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Foetal Alcohol Spectrum Disorders and Alterations in Brain and Behaviour

Consuelo Guerri[1,*], Alissa Bazinet[2] and Edward P. Riley[2]

[1]Department of Cell Pathology, Centro de Investigacion Principe Felipe, Avda Autopista del Saler, 46013-Valencia, Spain; [2]Department of Psychology and Center for Behavioral Teratology, San Diego State University, San Diego, CA 92120, USA

[*]Corresponding author: Centro de Investigacion Principe Felipe, Avda. Autopista del Saler 16, 46013-Valencia, Spain. Tel: +34-96-328-96-80; Fax: +34-96-328-97-01; E-mail: guerri@cipf.es

Abstract — The term 'Foetal Alcohol Spectrum Disorders (FASD)' refers to the range of disabilities that may result from prenatal alcohol exposure. This article reviews the effects of ethanol on the developing brain and its long-term structural and neurobehavioural consequences. Brain imaging, neurobehavioural and experimental studies demonstrate the devastating consequences of prenatal alcohol exposure on the developing central nervous system (CNS), identifying specific brain regions affected, the range of severity of effects and mechanisms involved. In particular, neuroimaging studies have demonstrated overall and regional volumetric and surface area reductions, abnormalities in the shape of particular brain regions, and reduced and increased densities for white and grey matter, respectively. Neurobehaviourally, FASD consists of a continuum of long-lasting deficits affecting multiple aspects of cognition and behaviour. Experimental studies have also provided evidence of the vulnerability of the CNS to the teratogenic effects of ethanol and have provided new insight on the influence of risk factors in the type and severity of observed brain abnormalities. Finally, the potential molecular mechanisms that underlie the neuroteratological effects of alcohol are discussed, with particular emphasis on the role of glial cells in long-term neurodevelopmental liabilities.

INTRODUCTION

The idea that ethanol affects the developing brain was apparent from the first modern clinical reports describing the offspring of alcohol-abusing women (Lemoine *et al*., 1968; Jones and Smith 1973), where a number of brain and behavioural deficits were noted. Since these reports, a large number of well-controlled experimental studies and other clinical reports have documented the vulnerability of the developing central nervous system (CNS) to ethanol. Ethanol is considered to be one of the most common substances that impact the developing brain, and prenatal alcohol exposure is a leading preventable cause of birth defects, mental retardation and neurodevelopmental disorders (American Academy of Pediatrics, 2000).

Among the most recognized consequences of prenatal alcohol exposure is the foetal alcohol syndrome (FAS; Jones and Smith, 1973), characterized by pre- and postnatal growth deficiencies, craniofacial anomalies and evidence of CNS dysfunction. FAS is now recognized as an important cause of intellectual disabilities and behaviour problems in many countries (Streissguth *et al*., 1991; Alati *et al*., 2006; Autti-Rämö *et al*., 2006; Ceccanti *et al*., 2007; Spohr *et al*., 2007). However, over the last 30 years, it has become clear that FAS is not the only outcome resulting from prenatal alcohol exposure. Indeed, the effects of prenatal alcohol exposure lie on a continuum of physical anomalies and behavioural and cognitive deficits. The term foetal alcohol spectrum disorders (FASD) has been adopted (Sokol *et al*., 2003) as a nondiagnostic umbrella term to describe this range of effects. The prevalence of FASD, which can be physical, mental or behavioural, is estimated to be ∼1% of all births (May and Gossage 2001).

Until recently, it was impossible to evaluate the effects of ethanol on human brain structure and function *in vivo*. However, novel neuroimaging techniques such as magnetic resonance imaging (MRI), functional MRI (fMRI), diffusion tensor imaging (DTI), magnetic resonance spectroscopy (MRS), positron emission tomography (PET) and single photon emission computed tomography (SPECT) have been employed on individuals with FASD to provide insights into the structural and functional alterations caused by prenatal ethanol exposure. Basic animal studies also provide evidence of the vulnerability of the CNS to the effects of ethanol, revealing that effects are not uniform and that some brain areas or cell populations are more vulnerable than others (Guerri, 1998, 2002).

This paper briefly reviews the current knowledge of the effects of ethanol on the developing CNS and their correlation, when appropriate, with the cognitive, behavioural and psychopathological deficits observed in individuals exposed prenatally to alcohol. Finally, potential mechanisms underlying the neuroteratological effects of ethanol, as well as the role of glial cells in long-term neurodevelopmental liabilities, are discussed.

Neurobehavioural disorders in individuals with FASD

Heavy prenatal alcohol exposure has been associated with widespread neuropsychological deficits across several domains, including general intelligence, memory, language, attention, learning, visuospatial abilities, executive functioning, fine and gross motor skills, and social and adaptive functioning (Mattson and Riley, 1998). The mean IQ of children with FAS is estimated in the low 70s (Mattson and Riley, 1998), and FAS is the leading preventable cause of mental retardation (Abel and Sokol, 1987). However, the majority of children with FAS are not mentally retarded, and IQ scores show a wide range (e.g. 20–120, Mattson and Riley, 1998). Individuals prenatally exposed to high levels of alcohol but without all the characteristics of FAS have IQs that average in the low 80s (Mattson and Riley, 1998). Children exposed to alcohol prenatally also have decreased academic achievement and higher rates of learning disabilities than non-exposed children (Howell *et al*., 2006), which may relate to impairments in verbal and nonverbal learning and memory (Mattson *et al*., 1996a; Mattson and Roebuck, 2002; Roebuck-Spencer and Mattson,

2004). Studies suggest that long-term retention of verbal information is intact in alcohol-exposed individuals, but initial encoding processes may be impaired (e.g. Mattson and Roebuck, 2002; Willford *et al*., 2004). In addition to nonverbal memory difficulties, individuals with FASD show visuospatial processing deficits (e.g., Mattson *et al.,* 1996a), suggesting abnormality in the frontal-subcortical pathway and greater damage to the left hemisphere. Attention deficits are also frequently cited in the FASD population (Coles *et al*., 2002; Mattson *et al*., 2006), and attention deficit hyperactivity disorder (ADHD) is the most frequent comorbid psychiatric disorder diagnosed in alcohol-exposed children (Fryer *et al.,* 2007a). Several studies have reported executive functioning deficits in individuals with FASD, including problems with response inhibition, set shifting, planning and concept formation, and verbal and nonverbal fluency (e.g. Kodituwakku *et al*., 1995; Mattson *et al*., 1999). Finally, motor dysfunction such as tremors, weak grasp, poor hand/eye coordination, and gait and balance difficulties were described in early reports of FAS (Jones and Smith, 1973) and supported in later studies (Mattson *et al*., 1998; Roebuck *et al*., 1998). Motor deficits persist into adulthood in heavily exposed populations (Connor *et al*., 2006).

