways in the immune system. Inflammopharmacology.

Kajimoto, K., Allan, A., and Cunningham, L.A. (2013). Fate Analysis of Adult Hippocampal Progenitors in a Murine Model of Fetal Alcohol Spectrum Disorder (FASD). PLoS One *8*: e73788.

Kalivas, P.W. (2004). Glutamate systems in cocaine addiction. Curr. Opin. Pharmacol. *4*: 23–29.

Kane, C.J.M., and Drew, P.D. (2016). Inflammatory responses to alcohol in the CNS: nuclear receptors as potential therapeutics for alcohol-induced neuropathologies. J. Leukoc. Biol. *100*: 951–959.

Kane, C.J.M., Phelan, K.D., Han, L., Smith, R.R., Xie, J., Douglas, J.C., et al. (2011). Protection of neurons and microglia against ethanol in a mouse model of fetal alcohol spectrum disorders by peroxisome proliferator-activated receptor-γ agonists. Brain. Behav. Immun. *25*: S137–S145.

Karlstetter, M., Lippe, E., Walczak, Y., Moehle, C., Aslanidis, A., Mirza, M., et al. (2011). Curcumin is a potent modulator of microglial gene expression and migration. J. Neuroinflammation *8*: 125.

Kataoka, Y., Shibata, K., Gomita, Y., and Ueki, S. (1982). The mammillary body is a potential site of antianxiety action of benzodiazepines. Brain Res. *241*: 374–377.

Keiver, K., Bertram, C.P., Orr, A.P., and Clarren, S. (2015). Salivary cortisol levels are elevated in the afternoon and at bedtime in children with prenatal alcohol exposure. Alcohol *49*: 79–87.

Kesner, R.P. (2018). An analysis of dentate gyrus function (an update). Behav. Brain Res. *354*: 84–91.

Keverne, E.B., Pfaff, D.W., and Tabansky, I. (2015). Epigenetic changes in the developing brain: Effects on behavior. Proc. Natl. Acad. Sci. *112*: 6789–6795.

Kierdorf, K., and Prinz, M. (2013). Factors regulating microglia activation. Front. Cell. Neurosci. *7*: 44.

Kim, M.-S., Akhtar, M.W., Adachi, M., Mahgoub, M., Bassel-Duby, R., Kavalali, E.T., et al. (2012). An Essential Role for Histone Deacetylase 4 in Synaptic Plasticity and Memory Formation. J. Neurosci. *32*: 10879–10886.

Kim, P., Park, J.H., Choi, C.S., Choi, I., Joo, S.H., Kim, M.K., et al. (2013). Effects of ethanol exposure during early pregnancy in hyperactive, inattentive and impulsive behaviors and MeCP2 expression in rodent offspring. Neurochem. Res. *38*: 620–631.

Kleiber, M.L., Diehl, E.J., Laufer, B.I., Mantha, K., Chokroborty-Hoque, A., Alberry, B., et al. (2014). Long-term genomic and epigenomic dysregulation as a consequence of prenatal alcohol exposure: a model for fetal alcohol spectrum disorders. Front. Genet. *5*: 161.

Kleiber, M.L., Laufer, B.I., Wright, E., Diehl, E.J., and Singh, S.M. (2012). Long-term alterations to the brain transcriptome in a maternal voluntary consumption model of fetal alcohol spectrum disorders. Brain Res. *1458*: 18–33.

Kleiber, M.L., Mantha, K., Stringer, R.L., and Singh, S.M. (2013). Neurodevelopmental alcohol exposure elicits long-term changes to gene expression that alter distinct molecular pathways dependent on timing of exposure. J. Neurodev. Disord. *5*: 6.

Kleiber, M.L., Wright, E., and Singh, S.M. (2011). Maternal voluntary drinking in C57BL/6J mice: Advancing a model for fetal alcohol spectrum disorders. Behav. Brain Res. *223*: 376–387.

Klintsova, A.Y., Hamilton, G.F., and Boschen, K.E. (2012). Long-term consequences of developmental alcohol exposure on brain structure and function: therapeutic benefits of physical activity. Brain Sci. *3*: 1–38.

Klintsova, A.Y., Helfer, J.L., Calizo, L.H., Dong, W.K., Goodlett, C.R., and Greenough, W.T. (2007). Persistent impairment of hippocampal neurogenesis in young adult rats following early postnatal alcohol exposure. Alcohol. Clin. Exp. Res. *31*: 2073–2082.

Komada, M., Hara, N., Kawachi, S., Kawachi, K., Kagawa, N., Nagao, T., et al. (2017). Mechanisms underlying neuro-inflammation and neurodevelopmental toxicity in the mouse neocortex following prenatal exposure to ethanol. Sci. Rep. *7*: 3–4.

Kong, H., Kuang, W., Li, S., and Xu, M. (2011). Activation of dopamine D3 receptors inhibits reward-related learning induced by cocaine. Neuroscience *176*: 152–161.

Koob, G.F., Arends, M.A., and Moal, M. Le (2014). Alcohol. In Drugs, Addiction, and the Brain, (Elsevier), pp 173–219.

Koob, G.F., and Moal, M. Le (2001). Drug addiction, dysregulation of reward, and allostasis. Neuropsychopharmacology *24*: 97–129.

Koob, G.F., and Moal, M. Le (2005). Plasticity of reward neurocircuitry and the 'dark side' of drug addiction. Nat. Neurosci. *8*: 1442–1444.

Koob, G.F., and Moal, M. Le (2008). Addiction and the brain antireward system. Annu. Rev. Psychol. *59*: 29–53.

Koob, G.F., and Volkow, N.D. (2010). Neurocircuitry of addiction. Neuropsychopharmacology *35*: 217–38.

Koreman, E., Sun, X., and Lu, Q.R. (2018). Chromatin remodeling and epigenetic regulation of oligodendrocyte myelination and myelin repair. Mol. Cell. Neurosci. *87*: 18–26.

Koren, G. (2002). Drinking alcohol while breastfeeding. Will it harm my baby? Can. Fam. Physician *48*: 39–41.

Kowiański, P., Lietzau, G., Czuba, E., Waśkow, M., Steliga, A., and Moryś, J. (2018). BDNF: A Key Factor with Multipotent Impact on Brain Signaling and Synaptic Plasticity. Cell. Mol. Neurobiol. *38*: 579–593.

Kozanian, O.O., Rohac, D.J., Bavadian, N., Corches, A., Korzus, E., and Huffman, K.J. (2018). Long-Lasting Effects of Prenatal Ethanol Exposure on Fear Learning and Development of the Amygdala. Front. Behav. Neurosci. *12*: 200.

Krahe, T.E., Wang, W., and Medina, A.E. (2009). Phosphodiesterase inhibition increases CREB phosphorylation and restores orientation selectivity in a model of fetal alcohol spectrum disorders. PLoS One *4*: e6643.

Krawczyk, M., Ramani, M., Dian, J., Florez, C.M., Mylvaganam, S., Brien, J., et al. (2016). Hippocampal hyperexcitability in fetal alcohol spectrum disorder: Pathological sharp waves and excitatory/inhibitory synaptic imbalance. Exp. Neurol. *280*: 70–79.

Krishnan, H.R., Sakharkar, A.J., Teppen, T.L., Berkel, T.D.M., and Pandey, S.C. (2014). The epigenetic landscape of alcoholism. Int. Rev. Neurobiol. *115*: 75–116.

Kuhn, P.L., Petroulakis, E., Zazanis, G. a., and McKinnon, R.D. (1995). Motor function analysis of myelin mutant mice using a rotarod. Int. J. Dev. Neurosci. *13*: 715–722.

Kundakovic, M., and Champagne, F.A. (2015). Early-life experience, Epigenetics, and the developing brain. Neuropsychopharmacology *40*: 141–153.

Kyzar, E.J., Floreani, C., Teppen, T.L., and Pandey, S.C. (2016). Adolescent Alcohol Exposure: Burden of Epigenetic Reprogramming, Synaptic Remodeling, and Adult Psychopathology. Front. Neurosci. *10*: 222.

Lalonde, R. (2002). The neurobiological basis of spontaneous alternation. Neurosci. Biobehav. Rev. *26*: 91–104.

Lange, S., Probst, C., Gmel, G., Rehm, J., Burd, L., and Popova, S. (2017a). Global Prevalence of Fetal Alcohol Spectrum Disorder Among Children and Youth: A Systematic Review and Meta-analysis. JAMA Pediatr. *171*: 948–956.

Lange, S., Probst, C., Rehm, J., and Popova, S. (2017b). Prevalence of binge drinking during pregnancy by country and World Health Organization region: Systematic review and meta-analysis. Reprod. Toxicol. *73*: 214–221.

Lange, S., Shield, K., Koren, G., Rehm, J., and Popova, S. (2014). A comparison of the prevalence of prenatal alcohol exposure obtained via maternal self-reports versus meconium testing: a systematic literature review and meta-analysis. BMC Pregnancy Childbirth *14*: 127.

Latham, J.A., and Dent, S.Y.R. (2007). Cross-regulation of histone modifications. Nat. Struct. Mol. Biol. *14*: 1017–1024.

Lau, A., and Tymianski, M. (2010). Glutamate receptors, neurotoxicity and neurodegeneration. Pflügers Arch. - Eur. J. Physiol. *460*: 525–542.

Laufer, B.I., Chater-Diehl, E.J., Kapalanga, J., and Singh, S.M. (2017). Long-term alterations to DNA methylation as a biomarker of prenatal alcohol exposure: From mouse models to human children with fetal alcohol spectrum disorders. Alcohol *60*: 67–75.

Lebel, C., Roussotte, F., and Sowell, E.R. (2011). Imaging the Impact of Prenatal Alcohol Exposure on the Structure of the Developing Human Brain. Neuropsychol. Rev. *21*: 102–118.

Lecca, S., Meye, F.J., and Mameli, M. (2014). The lateral habenula in addiction and depression: an anatomical, synaptic and behavioral overview. Eur. J. Neurosci. *39*: 1170–1178.

Leclercq, S., Saeger, C. De, Delzenne, N., Timary, P. De, and Stärkel, P. (2014). Role of inflammatory pathways, blood mononuclear cells, and gut-derived bacterial products in alcohol dependence. Biol. Psychiatry *76*: 725–733.

Lecuyer, M., Laquerrière, A., Bekri, S., Lesueur, C., Ramdani, Y., Jégou, S., et al. (2017). PLGF, a placental marker of fetal brain defects after in utero alcohol exposure. Acta Neuropathol. Commun. *5*: 44.

Lee, H.-I., McGregor, R.A., Choi, M.-S., Seo, K.-I., Jung, U.J., Yeo, J., et al. (2013). Low doses of curcumin protect alcohol-induced liver damage by modulation of the alcohol metabolic pathway, CYP2E1 and AMPK. Life Sci. *93*: 693–9.

Lee, S., Choi, I., Kang, S., and Rivier, C. (2008). Role of various neurotransmitters in mediating the long-term endocrine consequences of prenatal alcohol exposure. Ann. N. Y. Acad. Sci. *1144*: 176–88.

Leggio, G.M., Marco, R. Di, Gulisano, W., D'Ascenzo, M., Torrisi, S.A., Geraci, F., et al. (2019). Dopaminergic-GABAergic interplay and alcohol binge drinking. Pharmacol. Res. *141*: 384–391.

Legube, G., and Trouche, D. (2003). Regulating histone acetyltransferases and deacetylases. EMBO Rep. *4*: 944–7.

Lehnardt, S. (2010). Innate immunity and neuroinflammation in the CNS: The role of

microglia in toll-like receptor-mediated neuronal injury. Glia *58*: 253–263.

Lemoine, P., Harousseau, H., Borteyru, J., and Menuet, J. (1968). Les enfants de parents alcooliques: Anomalies observees a propos de 127 cas. Ouest Méd *21*: 476–482.

Lewohl, J.M., Wixey, J., Harper, C.G., and Dodd, P.R. (2005). Expression of MBP, PLP, MAG, CNP, and GFAP in the Human Alcoholic Brain. Alcohol. Clin. Exp. Res. *29*: 1698–1705.

Li, H., and Richardson, W.D. (2016). Evolution of the CNS myelin gene regulatory program. Brain Res. *1641*: 111–121.

Licciardi, P.V., Ververis, K., Tang, M.L., El-Osta, A., and Karagiannis, T.C. (2013). Immunomodulatory Effects of Histone Deacetylase Inhibitors. Curr. Mol. Med. *13*: 640–647.

Liddelow, S.A., Guttenplan, K.A., Clarke, L.E., Bennett, F.C., Bohlen, C.J., Schirmer, L., et al. (2017). Neurotoxic reactive astrocytes are induced by activated microglia. Nature *541*: 481–487.

Lim, G.P., Chu, T., Yang, F., Beech, W., Frautschy, S. a, and Cole, G.M. (2001). The curry spice curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse. J. Neurosci. *21*: 8370–8377.

