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Research report

Sensitivity of modified Biel-maze task, compared with Y-maze task, to measure spatial learning and memory deficits of ethanol teratogenicity in the guinea pig

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ABSTRACT

Ethanol consumption during pregnancy can produce a variety of teratogenic effects in offspring, termed Fetal Alcohol Spectrum Disorders (FASD). The most debilitating and permanent consequence of chronic prenatal ethanol exposure (CPEE) is neurobehavioral teratogenicity, which often manifests as cognitive and behavioral impairments, including deficits in spatial learning and memory. This study tested the hypothesis that a modified dry-land version of the multi-choice Biel-maze task is more sensitive than the rewarded-alternation Y-maze task for the determination of spatial learning and memory deficits of ethanol teratogenicity. Pregnant guinea pigs received ethanol (4 g/kg maternal body weight/day) or isocaloric-sucrose/pair-feeding (control) for 5 days/week throughout gestation. CPEE resulted in ethanol neurobehavioral teratogenicity in offspring, as demonstrated by increased spontaneous locomotor activity at postnatal day (PD) 10 and decreased brain weight at euthanasia (PD 150–200). On PD 21, offspring were randomly assigned to one of two tasks to assess spatial learning and memory performance: a dry-land version of the Biel maze or a rewarded-alternation Y-maze. Animals were habituated to the environment of their assigned task and performance of each CPEE or control offspring was measured. In the modified Biel maze, CPEE and control offspring were not different for percent completed trials or time to complete a trial. However, CPEE offspring made more errors (reversals and entering dead ends) in the Biel maze, demonstrating impaired spatial learning and memory. In contrast, CPEE offspring did not have impaired performance of the rewarded-alternation Y-maze task. Therefore, the modified dry-land version of the Biel-maze task, which measures cognitive performance using a complex multi-choice design, is more sensitive in demonstrating CPEE-induced spatial learning and memory deficits compared with a simple, rewarded-alternation Y-maze task.

1. Introduction

Prenatal alcohol (ethanol) exposure, via maternal consumption of alcohol, can lead to a wide range of adverse developmental outcomes in children, and is one of the leading causes of mental deficiency in the Western world [1,2]. The umbrella term Fetal Alcohol Spectrum Disorders (FASD) is used to describe the broad range of postnatal structural and functional defects produced by all types of prenatal ethanol exposure [2,3]. The estimated incidence of FASD is about 1 in 100 live births, and recent epidemiological studies indicate prevalence as high as 2–5% [4]. A study that examined key cost components estimated that the total adjusted annual cost associated with FASD in Canada is $5.3 billion [5].

Ethanol neurobehavioral teratogenicity is often the most debilitating and permanent manifestation of FASD [1]. Children exposed to ethanol prenatally may display cognitive, behavioral, social, and motor impairments [6–10]. Following chronic prenatal ethanol exposure (CPEE), rodent offspring show deficits in many spatial learning and memory tasks, including impaired performance of T-maze, Morris water maze, and radial arm maze tasks [11–16]. Behavioral impairments, including hyperactivity, increased perseveration responses, and increased disinhibition, have also been shown in CPEE offspring [14,17–23].

Although several rodent studies have shown that the Morris water maze is an effective tool to demonstrate learning and memory deficits in CPEE offspring [14–16], there are potential

Abbreviations: FASD, fetal alcohol spectrum disorders; CPEE, chronic prenatal ethanol exposure; GD, gestational day; PD, postnatal day.

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confounding factors when using this task to measure neurobehavioral and cognitive performance in the guinea pig [22,24–29]. The Morris water maze is a complex task that involves stress, visuospatial integration, and motor abilities [30]. Acute swim stress in task-naïve guinea pigs leads to increased saliva cortisol concentration in CPEE animals relative to isocaloric-sucrose/pair-fed controls [28]. In the guinea pig, CPEE increases thigmotaxic swimming in the Morris water maze without apparent impaired spatial mapping of the hidden escape platform [25]. Furthermore, CPEE guinea pigs demonstrate a different pattern of swimming and search behavior than nutritional control offspring [25]. Increased stress response could contribute to poor Morris-water-maze performance in CPEE guinea pigs, and may impair any interpretation of deficits in spatial learning or memory [25,26,29]. It was proposed that the food rewarded dry-land maze tasks used in the present study would distinguish learning and memory deficits from stress and fear responses, which commonly confound Morris-water-maze task performance in the guinea pig.

Optimally, one should determine the effects of CPEE in more than one learning and memory task to better understand and interpret the nature of performance deficits and cognitive impairment. This study tested the hypothesis that a modified dry-land version of the multi-choice Biel-maze task is more sensitive than the rewarded-alternation Y-maze task for the determination of spatial learning and memory deficits of ethanol teratogenicity. Guinea pig offspring were randomly assigned to undergo testing using one of two dry-land-maze tasks for spatial learning and memory: a complex modified Biel-maze [31] or a simple rewarded-alternation Y-maze task. Two tasks were used in this study because clinical investigations have shown that children with FASD may not demonstrate impairments in relatively simple tasks, but often show learning and memory deficits in complex, multi-choice tasks, for which higher-order executive functioning is required [7,32,33].

The guinea pig is a reliable animal model for the study of ethanol teratogenicity because the pattern of *in utero* development is more similar to the human compared with other rodent species with respect to its trimester-equivalent gestation [34] placental morphology [35], and extensive prenatal development, including the brain growth spurt [36]. Furthermore, the pharmacokinetics of ethanol in the maternal-fetal unit is very similar in the guinea pig and human [37].

