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Spatial learning deficits induced by chronic prenatal ethanol exposure can be overcome by non-spatial pre-training

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Abstract

This study tested the hypothesis that behavioural intervention, in the form of non-spatial pre-training, mitigates the deficits in spatial learning tasks induced in guinea pig offspring by chronic prenatal ethanol exposure (CPEE). Timed, pregnant guinea pigs were treated with ethanol (4 g/kg maternal body weight/day), isocaloric-sucrose/pair-feeding, or water throughout gestation. Offspring received non-spatial pre-training, in which animals were exposed to the procedural requirements of the water maze in the absence of distal spatial cues, and then were tested in both stationary-platform and moving-platform tasks with spatial cues. Saliva cortisol was quantified in non-trained and pre-trained animals before and after exposure to the water maze.

Results: CPEE offspring exhibited performance deficits in the stationary-platform task, and non-spatial pre-training improved performance of CPEE offspring to control levels. In contrast, non-spatial pre-training had no effect on the impaired performance of CPEE offspring in the moving-platform task. Non-trained CPEE offspring had elevated saliva cortisol concentration after water-maze exposure compared to control offspring. Moreover, pre-trained control animals exhibited a sensitization of the cortisol response after repeated exposure to the water maze, and this was not evident in pre-trained CPEE offspring.

Conclusions: These data demonstrate that CPEE produced deficits in spatial learning and memory processes that were partially overcome by non-spatial pre-training; however, more difficult tasks continued to reveal cognitive deficits. For repeated exposure to the water maze, CPEE offspring achieved a level of performance that was not different from control offspring, suggesting that it is the initial rate of acquisition of new learning, rather than the overall ability to learn, that is most adversely affected by CPEE.

Keywords: Fetal alcohol syndrome; Guinea pig; Cortisol; Stress; Water maze; Behavioural intervention; Pre-training

1. Introduction

Alcohol consumption during pregnancy can lead to a number of physical, behavioural, and psychological problems in affected children, and may result in the fetal alcohol syndrome (FAS). The FAS is defined by three principal characteristics in infants: damage to the brain, facial malformations, and growth delays [22]. Of these three principal features, it is the brain injury that is the most debilitating and persistent throughout life. The brain injury of FAS involves decreased brain size, and damage to specific regions including the hippocampus, a brain structure involved in learning, memory, and regulation of behaviour [2]. In the guinea pig, a reliable model of ethanol teratogenicity [25], chronic prenatal ethanol exposure (CPEE) induces hippocampal growth restriction and selective neuronal cell loss [11]. Behaviourally, CPEE offspring demonstrate performance deficits in spatial learning tasks such as the Morris water maze [18,30], a task sensitive to hippocampal injury [25]. Similarly, in the rat, deficits in spatial acquisition in the water

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maze have been found after chronic prenatal ethanol exposure [3,41], and after binge-like exposure to ethanol during the neonatal brain growth spurt [24,21].

Environmental intervention during postnatal life can accelerate development and facilitate recovery from brain injuries [31]. It is well established that environmental enrichment can provide improvement of performance deficits in the Morris water maze for CPEE rats compared with control rats [13]. For example, group housing of CPEE rat offspring with a variety of "toys" included in the home cage, together with daily handling, results in significant improvement in offspring performance in spatial learning paradigms such as the water maze [14]. Similarly, male CPEE rat offspring raised in home cages containing a running wheel demonstrate an improvement in spatial memory [7], suggesting that voluntary exercise also can improve the performance of CPEE offspring in learning tasks. Another approach that does not involve improving the home cage environment is behavioural intervention, or non-spatial pre-training. Non-spatial pre-training has been used as an intervention to overcome drug-induced water-maze performance deficits induced by NMDA antagonists [16], acute ethanol exposure [5] and brain lesions [17]. To the best of our knowledge, non-spatial pre-training has not previously been tested in CPEE-induced learning deficits. For non-spatial pre-training, animals swim in the water maze under conditions in which distal visuo-spatial cues are absent, and the hidden platform is moved to a new random location after every trial [28]. Thus, the animal is afforded the opportunity to acquire the general behavioural requirements of the water maze (swimming away from the walls, finding and climbing on to a hidden platform for escape) in the absence of information about the spatial environment. It is important to understand that performance deficits in the water maze can be a result of many factors, including impaired motor or sensory ability, swim stress associated with the task, impaired acquisition of the appropriate behavioural strategy, and/or impaired spatial navigation and acquisition. Optimally, one should determine the effects of prenatal treatment in a number of water-maze tasks to better understand and interpret the nature of performance deficits and cognitive impairment.

The objective of the present study was to test the hypothesis that a behavioural intervention, involving non-spatial pre-training of the animal in the environment to be encountered during testing, overcomes CPEE-induced deficits in water-maze performance in the guinea pig. More specifically, we were interested in determining whether the behavioural intervention would provide a global benefit in multiple water-maze paradigms. Moreover, we recently demonstrated that CPEE induces very high maternal cortisol concentration during gestation, and alters glucocorticoid receptor function in the hippocampus of the young adult guinea pig [19]. Ethanol consumption by the pregnant female rat increases glucocorticoid activity by increasing the set point of HPA axis function, thereby resulting in increased basal and stress-induced plasma corticosterone concentrations [37]. Moreover, this effect of CPEE is associated with altered adrenocortical development in young postnatal offspring [38], and HPA axis hyper-responsiveness to stressors in adult offspring [26,35,39]. We, therefore, also determined the effect of CPEE on the stress response of guinea pig offspring for first exposure in the water maze, and whether non-spatial pre-training caused adaptation in this stress response.

2. Methods

2.1. Experimental animals

The experimental protocol was approved by the Queen's University Animal Care Committee, and was conducted in accordance with the guidelines of the Canadian Council on Animal Care. Female, nulliparous Dunkin–Hartley-strain guinea pigs (Charles River Canada Inc.), approximately 600 g body weight, were bred using an established procedure [9]. Gestational day 0 was defined as the last day of full vaginal-membrane opening, and term is about gestational day 68. Animals were housed individually in plastic cages with a 12-h light/dark cycle at an ambient temperature of 23 °C. At postnatal day 17, animals were weaned from their mothers and separated by sex.

2.2. Animal treatment regimens

Pregnant animals were separated into one of three experimental groups and received via oral intubation: a) 4 g ethanol (30% v/v)/kg maternal body weight/day with ad libitum access to food and water; b) isocaloric-sucrose (42% w/v) with pair-feeding to an ethanol treated animal and ad libitum access to water; c) isovolumetric water with ad libitum access to food and water. Treatment was administered in two equally divided doses, 2 h apart every day throughout gestation (gestational day 2 to gestational day 67). A blood sample was taken on GD 57 via an ear blood vessel 1 h after the second divided dose. Blood ethanol concentration (BEC) was determined using an established gas–liquid chromatographic method [34].

2.3. Behavioural testing

The Morris water-maze test of spatial learning was adapted for guinea pigs [8], based on an established method [27]. The maze consisted of a pool (1.8-m diameter) filled with water that was made opaque by adding 500 ml of non-toxic white paint. Each trial in the water maze was recorded using a video camera for off-line analysis. All water-maze testing was conducted in the afternoon between 1300 and 1500 h. Offspring from each litter of each treatment group were randomly selected and separated into non-trained and pre-trained groups.

2.4. Non-spatial pre-training

Offspring from each individual litter were randomly assigned to the pre-trained and non-trained groups. Offspring assigned to the pre-trained groups underwent non-spatial pre-training over five consecutive days, in which each guinea pig was given two trials each day with a 3-h delay between trials.