Lim, K.-H., and Staudt, L.M. (2013). Toll-like receptor signaling. Cold Spring Harb. Perspect. Biol. *5*: a011247.

Lipinski, R.J., Hammond, P., O'Leary-Moore, S.K., Ament, J.J., Pecevich, S.J., Jiang, Y., et al. (2012). Ethanol-Induced Face-Brain Dysmorphology Patterns Are Correlative and Exposure-Stage Dependent. PLoS One *7*: e43067.

Lippai, D., Bala, S., Csak, T., Kurt-Jones, E.A., and Szabo, G. (2013a). Chronic Alcohol-Induced microRNA-155 Contributes to Neuroinflammation in a TLR4-Dependent Manner in Mice. PLoS One *8*: 1–10.

Lippai, D., Bala, S., Petrasek, J., Csak, T., Levin, I., Kurt-Jones, E. a, et al. (2013b). Alcohol-induced IL-1β in the brain is mediated by NLRP3/ASC inflammasome activation that amplifies neuroinflammation. J. Leukoc. Biol. *94*: 171–82.

Liu, J., and Casaccia, P. (2010). Epigenetic regulation of oligodendrocyte identity. Trends Neurosci. *33*: 193–201.

Liu, J., Moyon, S., Hernandez, M., and Casaccia, P. (2016). Epigenetic control of oligodendrocyte development: adding new players to old keepers. Curr. Opin. Neurobiol. *39*: 133–138.

Liu, S.B., Mi, W.L., and Wang, Y.Q. (2013). Research progress on the NLRP3

inflammasome and its role in the central nervous system. Neurosci Bull *29*: 779–787.

Liu, Y., Balaraman, Y., Wang, G., Nephew, K.P., and Zhou, F.C. (2009). Alcohol exposure alters DNA methylation profiles in mouse embryos at early neurulation. Epigenetics *4*: 500–11.

Liu, Z., Liu, Y., Gao, R., Li, H., Dunn, T., Wu, P., et al. (2014). Ethanol Suppresses PGC-1α Expression by Interfering with the cAMP-CREB Pathway in Neuronal Cells. PLoS One *9*: e104247.

Liyanage, V.R.B., Curtis, K., Zachariah, R.M., Chudley, A.E., and Rastegar, M. (2017). Overview of the Genetic Basis and Epigenetic Mechanisms that Contribute to FASD Pathobiology. Curr. Top. Med. Chem. *17*: 808–828.

Long, L., Li, Y., Wang, Y.D., He, Q.Y., Li, M., Cai, X.D., et al. (2010). The preventive effect of oral EGCG in a fetal alcohol spectrum disorder mouse model. Alcohol. Clin. Exp. Res. *34*: 1929–1936.

Lonze, B.E., and Ginty, D.D. (2002). Function and regulation of CREB family transcription factors in the nervous system. Neuron *35*: 605–23.

López-Arnau, R., Luján, M.A., Duart-Castells, L., Pubill, D., Camarasa, J., Valverde, O., et al. (2017). Exposure of adolescent mice to 3,4-methylenedioxypyrovalerone increases the psychostimulant, rewarding and reinforcing effects of cocaine in adulthood. Br. J. Pharmacol. *174*: 1161–1173.

Louth, E.L., Bignell, W., Taylor, C.L., and Bailey, C.D.C. (2016). Developmental Ethanol Exposure Leads to Long-Term Deficits in Attention and Its Underlying Prefrontal Circuitry. Eneuro *3*: ENEURO.0267-16.2016.

Louth, E.L., Luctkar, H.D., Heney, K.A., and Bailey, C.D.C. (2018). Developmental ethanol exposure alters the morphology of mouse prefrontal neurons in a layer-specific manner. Brain Res. *1678*: 94–105.

Lucas, B.R., Latimer, J., Pinto, R.Z., Ferreira, M.L., Doney, R., Lau, M., et al. (2014). Gross Motor Deficits in Children Prenatally Exposed to Alcohol: A Meta-analysis. Pediatrics *134*: e192–e209.

Luján, M.Á., Cantacorps, L., and Valverde, O. (2019). The pharmacological reduction of hippocampal neurogenesis attenuates the protective effects of cannabidiol on cocaine voluntary intake. Addict. Biol. e12778.

Luján, M.Á., Castro-Zavala, A., Alegre-Zurano, L., and Valverde, O. (2018). Repeated Cannabidiol treatment reduces cocaine seeking and modulates neural proliferation and CB1R expression in the hippocampus. Neuropharmacology *143*: 163–175.

Lunde-Young, R., Ramirez, J., Naik, V., Orzabal, M., Lee, J., Konganti, K., et al. (2019). Hippocampal transcriptome reveals novel targets of FASD pathogenesis.

Brain Behav. *9*: e01334.

Luo, J. (2012). Mechanisms of Ethanol-Induced Death of Cerebellar Granule Cells. The Cerebellum *11*: 145–154.

Lüscher, C. (2016). The Emergence of a Circuit Model for Addiction. Annu. Rev. Neurosci. *39*: 257–76.

Lüscher, C., and Bellone, C. (2008). Cocaine-evoked synaptic plasticity: a key to addiction? Nat. Neurosci. *11*: 737–8.

Lussier, A. a, Stepien, K. a, Neumann, S.M., Pavlidis, P., Kobor, M.S., and Weinberg, J. (2015). Prenatal alcohol exposure alters steady-state and activated gene expression in the adult rat brain. Alcohol. Clin. Exp. Res. *39*: 251–61.

Lussier, A.A., Weinberg, J., and Kobor, M.S. (2017). Epigenetics studies of fetal alcohol spectrum disorder: where are we now? Epigenomics *9*: 291–311.

Ma, Y.-Y. (2019). Striatal morphological and functional alterations induced by prenatal alcohol exposure. Pharmacol. Res. *142*: 262–266.

Maccari, S., Krugers, H.J., Morley-Fletcher, S., Szyf, M., and Brunton, P.J. (2014). The consequences of early-life adversity: Neurobiological, behavioural and epigenetic adaptations. J. Neuroendocrinol. *26*: 707–723.

Maccarrone, M., Valverde, O., Barbaccia, M.L., Castañé, A., Maldonado, R., Ledent, C., et al. (2002). Age-related changes of anandamide metabolism in CB1 cannabinoid receptor knockout mice: correlation with behaviour. Eur. J. Neurosci. *15*: 1178–86.

MacDonald, J.L., and Roskams, A.J. (2009). Epigenetic regulation of nervous system development by DNA methylation and histone deacetylation. Prog. Neurobiol. *88*: 170–183.

Macdowell, E.C., and Vicari, E.M. (1917). On the Growth and Fecundity of Alcoholized Rats. Proc. Natl. Acad. Sci. U. S. A. *3*: 577–9.

Macht, V.A., Kelly, S.J., and Gass, J.T. (2017). Sex-specific effects of developmental alcohol exposure on cocaine-induced place preference in adulthood. Behav. Brain Res. *332*: 259–268.

Mahnke, A.H., Miranda, R.C., and Homanics, G.E. (2017). Epigenetic mediators and consequences of excessive alcohol consumption. Alcohol *60*: 1–6.

Maître, T.W. Le, Dhanabalan, G., Bogdanovic, N., Alkass, K., and Druid, H. (2018). Effects of Alcohol Abuse on Proliferating Cells, Stem/Progenitor Cells, and Immature Neurons in the Adult Human Hippocampus. Neuropsychopharmacology *43*: 690–699.

Makinodan, M., Tatsumi, K., Manabe, T., Yamauchi, T., Makinodan, E., Matsuyoshi,

H., et al. (2008). Maternal immune activation in mice delays myelination and axonal development in the hippocampus of the offspring. J. Neurosci. Res. *86*: 2190–2200.

Mamluk, L., Edwards, H.B., Savović, J., Leach, V., Jones, T., Moore, T.H.M., et al. (2017). Low alcohol consumption and pregnancy and childhood outcomes: time to change guidelines indicating apparently 'safe' levels of alcohol during pregnancy? A systematic review and meta-analyses. BMJ Open *7*: e015410.

Mandal, C., Halder, D., Jung, K.H., and Chai, Y.G. (2017a). Gestational Alcohol Exposure Altered DNA Methylation Status in the Developing Fetus. Int. J. Mol. Sci. *18*: 1386.

Mandal, C., Halder, D., Jung, K.H., and Chai, Y.G. (2017b). In utero alcohol exposure and the alteration of histone marks in the developing fetus: An epigenetic phenomenon of maternal drinking. Int. J. Biol. Sci. *13*: 1100–1108.

Mandal, C., Park, K.S., Jung, K.H., and Chai, Y.G. (2015). Ethanol-related alterations in gene expression patterns in the developing murine hippocampus. Acta Biochim. Biophys. Sin. (Shanghai). *47*: 581–7.

Manning, M.A., and Hoyme, E. (2007). Fetal alcohol spectrum disorders: A practical clinical approach to diagnosis. Neurosci. Biobehav. Rev. *31*: 230–238.

Mantamadiotis, T., Lemberger, T., Bleckmann, S.C., Kern, H., Kretz, O., Villalba, A.M., et al. (2002). Disruption of CREB function in brain leads to neurodegeneration. Nat. Genet. *31*: 47–54.

Mantha, K., Laufer, B.I., and Singh, S.M. (2014). Molecular changes during neurodevelopment following second-trimester binge ethanol exposure in a mouse model of fetal alcohol spectrum disorder: From immediate effects to long-term adaptation. Dev. Neurosci. *36*: 29–43.

Marche, K., Danel, T., and Bordet, R. (2011). Fetal alcohol-induced hyperactivity is reversed by treatment with the PPARα agonist fenofibrate in a rat model. Psychopharmacology (Berl). *214*: 285–296.

Marcu, M.G., Jung, Y.-J., Lee, S., Chung, E.-J., Lee, M.-J., Trepel, J., et al. (2006). Curcumin is an inhibitor of p300 histone acetylatransferase. Med. Chem. *2*: 169–74.

Marjonen, H., Sierra, A., Nyman, A., Rogojin, V., Gr??hn, O., Linden, A.M., et al. (2015). Early maternal alcohol consumption alters hippocampal DNA methylation, gene expression and volume in a mouse model. PLoS One *10*: 1–20.

Marquardt, K., and Brigman, J.L. (2016). The impact of prenatal alcohol exposure on social, cognitive and affective behavioral domains: Insights from rodent models. Alcohol *51*: 1–15.

Marquardt, K., Sigdel, R., Caldwell, K., and Brigman, J.L. (2014). Prenatal ethanol

exposure impairs executive function in mice into adulthood. Alcohol. Clin. Exp. Res. *38*: 2962–8.

Massaad, C.A., and Klann, E. (2011). Reactive Oxygen Species in the Regulation of Synaptic Plasticity and Memory. Antioxid. Redox Signal. *14*: 2013–2054.

Matsumoto, M., and Hikosaka, O. (2007). Lateral habenula as a source of negative reward signals in dopamine neurons. Nature *447*: 1111–1115.

Mattson, S.N., Roesch, S.C., Glass, L., Deweese, B.N., Coles, C.D., Kable, J.A., et al. (2013). Further Development of a Neurobehavioral Profile of Fetal Alcohol Spectrum Disorders. Alcohol. Clin. Exp. Res. *37*: 517–528.

May, P.A., Baete, A., Russo, J., Elliott, A.J., Blankenship, J., Kalberg, W.O., et al. (2014). Prevalence and Characteristics of Fetal Alcohol Spectrum Disorders. Pediatrics *134*: 855–866.

May, P.A., Blankenship, J., Marais, A.S., Gossage, J.P., Kalberg, W.O., Joubert, B., et al. (2013). Maternal alcohol consumption producing fetal alcohol spectrum disorders (FASD): Quantity, frequency, and timing of drinking. Drug Alcohol Depend. *133*: 502–512.

May, P.A., Chambers, C.D., Kalberg, W.O., Zellner, J., Feldman, H., Buckley, D., et al. (2018). Prevalence of fetal alcohol spectrum disorders in 4 US communities. JAMA - J. Am. Med. Assoc. *319*: 474–482.

May, P.A., and Gossage, J.P. (2011). Maternal risk factors for fetal alcohol spectrum disorders: not as simple as it might seem. Alcohol Res. Health *34*: 15–26.

May, P.A., Hasken, J.M., Blankenship, J., Marais, A.S., Joubert, B., Cloete, M., et al. (2016). Breastfeeding and maternal alcohol use: Prevalence and effects on child outcomes and fetal alcohol spectrum disorders. Reprod. Toxicol. *63*: 13–21.

Mayfield, J., Ferguson, L., and Harris, R.A. (2013). Neuroimmune signaling: a key component of alcohol abuse. Curr. Opin. Neurobiol. *23*: 513–20.

McClung, C.A., and Nestler, E.J. (2003). Regulation of gene expression and cocaine reward by CREB and ΔFosB. Nat. Neurosci. *6*: 1208–1215.