Influence of prenatal alcohol exposure on brain structure

Before the use of neuroimaging techniques, autopsies provided the only information about specific brain abnormalities evidenced in humans with FAS. In their initial report, Jones and Smith (1973) noted widespread damage in the brain of an infant with FAS, including microcephaly, agenesis of the corpus callosum (CC) and anterior commissure, errors in migration, and anomalies in the cerebellum and brainstem. Subsequent reports confirmed these findings and added ventricular abnormalities, CNS disorganization, and abnormalities in the basal ganglia, hippocampus, pituitary gland and optic nerve (Jones and Smith, 1975; Clarren *et al*., 1978; Clarren, 1986; Coulter *et al*., 1993).

Despite demonstrating effects of prenatal alcohol on the brain, results from autopsy studies may not be typical. Since 1992, various imaging modalities have been used to study living individuals with FASD, which has allowed the identification of more subtle brain alterations occurring in FASD. Consistent with autopsy findings, MRI studies have reported overall volume reductions in the cranial, cerebral and cerebellar vaults in FASD (Mattson *et al.,* 1992, 1994, 1996c; Swayze II *et al*., 1997; Archibald *et al.,* 2001; Sowell *et al.,* 2001a, 2002a; Autti-Rämö *et al.,* 2002). Furthermore, other studies have suggested that this decrease is not uniform but rather that the parietal lobe (Archibald *et al*., 2001; Sowell *et al*., 2001a, 2002a, 2002b), portions of the frontal lobe (Sowell *et al*., 2002a) and specific areas of the cerebellum (Sowell *et al*., 1996; Autti-Rämö *et al*., 2002; O'Hare *et al*., 2005) appear to be especially sensitive to alcohol insult.

Abnormalities of the CC, including complete and partial agenesis (Riley *et al*., 1995; Swayze II *et al*., 1997; Bhatara *et al*., 2002), hypoplasia (Mattson *et al.,* 1992; Swayze II *et al*., 1997; Autti-Rämö *et al*., 2002; Bhatara *et al.,* 2002), displacement in posterior regions (Sowell *et al*., 2001b), regional surface area reductions in anterior and posterior areas (Riley *et al*., 1995; Sowell *et al*., 2001b) and increased variability in shape (Bookstein *et al*., 2002a, 2002b) have also been reported. More recently, DTI has been used to examine the white matter integrity of the CC (Ma *et al*., 2005; Wozniak *et al*., 2006). One study of young adults found that individuals with FAS had decreased white matter integrity in the genu and splenium of the CC when compared to controls (Ma *et al*., 2005). However, another study using individuals with more moderate levels of exposure found only diffusion differences in the isthmus of the CC (Wozniak *et al*., 2006). Importantly, CC abnormalities have been associated with neuropsychological deficits commonly observed in alcohol-exposed populations, such as bimanual coordination (Roebuck-Spencer and Mattson, 2004), attention (Coles *et al*., 2002), verbal learning ability (Sowell *et al*., 2001b) and executive functioning (Kodituwakku *et al*., 2001; Bookstein *et al*., 2002b).

The cerebellum has also been identified as particularly vulnerable to prenatal alcohol exposure. Specifically, individuals with FASD show reduced cerebellar volume (Mattson *et al*., 1994; Archibald *et al*., 2001) and surface area (Autti-Rämö *et al*., 2002) as well as reduced volume and displacement of the anterior vermis of the cerebellum (Sowell *et al*., 1996; Autti-Rämö *et al*., 2002; O'Hare *et al*., 2005). Two studies have confirmed that the volume of the anterior cerebellar vermis, an early developing part of the cerebellum, is reduced in FASD relative to controls even after controlling for overall brain size (Sowell *et al*., 1996; Autti-Rämö *et al*., 2002). FAS subjects also show more severe displacement in the superior and anterior edges of the anterior vermis than do non-dysmorphic FASD individuals (O'Hare *et al*., 2005). Given that the cerebellum is responsible for the execution of motor behaviour such as posture, balance and coordination, the structural abnormalities observed may help to explain motor deficits often seen in individuals with FASD (Roebuck *et al*., 1998; Roebuck-Spencer and Mattson, 2004). The cerebellum is also involved in other functions, such as attention regulation (e.g. Akshoomoff *et al*., 1997) and classical conditioning (Woodruff-Pak *et al*., 2000). In accordance with this, deficits in classically conditioned eyeblink responses are noted in children with FAS (Coffin *et al*., 2005) and cerebellar vermis displacement is negatively correlated with verbal learning and memory in FASD (O'Hare *et al*., 2005).

Several studies report volumetric reductions in the basal ganglia, although only the caudate is disproportionately reduced when overall brain size is accounted for (Mattson *et al*., 1992, 1994, 1996b; Archibald *et al*., 2001). In fact, the caudate has been reported to be one of the most sensitive brain areas to prenatal alcohol insult. Abnormalities in the caudate region are hypothesized to be related to deficits in executive functioning, attention and response inhibition (Mattson *et al*., 1996b).

Abnormalities of grey and white matter distribution and density within specific brain regions can occur in FASD. Archibald *et al*. (2001) concluded that white matter decreases are greater than grey matter decreases within the cerebrum, while Sowell *et al*. (2002a) discovered that grey matter density was significantly increased and white matter was decreased in the perisylvian cortices and inferior parietal regions, particularly in the left hemisphere. Structural irregularities may relate to these grey and white matter abnormalities, as the parietal region is also reduced in volume in alcohol-exposed individuals (Sowell *et al*., 2001a; 2002a; 2002b). Sowell *et al*. (2002a) suggest that the increased grey matter density observed in FASD may

be caused by an irregular cortical thinning process that occurs during development.

