BRAIN RESEARCH 1384 (2011) 29–41

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Altered adult hippocampal neuronal maturation in a rat model of fetal alcohol syndrome

Joana Gil-Mohapel[a], *Fanny Boehme*[a,b], *Anna Patten*[a,b], *Adrian Cox*[a,b], *Leah Kainer*[a], *Erica Giles*[a], *Patricia S. Brocardo*[a], *Brian R. Christie*[a,b,c,d,*]

[a]*Division of Medical Sciences, Island Medical Program, University of Victoria, Victoria, British Columbia, Canada*
[b]*Department of Biology, University of Victoria, Victoria, British Columbia, Canada*
[c]*The Brain Research Centre and Program in Neuroscience, University of British Columbia, Vancouver, British Columbia, Canada*
[d]*Department of Cellular and Physiological Sciences, University of British Columbia, Vancouver, British Columbia, Canada*

ARTICLE INFO

Article history:
Accepted 31 January 2011

Keywords:
Adult hippocampal neurogenesis
Dentate gyrus
Fetal alcohol syndrome
Fetal alcohol sprectrum disorders
Hippocampus
Rodent model

ABSTRACT

Exposure to ethanol during pregnancy can be devastating to the developing nervous system, leading to significant central nervous system dysfunction. The hippocampus, one of the two brain regions where neurogenesis persists into adulthood, is particularly sensitive to the teratogenic effects of ethanol. In the present study, we tested a rat model of fetal alcohol syndrome (FAS) with ethanol administered via gavage throughout all three trimester equivalents. Subsequently, we assessed cell proliferation, as well as neuronal survival, and differentiation in the dentate gyrus of the hippocampus of adolescent (35 days old), young adult (60 days old) and adult (90 days old) Sprague–Dawley rats. Using both extrinsic (bromodeoxyuridine) and intrinsic (Ki-67) markers, we observed no significant alterations in cell proliferation and survival in ethanol-exposed animals when compared with their pair-fed and ad libitum controls. However, we detected a significant increase in the number of new immature neurons in animals that were exposed to ethanol throughout all three trimester equivalents. This result might reflect a compensatory mechanism to counteract the deleterious effects of prenatal ethanol exposure or an ethanol-induced arrest of the neurogenic process at the early neuronal maturation stages. Taken together these results indicate that exposure to ethanol during the period of brain development causes a long-lasting dysregulation of the neurogenic process, a mechanism that might contribute, at least in part, to the hippocampal deficits that have been reported in rodent models of FAS.

* *Corresponding author at:* Division of Medical Sciences, Island Medical Program, University of Victoria, Victoria, British Columbia, V8W 2Y2, Canada. Fax: +1 250 772 5505.
E-mail address: brain64@uvic.ca (B.R. Christie).

Abbreviations: ANOVA, analysis of variance; ARBD, alcohol-related birth defects; ARND, alcohol-related neurological disorders; BAC, blood alcohol concentration; BrdU, bromodeoxyuridine; CNS, central nervous system; CORT, corticosterone; DAB, 2,2-diaminobenzedine; DCX, doublecortin; DG, dentate gyrus; FAS, fetal alcohol syndrome; FASD, fetal alcohol spectrum disorders; GABA, gamma-aminobutyric acid; GCL, granule cell layer; GD, gestational day; HPA, hypothalamus–pituitary–adrenal axis; i.p., intraperitoneal; PFA, paraformaldehyde; PD, post-natal day; RT, room temperature; s.c., sub-cutaneous; SEM, standard error of the mean; SGZ, sub-granular zone; TBS, Tris-buffered saline

0006-8993/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2011.01.116

1. Introduction

Ethanol consumption during pregnancy can result in a wide range of adverse effects in the developing fetus. The severity of fetal damage due to ethanol exposure depends on a number of factors that include the timing, pattern, and amount of ethanol consumed. Furthermore, genetic variation and differences in the rate of ethanol metabolism also impact the severity of the effects of this teratogen (for review, see Gil-Mohapel et al., 2010). The term fetal alcohol spectrum disorders (FASD) encompasses the vast range of pathological conditions that can occur when ethanol is consumed during different periods of the pregnancy (Sokol et al., 2003). These include alcohol-related birth defects (ARBD), alcohol-related neurological disorders (ARND), (Burd and Martsolf, 1989; May et al., 2009; Oesterheld et al., 1998), and fetal alcohol syndrome (FAS). FAS, the most severe condition that results from in utero ethanol exposure, is defined by a pattern of cranio-facial dysmorphologies (Jones and Smith, 1973; Sokol and Clarren, 1989), growth retardation (Sokol and Clarren, 1989), and dysfunction of the central nervous system (CNS). In particular, CNS dysfunction may be manifested as mental retardation, cognitive and/or behavioral deficits (including impaired spatial memory performance) (Coles et al., 1991; Hamilton et al., 2003; Streissguth et al., 1989; Uecker and Nadel, 1996), as well as impaired motor performance and communication skills that persist into adulthood (Jones and Smith, 1973; Sowell et al., 2002). These deficits are generally accompanied by both structural and functional brain damage (Chen et al., 2003; Kerns et al., 1997; Streissguth and LaDue, 1987; West et al., 1984). Indeed, neuroimaging studies have revealed reduced volume of several brain structures (including the cerebral cortex, amygdaloid body, basal ganglia, corpus callosum, cerebellum, and the hippocampus) with FASD (Archibald et al., 2001; Autti-Ramo, 2002; Ikonomidou et al., 2000; Klintsova et al., 2007; Roebuck et al., 1998).