McCoy, M.K., and Tansey, M.G. (2008). TNF signaling inhibition in the CNS: implications for normal brain function and neurodegenerative disease. J. Neuroinflammation *5*: 45.

McFarland, K., Lapish, C.C., and Kalivas, P.W. (2003). Prefrontal Glutamate Release into the Core of the Nucleus Accumbens Mediates Cocaine-Induced Reinstatement of Drug-Seeking Behavior. J. Neurosci. *23*: 3531–3537.

McGowan, P.O., and Roth, T.L. (2015). Epigenetic pathways through which

experiences become linked with biology. Dev. Psychopathol. *27*: 637–48.

McGowan, P.O., Suderman, M., Sasaki, A., Huang, T.C.T., Hallett, M., Meaney, M.J., et al. (2011). Broad epigenetic signature of maternal care in the brain of adult rats. PLoS One *6*: e14739.

McKenzie, I.A., Ohayon, D., Li, H., Faria, J.P. de, Emery, B., Tohyama, K., et al. (2014). Motor skill learning requires active central myelination. Science *346*: 318–22.

McLachlan, K., Rasmussen, C., Oberlander, T.F., Loock, C., Pei, J., Andrew, G., et al. (2016). Dysregulation of the cortisol diurnal rhythm following prenatal alcohol exposure and early life adversity. Alcohol *53*: 9–18.

Meaney, M.J., and Szyf, M. (2005). Maternal care as a model for experience-dependent chromatin plasticity? Trends Neurosci. *28*: 456–63.

Medina, A.E., Krahe, T.E., and Ramoa, A.S. (2006). Restoration of Neuronal Plasticity by a Phosphodiesterase Type 1 Inhibitor in a Model of Fetal Alcohol Exposure. J. Neurosci. *26*: 1057–1060.

Mémet, S. (2006). NF-κB functions in the nervous system: From development to disease. Biochem. Pharmacol. *72*: 1180–1195.

Mennella, J. (2001). Alcohol's effect on lactation. Alcohol Res. Health *25*: 230–4.

Miñana, R., Climent, E., Barettino, D., Segui, J.M., Renau-Piqueras, J., and Guerri, C. (2002). Alcohol Exposure Alters the Expression Pattern of Neural Cell Adhesion Molecules During Brain Development. J. Neurochem. *75*: 954–964.

Mitew, S., Hay, C.M., Peckham, H., Xiao, J., Koenning, M., and Emery, B. (2014). Mechanisms regulating the development of oligodendrocytes and central nervous system myelin. Neuroscience *276*: 29–47.

Montagud-Romero, S., Cantacorps, L., and Valverde, O. (2019). Histone deacetylases inhibitor trichostatin A reverses anxiety-like symptoms and memory impairments induced by maternal binge alcohol drinking in mice. J. Psychopharmacol. 026988111985720.

Montagud-Romero, S., Montesinos, J., Pascual, M., Aguilar, M.A., Roger-Sanchez, C., Guerri, C., et al. (2016). 'Up-regulation of histone acetylation induced by social defeat mediates the conditioned rewarding effects of cocaine. Prog. Neuro-Psychopharmacology Biol. Psychiatry *70*: 39–48.

Montesinos, J., Alfonso-Loeches, S., and Guerri, C. (2016a). Impact of the Innate Immune Response in the Actions of Ethanol on the Central Nervous System. Alcohol. Clin. Exp. Res. *40*: 2260–2270.

Montesinos, J., Pascual, M., Pla, A., Maldonado, C., Rodríguez-Arias, M., Miñarro, J.,

et al. (2015). TLR4 elimination prevents synaptic and myelin alterations and long-term cognitive dysfunctions in adolescent mice with intermittent ethanol treatment. Brain. Behav. Immun. *45*: 233–44.

Montesinos, J., Pascual, M., Rodríguez-Arias, M., Miñarro, J., and Guerri, C. (2016b). Involvement of TLR4 in the long-term epigenetic changes, rewarding and anxiety effects induced by intermittent ethanol treatment in adolescence. Brain. Behav. Immun. *53*: 159–171.

Moonat, S., Sakharkar, A.J., Zhang, H., Tang, L., and Pandey, S.C. (2013). Aberrant histone deacetylase2-mediated histone modifications and synaptic plasticity in the amygdala predisposes to anxiety and alcoholism. Biol. Psychiatry *73*: 763–773.

Mooney, S.M., and Miller, M.W. (2001). Effects of prenatal exposure to ethanol on the expression of bcl-2, bax and caspase 3 in the developing rat cerebral cortex and thalamus. Brain Res. *911*: 71–81.

Moore, E.M., Migliorini, R., Infante, M.A., and Riley, E.P. (2014). Fetal Alcohol Spectrum Disorders: Recent Neuroimaging Findings. Curr. Dev. Disord. Reports *1*: 161–172.

Moore, K.L., and Persaud, T.V.. (1993). The developing human: clinically oriented embryology. (Philadelphia: WB Saunders).

Moreno-Jiménez, E.P., Flor-García, M., Terreros-Roncal, J., Rábano, A., Cafini, F., Pallas-Bazarra, N., et al. (2019). Adult hippocampal neurogenesis is abundant in neurologically healthy subjects and drops sharply in patients with Alzheimer's disease. Nat. Med. *25*: 554–560.

Moresco, E.M.Y., LaVine, D., and Beutler, B. (2011). Toll-like receptors. Curr. Biol. *21*: R488-93.

Morris, S. a, Eaves, D.W., Smith, A.R., and Nixon, K. (2010). Alcohol inhibition of neurogenesis: a mechanism of hippocampal neurodegeneration in an adolescent alcohol abuse model. Hippocampus *20*: 596–607.

Moscoso-Castro, M., Gracia-Rubio, I., Ciruela, F., and Valverde, O. (2016). Genetic blockade of adenosine A2A receptors induces cognitive impairments and anatomical changes related to psychotic symptoms in mice. Eur. Neuropsychopharmacol. *26*: 1227–40.

Moscoso-Castro, M., López-Cano, M., Gracia-Rubio, I., Ciruela, F., and Valverde, O. (2017). Cognitive impairments associated with alterations in synaptic proteins induced by the genetic loss of adenosine A2Areceptors in mice. Neuropharmacology *126*: 48–57.

Most, D., Ferguson, L., and Harris, R.A. (2014). Molecular basis of alcoholism. Handb. Clin. Neurol. *125*: 89–111.

Muñoz-Villegas, P., Rodríguez, V.M., Giordano, M., and Juárez, J. (2017). Risk-taking, locomotor activity and dopamine levels in the nucleus accumbens and medial prefrontal cortex in male rats treated prenatally with alcohol. Pharmacol. Biochem. Behav. *153*: 88–96.

Muralidharan, P., Connors, C.T., Mohammed, A.S., Sarmah, S., Marrs, K., Marrs, J.A., et al. (2017). Turmeric Extract Rescues Ethanol-Induced Developmental Defect in the Zebrafish Model for Fetal Alcohol Spectrum Disorder (FASD). J. Food Sci. *82*: 2221–2225.

Murawski, N.J., Moore, E.M., Thomas, J.D., and Riley, E.P. (2015). Advances in Diagnosis and Treatment of Fetal Alcohol Spectrum Disorders: From Animal Models to Human Studies. Alcohol Res. *37*: 97–108.

Murray, J., Burgess, S., Zuccolo, L., Hickman, M., Gray, R., and Lewis, S.J. (2016). Moderate alcohol drinking in pregnancy increases risk for children's persistent conduct problems: Causal effects in a Mendelian randomisation study. J. Child Psychol. Psychiatry Allied Discip. *57*: 575–584.

Nanji, A.A., Jokelainen, K., Tipoe, G.L., Rahemtulla, A., Thomas, P., and Dannenberg, A.J. (2003). Curcumin prevents alcohol-induced liver disease in rats by inhibiting the expression of NF-κB-dependent genes. Am. J. Physiol. Liver Physiol. *284*: G321–G327.

Nayak, D., Roth, T.L., and McGavern, D.B. (2014). Microglia Development and Function. Annu. Rev. Immunol. *32*: 367–402.

Nelson, E.D., and Monteggia, L.M. (2011). Epigenetics in the mature mammalian brain: Effects on behavior and synaptic transmission. Neurobiol. Learn. Mem. *96*: 53–60.

Nestler, E.J. (2005). Is there a common molecular pathway for addiction? Nat. Neurosci. *8*: 1445–1449.

Nestler, E.J. (2008). Transcriptional mechanisms of addiction: role of FosB. Philos. Trans. R. Soc. B Biol. Sci. *363*: 3245–3255.

Newville, J., Valenzuela, C.F., Li, L., Jantzie, L.L., and Cunningham, L.A. (2017). Acute oligodendrocyte loss with persistent white matter injury in a third trimester equivalent mouse model of fetal alcohol spectrum disorder. Glia *65*: 1317–1332.

Ngai, Y.F., Sulistyoningrum, D.C., O'Neill, R., Innis, S.M., Weinberg, J., and Devlin, A.M. (2015). Prenatal alcohol exposure alters methyl metabolism and programs serotonin transporter and glucocorticoid receptor expression in brain. Am. J. Physiol. Regul. Integr. Comp. Physiol. *309*: R613-22.

Nguyen, T.T., Coppens, J., and Riley, E.P. (2010). Prenatal Alcohol Exposure, FAS, and FASD: An Introduction. pp 1–13.

NIAAA (2016). Drinking levels defined.

NIAAA (2019). What is a standard drink?

Nice, L.B. (1912). Comparative studies on the effects of alcohol, nicotine, tobacco smoke and caffeine on white mice. I. Effects on reproduction and growth. J. Exp. Zool. *12*: 133–152.

Nice, L.B. (1917). Further Observations on the Effects of Alcohol on White Mice. Am. Nat. *51*: 596–607.

Nickel, M., and Gu, C. (2018). Regulation of Central Nervous System Myelination in Higher Brain Functions. Neural Plast. *2018*: 1–12.

Nicloux, M. (1900). Passage de l'alcool ingéré de la mere au foetus et passage de l'alcool ingéré dans le lait, en paticulier chez la femme. Obstetrique. *5*: 97–132.

Nirgudkar, P., Taylor, D.H., Yanagawa, Y., and Valenzuela, C.F. (2016). Ethanol exposure during development reduces GABAergic/glycinergic neuron numbers and lobule volumes in the mouse cerebellar vermis. Neurosci. Lett. *632*: 86–91.

Nixon, K. (2006). Alcohol and adult neurogenesis: roles in neurodegeneration and recovery in chronic alcoholism. Hippocampus *16*: 287–95.

Nixon, K., and Crews, F.T. (2002). Binge ethanol exposure decreases neurogenesis in adult rat hippocampus. J. Neurochem. *83*: 1087–1093.

Norden, D.M., Trojanowski, P.J., Villanueva, E., Navarro, E., and Godbout, J.P. (2016). Sequential activation of microglia and astrocyte cytokine expression precedes increased Iba-1 or GFAP immunoreactivity following systemic immune challenge. Glia *64*: 300–16.

Norman, A., Crocker, N., Mattson, S., and Riley, E. (2009). Neuroimaging and fetal alcohol spectrum disorders. Dev. Disabil. Res. Rev. *15*: 209–217.

Nunes, F., Ferreira-Rosa, K., Pereira, M.D.S., Kubrusly, R.C., Manhães, A.C., Abreu-Villaça, Y., et al. (2011). Acute administration of vinpocetine, a phosphodiesterase type 1 inhibitor, ameliorates hyperactivity in a mice model of fetal alcohol spectrum disorder. Drug Alcohol Depend. *119*: 81–7.

O'Neill, L.A., and Kaltschmidt, C. (1997). NF-kappa B: a crucial transcription factor for glial and neuronal cell function. Trends Neurosci. *20*: 252–8.

Oak, S., Mandrekar, P., Catalano, D., Kodys, K., and Szabo, G. (2006). TLR2- and TLR4-mediated signals determine attenuation or augmentation of inflammation by acute alcohol in monocytes. J. Immunol. (Baltimore, Md. 1950) *176*: 7628–7635.

Oever, M.C. Van den, Spijker, S., Smit, A.B., and Vries, T.J. De (2010). Prefrontal cortex plasticity mechanisms in drug seeking and relapse. Neurosci. Biobehav. Rev.

35: 276–284.

Ohta, K.I., Sakata-Haga, H., and Fukui, Y. (2010). Alteration in anxiety-related behaviors and reduction of serotonergic neurons in raphe nuclei in adult rats prenatally exposed to ethanol. Congenit. Anom. (Kyoto). *50*: 105–114.

Olateju, O.I., Bhagwandin, A., Ihunwo, A.O., and Manger, P.R. (2017). Changes in the Cholinergic, Catecholaminergic, Orexinergic and Serotonergic Structures Forming Part of the Sleep Systems of Adult Mice Exposed to Intrauterine Alcohol. Front. Neuroanat. *11*: 1–15.