In addition to commonly observed structural abnormalities as described above, research is beginning to outline functional deficits in the brains of individuals with FASD. For example, a PET study has reported a subtle reduction in glucose metabolism in the thalamus and caudate (Clark *et al*., 2000) in young adults with FAS. Further, cerebral blood flow (CBF) as well as dopaminergic and serotonergic neurotransmission has been studied using SPECT. Decreased CBF in the left parietooccipital region was reduced while CBF in the right frontal region was increased in FASD (Riikonen *et al*., 1999). There were also reduced serotonin levels in the medial frontal cortex and an increase in striatal dopamine transporter binding (Riikonen *et al*., 2005). Another study demonstrated reduced CBF in the temporal region relative to the cerebellum (Bhatara *et al*., 2002).

fMRI and MRS have also been applied to the study of prenatal alcohol exposure. Malisza and colleagues (2005) conducted an fMRI study using a spatial working memory paradigm to examine haemodynamic brain activation in exposed and non-exposed adults and children. Individuals with FASD showed increased functional activity in the inferior and middle frontal cortices when compared with matched controls. In addition, controls showed increased frontal lobe activity with increasing task difficulty, while FASD participants did not. However, Bookheimer and Sowell (2005) note that the observed activation patterns cannot be attributed to fundamental differences in brain function between groups, as alcohol-exposed individuals were less accurate and had slower reaction times than controls, and results may instead be related to performance factors. Another study of alcohol-exposed children examined activation during a response inhibition task and found that individuals with FASD displayed increased frontal and decreased caudate activation in comparison to controls despite equivalent task performance (Fryer *et al*., 2007b). A third study examined activation patterns during a verbal paired associates learning task. Alcohol-exposed children showed significantly less activation in left medial and posterior temporal regions and significantly more activation in right dorsal frontal regions relative to controls, even when group differences in performance were statistically controlled (Sowell et al., 2007).

Two studies have examined functional brain metabolism in alcohol-exposed populations using MRS techniques. Results of one study suggest that prenatal alcohol exposure is associated with altered brain metabolism in several anatomic regions, including lower *N*-acetylaspartate/choline (NAA/Cho) and lower NAA/creatine (NAA/Cr) ratios in parietal and frontal cortices, frontal white matter, CC, thalamus and cerebellar dentate nucleus. Also, individuals with FASD had higher absolute levels of Cho and Cr in comparison to controls, but showed no difference in levels of NAA (Fagerlund *et al*., 2006). These data are indicative of a metabolic alteration in the glial cell population, rather than the neuronal cell population, associated with prenatal alcohol exposure; also, brain metabolism appears to be permanently affected (Fagerlund *et al*., 2006). In contrast, a second MRS study uncovered a higher metabolite ratio of NAA/Cr in the left caudate nucleus of individuals with FASD as compared to controls. Analysis of absolute concentrations suggested that this increase was due to an increase in the NAA concentration alone (Cortese *et al*., 2006).

Factors influencing the effects of prenatal ethanol exposure on the developing brain

The clinical data indicate considerable variability in the range and magnitude of prenatal ethanol-induced effects on brain abnormalities and behavioural outcomes. Several biological and environmental factors are known to influence the effects of ethanol on the developing brain, including dose of alcohol and exposure pattern, developmental timing of exposure, genetic background of the mother and foetus, maternal nutrition, maternal age, socioeconomic status and synergistic reactions with other drugs. Among these factors, the levels of alcohol reaching the foetal brain and the duration of exposure markedly influence the type and the extent of the damage (Maier *et al*., 1996). In addition, the genetic background of the mother and foetus, such as variations in ethanol metabolism, also influence the risk of ethanol-induced malformations in the foetus (Thomas *et al*., 1998). The more efficient alcohol dehydrogenase (ADH) allele, ADH 1B*3, affords protection for FASD outcomes (McCarver *et al*., 1997), while the maternal and foetal ADH1B*2 allele reduced the risk for FAS in a South African population (in comparison with ADH1B*1) (see rev., Warren and Li, 2005).

The specific brain structure affected and the magnitude of the damage are also strongly influenced by the developmental timing of ethanol exposure. For example, facial dysmorphology, a salient feature of FAS (Jones and Smith 1973), appears to arise only when high-peak blood alcohol levels occur during the embryonic stage of gastrulation (Sulik, 2005). Mice exposed to ethanol on embryonic day 7 (E7) or E8 (Sulik, 2005) and macaques exposed to ethanol on E19 or E20 (Astley *et al*., 1999) exhibit FAS-associated facial dysmorphia. Exposure to alcohol during gastrulation also has a negative impact on the developing brain, reducing the neural progenitors pool (Rubert *et al*., 2006) and causing long-term effects on the forebrain (Ashwell and Zhang, 1996; Miller, 2007) as well as on mature brainstem nuclei structures (Mooney and Miller, 2007). Human studies demonstrate that binge drinking as well as chronic alcohol abuse during the early stage of human embryogenesis (corresponding to the third week of human gestation) is associated with a greater incidence of craniofacial defects and mental disabilities (Ernhart *et al*., 1987).

Alcohol also affects other ontogenetic stages of brain development. For example, a second critical period is the stage of neuroepithelial cell proliferation and migration, which occurs in humans from 7 to 20 weeks of gestation (Suzuki, 2007) and from gestation day 12 (G12) to G20–21 in the rat. At this stage, most of the areas of the nervous system (except the cerebellum) begin to differentiate. Experimental studies report that ethanol exposure at this stage alters neuronal migration and affects the timing and the pattern of cell generation by reducing the number of neurons and glial cells in the neocortex, hippocampus and sensory nucleus (Gressens *et al*., 1992; Miller, 1995a, 1995b; Valles *et al*., 1997; Rubert *et al*., 2006). As with other neurodevelopmental disorders (Suzuki, 2007), perturbation of neuroglial proliferation and migration by ethanol could cause long-term abnormalities in the cerebral cortex and in brain size, as noted in individuals with FAS and FASD (e.g. Archibald

et al., 2001; Sowell *et al*., 2001a, 2002a), and likely contribute to the cognitive defects observed in adults with FASD. Additionally, since formation of the CC and glial midline starts around 7 weeks in humans (Paul *et al*., 2007; Richards *et al*., 2004), exposure to alcohol at this stage might disrupt the early events in callosal formation, and later on might dysregulate axonal pruning, leading to agenesis, hypoplasia or abnormalities in the CC (e.g. Riley *et al*., 1995; Swayze II *et al*., 1997; Bhatara *et al*., 2002). In fact, administration of a single high dose of ethanol to neonatal rats caused a marked reduction in the thickness of the CC (Olney, 2004), possibly resulting from the loss of cerebro-cortical neurons whose axons projections comprise much of the mass of the CC.

