Behavioural Brain Research 252 (2013) 326–333

Contents lists available at SciVerse ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Early gestational exposure to moderate concentrations of ethanol alters adult behaviour in C57BL/6J mice

Michelle C. Sanchez Vega[a], Suyinn Chong[b], Thomas H.J. Burne[a,c,*]

[a] Queensland Brain Institute, The University of Queensland, St. Lucia, Queensland, Australia
[b] Mater Research, South Brisbane, Queensland, Australia
[c] Queensland Centre for Mental Health Research, The Park Centre for Mental Health, Richlands, Queensland, Australia

HIGHLIGHTS

- We examined the effects of a moderate dose ethanol exposure during the first eight days of gestation in mice.
- Ethanol-exposed mice showed increased hyperlocomotion at postnatal days 14, 21 and 70.
- Ethanol-exposed mice showed a significant improvement in memory in the water maze.
- Moderate prenatal ethanol exposure leads to persistent behavioural alterations into adulthood.

ARTICLE INFO

Article history:
Received 26 April 2013
Received in revised form 31 May 2013
Accepted 1 June 2013
Available online 10 June 2013

Keywords:
Ethanol
Brain function
Fetal alcohol exposure
Animal model
Learning
Memory

ABSTRACT

Alcohol consumption during pregnancy has deleterious effects on the developing foetus ranging from subtle physical deficits to severe behavioural abnormalities and is encompassed under a broad umbrella term, foetal alcohol spectrum disorders (FASD). High levels of exposure show distinct effects, whereas the consequences of moderate exposures have been less well studied. The aim of this study was to examine the effects of a moderate dose ethanol exposure using an ad libitum drinking procedure during the first eight days of gestation in mice on the behavioural phenotype of adult offspring. Adult female C57Bl/6J mice were mated and exposed to either 10% (v/v) ethanol or water for the first 8 days of gestation (GD 0–8), and then offered water for the rest of gestation. Early developmental milestone achievement was assessed in offspring at postnatal days (P) 7, 14 and 21. Adult offspring underwent a comprehensive battery of behavioural tests to examine a range of behavioural domains including locomotion, exploration, anxiety, social behaviour, learned helplessness, sensorimotor gating, and nociception, as well as spatial memory in a water maze. Ethanol-exposed mice had similar postnatal developmental trajectories to water-exposed mice. However, the ethanol-exposed mice showed increased hyperlocomotion at P 14, 21 and 70 ($p < 0.05$). Increased exploration and heightened motivation were also observed in adult mice. Furthermore, ethanol-exposed mice showed a significant improvement in memory in the water maze. The main findings were that mice had persistent and long lasting alterations in behaviour, including hyperactivity and enhanced spatial memory. These data suggest that even moderate dose ethanol exposure in early gestation has long term consequences on brain function and behaviour in mice.

1. Introduction

Alcohol consumption is a widespread practice around the world, although its consumption during pregnancy has long-lasting consequences for the developing foetus. The anomalies provoked by the deleterious effects of prenatal alcohol consumption were established in 1973 with the term Foetal alcohol syndrome (FAS) [1,2]. FAS is characterized by three main clinical features, including growth restriction, craniofacial abnormalities in addition to structural and/or functional deficits in the brain. It is the most severe form of alcohol related disorders within the non-diagnostic umbrella term foetal alcohol spectrum disorders (FASD) [3,4]. Although the severity can vary among individuals, it is mostly associated with the level of exposure, in which heavy drinking patterns are more likely to cause FAS. Moderate and light drinking are commonly responsible for milder forms of FASD, which lack the presence of dysmorphic features typical of FAS [5] but exhibit neurobehavioural and cognitive impairment [6]. For example, several studies have reported FAS children to be hyperactive, irritable and experience difficulties in tasks of vigilance, reaction time and information processing [7–10] and

* Corresponding author at: Queensland Brain Institute, University of Queensland, St. Lucia, Queensland 4076, Australia. Tel.: +61 7 3346 6371; fax: +61 7 3346 6301.
E-mail address: t.burne@uq.edu.au (T.H.J. Burne).