Olateju, O.I., Spocter, M.A., Patzke, N., Ihunwo, A.O., and Manger, P.R. (2018). Hippocampal neurogenesis in the C57BL/6J mice at early adulthood following prenatal alcohol exposure. Metab. Brain Dis. *33*: 397–410.

Olney, J.W., Tenkova, T., Dikranian, K., Muglia, L.J., Jermakowicz, W.J., D'Sa, C., et al. (2002a). Ethanol-induced caspase-3 activation in the in vivo developing mouse brain. Neurobiol. Dis. *9*: 205–19.

Olney, J.W., Tenkova, T., Dikranian, K., Qin, Y.-Q., Labruyere, J., and Ikonomidou, C. (2002b). Ethanol-induced apoptotic neurodegeneration in the developing C57BL/6 mouse brain. Dev. Brain Res. *133*: 115–126.

Omelchenko, N., Bell, R., and Sesack, S.R. (2009). Lateral habenula projections to dopamine and GABA neurons in the rat ventral tegmental area. Eur. J. Neurosci. *30*: 1239–1250.

Ozer, E., Sarioglu, S., and Güre, A. (2000). Effects of prenatal ethanol exposure on neuronal migration, neuronogenesis and brain myelination in the mice brain. Clin. Neuropathol. *19*: 21–5.

Paley, B., and O'Connor, M.J. (2011). Behavioral interventions for children and adolescents with fetal alcohol spectrum disorders. Alcohol Res. Health *34*: 64–75.

Palmisano, M., and Pandey, S.C. (2017). Epigenetic mechanisms of alcoholism and stress-related disorders. Alcohol *60*: 7–18.

Panahi, Y., Ahmadi, Y., Teymouri, M., Johnston, T.P., and Sahebkar, A. (2018). Curcumin as a potential candidate for treating hyperlipidemia: A review of cellular and metabolic mechanisms. J. Cell. Physiol. *233*: 141–152.

Pandey, S.C., Kyzar, E.J., and Zhang, H. (2017). Epigenetic basis of the dark side of alcohol addiction. Neuropharmacology *122*: 74–84.

Pandey, S.C., Ugale, R., Zhang, H., Tang, L., and Prakash, A. (2008). Brain Chromatin Remodeling: A Novel Mechanism of Alcoholism. J. Neurosci. *28*: 3729–3737.

Paolicelli, R.C., Bolasco, G., Pagani, F., Maggi, L., Scianni, M., Panzanelli, P., et al.

(2011). Synaptic pruning by microglia is necessary for normal brain development. Science *333*: 1456–8.

Paredes, M.F., Sorrells, S.F., Cebrian-Silla, A., Sandoval, K., Qi, D., Kelley, K.W., et al. (2018). Does Adult Neurogenesis Persist in the Human Hippocampus? Cell Stem Cell *23*: 780–781.

Park, C., Rehrauer, H., and Mansuy, I.M. (2013). Genome-wide analysis of H4K5 acetylation associated with fear memory in mice. BMC Genomics *14*: 539.

Park, H., and Poo, M. (2013). Neurotrophin regulation of neural circuit development and function. Nat. Rev. Neurosci. *14*: 7–23.

Park, P.-H., Lim, R.W., and Shukla, S.D. (2005). Involvement of histone acetyltransferase (HAT) in ethanol-induced acetylation of histone H3 in hepatocytes: potential mechanism for gene expression. Am. J. Physiol. Gastrointest. Liver Physiol. *289*: G1124–G1136.

Parker, M.O., Evans, A.M.-D., Brock, A.J., Combe, F.J., Teh, M.-T., and Brennan, C.H. (2016). Moderate alcohol exposure during early brain development increases stimulus-response habits in adulthood. Addict. Biol. *21*: 49–60.

Parolin, M., Simonelli, A., Mapelli, D., Sacco, M., and Cristofalo, P. (2016). Parental substance abuse as an early traumatic event. Preliminary findings on neuropsychological and personality functioning in young drug addicts exposed to drugs early. Front. Psychol. *7*: 1–15.

Pascual, M., Baliño, P., Alfonso-Loeches, S., Aragón, C.M.G., and Guerri, C. (2011). Impact of TLR4 on behavioral and cognitive dysfunctions associated with alcohol-induced neuroinflammatory damage. Brain. Behav. Immun. *25*: 80–91.

Pascual, M., Baliño, P., Aragón, C.M.G., and Guerri, C. (2014a). Cytokines and chemokines as biomarkers of ethanol-induced neuroinflammation and anxiety-related behavior: Role of TLR4 and TLR2. Neuropharmacology *89C*: 352–359.

Pascual, M., Boix, J., Felipo, V., and Guerri, C. (2009). Repeated alcohol administration during adolescence causes changes in the mesolimbic dopaminergic and glutamatergic systems and promotes alcohol intake in the adult rat. J. Neurochem. *108*: 920–931.

Pascual, M., Montesinos, J., Montagud-Romero, S., Forteza, J., Rodríguez-Arias, M., Miñarro, J., et al. (2017). TLR4 response mediates ethanol-induced neurodevelopment alterations in a model of fetal alcohol spectrum disorders. J. Neuroinflammation *14*: 145.

Pascual, M., Pla, A., Miñarro, J., and Guerri, C. (2014b). Neuroimmune Activation and Myelin Changes in Adolescent Rats Exposed to High-Dose Alcohol and Associated Cognitive Dysfunction: A Review with Reference to Human Adolescent Drinking.

Alcohol Alcohol. *49*: 187–192.

Pascual, M., Valles, S.L., Renau-Piqueras, J., and Guerri, C. (2003). Ceramide pathways modulate ethanol-induced cell death in astrocytes. J. Neurochem. *87*: 1535–1545.

Patten, A., Brocardo, P.S., Gil-Mohapel, J., and Christie, B.R. (2014a). Oxidative Stress in Fetal Alcohol Spectrum Disorders – Insights for the Development of Antioxidant-Based Therapies. In Systems Biology of Free Radicals and Antioxidants, (Berlin, Heidelberg: Springer Berlin Heidelberg), pp 645–667.

Patten, A.R., Brocardo, P.S., and Christie, B.R. (2013). Omega-3 supplementation can restore glutathione levels and prevent oxidative damage caused by prenatal ethanol exposure. J. Nutr. Biochem. *24*: 760–769.

Patten, A.R., Fontaine, C.J., and Christie, B.R. (2014b). A comparison of the different animal models of fetal alcohol spectrum disorders and their use in studying complex behaviors. Front. Pediatr. *2*: 93.

Peixoto, L., and Abel, T. (2013). The role of histone acetylation in memory formation and cognitive impairments. Neuropsychopharmacology *38*: 62–76.

Peleg, S., Sananbenesi, F., Zovoilis, A., Burkhardt, S., Bahari-Javan, S., Agis-Balboa, R.C., et al. (2010). Altered Histone Acetylation Is Associated with Age-Dependent Memory Impairment in Mice. Science (80-.). *328*: 753–756.

Peng, C., Zhang, W., Zhao, W., Zhu, J., Huang, X., and Tian, J. (2015). Alcohol-induced histone H3K9 hyperacetylation and cardiac hypertrophy are reversed by a histone acetylases inhibitor anacardic acid in developing murine hearts. Biochimie *113*: 1–9.

Penney, J., and Tsai, L.-H. (2014). Histone deacetylases in memory and cognition. Sci. Signal. *7*: re12–re12.

Pepino, M.Y., Abate, P., Spear, N.E., and Molina, J.C. (2002). Disruption of maternal behavior by alcohol intoxication in the lactating rat: a behavioral and metabolic analysis. Alcohol. Clin. Exp. Res. *26*: 1205–1214.

Perez, M.J., Velasco, E., Monte, M.J., Gonzalez-Buitrago, J.M., and Marin, J.J.G. (2006). Maternal ethanol consumption during pregnancy enhances bile acid-induced oxidative stress and apoptosis in fetal rat liver. Toxicology *225*: 183–94.

Perkins, A., Lehmann, C., Lawrence, R.C., and Kelly, S.J. (2013). Alcohol exposure during development: Impact on the epigenome. Int. J. Dev. Neurosci. *31*: 391–397.

Petrelli, B., Weinberg, J., and Hicks, G.G. (2018). Effects of prenatal alcohol exposure (PAE): insights into FASD using mouse models of PAE. Biochem. Cell Biol. *96*: 131–147.

Petrenko, C.L.M., and Alto, M.E. (2017). Interventions in fetal alcohol spectrum disorders: An international perspective. Eur. J. Med. Genet. *60*: 79–91.

Pfinder, M., Kunst, A.E., Feldmann, R., Eijsden, M. van, and Vrijkotte, T.G.M. (2014). Educational Differences in Continuing or Restarting Drinking in Early and Late Pregnancy: Role of Psychological and Physical Problems. J. Stud. Alcohol Drugs *75*: 47–55.

Philibin, S.D., Hernandez, A., Self, D.W., and Bibb, J.A. (2011). Striatal Signal Transduction and Drug Addiction. Front. Neuroanat. *5*: 1–15.

Pierce, R.C., and Kumaresan, V. (2006). The mesolimbic dopamine system: The final common pathway for the reinforcing effect of drugs of abuse? Neurosci. Biobehav. Rev. *30*: 215–238.

Ponomarev, I. (2013). Epigenetic control of gene expression in the alcoholic brain. Alcohol Res. *35*: 69–76.

Popoola, D.O., Borrow, A.P., Sanders, J.E., Nizhnikov, M.E., and Cameron, N.M. (2015). Can low-level ethanol exposure during pregnancy influence maternal care? An investigation using two strains of rat across two generations. Physiol. Behav. *148*: 111–121.

Popova, S., Lange, S., Probst, C., Gmel, G., and Rehm, J. (2017a). Estimation of national, regional, and global prevalence of alcohol use during pregnancy and fetal alcohol syndrome: a systematic review and meta-analysis. Lancet Glob. Heal. *5*: e290–e299.

Popova, S., Lange, S., Probst, C., Gmel, G., and Rehm, J. (2018). Global prevalence of alcohol use and binge drinking during pregnancy, and fetal alcohol spectrum disorder. Biochem. Cell Biol. *96*: 237–240.

Popova, S., Lange, S., Probst, C., Parunashvili, N., and Rehm, J. (2017b). Prevalence of alcohol consumption during pregnancy and Fetal Alcohol Spectrum Disorders among the general and Aboriginal populations in Canada and the United States. Eur. J. Med. Genet. *60*: 32–48.

Popova, S., Lange, S., Shield, K., Burd, L., and Rehm, J. (2019). Prevalence of fetal alcohol spectrum disorder among special subpopulations: a systematic review and meta-analysis. Addiction 1150–1172.

Popova, S., Lange, S., Shield, K., Mihic, A., Chudley, A.E., Mukherjee, R.A.S., et al. (2016). Comorbidity of fetal alcohol spectrum disorder: A systematic review and meta-analysis. Lancet *387*: 978–987.

Popović, M., Caballero-Bleda, M., and Guerri, C. (2006). Adult rat's offspring of alcoholic mothers are impaired on spatial learning and object recognition in the Can test. Behav. Brain Res. *174*: 101–11.

Poulos, S.G., Richie, W.D., Bailey, R.K., Lee, A., Peña, I. Dela, Sanberg, P.R., et al. (2014). The potential of neural stem cell transplantation for the treatment of fetal alcohol spectrum disorder. Prog. Neuropsychopharmacol. Biol. Psychiatry *54C*: 149–156.

Pradier, B., Erxlebe, E., Markert, A., and Rácz, I. (2018). Microglial IL-1β progressively increases with duration of alcohol consumption. Naunyn. Schmiedebergs. Arch. Pharmacol. *391*: 455–461.

Puga, F., Barrett, D.W., Bastida, C.C., and Gonzalez-Lima, F. (2007). Functional networks underlying latent inhibition learning in the mouse brain. Neuroimage *38*: 171–183.

Purger, D., Gibson, E.M., and Monje, M. (2016). Myelin plasticity in the central nervous system. Neuropharmacology *110*: 563–573.

Qiao, Y., Wang, P., Qi, J., Zhang, L., and Gao, C. (2012). TLR-induced NF-κB activation regulates NLRP3 expression in murine macrophages. FEBS Lett. *586*: 1022–6.

Qin, L., and Crews, F.T. (2012). NADPH oxidase and reactive oxygen species contribute to alcohol-induced microglial activation and neurodegeneration. J. Neuroinflammation *9*: 5.

Qin, L., He, J., Hanes, R.N., Pluzarev, O., Hong, J.-S., and Crews, F.T. (2008). Increased systemic and brain cytokine production and neuroinflammation by endotoxin following ethanol treatment. J. Neuroinflammation *5*: 10.