Finally, alcohol also interferes with the 'brain growth spurt' neonatal stage in the rat (roughly the equivalent of the third trimester of human gestation), a period characterized by glial development (astroglial and oligodendroglia), synaptogenesis and development of the cerebellum. Ethanol exposure induces microcephaly, causes neuronal cell loss in both the hippocampus and cerebellum (Goodlett and Lundahl, 1996; Dikranian *et al*., 2005), alters synaptogenesis and glial development (Guerri and Renau-Piqueras, 1997) and can cause learning/memory deficits, as well as long-term and neurobehavioural dysfunctions (Wozniak *et al*., 2004; Popovic *et al*., 2006). In addition, administration of a single high or moderate dose of ethanol to neonatal mice throughout the period of early synaptogenesis triggers a significant neuroapoptosis in several brain regions (e.g. forebrain, midbrain, brainstem, cerebellum, spinal cord and retina) (Ikonomidou *et al*., 2000; Dikranian *et al*., 2005; Tenkova *et al*., 2003), with the caudate nucleus and the frontal and parietal cortices the most vulnerable regions to the neuroapoptotic effects of ethanol (Young and Olney, 2005). In line with these data, alterations in the caudate nucleus and in the frontal cortices have also been noted in individuals with FASD (Cortese *et al*, 2006; Fagerlund *et al*., 2006).

Collectively, experimental data indicate that although ethanol can interfere with important ontogenetic stages of the mammalian brain, the levels of ethanol reaching the foetal brain and the developmental timing of ethanol exposure are important determinants of the specific brain structures affected and the resulting degree of damage.

Potential mechanisms involved in ethanol-induced brain damage during development

Several molecular mechanisms have been identified as potential candidates responsible for the range of FASD phenotypes (Guerri 1998, 2002; Goodlett *et al*., 2005). These mechanisms are likely to participate at different stages of development and/or with different doses of ethanol. They include (1) alterations in the regulation of gene expression (e.g. reduced retinoic acid signalling, homeobox gene expression, altered DNA methylation; Deltour *et al*., 1996; Rifas *et al*., 1997), (2) interference with mitogenic and growth factor responses involved in neural stem cell proliferation, migration and differentiation (see revs., Miller, 2006; Mooney *et al*., 2006; Siegenthaler and Miller, 2006), (3) disturbances in molecules that mediate cell–cell interactions (L1, NCAM, loss of trophic support; e.g. Wilkemeyer *et al*., 2002; Miñana *et al*., 2000), (4) activation of molecular signalling controlling cell survival or death (growth factors deprivation, oxidative stress, apoptotic signalling and caspase-3 activation, suppression of NMDA glutamate and GABAA receptors, withdrawal-induced glutamatergic excitotoxicity, e.g. Guerri *et al*., 1994; Pascual *et al*., 2003; Olney, 2004; Thomas *et al*., 2004; Young *et al*., 2005) and (5) derangements in glial proliferation, differentiation and functioning (Guerri *et al*., 2001, 2006). This last mechanism is supported by neuroimaging studies revealing global white matter reductions and abnormalities in FASD (Archibald *et al*., 2001; Sowell *et al*., 2002a). Due to the potential contribution of this mechanism to the brain damage observed in young adults with FASD, we will describe the evidence demonstrating that glial cells are an important target of alcohol teratogenesis.

Role of glial cells in the ethanol-induced brain abnormalities

Alterations in glial development are suspected to contribute to the adverse effects of alcohol on the developing brain because neuroglial heterotopias, alterations in neuronal migration and agenesis of the CC and anterior commissure (areas originally formed by glial cells) have been observed in postmortem studies of children with FAS (Clarren *et al*., 1978).

Glial cells are present throughout CNS development and play critical roles in multiple developmental events. During embryogenesis, radial glia (RG) provides physical and chemical guidance for the migration of young neurons, and new data indicate that RG serves as a multipotential neural precursor cell, having the potential to self-renew and to generate neurons (e.g. Sanai *et al*., 2004) and oligodendrocytes (Ventura and Goldman, 2007). During synaptogenesis, glial cells and glial-derived factors promote the formation of mature functional synapses (Ullian *et al*., 2001), regulate neurotransmitters and energy in the brain (Magistretti, 2006) and play key roles in synaptic function (Haydon, 2001; Fields and Stevens-Graham, 2002). Disturbances of glia or of neuronal–glial communication during the well-established critical periods of brain development can cause irreversible deficits in CNS function (Lammens, 2000; Ross and Walsh, 2001; Crespel *et al*., 2002). Alteration of astrocytes and of neuronal–glial interactions could also affect the later generation of oligodendrocytes and myelin (Ganat *et al*., 2006, Ishibashi *et al*., 2006), causing abnormalities in white matter.

Clinical and experimental studies provide compelling evidence that foetal and/or neonatal exposure to ethanol profoundly affects glial functions (Guerri and Renau-Piqueras, 1997; Guerri *et al*., 2001; 2006). Experimental studies demonstrate that ethanol exposure during embryogenesis reduces the telencephalic RG progenitor pool and its transformation into neurons and astrocytes (Rubert *et al*., 2006) and impairs RG morphology. Ethanol also impairs astroglial proliferation, survival and functions (Guerri *et al*., 2001, 2006) that can affect many developmental processes, such as the availability of trophic support molecules involved in neuronal survival, modulation of the formation of mature synapses and regulation of synaptic transmission (Pascual and Guerri, 2007). Neuroimaging studies of individuals with FASD have shown more important losses in white matter structures (mainly composed of astrocytes, oligodendrocytes and myelin) than in grey matter structures (Riikonen *et al*., 1999; Archibald *et al*., 2001; Sowell *et al*., 2001a, 2002b). Changes in brain metabolism