It has long been known that the hippocampus is a brain area that is particularly sensitive to the effects of ethanol during its development (Ho et al., 1972). The type and extent of neuronal loss in this brain structure and related cognitive impairments seem to depend on the developmental timing as well as the dose and extent of prenatal ethanol exposure (for review, see Gil-Mohapel et al., 2010). The hippocampus is also one of the few regions in the mammalian brain where neurogenesis (i.e., the generation of new neurons) continues to occur throughout adulthood, and the newly generated neurons are believed to be crucial to the functioning of this structure (for review, see Zhao et al., 2008). Indeed, correlative studies have shown that hippocampal neurogenesis can be modulated by learning and behavioral experience and that loss of hippocampal neurogenic function can have consequences on memory formation (for review see Bruel-Jungerman et al., 2007). Given the fact that cognitive deficits (including learning and memory impairments) are often associated with FASD, it is of interest to investigate whether exposure to ethanol during the period of brain development can have long-term effects on adult hippocampal neurogenesis.

To date, there is still a paucity of data on whether prenatal and/or early post-natal ethanol exposure affects adult hippocampal neurogenesis (for review, see Gil-Mohapel et al., 2010). With the exception of a few studies where mice received an acute dose of ethanol at post-natal day (PD) 7 (Ieraci and Herrera, 2007; Wozniak et al., 2004), in most cases researchers have used one of two different time frames of ethanol exposure: either the first and second trimester equivalent together [approximately from gestational days (GDs) 1 to 23] (Choi et al., 2005; Redila et al., 2006), or the third trimester equivalent alone (approximately from PDs 4–10) (Helfer et al., 2009; Klintsova et al., 2007). Furthermore, distinct modes or routes of ethanol administration have been used in the different studies. A sub-cutaneous (s.c.) injection of ethanol has been used in the acute models (Ieraci and Herrera, 2007; Wozniak et al., 2004), while a liquid diet has been the method of choice in the prenatal studies (Choi et al., 2005; Redila et al., 2006). Conversely, intragastric intubation (gavage) has been preferred for post-natal studies (Helfer et al., 2009; Klintsova et al., 2007). Because of the disparity in time points and routes of administration, it has been difficult to draw generalized conclusions regarding whether pre- and post-natal ethanol exposure affects the process of neurogenesis in the adult brain (for review, see Gil-Mohapel et al., 2010).

In the present study we evaluated the effects of ethanol exposure during all three trimester equivalents (i.e., during the entire gestation and during early post-natal life) on adult hippocampal neurogenesis. We used the intragastric intubation technique to deliver ethanol in a highly controlled fashion to both pregnant rat dams and their offspring. Moreover, since most behavioral deficits associated with FASD are observed later on in life (typically when children reach their educational years) and commonly persist into adulthood, we choose to analyze the effects of early ethanol exposure on adult hippocampal neurogenesis when animals reached one of the three following stages: adolescence, early adulthood, and adulthood. With this highly controlled model of FAS we show that exposure to ethanol during the entire period of brain development has long-term effects on the process of adult hippocampal neurogenesis that persist into adulthood.

2. Results

2.1. *Characterization of the rat intragastric intubation model*

The food consumption of the pregnant dams was recorded on a daily basis, in order to control for a potential ethanol-induced reduction in caloric intake. Pair-fed animals had their daily food allotments matched to the amount of food consumed by the ethanol-exposed group in each particular day of gestation; as expected, repeated measures ANOVA detected an effect of *treatment* on food consumption [$F(2, 50)=142.33$, $p<0.001$; with ad libitum>pair-fed $p<0.001$ and ad libitum>ethanol-exposed, $p>0.001$] with no significant differences between pair-fed and ethanol-exposed groups ($p=0.50$) (Fig. 2A).

The same effect of *treatment* on body weight was observed when weight gain throughout gestation was analyzed [repeated measures ANOVA, $F(2, 67)=30.75$, $p<0.001$; with ad libitum> pair-fed, $p<0.001$, ad libitum>ethanol-exposed, $p<0.001$, and