Raineki, C., Bodnar, T.S., Holman, P.J., Baglot, S.L., Lan, N., and Weinberg, J. (2017). Effects of early-life adversity on immune function are mediated by prenatal environment: Role of prenatal alcohol exposure. Brain. Behav. Immun. *66*: 210–220.

Raineki, C., Chew, L., Mok, P., Ellis, L., and Weinberg, J. (2016). Short- and long-term effects of stress during adolescence on emotionality and HPA function of animals exposed to alcohol prenatally. Psychoneuroendocrinology *74*: 13–23.

Raineki, C., Hellemans, K.G.C., Bodnar, T., Lavigne, K.M., Ellis, L., Woodward, T.S., et al. (2014). Neurocircuitry underlying stress and emotional regulation in animals prenatally exposed to alcohol and subjected to chronic mild stress in adulthood. Front. Endocrinol. (Lausanne). *5*: 1–14.

Rajakrishnan, V., Viswanathan, P., Rajasekharan, K.N., and Menon, V.P. (1999). Neuroprotective role of curcumin from Curcuma longa on ethanol-induced brain damage. Phyther. Res. *13*: 571–574.

Ramachandran, V., Perez, A., Chen, J., Senthil, D., Schenker, S., and Henderson, G.I. (2001). In Utero Ethanol Exposure Causes Mitochondrial Dysfunction, Which Can Result in Apoptotic Cell Death in Fetal Brain: A Potential Role for 4-Hydroxynonenal.

Alcohol. Clin. Exp. Res. *25*: 862–871.

Ramsay, M. (2010). Genetic and epigenetic insights into fetal alcohol spectrum disorders. Genome Med. *2*: 27.

Reddy, V.D., Padmavathi, P., Kavitha, G., Saradamma, B., and Varadacharyulu, N. (2013). Alcohol-induced oxidative/nitrosative stress alters brain mitochondrial membrane properties. Mol. Cell. Biochem. *375*: 39–47.

Redila, V. a., Olson, A.K., Swann, S.E., Mohades, G., Webber, A.J., Weinberg, J., et al. (2006). Hippocampal cell proliferation is reduced following prenatal ethanol exposure but can be rescued with voluntary exercise. Hippocampus *16*: 305–311.

Reis, K.P., Heimfarth, L., Pierozan, P., Ferreira, F., Loureiro, S.O., Fernandes, C.G., et al. (2015). High postnatal susceptibility of hippocampal cytoskeleton in response to ethanol exposure during pregnancy and lactation. Alcohol *49*: 665–74.

Resendiz, M., Mason, S., Lo, C.L., and Zhou, F.C. (2014). Epigenetic regulation of the neural transcriptome and alcohol interference during development. Front. Genet. *5*: 285.

Rhodes, J.S., Best, K., Belknap, J.K., Finn, D. a, and Crabbe, J.C. (2005). Evaluation of a simple model of ethanol drinking to intoxication in C57BL/6J mice. Physiol. Behav. *84*: 53–63.

Rhodes, J.S., Ford, M.M., Yu, C.H., Brown, L.L., Finn, D.A., Garland, T., et al. (2007). Mouse inbred strain differences in ethanol drinking to intoxication. Genes, Brain Behav. *6*: 1–18.

Rice, J., and Gu, C. (2019). Function and Mechanism of Myelin Regulation in Alcohol Abuse and Alcoholism. Bioessays *41*: e1800255.

Rice, J.P., Suggs, L.E., Lusk, A. V., Parker, M.O., Candelaria-Cook, F.T., Akers, K.G., et al. (2012). Effects of exposure to moderate levels of ethanol during prenatal brain development on dendritic length, branching, and spine density in the nucleus accumbens and dorsal striatum of adult rats. Alcohol *46*: 577–584.

Riha, P.D., Rojas, J.C., and Gonzalez-Lima, F. (2011). Beneficial network effects of methylene blue in an amnestic model. Neuroimage *54*: 2623–2634.

Riley, E.P., Infante, M.A., and Warren, K.R. (2011). Fetal Alcohol Spectrum Disorders: An Overview. Neuropsychol. Rev. *21*: 73–80.

Robinson, G., Most, D., Ferguson, L.B., Mayfield, J., Harris, R.A., and Blednov, Y.A. (2014). Neuroimmune pathways in alcohol consumption: evidence from behavioral and genetic studies in rodents and humans. Int. Rev. Neurobiol. *118*: 13–39.

Rodríguez-Martos Dauer, A., Gual Solé, A., and Llopis Llácer, J.J. (1999). [The

'standard drink unit' as a simplified record of alcoholic drink consumption and its measurement in Spain]. Med. Clin. (Barc). *112*: 446–50.

Rodriguez, C.I., Davies, S., Calhoun, V., Savage, D.D., and Hamilton, D.A. (2016). Moderate Prenatal Alcohol Exposure Alters Functional Connectivity in the Adult Rat Brain. Alcohol. Clin. Exp. Res. *40*: 2134–2146.

Roger-Sánchez, C., Aguilar, M.A., Rodríguez-Arias, M., Aragon, C.M., and Miñarro, J. (2012). Age- and sex-related differences in the acquisition and reinstatement of ethanol CPP in mice. Neurotoxicol. Teratol. *34*: 108–115.

Rogers, B.P., Parks, M.H., Nickel, M.K., Katwal, S.B., and Martin, P.R. (2012). Reduced Fronto-Cerebellar Functional Connectivity in Chronic Alcoholic Patients. Alcohol. Clin. Exp. Res. *36*: 294–301.

Roozen, S., Black, D., Peters, G.-J.Y., Kok, G., Townend, D., Nijhuis, J.G., et al. (2016a). Fetal Alcohol Spectrum Disorders (FASD): an Approach to Effective Prevention. Curr. Dev. Disord. Reports *3*: 229–234.

Roozen, S., Peters, G.-J.Y., Kok, G., Townend, D., Nijhuis, J., and Curfs, L. (2016b). Worldwide Prevalence of Fetal Alcohol Spectrum Disorders: A Systematic Literature Review Including Meta-Analysis. Alcohol. Clin. Exp. Res. *40*: 18–32.

Ros-Simó, C., and Valverde, O. (2012). Early-life social experiences in mice affect emotional behaviour and hypothalamic-pituitary-adrenal axis function. Pharmacol. Biochem. Behav. *102*: 434–41.

Ross, E.J., Graham, D.L., Money, K.M., and Stanwood, G.D. (2015). Developmental consequences of fetal exposure to drugs: what we know and what we still must learn. Neuropsychopharmacology *40*: 61–87.

Royal College of Physicians of London (1726). Royal College of Physicians.

Rubert, G., Miñana, R., Pascual, M., and Guerri, C. (2006). Ethanol exposure during embryogenesis decreases the radial glial progenitor pool and affects the generation of neurons and astrocytes. J. Neurosci. Res. *84*: 483–496.

Rubio, S., Begega, A., Santín, L.J., and Arias, J.L. (1996). Ethanol- and diazepam-induced cytochrome oxidase activity in mammillary bodies. Pharmacol. Biochem. Behav. *55*: 309–314.

Runegaard, A.H., Sørensen, A.T., Fitzpatrick, C.M., Jørgensen, S.H., Petersen, A. V, Hansen, N.W., et al. (2018). Locomotor- and Reward-Enhancing Effects of Cocaine Are Differentially Regulated by Chemogenetic Stimulation of Gi-Signaling in Dopaminergic Neurons. Eneuro *5*: ENEURO.0345-17.2018.

Russo, S.J., and Nestler, E.J. (2014). The Brain Reward Circuitry in Mood Disorders. Nat. Rev. Neurosci. *14*: 1–34.

Sadrian, B., Lopez-Guzman, M., Wilson, D. a, and Saito, M. (2014). Distinct neurobehavioral dysfunction based on the timing of developmental binge-like alcohol exposure. Neuroscience *280*: 204–19.

Sadrian, B., Subbanna, S., Wilson, D.A., Basavarajappa, B.S., and Saito, M. (2012). Lithium prevents long-term neural and behavioral pathology induced by early alcohol exposure. Neuroscience *206*: 122–35.

Saito, M., Chakraborty, G., Hui, M., Masiello, K., and Saito, M. (2016). Ethanol-Induced Neurodegeneration and Glial Activation in the Developing Brain. Brain Sci. *6*: 31.

Salami, M., Aghanouri, Z., Akbar Rashidi, A., and Keshavarz, M. (2004). Prenatal Alcohol Exposure and Dysfunction of Hippocampal Formation in Cognition. Int. J. Reprod. Med. *2*: 43–50.

Samudio-Ruiz, S.L., Allan, A.M., Sheema, S., and Caldwell, K.K. (2010). Hippocampal N-methyl-d-aspartate receptor subunit expression profiles in a mouse model of prenatal alcohol exposure. Alcohol. Clin. Exp. Res. *34*: 342–353.

Samudio-Ruiz, S.L., Allan, A.M., Valenzuela, C.F., Perrone-Bizzozero, N.I., and Caldwell, K.K. (2009). Prenatal ethanol exposure persistently impairs NMDA receptor-dependent activation of extracellular signal-regulated kinase in the mouse dentate gyrus. J. Neurochem. *109*: 1311–1323.

Sanchez Vega, M.C., Chong, S., and Burne, T.H.J. (2013). Early gestational exposure to moderate concentrations of ethanol alters adult behaviour in C57BL/6J mice. Behav. Brain Res. *252*: 326–33.

Sanei, M., and Saberi-Demneh, A. (2019). Effect of curcumin on memory impairment: A systematic review. Phytomedicine *52*: 98–106.

Sarker, M.R., Franks, S., Sumien, N., Thangthaeng, N., Filipetto, F., and Forster, M. (2015). Curcumin mimics the neurocognitive and anti-inflammatory effects of caloric restriction in a mouse model of midlife obesity. PLoS One *10*: 1–18.

Sarker, M.R., and Franks, S.F. (2018). Efficacy of curcumin for age-associated cognitive decline: a narrative review of preclinical and clinical studies. GeroScience *40*: 73–95.

Sarman, I. (2018). Review shows that early fetal alcohol exposure may cause adverse effects even when the mother consumes low levels. Acta Paediatr. *140*: 874–888.

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

Savage, D.D., Rosenberg, M.J., Wolff, C.R., Akers, K.G., El-Emawy, A., Staples, M.C.,

et al. (2010). Effects of a novel cognition-enhancing agent on fetal ethanol-induced learning deficits. Alcohol. Clin. Exp. Res. *34*: 1793–1802.

Schambra, U.B., Nunley, K., Harrison, T.A., and Lewis, C.N. (2016). Consequences of low or moderate prenatal ethanol exposures during gastrulation or neurulation for open field activity and emotionality in mice. Neurotoxicol. Teratol. *57*: 39–53.

Schneider, M.L., Moore, C.F., and Adkins, M.M. (2011). The effects of prenatal alcohol exposure on behavior: rodent and primate studies. Neuropsychol. Rev. *21*: 186–203.

Schneider, M.L., Moore, C.F., Barnhart, T.E., Larson, J. a, DeJesus, O.T., Mukherjee, J., et al. (2005). Moderate-level prenatal alcohol exposure alters striatal dopamine system function in rhesus monkeys. Alcohol. Clin. Exp. Res. *29*: 1685–1697.

Schneider, R.D., and Thomas, J.D. (2016). Adolescent Choline Supplementation Attenuates Working Memory Deficits in Rats Exposed to Alcohol During the Third Trimester Equivalent. Alcohol. Clin. Exp. Res. *40*: 897–905.

Scholz, J., Niibori, Y., W Frankland, P., and P Lerch, J. (2015). Rotarod training in mice is associated with changes in brain structure observable with multimodal MRI. Neuroimage *107*: 182–189.

Schramm-Sapyta, N.L., Olsen, C.M., and Winder, D.G. (2006). Cocaine self-administration reduces excitatory responses in the mouse nucleus accumbens shell. Neuropsychopharmacology *31*: 1444–1451.

Sebastiani, G., Borrás-Novell, C., Casanova, M.A., Pascual Tutusaus, M., Ferrero Martínez, S., Gómez Roig, M.D., et al. (2018). The Effects of Alcohol and Drugs of Abuse on Maternal Nutritional Profile during Pregnancy. Nutrients *10*: 1–17.

Seleverstov, O., Tobiasz, A., Jackson, J.S., Sullivan, R., Ma, D., Sullivan, J.P., et al. (2017). Maternal alcohol exposure during mid-pregnancy dilates fetal cerebral arteries via endocannabinoid receptors. Alcohol *61*: 51–61.

Semple, B.D., Blomgren, K., Gimlin, K., Ferriero, D.M., and Noble-Haeusslein, L.J. (2013). Brain development in rodents and humans: Identifying benchmarks of maturation and vulnerability to injury across species. Prog. Neurobiol. *106–107*: 1–16.

Sen, N. (2015). Epigenetic Regulation of Memory by Acetylation and Methylation of Chromatin: Implications in Neurological Disorders, Aging, and Addiction. NeuroMolecular Med. *17*: 97–110.