0166-4328/$ – see front matter.
http://dx.doi.org/10.1016/j.bbr.2013.06.003

life-long impairments can include impulsivity, hyperactivity, social ineptness, poor judgement and learning disabilities [11].

Australian survey data indicate that up to 80% of women have consumed alcohol in the three months prior to conception, and more than 50% of women continued to drink during pregnancy [12–15]. Although the consumption rate is clearly lower in pregnant women, up to 50% of pregnancies are unplanned [13]. Therefore, it is possible that the foetus may undergo inadvertent exposure before the pregnancy is confirmed.

Rodents have been used to investigate the effect of prenatal ethanol exposure on brain function and behaviour [16,17]. C57BL/6J mice exhibit a propensity to voluntarily consume alcohol and, using a voluntary drinking paradigm minimizes confounding effects of maternal stress caused by ethanol injections [18]. A recently established mouse model, [19], utilised ad libitum ethanol exposure (moderate levels of 10% v/v) during the first eight days of gestation (GD 0-8), which in terms of development, is equivalent to the first 3–4 weeks of a human pregnancy [20] mimicking the period of time during which mothers are unaware of pregnancy. Using this procedure, adult coat colour changes were detected in C57Bl/6 mice carrying the epigenetically regulated allele, *Agouti viable yellow* (A^{vy}). Given that adult coat colour is known to be linked to the A^{vy} allele, changes in this phenotype show that moderate prenatal alcohol exposure (GD 0–8) could alter gene expression and DNA methylation [21]. Moreover, this study showed, for the first time, that moderate prenatal alcohol exposure could affect the adult phenotype by modifying the epigenotype of the early embryo [19,20]. However, no detailed behavioural phenotype has been published using this model of moderate dose ethanol exposure in mice [22].

The aim of this study was to characterise the effects of moderate ethanol exposure early in gestation (GD 0–8) on brain function and behaviour in adult offspring. The initial aim was to examine the generalized behavioural profile as well as more specific behavioural domains, such as learning and memory. For the purposes of this study, the C57BL/6J mouse strain was selected because of the well characterized genetic background and alcohol preference which allows for a less invasive alcohol administration using an ad libitum drinking procedure during the first 8 days of pregnancy (GD 0–8) [19]. We hypothesised that moderate dose, prenatal ethanol exposure would not cause significant abnormalities on early stages (P 7, 14, 21) of offspring development but would have long-term effects on adult offspring behaviour, including increased locomotion and anxiety-related behaviour, and impaired spatial learning and memory.

2. Materials and methods

2.1. Animals and housing

Ten adult male and 60 adult female C57BL/6J mice were purchased (ARC, Perth, WA) at postnatal day 70 (P 70) and housed in individually ventilated OptiMice cages (Animal Care Systems, CO, USA) at 21 ± 1 °C, 40–60% humidity and 12 h light dark cycle (lights on 0700 h) with ad libitum access to pelleted food (Mouse Breeder Grower Diet, Specialty Feeds, WA) and water. The mice were habituated to the QBI animal house for 4–5 days and the females were divided across six breeding waves of 10 females each, and separate waves of mice arrived every fortnight. The same sires were used throughout the six waves to reduce variability from different sires. Males were caged with a single, nulliparous female in the afternoon and checked each morning between 0830 and 0900 h for the presence of a vaginal plug, which indicated that mating had taken place. The day of plug detection is considered gestational day 0 (GD 0), at which the female was removed from the cage and housed individually.

2.2. Maternal model of ethanol exposure

We conducted a pilot study to ensure that mice would voluntarily consume 10% (v/v) non-denatured ethanol without an acclimatization period. Time mated female mice ($n = 4$) had access to 2 bottles (10% ethanol or water) and based on the volume consumed we found that they had a preference (67% ± 2, SEM) for the bottle containing 10% ethanol when exposed from GD 0–8. We therefore exposed mice used in the present study to a single bottle as described previously [19]. Half of the females were randomly assigned to the ethanol group and they had access to one bottle containing 10% ethanol. The remaining mice comprised the control group which had access to a bottle containing tap water. All animals had ad libitum access to the drinking bottles and food. Every 24 h the contents of the water bottles were replaced with fresh solution and consumption (ml) measured. The average daily consumption of 10% ethanol during GD 0–8 was 4.2 ± 0.2 (SEM) ml/mouse/day (or 16 g ethanol/kg body weight/day). It has been shown that female mice voluntarily consuming 10% ethanol at 14 g ethanol/kg body weight/day produces average peak blood alcohol levels of ca. 120 mg/dl [23]. On the last day of exposure, GD 8, the bottle containing ethanol was replaced with a bottle containing water. All dams were subjected to only one cycle of ethanol exposure, and all other environmental factors (e.g. cage type, environmental enrichment) were kept the same between groups.