have also been demonstrated in adolescents and young adults with FASD (Fagerlund *et al*. 2006), using MRS to evaluate neuronal and glial markers. The metabolic anomalies observed in different brain structures (parietal and frontal cortices, frontal white matter, CC, thalamus and cerebellar dentate nucleus) of FASD individuals are consistent with abnormalities in the glial cell pool, rather than in the neurons. Because astroglial cells play critical roles in the metabolic processes linked to neuronal activity such as blood flow, energy and glucose utilization (e.g. Magistretti, 2006), the results suggest that ethanol-induced glial impairment during brain development could affect neuronal activity, leading to permanent metabolic and structural brain alterations. Overwhelming evidence derived from clinical and experimental data highlights the vulnerability of human glial cells to the teratogenic effects of ethanol and the permanent consequences of these effects. Nevertheless, there may also be some neuronal-mediated effects, since some studies reported increased neuronal death as a primary effect of ethanol administration to neonatal rats (e.g. Goodlett and Lundahl, 1996; Ikonomidou *et al*., 2000; Olney, 2004).

SUMMARY AND CONCLUSIONS

A substantial body of evidence has emerged for the devastating consequences of prenatal alcohol exposure on the developing CNS, allowing a clearer appreciation of the specific brain regions affected, mechanisms involved and the range of effects. Prenatal alcohol exposure causes structural alterations to the shape, volume and surface area of the overall brain and particular brain regions, as well as reduced white matter and increased grey matter densities in corresponding areas. In addition, studies are now beginning to describe functional brain activation abnormalities in alcohol-exposed populations that may provide a better understanding of the underlying neural processes associated with the neuropsychological impairments that have consistently been reported in such individuals. An overall goal of this research is to provide a comprehensive picture of the associations among structural and functional brain abnormalities and observed behaviour in individuals with FASD. Another aim of this work is to identify factors that mediate the relationship between prenatal alcohol exposure and the type and severity of brain abnormalities and behavioural deficits. To this aim, experimental studies have begun to outline dosage and timing factors of alcohol use during pregnancy that contribute to changes in critical CNS developmental processes and determine, in part, the range of effects a given individual may later display. Finally, it is important to understand the molecular mechanisms that underlie alcohol's neuroteratological effects, and research has pointed to abnormalities in glial cell proliferation, differentiation and function as one significant mechanism responsible for the adverse effects of alcohol on the developing brain. While the evidence for a neurocognitive phenotype of FASD is becoming more consistent, future research is needed to strengthen the current findings, as well as to identify additional factors that play a role in the variation of alcohol-related effects.

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REFERENCES

Abel EL, Sokol RJ. (1987) Incidence of fetal alcohol syndrome and economic impact of FAS-related anomalies. *Drug Alcohol Depend* **19**:51–70.

Akshoomoff NA, Courchesne E, Townsend JP. (1997) Attention coordination and anticipatory control. *Int Rev Neurobiol* **41**:575–98.

Alati R, Al Mamun A, Williams GM *et al.* (2006) In utero alcohol exposure and prediction of alcohol disorders in early adulthood: a birth cohort study. *Arch Gen Psychiatry* **63**:1009–16.

American Academy of Pediatrics. (2000) Committee on Substance Abuse and Committee on Children with Disabilities. Fetal alcohol syndrome and alcohol-related neurodevelopmental disorders. *Pediatrics* **106**:358–61.

Archibald SL, Fennema-Notestine C, Gamst A *et al.* (2001) Brain dysmorphology in individuals with severe prenatal alcohol exposure. *Dev. Med. Child Neurol.* **43**:148–54.

Ashwell KW, Zhang LI. (1996) Forebrain hypoplasia following prenatal ethanol exposure: quantitative analysis of effects on specific forebrain nuclei. *Pathology* **28**:161–6.

Astley SJ, Magnuson SI, Omnell LM *et al.* (1999) Fetal alcohol syndrome: changes in craniofacial form with age, cognition, and timing of ethanol exposure in the macaque. *Teratology* **59**:163–72.

Autti-Rämö I, Autti T, Korkman M *et al.* (2002) MRI findings in children with school problems who had been exposed prenatally to alcohol. *Dev. Med. Child Neurol.* **44**:98–106.

Autti-Rämö I, Fagerlund A, Ervalahti N *et al.* (2006) Fetal alcohol spectrum disorders in Finland: clinical delineation of 77 older children and adolescents. *Am J Med Genet A* **140**:137–43.

Bhatara VS, Lovrein F, Kirkeby J *et al.* (2002). Brain function in fetal alcohol syndrome assessed by single photon emission computed tomography. *S D J Med* **55**:59–62.

Bookheimer SY, Sowell ER. (2005). Brain imaging in FAS: commentary on the article by Malisza et al. *Pediatr Res* **58**:1148–9.

Bookstein FL, Sampson PD, Connor PD *et al.* (2002a) Midline corpus callosum is a neuroanatomical focus of fetal alcohol damage. *Anat Rec* **269**:162–74.

Bookstein FL, Streissguth AP, Sampson PD *et al.* (2002b) Corpus callosum shape and neuropsychological deficits in adult males with heavy fetal alcohol exposure. *NeuroImage* **15**:233–51.

Ceccanti M, Alessandra Spagnolo P, Tarani L *et al.* (2007) Clinical delineation of fetal alcohol spectrum disorders (FASD) in Italian children: comparison and contrast with other racial/ethnic groups and implications for diagnosis and prevention. *Neurosci Biobehav Rev* **31**:270–7.

Clark CM, Li D, Conry J *et al.* (2000) Structural and functional brain integrity of fetal alcohol syndrome in nonretarded cases. *Pediatrics* **105**:1096–9.

Clarren SK. (1986). Neuropathology in fetal alcohol syndrome. In West J R (ed.). *Alcohol and Brain Development* New York: Oxford University Press, 158–66.

Clarren SK, Alvord EC Jr, Sumi SM *et al.* (1978) Brain malfunctions related to prenatal exposure to alcohol. *J Pediatr* **92**:64–7.

Coffin JM, Baroody S, Schneider K *et al.* (2005) Impaired cerebellar learning in children with prenatal alcohol exposure: a comparative study of eyeblink conditioning in children with ADHD and dyslexia. *Cortex* **41**:389–98.