Servais, L., Hourez, R., Bearzatto, B., Gall, D., Schiffmann, S.N., and Cheron, G. (2007). Purkinje cell dysfunction and alteration of long-term synaptic plasticity in fetal alcohol syndrome. Proc. Natl. Acad. Sci. U. S. A. *104*: 9858–9863.

Shah, A., Zuo, W., Kang, S., Li, J., Fu, R., Zhang, H., et al. (2017). The lateral habenula

and alcohol: Role of glutamate and M-type potassium channels. Pharmacol. Biochem. Behav. *162*: 94–102.

Shah, S.A., Yoon, G.H., and Kim, M.O. (2015). Protection of the Developing Brain with Anthocyanins Against Ethanol-Induced Oxidative Stress and Neurodegeneration. Mol. Neurobiol. *51*: 1278–1291.

Shakiba, A. (2014). The role of the cerebellum in neurobiology of psychiatric disorders. Neurol. Clin. *32*: 1105–15.

Shao, J., and Tu, D. (1995). The Jackknife and Bootstrap. Springer-Verlag, New York.

Shastri, A., Bonifati, D.M., and Kishore, U. (2013). Innate Immunity and Neuroinflammation. Mediators Inflamm. *2013*: 1–19.

Shawa, H. El, Abbott, C.W., and Huffman, K.J. (2013). Prenatal ethanol exposure disrupts intraneocortical circuitry, cortical gene expression, and behavior in a mouse model of FASD. J. Neurosci. *33*: 18893–905.

Shen, R.-Y., and Choong, K.-C. (2006). Different adaptations in ventral tegmental area dopamine neurons in control and ethanol exposed rats after methylphenidate treatment. Biol. Psychiatry *59*: 635–42.

Shenoda, B.B. (2017). An Overview of the Mechanisms of Abnormal GABAergic Interneuronal Cortical Migration Associated with Prenatal Ethanol Exposure. Neurochem. Res. *42*: 1279–1287.

Shiotsuki, H., Yoshimi, K., Shimo, Y., Funayama, M., Takamatsu, Y., Ikeda, K., et al. (2010). A rotarod test for evaluation of motor skill learning. J. Neurosci. Methods *189*: 180–185.

Shirasaka, T., Hashimoto, E., Ukai, W., Yoshinaga, T., Ishii, T., Tateno, M., et al. (2012). Stem cell therapy: social recognition recovery in a FASD model. Transl. Psychiatry *2*: e188.

Shrestha, U., and Singh, M. (2013). Effect of folic acid in prenatal alcohol induced behavioral impairment in Swiss albino mice. Ann. Neurosci. *20*: 134–138.

Shukla, S.D., Velazquez, J., French, S.W., Lu, S.C., Ticku, M.K., and Zakhari, S. (2008). Emerging role of epigenetics in the actions of alcohol. Alcohol. Clin. Exp. Res. *32*: 1525–1534.

Sikora, E., Scapagnini, G., and Barbagallo, M. (2010). Curcumin, inflammation, ageing and age-related diseases. Immun. Ageing *7*: 1.

Simi, a, Tsakiri, N., Wang, P., and Rothwell, N.J. (2007). Interleukin-1 and inflammatory neurodegeneration. Biochem. Soc. Trans. *35*: 1122–1126.

Singh, S., and Aggarwal, B.B. (1995). Activation of Transcription Factor NF-κB Is

Suppressed by Curcumin (Diferuloylmethane). J. Biol. Chem. *270*: 24995–25000.

Sisk, C.L., and Foster, D.L. (2004). The neural basis of puberty and adolescence. Nat. Neurosci. *7*: 1040–1047.

Skorput, a. G.J., Gupta, V.P., Yeh, P.W.L., and Yeh, H.H. (2015). Persistent Interneuronopathy in the Prefrontal Cortex of Young Adult Offspring Exposed to Ethanol In Utero. J. Neurosci. *35*: 10977–10988.

Smiley, J.F., Saito, M., Bleiwas, C., Masiello, K., Ardekani, B., Guilfoyle, D.N., et al. (2015). Selective reduction of cerebral cortex GABA neurons in a late gestation model of fetal alcohol spectrum disorder. Alcohol *49*: 571–580.

Smith, A.M., Zeve, D.R., Grisel, J.J., and Chen, W.-J.A. (2005). Neonatal alcohol exposure increases malondialdehyde (MDA) and glutathione (GSH) levels in the developing cerebellum. Brain Res. Dev. Brain Res. *160*: 231–8.

Smith, S.M., Garic, A., Flentke, G.R., and Berres, M.E. (2014). Neural crest development in fetal alcohol syndrome. Birth Defects Res. Part C - Embryo Today Rev. *102*: 210–20.

Sobrian, S.K., Jones, B.L., James, H., Kamara, F.N., and Holson, R.R. (2005). Prenatal ethanol preferentially enhances reactivity of the dopamine D 1 but not D2 or D3 receptors in offspring. Neurotoxicol. Teratol. *27*: 73–93.

Sokol, R.J., Delaney-Black, V., and Nordstrom, B. (2003). Fetal alcohol spectrum disorder. JAMA *290*: 2996–9.

Sorce, S., and Krause, K.-H. (2009). NOX Enzymes in the Central Nervous System: From Signaling to Disease. Antioxid. Redox Signal. *11*: 2481–2504.

Soria, G., Barbano, M.F., Maldonado, R., and Valverde, O. (2008). A reliable method to study cue-, priming-, and stress-induced reinstatement of cocaine self-administration in mice. Psychopharmacology (Berl). *199*: 593–603.

Sorrells, S.F., Paredes, M.F., Cebrian-Silla, A., Sandoval, K., Qi, D., Kelley, K.W., et al. (2018). Human hippocampal neurogenesis drops sharply in children to undetectable levels in adults. Nature *555*: 377–381.

Sowell, E.R., Johnson, A., Kan, E., Lu, L.H., Horn, J.D. Van, Toga, A.W., et al. (2008). Mapping white matter integrity and neurobehavioral correlates in children with fetal alcohol spectrum disorders. J Neurosci *28*: 1313–1319.

Spalding, K.L., Bergmann, O., Alkass, K., Bernard, S., Salehpour, M., Huttner, H.B., et al. (2013). Dynamics of Hippocampal Neurogenesis in Adult Humans. Cell *153*: 1219–1227.

Spanagel, R. (2009). Alcoholism: a systems approach from molecular physiology to

addictive behavior. Physiol. Rev. *89*: 649–705.

Spanagel, R., Noori, H.R., and Heilig, M. (2014). Stress and alcohol interactions: Animal studies and clinical significance. Trends Neurosci. *37*: 219–227.

Spear, N.E., and Molina, J.C. (2005). Fetal or infantile exposure to ethanol promotes ethanol ingestion in adolescence and adulthood: a theoretical review. Alcohol. Clin. Exp. Res. *29*: 909–929.

Stamatakis, A.M., Jennings, J.H., Ung, R.L., Blair, G.A., Weinberg, R.J., Neve, R.L., et al. (2013). A Unique Population of Ventral Tegmental Area Neurons Inhibits the Lateral Habenula to Promote Reward. Neuron *80*: 1039–1053.

Stockard, C., and Papanicolaou, G. (1916). A Further Analysis of the Hereditary Transmission of Degeneracy and Deformities by the Descendants of Alcoholized Mammals. Am. Nat. *50*: 65–88.

Strata, P. (2015). The emotional cerebellum. Cerebellum *14*: 570–7.

Stratton, K., Howe, C., and Battaglia, F. (1996). Fetal Alcohol Syndrome: Diagnosis, Epidemiology, Prevention, and Treatment. (Washington, D.C.: National Academies Press).

Streissguth, A.P., Bookstein, F.L., Barr, H.M., Sampson, P.D., O'Malley, K., and Young, J.K. (2004). Risk factors for adverse life outcomes in fetal alcohol syndrome and fetal alcohol effects. J. Dev. Behav. Pediatr. *25*: 228–38.

Streit, W.J., Mrak, R.E., and Griffin, W.S.T. (2004). Microglia and neuroinflammation: a pathological perspective. J. Neuroinflammation *1*: 14.

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

Subbanna, S., Nagre, N.N., Shivakumar, M., Umapathy, N.S., Psychoyos, D., and Basavarajappa, B.S. (2014a). Ethanol induced acetylation of histone at G9a exon1 and G9a-mediated histone H3 dimethylation leads to neurodegeneration in neonatal mice. Neuroscience *258*: 422–32.

Subbanna, S., Nagre, N.N., Umapathy, N.S., Pace, B.S., and Basavarajappa, B.S. (2014b). Ethanol exposure induces neonatal neurodegeneration by enhancing CB1R Exon1 histone H4K8 acetylation and up-regulating CB1R function causing neurobehavioral abnormalities in adult mice. Int. J. Neuropsychopharmacol. *18*: pyu028.

Subramoney, S., Eastman, E., Adnams, C., Stein, D.J., and Donald, K.A. (2018). The Early Developmental Outcomes of Prenatal Alcohol Exposure: A Review. Front. Neurol. *9*: 1108.

Suh, H.-S., Choi, S., Khattar, P., Choi, N., and Lee, S.C. (2010). Histone Deacetylase Inhibitors Suppress the Expression of Inflammatory and Innate Immune Response Genes in Human Microglia and Astrocytes. J. Neuroimmune Pharmacol. *5*: 521–532.

Sulik, K.K. (2005). Genesis of alcohol-induced craniofacial dysmorphism. Exp. Biol. Med. (Maywood). *230*: 366–75.

Sulik, K.K., Johnston, M.C., and Webb, M.A. (1981). Fetal alcohol syndrome: embryogenesis in a mouse model. Science *214*: 936–8.

Sullivan, W.C. (1899). A Note on the Influence of Maternal Inebriety on the Offspring. J. Ment. Sci. *45*: 489–503.

Summers, B.L., Henry, C.M. a, Rofe, A.M., and Coyle, P. (2008). Dietary zinc supplementation during pregnancy prevents spatial and object recognition memory impairments caused by early prenatal ethanol exposure. Behav. Brain Res. *186*: 230–8.

Suzuki, K. (2007). Neuropathology of developmental abnormalities. Brain Dev. *29*: 129–141.

Symons, M., Pedruzzi, R.A., Bruce, K., and Milne, E. (2018). A systematic review of prevention interventions to reduce prenatal alcohol exposure and fetal alcohol spectrum disorder in indigenous communities. BMC Public Health *18*: 1227.

Taffe, M. a, Kotzebue, R.W., Crean, R.D., Crawford, E.F., Edwards, S., and Mandyam, C.D. (2010). Long-lasting reduction in hippocampal neurogenesis by alcohol consumption in adolescent nonhuman primates. Proc. Natl. Acad. Sci. U. S. A. *107*: 11104–11109.

Tajuddin, N., Moon, K.-H., Marshall, S.A., Nixon, K., Neafsey, E.J., Kim, H.-Y., et al. (2014). Neuroinflammation and neurodegeneration in adult rat brain from binge ethanol exposure: abrogation by docosahexaenoic acid. PLoS One *9*: e101223.

Takeda, K., and Akira, S. (2015). Toll-Like Receptors. In Current Protocols in Immunology, (Hoboken, NJ, USA: John Wiley & Sons, Inc.), pp 14.12.1-14.12.10.

Tan, C.H., Denny, C.H., Cheal, N.E., Sniezek, J.E., and Kanny, D. (2015). Alcohol use and binge drinking among women of childbearing age - United States, 2011-2013. MMWR. Morb. Mortal. Wkly. Rep. *64*: 1042–6.

Tay, T.L., Savage, J.C., Hui, C.W., Bisht, K., and Tremblay, M.-È. (2017). Microglia across the lifespan: from origin to function in brain development, plasticity and cognition. J. Physiol. *595*: 1929–1945.

Terasaki, L.S., and Schwarz, J.M. (2016). Effects of Moderate Prenatal Alcohol Exposure during Early Gestation in Rats on Inflammation across the Maternal-Fetal-Immune Interface and Later-Life Immune Function in the Offspring. J.

Neuroimmune Pharmacol. *11*: 680–692.

Thiele, T.E., Crabbe, J.C., and Boehm, S.L. (2014). 'Drinking in the dark' (DID): A simple mouse model of binge-like alcohol intake. Curr. Protoc. Neurosci. *68*: 1–17.

Thiele, T.E., and Navarro, M. (2014). 'Drinking in the dark' (DID) procedures: a model of binge-like ethanol drinking in non-dependent mice. Alcohol *48*: 235–41.

Thomas, J.D., Fleming, S.L., and Riley, E.P. (2002). Administration of Low Doses of MK-801 During Ethanol Withdrawal in the Developing Rat Pup Attenuates Alcohol's Teratogenic Effects. Alcohol. Clin. Exp. Res. *26*: 1307–1313.