2.3. Monitoring early development

To determine whether there were obvious developmental differences between exposure groups (ethanol-exposed, control), a total of 45 animals (control, $n = 15$; ethanol-exposed, $n = 30$) were examined for offspring development at P7, 14 and 21. The parameters measured were weight, length, ear folding and eye opening (scale 0–2), teeth eruption (scale 0–3), fur development (scale 0–4) and righting reflex (scale 0–1) (adapted from [24] and [25]). In addition, the number of steps made in 30 s was recorded as a measure of locomotion. These mice were not use for any subsequent behavioural observations.

2.4. Behavioural testing

Behavioural experiments began on mice naïve to handling once the offspring reached adulthood (P70). There were two broad approaches used including, (1) a comprehensive behavioural phenotyping screen, as well as more detailed assessment of (2) spatial learning and memory.

The first section consisted of a battery of generalized tests, which provided an assessment of the broad behavioural domains, including locomotion, exploratory behaviour, anxiety-related behaviour, avoidance learning and pain tolerance. Even though these are not specific tests for behaviours observed in FASD models, they are very sensitive measures of altered brain function and behaviour [26].

All mice were assessed for locomotor activity on the first day of testing (P 70). The behavioural screen was conducted in the following order on half of the adult offspring from two separate breeding waves (control, $n = 26$; ethanol-exposed, $n = 25$). Each test was performed on a separate day, from least to most stressful, in the following order: elevated plus maze (P 71), holeboard (P 72), light/dark transition (P 73), sucrose preference (P 74), active avoidance (P 75), hot plate and tail flick (P 76), forced swim test (P 77) and prepulse inhibition (P 87).

2.4.1. Open field

Each mouse was placed in a clear open field (45 cm × 45 cm × 45 cm, Med Associates Inc., USA) within a sound attenuated chamber and activity levels were recorded for 30 min. The light level was set at 18 lx. As a measure of spontaneous activity, distance travelled was calculated using activity monitor tracking software based on beam breaks from three 16 beam infrared arrays and was sampled in 1 min time bins [27,28].

2.4.2. Elevated plus maze

Each mouse was placed on a cross-shaped platform made with opaque grey acrylic; the platform consisted of two opposing pairs of arms, one open (5 cm × 30 cm) and one closed (5 cm × 30 cm × 30 cm high) extending from a central platform (5 cm × 5 cm) that was positioned 50 cm above the ground. During the 10 min test, the amount of time spent in each of the arms as well as in the central platform and the latency to enter the open arms was recorded. The position of the mouse within the maze was determined by the point location of the centre of mass within Ethovision software. The number of arm changes was also recorded as a measure of general activity. The percentage of time that animals spent on the open arms of the maze, relative to closed arms, was used as the primary measure of anxiety-related behaviour [29].

2.4.3. Holeboard

Each mouse was placed in an opaque white acrylic box (30 cm × 30 cm × 30 cm) with a raised (2.5 cm) floor insert containing four holes (2.5 cm diameter, 5.3 cm from each corner) for 10 min. The frequency of head dipping into any of the four holes was used as the primary measure of exploration [30].

2.4.4. Light/dark transition

A dark acrylic insert with a small entrance was placed over half of the open field arena (45 cm × 45 cm × 45 cm), creating a lit area (100 lx) and a dark area (1 lx). Each mouse was placed in the lit area and its activity was recorded for 30 min using activity tracking software with three 16 beam infrared arrays (Med Associates Inc., USA). The proportion of time spent in each area was then analysed as a measure of anxiety-related behaviours [31].