Coles CD, Platzman KA, Lynch ME *et al.* (2002) Auditory and visual sustained attention in adolescents prenatally exposed to alcohol. *Alcohol Clin Exp Res* **26**:263–71.

Connor PD, Sampson PD, Streissguth AP *et al.* (2006) Effects of prenatal alcohol exposure on fine motor coordination and balance: a study of two adult samples. *Neuropsychologia* **44**:744–51.

Cortese BM, Moore GJ, Bailey BA *et al.* (2006) Magnetic resonance and spectroscopic imaging in prenatal alcohol-exposed children: preliminary findings in the caudate nucleus. *Neurotoxicol Teratol* **28**:597–606.

Coulter CL, Leech RW, Schafer B *et al.* (1993) Midline cerebral dysgenesis, dysfunction of the hypothalamic pituitary axis, and fetal alcohol effects. *Arch Neurol* **50**:771–5.

Crespel A, Coubes P, Rousset MC *et al.* (2002) Immature-like astrocytes are associated with dendate granule cell migration in human temporal lobe epilepsy. *Neurosci Lett* **330**:114–8.

Deltour L, Ang HL, Duester G. (1996) Ethanol inhibition of retinoic acid synthesis as a potential mechanism for fetal alcohol syndrome. *FASEB J* **10**:1050–7.

Dikranian K, Qin YQ, Labruyere J *et al.* (2005) Ethanol-induced neuroapoptosis in the developing rodent cerebellum and related brain stem structures. *Brain Res Dev Brain Res* **155**:1–13.

Ernhart CB, Sokol RJ, Martier S *et al.* (1987) Alcohol teratogenicity in the human: a detailed assessment of specificity, critical period, and threshold. *Am J Obstet Gynecol* **156**:33–9.

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

Fields RD, Stevens-Graham B. (2002) New insights into neuron-glia communication. *Science* **298**:556–62.

Fryer SL, McGee CL, Matt GE *et al.* (2007a) Evaluation of psychopathological conditions in children with heavy prenatal alcohol exposure. *Pediatrics* **119**:733–41.

Fryer SL, Tapert SF, Mattson SN *et al.* (2007b) Prenatal alcohol exposure affects frontal–striatal BOLD response during inhibitory control. *Alcohol Clin Exp Res* **31**:1415–24.

Ganat YM, Silbereis J, Cave C. *et al*. (2006) Early postnatal astroglial cells produce multilineage precursors and neural stem cells in vivo. *J Neurosci* **26**:8609–21.

Goodlett CR, Horn KH, Zhou FC. (2005) Alcohol teratogenesis: mechanisms of damage and strategies for intervention. *Exp Biol Med* **230**:394–406.

Goodlett CR, Lundahl KR. (1996) Temporal determinants of neonatal alcohol-induced cerebellar damage and motor performance deficits. *Pharmacol Biochem Behav* **55**:531–40.

Gressens P, Lammens M, Picard JJ *et al.* (1992) Ethanol-induced disturbances of gliogenesis and neurogenesis in the developing murine brain: an in vitro and in vivo inmunohistochemical and ultrastructural study. *Alcohol Alcohol* **27**:219–26.

Guerri C. (1998) Neuroanatomical and neurophysiological mechanisms involved in central nervous system dysfunctions induced by prenatal alcohol exposure. *Alcohol Clin Exp Res* **22**:304–12.

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

Guerri C, Montoliu C, Renau-Piqueras J. (1994) Involvement of free radical mechanism in the toxic effects of alcohol: implications for fetal alcohol syndrome. *Adv Exp Med Biol* **366**:291–305.

Guerri C, Pascual M, Renau-Piqueras J. (2001) Glia and fetal alcohol syndrome. *Neurotoxicology* **22**:593–9.

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

Guerri C, Rubert G, Pascual M. (2006) Glial targets of developmental exposure to ethanol. In Miller MW (ed.). *Brain Development*. Oxford: Oxford University Press, 295–312.

Haydon PG. (2001) Glia: listening and talking to the synapse. *Nat Rev Neurosci* **2**:185–93.

Howell KK, Lynch ME, Platzman KA *et al.* (2006) Prenatal alcohol exposure and ability, academic achievement, and school functioning in adolescence: a longitudinal follow-up. *J Pediatr Psychol* **31**:116–26.

Ikonomidou C, Bittigau P, Ishimaru MJ *et al.* (2000) Ethanol-induced apoptotic neurodegeneration and fetal alcohol syndrome. *Science* **287**:1056–60.

Ishibashi T, Dakin KA, Stevens B *et al.* (2006) Astrocytes promote myelination in response to electrical impulses. *Neuron* **49**:823–32.

Jones KL, Smith DW. (1973) Recognition of the fetal alcohol syndrome in early infancy. *Lancet* **2**:999–1001.

Jones KL, Smith DW. (1975) The fetal alcohol syndrome. *Teratology* **12**:1–10.

Kodituwakku PW, Handmaker NS, Cutler SK *et al*. (1995) Specific impairments in self-regulation in children exposed to alcohol prenatally. *Alcohol Clin Exp Res* **19**:1558–64.

Kodituwakku PW, Kalberg W, May PA. (2001) The effects of prenatal alcohol exposure on executive functioning. *Alcohol Res Health* **25**:192–8.

Lammens M. (2000) Neuronal migration disorders in man. *Eur J Morphol* **38**:327–33.

Lemoine P, Harousseau H, Borteyru J-P *et al.* (1968) Les enfants de parents alcooliques. Anomalies observees. A propos de 127 cas. [Children of alcoholic parents. Abnormalities observed in 127 cases]. *Ouest Medical* **21**:476–82.

Ma X, Coles CD, Lynch ME *et al.* (2005) Evaluation of corpus callosum anisotropy in young adults with fetal alcohol syndrome according to diffusion tensor imaging. *Alcohol Clin Exp Res* **29**:1214–22.

Magistretti PJ. (2006) Neuron-glia metabolic coupling and plasticity. *J Exp Biol* **209**:2304–11.

Maier SE, Chen W, West JR. (1996) The effects of timing and duration of alcohol exposure on development of the fetal brain. In Abel EL (ed). *Fetal Alcohol Syndrome: From Mechanism to Prevention*. Boca Raton, FL: CRC Press, 27–50.