Thomas, J.D., Leany, B.D., and Riley, E.P. (2003). Differential vulnerability to motor deficits in second replicate HAS and LAS rats following neonatal alcohol exposure. Pharmacol. Biochem. Behav. *75*: 17–24.

Thomas, M.J., Kalivas, P.W., and Shaham, Y. (2008). Neuroplasticity in the mesolimbic dopamine system and cocaine addiction. Br. J. Pharmacol. *154*: 327–342.

Thompson, R.A., and Nelson, C.A. (2001). Developmental science and the media: Early brain development. Am. Psychol. *56*: 5–15.

Tian, L., Ma, L., Kaarela, T., and Li, Z. (2012). Neuroimmune crosstalk in the central nervous system and its significance for neurological diseases. J. Neuroinflammation *9*: 594.

Timary, P. de, Stärkel, P., Delzenne, N.M., and Leclercq, S. (2017). A role for the peripheral immune system in the development of alcohol use disorders? Neuropharmacology *122*: 148–160.

Tiwari, V., Arora, V., and Chopra, K. (2012). Attenuation of NF-κβ mediated apoptotic signaling by tocotrienol ameliorates cognitive deficits in rats postnatally exposed to ethanol. Neurochem. Int. *61*: 310–20.

Tiwari, V., and Chopra, K. (2012). Attenuation of oxidative stress, neuroinflammation, and apoptosis by curcumin prevents cognitive deficits in rats postnatally exposed to ethanol. Psychopharmacology (Berl). *224*: 519–535.

Tiwari, V., and Chopra, K. (2013). Protective effect of curcumin against chronic alcohol-induced cognitive deficits and neuroinflammation in the adult rat brain. Neuroscience *244*: 147–58.

Toga, A.W., Thompson, P.M., and Sowell, E.R. (2006). Mapping brain maturation. Trends Neurosci. *29*: 148–59.

Tong, M., Ziplow, J., Chen, W.C., Nguyen, Q.-G., Kim, C., and la Monte, S.M. de (2013). Motor Function Deficits Following Chronic Prenatal Ethanol Exposure are Linked to Impairments in Insulin/IGF, Notch and Wnt Signaling in the Cerebellum.

J. Diabetes Metab. *4*: 238.

Toosi, A., Shajiee, H., Khaksari, M., Vaezi, G., and Hojati, V. (2019). Obestatin improve spatial memory impairment in a rat model of fetal alcohol spectrum disorders via inhibiting apoptosis and neuroinflammation. Neuropeptides *74*: 88–94.

Topper, L.A., Baculis, B.C., and Valenzuela, C.F. (2015). Exposure of neonatal rats to alcohol has differential effects on neuroinflammation and neuronal survival in the cerebellum and hippocampus. J. Neuroinflammation *12*: 160.

Toso, L., Poggi, S.H., Abebe, D., Roberson, R., Dunlap, V., Park, J., et al. (2005). N-Methyl-D-aspartate subunit expression during mouse development altered by in utero alcohol exposure. Am. J. Obstet. Gynecol. *193*: 1534–1539.

Toso, L., Roberson, R., Woodard, J., Abebe, D., and Spong, C.Y. (2006). Prenatal alcohol exposure alters GABA Aα 5 expression: A mechanism of alcohol-induced learning dysfunction. Am. J. Obstet. Gynecol. *195*: 522–527.

Tschopp, J., and Schroder, K. (2010). NLRP3 inflammasome activation: the convergence of multiple signalling pathways on ROS production? Nat. Rev. Immunol. *10*: 210–215.

Uban, K. a, Sliwowska, J.H., Lieblich, S., Ellis, L. a, Yu, W.K., Weinberg, J., et al. (2010). Prenatal alcohol exposure reduces the proportion of newly produced neurons and glia in the dentate gyrus of the hippocampus in female rats. Horm. Behav. *58*: 835–43.

Ungerer, M., Knezovich, J., and Ramsay, M. (2013). In utero alcohol exposure, epigenetic changes, and their consequences. Alcohol Res. *35*: 37–46.

UNODC (2019). World Drug Report.

Valenzuela, C.F., Morton, R. a., Diaz, M.R., and Topper, L. (2012). Does moderate drinking harm the fetal brain? Insights from animal models. Trends Neurosci. *35*: 284–292.

Vallés, S.L., Blanco, A.M., Pascual, M., and Guerri, C. (2004). Chronic ethanol treatment enhances inflammatory mediators and cell death in the brain and in astrocytes. Brain Pathol. *14*: 365–71.

Vallone, D., Picetti, R., and Borrelli, E. (2000). Structure and function of dopamine receptors. Neurosci. Biobehav. Rev. *24*: 125–132.

Valverde, O., Mantamadiotis, T., Torrecilla, M., Ugedo, L., Pineda, J., Bleckmann, S., et al. (2004). Modulation of Anxiety-Like Behavior and Morphine Dependence in CREB-Deficient Mice. Neuropsychopharmacology *29*: 1122–1133.

Vargas, W.M., Bengston, L., Gilpin, N.W., Whitcomb, B.W., and Richardson, H.N.

(2014). Alcohol binge drinking during adolescence or dependence during adulthood reduces prefrontal myelin in male rats. J. Neurosci. *34*: 14777–82.

Veazey, K.J., Parnell, S.E., Miranda, R.C., and Golding, M.C. (2015). Dose-dependent alcohol-induced alterations in chromatin structure persist beyond the window of exposure and correlate with fetal alcohol syndrome birth defects. Epigenetics Chromatin *8*: 39.

Velasquez, K.M., Molfese, D.L., and Salas, R. (2014). The role of the habenula in drug addiction. Front. Hum. Neurosci. *8*: 174.

Vengeliene, V., Bilbao, A., Molander, A., and Spanagel, R. (2009). Neuropharmacology of alcohol addiction. Br. J. Pharmacol. *154*: 299–315.

Verdin, E., and Ott, M. (2015). 50 years of protein acetylation: from gene regulation to epigenetics, metabolism and beyond. Nat. Rev. Mol. Cell Biol. *16*: 258–264.

Vertes, R.P. (2004). Differential Projections of the Infralimbic and Prelimbic Cortex in the Rat. Synapse *51*: 32–58.

Vetreno, R.P., and Crews, F.T. (2012). Adolescent binge drinking increases expression of the danger signal receptor agonist HMGB1 and toll-like receptors in the adult prefrontal cortex. Neuroscience *226*: 475–488.

Villain, H., Florian, C., and Roullet, P. (2016). HDAC inhibition promotes both initial consolidation and reconsolidation of spatial memory in mice. Sci. Rep. *6*: 27015.

Volkow, N.D., Koob, G.F., and McLellan, A.T. (2016). Neurobiologic Advances from the Brain Disease Model of Addiction. N. Engl. J. Med. *374*: 363–71.

Volkow, N.D., Wiers, C.E., Shokri-Kojori, E., Tomasi, D., Wang, G.J., and Baler, R. (2017). Neurochemical and metabolic effects of acute and chronic alcohol in the human brain: Studies with positron emission tomography. Neuropharmacology *122*: 175–188.

Volmar, C.H., and Wahlestedt, C. (2015). Histone deacetylases (HDACs) and brain function. Neuroepigenetics *1*: 20–27.

Waddell, J., and Mooney, S. (2017). Choline and Working Memory Training Improve Cognitive Deficits Caused by Prenatal Exposure to Ethanol. Nutrients *9*: 1080.

Wagner, J.L., Zhou, F.C., and Goodlett, C.R. (2014). Effects of one- and three-day binge alcohol exposure in neonatal C57BL/6 mice on spatial learning and memory in adolescence and adulthood. Alcohol *48*: 99–111.

Wang, G., Shi, Y., Jiang, X., Leak, R.K., Hu, X., Wu, Y., et al. (2015a). HDAC inhibition prevents white matter injury by modulating microglia/macrophage polarization through the GSK3β/PTEN/Akt axis. Proc. Natl. Acad. Sci. U. S. A. *112*:

2853–8.

Wang, J., Haj-Dahmane, S., and Shen, R.-Y. (2006). Effects of prenatal ethanol exposure on the excitability of ventral tegmental area dopamine neurons in vitro. J. Pharmacol. Exp. Ther. *319*: 857–863.

Wang, L.L., Zhang, Z., Li, Q., Yang, R., Pei, X., Xu, Y., et al. (2009). Ethanol exposure induces differential microRNA and target gene expression and teratogenic effects which can be suppressed by folic acid supplementation. Hum. Reprod. *24*: 562–579.

Wang, P., Liu, B.-Y., Wu, M.-M., Wei, X.-Y., Sheng, S., You, S.-W., et al. (2019a). Moderate prenatal alcohol exposure suppresses the TLR4-mediated innate immune response in the hippocampus of young rats. Neurosci. Lett. *699*: 77–83.

Wang, R., Hausknecht, K.A., Shen, Y.-L., Haj-Dahmane, S., Vezina, P., and Shen, R.-Y. (2018). Environmental enrichment reverses increased addiction risk caused by prenatal ethanol exposure. Drug Alcohol Depend. *191*: 343–347.

Wang, R., Shen, Y.-L., Hausknecht, K.A., Chang, L., Haj-Dahmane, S., Vezina, P., et al. (2019b). Prenatal ethanol exposure increases risk of psychostimulant addiction. Behav. Brain Res. *356*: 51–61.

Wang, X., Chu, G., Yang, Z., Sun, Y., Zhou, H., Li, M., et al. (2015b). Ethanol directly induced HMGB1 release through NOX2/NLRP1 inflammasome in neuronal cells. Toxicology *334*: 104–110.

Warner, L.A. (1995). Prevalence and Correlates of Drug Use and Dependence in the United States. Arch. Gen. Psychiatry *52*: 219.

Warner, R.H., and Rosett, H.L. (1975). The effects of drinking on offspring: an historical survey of the American and British literature. J. Stud. Alcohol *36*: 1395–1420.

Warren, K., and Li, T. (2005). Genetic polymorphisms: impact on the risk of fetal alcohol spectrum disorders. Birth Defects Res. A. Clin. Mol. Teratol. *73*: 195–203.

Weaver, I.C.G., Cervoni, N., Champagne, F. a, D'Alessio, A.C., Sharma, S., Seckl, J.R., et al. (2004). Epigenetic programming by maternal behavior. Nat. Neurosci. *7*: 847–854.

Wechsler, H., and Nelson, T.F. (2001). Binge drinking and the American college student: what's five drinks? Psychol. Addict. Behav. *15*: 287–91.

Weinberg, J., Sliwowska, J.H., Lan, N., and Hellemans, K.G.C. (2008). Prenatal Alcohol Exposure: Foetal Programming, the Hypothalamic-Pituitary-Adrenal Axis and Sex Differences in Outcome. J. Neuroendocrinol. *20*: 470–488.

Werts, R.L., Calcar, S.C. Van, Wargowski, D.S., and Smith, S.M. (2014). Inappropriate

feeding behaviors and dietary intakes in children with fetal alcohol spectrum disorder or probable prenatal alcohol exposure. Alcohol. Clin. Exp. Res. *38*: 871–8.

Wieczorek, L., Fish, E.W., O'Leary-Moore, S.K., Parnell, S.E., and Sulik, K.K. (2015). Hypothalamic-pituitary-adrenal axis and behavioral dysfunction following early binge-like prenatal alcohol exposure in mice. Alcohol *49*: 207–217.

Wilcox, M. V, Cuzon Carlson, V.C., Sherazee, N., Sprow, G.M., Bock, R., Thiele, T.E., et al. (2014). Repeated binge-like ethanol drinking alters ethanol drinking patterns and depresses striatal GABAergic transmission. Neuropsychopharmacology *39*: 579–94.

Wilhelm, C.J., and Guizzetti, M. (2015). Fetal Alcohol Spectrum Disorders: An Overview from the Glia Perspective. Front. Integr. Neurosci. *9*: 65.

Wilhoit, L.F., Scott, D.A., and Simecka, B.A. (2017). Fetal Alcohol Spectrum Disorders: Characteristics, Complications, and Treatment. Community Ment. Health J. *53*: 711–718.

Williams-Hemby, L., Grant, K.A., J. Gatto, G., and J. Porrino, L. (1996). Metabolic mapping of the effects of chronic voluntary ethanol consumption in rats. Pharmacol. Biochem. Behav. *54*: 415–423.

Wilsnack, R.W., Wilsnack, S.C., Kristjanson, A.F., Vogeltanz-Holm, N.D., and Gmel, G. (2009). Gender and alcohol consumption: patterns from the multinational GENACIS project. Addiction *104*: 1487–1500.

Wolf, M.E. (2002). Addiction: making the connection between behavioral changes and neuronal plasticity in specific pathways. Mol. Interv. *2*: 146–57.

Wolf, M.E. (2012). Addiction. In Basic Neurochemistry, (Elsevier), pp 1037–1055.