Malisza KL, Allman AA, Shiloff D *et al.* (2005) Evaluation of spatial working memory function in children and adults with fetal alcohol spectrum disorders: a functional magnetic resonance imaging study. *Pediatr Res* **58**:1150–7.

Mattson SN, Calarco KE, Lang AR. (2006) Focused and shifting attention in children with heavy prenatal alcohol exposure. *Neuropsychology* **20**:361–9.

Mattson SN, Goodman AM, Caine C *et al.* (1999) Executive functioning in children with heavy prenatal alcohol exposure. *Alcohol Clin Exp Res* **23**:1808–15.

Mattson SN, Gramling L, Delis D *et al.* (1996a) Global–local processing in children prenatally exposed to alcohol. *Child Neuropsychol* **2**:165–75.

Mattson SN, Riley EP. (1998) A review of the neurobehavioral deficits in children with fetal alcohol syndrome or prenatal exposure to alcohol. *Alcohol Clin Exp Res* **22**:279–94.

Mattson SN, Riley EP, Delis DC *et al.* (1996b) Verbal learning and memory in children with fetal alcohol syndrome. *Alcohol Clin Exp Res* **20**:810–6.

Mattson SN, Riley EP, Gramling LJ *et al.* (1998) Neuropsychological comparison of alcohol-exposed children with or without physical features of fetal alcohol syndrome. *Neuropsychology* **12**:146–53.

Mattson SN, Riley EP, Jernigan TL *et al.* (1992) Fetal alcohol syndrome: a case report of neuropsychological, MRI and EEG assessment of two children. *Alcohol Clin Exp Res* **16**:1001–3.

Mattson SN, Riley EP, Jernigan TL *et al.* (1994) A decrease in the size of the basal ganglia following prenatal alcohol exposure: a preliminary report. *Neurotoxicol Teratol* **16**:283–9.

Mattson SN, Riley EP, Sowell ER *et al.* (1996c) A decrease in the size of the basal ganglia in children with fetal alcohol syndrome. *Alcohol Clin Exp Res* **20**:1088–93.

Mattson SN, Roebuck TM. (2002) Acquisition and retention of verbal and nonverbal information in children with heavy prenatal alcohol exposure. *Alcohol Clin Exp Res* **26**:875–82.

May PA, Gossage JP. (2001) Estimating the prevalence of fetal alcohol syndrome: a summary. *Alcohol Res Health* **25**:159–67.

McCarver DG, Thomasson HR, Martier SS *et al.* (1997) Alcohol dehydrogenase-2′3 allele protects against alcohol-related birth defects among African Americans. *J Pharmacol Exp Ther* **283**:1095–110.

Miller MW. (1995a) Generation of neurons in the rat dendate gyrus and hippocampus: effect of prenatal and postnatal treatment with ethanol. *Alcohol Clin Exp Res* **19**:1500–9.

Miller MW. (1995b) Effect of pre- or postnatal exposure to ethanol on the total number of neurons in the trigeminal nerve: cell proliferation and neuronal death. *Alcohol Clin Exp Res* **19**:1359–63.

Miller MW. (2006) Early exposure to ethanol affects the proliferation of neuronal precursors. In Miller MW (ed.). *Brain Development. Normal Processes and Effects of Alcohol and Nicotine*. Oxford: Oxford University Press, 182–98.

Miller MW. (2007) Exposure to ethanol during gastrulation alters somatosensory-motor cortices and the underlying white matter in the macaque. *Cereb Cortex* **17**:2961–71.

Miñana R, Climent E, Barettino D *et al.* (2000) Alcohol exposure alters the expression pattern of neural cell adhesion molecules during brain development. *J Neurochem* **75**:954–64.

Mooney SM, Miller MW. (2007) Time-specific effects of ethanol exposure on cranial nerve nuclei: gastrulation and neuronogenesis. *Exp Neurol* **205**:56–63.

Mooney SM, Miller MW, Henderson GI. (2006) Intracellular events in ethanol-induced neuronal death. In Miller MW (ed.). *Brain Development. Normal Processes and Effects of Alcohol and Nicotine*. Oxford: Oxford University Press, 267–78.