Wolf, M.E. (2016). Synaptic mechanisms underlying persistent cocaine craving. Nat. Rev. Neurosci. *17*: 351–65.

Wolstenholme, J.T., Mahmood, T., Harris, G.M., Abbas, S., and Miles, M.F. (2017). Intermittent Ethanol during Adolescence Leads to Lasting Behavioral Changes in Adulthood and Alters Gene Expression and Histone Methylation in the PFC. Front. Mol. Neurosci. *10*: 307.

Wong-Riley, M.T.T. (1989). Cytochrome oxidase: an endogenous marker for neuron activity. Trends Neurosci. *12*: 94–101.

Woodward Hopf, F., and Mangieri, R.A. (2018). Do Alcohol-Related AMPA-Type Glutamate Receptor Adaptations Promote Intake? Handb. Exp. Pharmacol. *248*: 157–186.

Workman, J.L., Raineki, C., Weinberg, J., and Galea, L.A.M. (2015). Alcohol and pregnancy: Effects on maternal care, HPA axis function, and hippocampal

neurogenesis in adult females. Psychoneuroendocrinology *57*: 37–50.

World Health Organization, W. (2018). Global status report on alcohol and health 2018 (World Health Organization).

Wozniak, D.F., Hartman, R.E., Boyle, M.P., Vogt, S.K., Brooks, A.R., Tenkova, T., et al. (2004). Apoptotic neurodegeneration induced by ethanol in neonatal mice is associated with profound learning/memory deficits in juveniles followed by progressive functional recovery in adults. Neurobiol. Dis. *17*: 403–414.

Wozniak, J.R., Fuglestad, A.J., Eckerle, J.K., Fink, B.A., Hoecker, H.L., Boys, C.J., et al. (2015). Choline supplementation in children with fetal alcohol spectrum disorders: a randomized, double-blind, placebo-controlled trial. Am. J. Clin. Nutr. *102*: 1113–25.

Wu, Y., Lousberg, E.L., Moldenhauer, L.M., Hayball, J.D., Coller, J.K., Rice, K.C., et al. (2012). Inhibiting the TLR4-MyD88 signalling cascade by genetic or pharmacological strategies reduces acute alcohol-induced sedation and motor impairment in mice. Br. J. Pharmacol. *165*: 1319–1329.

Xu, C., and Shen, R.Y. (2001). Amphetamine normalizes the electrical activity of dopamine neurons in the ventral tegmental area following prenatal ethanol exposure. J. Pharmacol. Exp. Ther. *297*: 746–52.

Xu, Y., Lin, D., Li, S., Li, G., Shyamala, S.G., Barish, P.A., et al. (2009). Curcumin reverses impaired cognition and neuronal plasticity induced by chronic stress. Neuropharmacology *57*: 463–71.

Yan, X., Pan, B., Lv, T., Liu, L., Zhu, J., Shen, W., et al. (2017). Inhibition of histone acetylation by curcumin reduces alcohol-induced fetal cardiac apoptosis. J. Biomed. Sci. *24*: 1.

Yang, J.-Y., Xue, X., Tian, H., Wang, X.-X., Dong, Y.-X., Wang, F., et al. (2014). Role of microglia in ethanol-induced neurodegenerative disease: Pathological and behavioral dysfunction at different developmental stages. Pharmacol. Ther. *144*: 321–37.

Yates, W.R., Cadoret, R.J., Troughton, E.P., Stewart, M., and Giunta, T.S. (1998). Effect of fetal alcohol exposure on adult symptoms of nicotine, alcohol, and drug dependence. Alcohol. Clin. Exp. Res. *22*: 914–20.

Yoon, T., Okada, J., Jung, M., and Kim, J. (2008). Prefrontal cortex and hippocampus subserve different components of working memory in rats. Learn. Mem. *15*: 97–105.

Young, C., Klocke, B.J., Tenkova, T., Choi, J., Labruyere, J., Qin, Y.-Q., et al. (2003). Ethanol-induced neuronal apoptosis in vivo requires BAX in the developing mouse brain. Cell Death Differ. *10*: 1148–1155.

Young, C., Roth, K.A., Klocke, B.J., West, T., Holtzman, D.M., Labruyere, J., et al.

(2005). Role of caspase-3 in ethanol-induced developmental neurodegeneration. Neurobiol. Dis. *20*: 608–614.

Youngentob, S.L., and Glendinning, J.I. (2009). Fetal ethanol exposure increases ethanol intake by making it smell and taste better. Proc. Natl. Acad. Sci. U. S. A. *106*: 5359–5364.

Yu, S.Y., Gao, R., Zhang, L., Luo, J., Jiang, H., and Wang, S. (2013a). Curcumin ameliorates ethanol-induced memory deficits and enhanced brain nitric oxide synthase activity in mice. Prog. Neuro-Psychopharmacology Biol. Psychiatry *44*: 210–216.

Yu, S.Y., Zhang, M., Luo, J., Zhang, L., Shao, Y., and Li, G. (2013b). Curcumin ameliorates memory deficits via neuronal nitric oxide synthase in aged mice. Prog. Neuro-Psychopharmacology Biol. Psychiatry *45*: 47–53.

Zachariou, V., Sgambato-Faure, V., Sasaki, T., Svenningsson, P., Berton, O., Fienberg, A.A., et al. (2006). Phosphorylation of DARPP-32 at Threonine-34 is Required for Cocaine Action. Neuropsychopharmacology *31*: 555–562.

Zakhari, S. (2006). Overview: how is alcohol metabolized by the body? Alcohol Res. Health *29*: 245–254.

Zakhari, S. (2013). Alcohol metabolism and epigenetics changes. Alcohol Res. *35*: 6–16.

Zeisel, S.H. (2011). What choline metabolism can tell us about the underlying mechanisms of fetal alcohol spectrum disorders. Mol. Neurobiol. *44*: 185–191.

Zhang, L., Fang, Y., Xu, Y., Lian, Y., Xie, N., Wu, T., et al. (2015). Curcumin Improves Amyloid β-Peptide (1-42) Induced Spatial Memory Deficits through BDNF-ERK Signaling Pathway. PLoS One *10*: e0131525.

Zhang, L., Wu, C., Zhao, S., Yuan, D., Lian, G., Wang, X., et al. (2010). Demethoxycurcumin, a natural derivative of curcumin attenuates LPS-induced pro-inflammatory responses through down-regulation of intracellular ROS-related MAPK/NF-kappaB signaling pathways in N9 microglia induced by lipopolysaccharide. Int. Immunopharmacol. *10*: 331–8.

Zhang, T., Yanagida, J., Kamii, H., Wada, S., Domoto, M., Sasase, H., et al. (2019). Glutamatergic neurons in the medial prefrontal cortex mediate the formation and retrieval of cocaine-associated memories in mice. Addict. Biol. 1–11.

Zhang, W., Peng, C., Zheng, M., Gao, W., Zhu, J., Lv, T., et al. (2014). Prenatal alcohol exposure causes the over-expression of DHAND and EHAND by increasing histone H3K14 acetylation in C57 BL/6 mice. Toxicol. Lett. *228*: 140–6.

Zhang, X., Sliwowska, J.H., and Weinberg, J. (2005). Prenatal alcohol exposure and fetal programming: effects on neuroendocrine and immune function. Exp. Biol. Med.

(Maywood). *230*: 376–88.

Zhang, Y., Wang, H., Li, Y., and Peng, Y. (2018). A review of interventions against fetal alcohol spectrum disorder targeting oxidative stress. Int. J. Dev. Neurosci. *71*: 140–145.

Zheng, D., Li, Y., He, L., Tang, Y., Li, X., Shen, Q., et al. (2014). The protective effect of astaxanthin on fetal alcohol spectrum disorder in mice. Neuropharmacology *84*: 13–18.

Zhou, F.C., Sari, Y., Zhang, J.K., Goodlett, C.R., and Li, T.K. (2001). Prenatal alcohol exposure retards the migration and development of serotonin neurons in fetal C57BL mice. Dev. Brain Res. *126*: 147–155.

Zhou, R., Wang, S., and Zhu, X. (2010). Prenatal ethanol exposure attenuates GABAergic inhibition in basolateral amygdala leading to neuronal hyperexcitability and anxiety-like behavior of adult rat offspring. Neuroscience *170*: 749–57.

Zhou, R., Wang, S., and Zhu, X. (2012). Prenatal Ethanol Exposure Alters Synaptic Plasticity in the Dorsolateral Striatum of Rat Offspring via Changing the Reactivity of Dopamine Receptor. PLoS One *7*: e42443.

Zou, J.Y., and Crews, F.T. (2005). TNFα potentiates glutamate neurotoxicity by inhibiting glutamate uptake in organotypic brain slice cultures: neuroprotection by NFκB inhibition. Brain Res. *1034*: 11–24.

Zou, J.Y., and Crews, F.T. (2006). CREB and NF-kappaB transcription factors regulate sensitivity to excitotoxic and oxidative stress induced neuronal cell death. Cell. Mol. Neurobiol. *26*: 385–405.

Zou, J.Y., and Crews, F.T. (2010). Induction of innate immune gene expression cascades in brain slice cultures by ethanol: key role of NF-κB and proinflammatory cytokines. Alcohol. Clin. Exp. Res. *34*: 777–89.

Zou, J.Y., and Crews, F.T. (2014). Release of neuronal HMGB1 by ethanol through decreased HDAC activity activates brain neuroimmune signaling. PLoS One *9*: e87915.

Zuccolo, L., Lewis, S.J., Smith, G.D., Saya, K., Draper, E.S., Fraser, R., et al. (2013). Prenatal alcohol exposure and offspring cognition and school performance. A mendelian randomization natural experiment. Int. J. Epidemiol. *42*: 1358–1370.

ANNEX

During my PhD in the Neurobiology of Behaviour research group (GReNeC-NeuroBio) and during my research stay in the laboratory of Rainer Spanagel (Mannheim, Germany), I had the opportunity to be involved in other collaborative projects that have led to the following publications:

Article 1

Esteve-Arenys A, Gracia-Rubio I, **Cantacorps L**, Pozo OJ, Marcos J, Rodríguez-Árias M, Miñarro J, Valverde O. Binge ethanol drinking during adolescence modifies cocaine responses in mice. J Psychopharmacol. 2017 Jan;31(1):86-95. doi: 10.1177/0269881116681457.

Article 2

Blanco-Gandía MC, **Cantacorps L**, Aracil-Fernández A, Montagud-Romero S, Aguilar MA, Manzanares J, Valverde O, Miñarro J, Rodríguez-Arias M. Effects of bingeing on fat during adolescence on the reinforcing effects of cocaine in adult male mice. Neuropharmacology. 2017 Feb;113(Pt A):31-44. doi: 10.1016/j.neuropharm.2016.09.020.

Article 3

Portero-Tresserra M, Gracia-Rubio I, **Cantacorps L**, Pozo OJ, Gómez-Gómez A, Pastor A, López-Arnau R, de la Torre R, Valverde O. Maternal separation increases alcohol-drinking behaviour and reduces endocannabinoid levels in the mouse striatum and prefrontal cortex. Eur Neuropsychopharmacol. 2018 Feb 22. doi: 10.1016/j.euroneuro.2018.02.003.

Article 4

De Backer JF, Monlezun S, Detraux B, Gazan A, Vanopdenbosch L, Cheron J, Cannaza G, Valverde S, **Cantacorps L**, Nassar M, Venance L, Valverde O, Faure P, Zoli M, De Backer O, Gall D, Schiffmann S, de Kerchove d'Exaerde A. Deletion of Maged1 in mice abolishes locomotor and reinforcing effects of cocaine. EMBO rep. 2018 doi: 10.15252/embr.201745089

Article 5

Luján MÁ, **Cantacorps L**, Valverde O. The pharmacological reduction of hippocampal neurogenesis attenuates the protective effects of cannabidiol on cocaine voluntary intake. Addict Biol. 2019 Jun 4: e12778. doi: 10.1111/adb.12778.

Article 6

Bilbao A, Leixner S, Shoupeng W, **Cantacorps L**, Valverde O, Spanagel R. Reduced sensitivity to ethanol and excessive drinking in a mouse model of neuropathic pain. Addict Biol. 2019 Jun 25: e12784. doi: 10.1111/adb.12784.

Article 7

Montagud-Romero S, **Cantacorps L**, Valverde O. The HDAC inhibitor Trichostatin A reverses emotional and cognitive impairments induced by maternal binge alcohol drinking in mice. J Psychopharmacol. 2019 Jul 11:269881119857208. doi: 10.1177/0269881119857208.

Article 8

Montagud-Romero S, Fernández-Gómez FJ, **Cantacorps L**, Núñez C, Miñarro J, Rodríguez-Arias M, Milanés MV, Valverde O. Alcohol exposure during prenatal and lactational periods enhances reinforcing effects of alcohol through a mechanism involving glutamatergic neuroplasticity. Eur Neuropsychopharmacol. *(under review)*