O'Hare ED, Kan E, Yoshii J *et al.* (2005) Mapping cerebellar vermal morphology and cognitive correlates in prenatal alcohol exposure. *NeuroReport* **16**:1285–90.
Olney JW. (2004) Fetal alcohol syndrome at the cellular level. *Addict Biol* **9**:137–49.
Pascual M, Guerri C. (2007) The peptide NAP promotes neuronal growth and differentiation through extracellular signal-regulated protein kinase and Akt pathways, and protects neurons co-cultured with astrocytes damaged by ethano. *J Neurochem* **103**:557–68.
Pascual M, Valles SL, Renau-Piqueras J *et al.* (2003) Ceramide pathways modulate ethanol-induced cell death in astrocytes. *J Neurochem* **87**:1535–45.
Paul LK, Brown WS, Adolphs R *et al.* (2007) Agenesis of the corpus callosum: genetic, developmental and functional aspects of connectivity. *Nat Rev Neurosci* **8**:287–99.
Popovic M, Caballero-Bleda M, 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.
Richards LJ, Plachez C, Ren T. (2004) Mechanisms regulating the development of the corpus callosum and its agenesis in mouse and human. *Clin Genet* **66**:276–89.
Rifas L, Towler DA, Avioli LV. (1997) Gestational exposure to ethanol suppresses msx2 expression in developing mouse embryos. *Proc Natl Acad Sci USA* **94**:7549–54.
Riikonen R, Salonen I, Partanen K *et al.* (1999) Brain perfusion SPECT and MRI in foetal alcohol syndrome. *Dev Med Child Neurol* **41**:652–9.
Riikonen RS, Nokelainen P, Valkonen K *et al.* (2005) Deep serotonergic and dopaminergic structures in fetal alcoholic syndrome: a study with nor-β-CIT-single-photon emission computed tomography and magnetic resonance imaging volumetry. *Biol Psychiatry* **57**:1565–72.
Riley EP, Mattson SN, Sowell ER *et al.* (1995) Abnormalities of the corpus callosum in children prenatally exposed to alcohol. *Alcohol Clin Exp Res* **19**:1198–202.
Roebuck TM, Simmons RW, Mattson SN *et al.* (1998) Prenatal exposure to alcohol affects the ability to maintain postural balance. *Alcohol Clin Exp Res* **22**:252–8.
Roebuck-Spencer TM, Mattson SN. (2004) Implicit strategy affects learning in children with heavy prenatal alcohol exposure. *Alcohol Clin Exp Res* **28**:1424–31.
Ross ME, Walsh CA. (2001) Human brain malformations and their lessons for neuronal migration. *Annu Rev Neurosci* **24**:1041–70.
Rubert G, Miñana R, Pascual M *et al.* (2006) Ethanol exposure during embryogenesis decreases the radial glial progenitorpool and affects the generation of neurons and astrocytes. *J Neurosci Res* **84**:483–96.
Sanai N, Tramontin AD, Quiñones-Hinojosa A *et al.* (2004) Unique astrocytes ribbon in adult human brain contains neural stem cells but lacks chain migration. *Nature* **427**:740–4.
Siegenthaler JA, Miller MW. (2006) Mechanisms of ethanol-induced alterations in neuronal migration. In Miller MW (ed.). *Brain Development. Normal Processes and Effects of Alcohol and Nicotine*. Oxford: Oxford University Press, 216–29.
Sokol RG, Delaney-Black V, Nordstrom B. (2003) Fetal alcohol spectrum disorder. *JAMA* **290**:2996–9.
Sowell ER, Jernigan TL, Mattson SN *et al.* (1996) Abnormal development of the cerebellar vermis in children prenatally exposed to alcohol: size reduction in lobules I-V. *Alcohol Clin Exp Res* **20**:31–4.
Sowell ER, Lu LH, O'Hare ED *et al.* (2007) Functional magnetic resonance imaging of verbal learning in children with heavy prenatal alcohol exposure. *Neuro Report* **18**:635–9.
Sowell ER, Mattson SN, Thompson PM *et al.* (2001b) Mapping callosal morphology and cognitive correlates: effects of heavy prenatal alcohol exposure. *Neurology* **57**:235–44.
Sowell ER, Thompson PM, Mattson SN *et al.* (2001a) Voxel-based morphometric analyses of the brain in children and adolescents prenatally exposed to alcohol. *Neuro Report* **12**:515–23.
Sowell ER, Thompson PM, Mattson SN *et al.* (2002a) Regional brain shape abnormalities persist into adolescence after heavy prenatal alcohol exposure. *Cereb Cortex* **12**:856–65.
Sowell ER, Thompson PM, Peterson BS *et al.* (2002b) Mapping cortical gray matter asymmetry patterns in adolescents with heavy prenatal alcohol exposure. *Neuro Image* **17**:1807–19.
Spohr HL, Willms J, Steinhausen HC. (2007) Fetal alcohol spectrum disorders in young adulthood. *J Pediatr* **150**:175–9
Streissguth AP, Aase JM, Clarren SK *et al.* (1991) Fetal alcohol syndrome in adolescents and adults. *JAMA* **265**:1961–7.
Sulik KK. (2005) Genesis of alcohol-induced craniofacial dysmorphism. *Exp Biol Med* **230**:366–275.
Suzuki K. (2007) Neuropathology of developmental abnormalities. *Brain Dev* **29**:129–41.
Swayze VW, Johnson VP, Hanson JW *et al.* (1997) Magnetic resonance imaging of brain anomalies in fetal alcohol syndrome. *Pediatrics* **99**:232–40.
Tenkova T, Young C, Dikranian K *et al.* (2003) Ethanol-induced apoptosis in the developing visual system during synaptogenesis. *Invest Ophthalmol Vis Sci* **44**:2809–17.
Thomas JD, Garcia GG, Dominguez HD *et al.* (2004) Administration of eliprodil during ethanol withdrawal in the neonatal rat attenuates ethanol-induced learning deficits. *Psychopharmacology* **175**:189–95.
Thomas JD, Melcer T, Weinert S *et al.* (1998) Neonatal alcohol exposure produces hyperactivity in high alcohol sensitive (HAS) but not in low alcohol sensitive (LAS) rats. *Alcohol* **16**:237–42.
Ullian EM, Sapperstein SK, Christopherson KS *et al.* (2001) Control of synapse number by glia. *Science* **291**:657–61.
Vallés S, Pitarch J, Renau-Piqueras J *et al.* (1997) Ethanol exposure affects glial fibrillary acidic protein gene expression and transcription during rat brain development. *J Neurochem* **69**:2484–93.
Ventura RE, Goldman JE. (2007) Dorsal radial glia generate olfactory bulb interneurons in the postnatal murine brain. *J Neurosci* **27**:4297–302
Warren KR, Li TK. (2005) Genetic polymorphisms: impact on the risk of fetal alcohol spectrum disorders. *Birth Defects Re A Clin Mol Teratol* **73**:195–203.
Wilkemeyer MF, Menkari CE, Charness ME. (2002) Novel antagonists of alcohol inhibition of L1-mediated cell adhesion: multiple mechanisms of action. *Mol Pharmacol* **62**:1053–60.
Willford JA, Richardson GA, Leech SL *et al.* (2004) Verbal and visuospatial learning and memory function in children with moderate prenatal alcohol exposure. *Alcohol Clin Exp Res* **28**:497–507.
Woodruff-Pak DS, Goldenberg G, Downey-Lamb MM *et al.* (2000) Cerebellar volume in humans related to magnitude of classical conditioning. *Neuro Report* **11**:609–15.
Wozniak DF, Hartman RE, Boyle MP *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**:403–14.
Wozniak JR, Mueller BA, Chang P *et al.* (2006) Diffusion tensor imaging in children with fetal alcohol spectrum disorders. *Alcohol Clin Exp Res* **30**:1799–806.
Young C, Olney JW. (2005) Neuroapoptosis in the infant mouse brain triggered by a transient small increase in blood alcohol concentration. *Neurobiol Dis* **22**:548–54.
Young C, Roth KA, Klocke BJ *et al.* (2005) Role of caspase-3 in ethanol-induced developmental neurodegeneration. *Neurobiol Dis* **20**:608–14.