2. Material and methods

2.1. Experimental animals

Female, nulliparous Dunkin-Hartley-strain guinea pigs (Charles River Canada Inc., St Constant, QC), body weight between 550 g and 650 g, were bred with male guinea pigs using an established procedure [38]. Gestational day (GD) 0 was defined as the last day of full vaginal-membrane opening, and term was approximately GD 68. Pregnant animals were housed individually in plastic cages at an ambient temperature of 23 °C with a 12-h light/dark cycle (lights on from 07:00 to 19:00 h). All animals were cared for according to the principles and guidelines of the Canadian Council on Animal Care, and the experimental protocol was approved by the Queen's University Animal Care Committee.

2.2. Animal treatment regimens

Pregnant dams were randomly assigned to one of two maternal treatment groups: ethanol or nutritional control (sucrose). Each pregnant animal in the ethanol group received 4 g ethanol/kg maternal body weight/day as an aqueous ethanol solution (30%, v/v, prepared in tap water), 5 days/week throughout treatment, with ad libitum access to standard guinea pig chow (Lab Diet 5025, Purina, St Louis, MO). Previous studies have shown that this ethanol regimen reliably produces hippocampal injury and neurobehavioral teratogenicity in offspring with minimal maternal or fetal death [19,29,39]. Each pregnant dam in the nutritional control group was paired to an individual ethanol-treated pregnant animal and received isocaloric sucrose (42%, w/v, prepared in tap water) and food in the amount consumed daily by the ethanol-treated animal. Aqueous ethanol or sucrose solution was administered into the oral cavity of the pregnant animal with a syringe, followed by swallowing, on each treatment day in two equally divided doses, given 2 h apart starting at 09:00 h.

2.3. Maternal blood ethanol concentration (BEC)

On GD 57 or 58, 120 μL of blood was collected from the marginal ear vein of each pregnant guinea pig and treated with 38% (w/v) aqueous sodium citrate (anticoagulant). The blood sample was taken 1 h after the second divided dose of ethanol or sucrose. Maternal BEC was determined in plasma by an established gas–liquid chromatographic procedure using headspace-gas analysis [40].

2.4. Spontaneous locomotor activity

Starting at postnatal day (PD) 1, offspring were weighed daily and monitored for general health. Spontaneous locomotor activity of each individual offspring was measured at PD 10 in an open-field apparatus using an established protocol [22]. Each guinea pig was placed in a Plexiglass® open-field apparatus (45 cm × 45 cm × 25 cm), with surrounding infrared beams to measure horizontal and vertical movements using a software-driven data acquisition system (TSE Actimot, Scientific Products and Equipment, Concord, ON). Testing occurred between 12:00 h and 16:00 h in a quiet room at 22 °C with standard lighting. Each animal was placed in the middle of the open field and was left undisturbed for 30 min. The time spent moving, distance traveled and time hyperactive (defined as moving at speeds greater than 20 cm/s) were determined at 5-min intervals.

2.5. Spatial learning and memory tasks

Offspring from each litter were weaned, separated by sex, and housed in cages of 2–5 animals at PD 21, at which time they were randomly assigned to undergo testing using one of two dry-land maze tasks for spatial learning and memory. Half of the animals underwent cognitive testing in a dry-land version of the Biel water maze [31]. Cognitive performance was assessed in the remaining offspring using a one-choice rewarded alternation Y-maze task [41].

2.5.1. Biel-maze task

Prior to testing, offspring underwent habituation training in the dry-land maze. Starting at PD 21, Biel maze offspring had access to a food reward (Froot Loop™) in their home cage in order to habituate them to its taste and eliminate hyponeophagia, the unconditioned inhibition of feeding in a novel setting. On PD 24, each guinea pig assigned to the Biel maze began habituation training in the dry-land maze with 1 to 3 same-sex cagemates. The dry-land maze was constructed using opaque black corrugated plastic, with various lane lengths (50 centimeters (cm)–1100 cm), right and left turns, and dead ends; all walls were 30 cm high and lanes were 12 cm wide (Fig. 1A). Each group of animals had two 15 min habituation trials in the dry-land maze from PD 24 to PD 44, and the intertrial interval was approximately 3 h. A food reward was placed at 10 equidistant checkpoints, 60 cm apart throughout the full length of the maze, to provide incentive for exploration. In addition, the dry-land maze environment was filled with various toys (e.g. wooden blocks, ping pong balls, and other small plastic toys), three tunnels, and a ramp, to promote physical activity and exploration. A camcorder was used to record each trial, and the time spent moving (in seconds) by each group of animals was measured.

Cognitive performance of CPEE offspring compared with nutritional control offspring was measured in the Biel maze twice daily from PD 27 to PD 44 (Fig. 1A). A food reward (Froot Loop™) was placed at the designated end of the maze, and an individual animal was placed at the opposite end. All tunnels and toys were removed from the Biel maze prior to each trial, and all trials were video recorded for offline analysis. The time taken for each animal to complete the maze and reach the reward was measured. Errors in the Biel maze were measured using two methods: reversals and dead end entries. A reversal was defined as any time an animal made a directional change to return to the start position of the maze. A dead end entry was defined as a choice dynamic when the animal came to a position in the maze where there were 2 or more paths that could be selected. If the animal did not select the correct path to the food reward, a dead end entry was counted. The percent completed trials, the number of reversals, and the number of dead ends entered were recorded for each trial in the Biel maze.

2.5.2. Rewarded alternation Y-maze task

The rewarded alternation Y-maze task was modified for the guinea pig from an established rat protocol [41]. All offspring underwent habituation to the Y-maze prior to commencing trials. Similar to the Biel maze, the Y-maze offspring had access to a food reward (Froot Loop™) in their home cage prior to commencing the task in order to habituate them to its taste and eliminate hyponeophagia. Animals underwent 2 days of habituation in the Y-maze on PD 46 and PD 47, when animals were placed in the Y-maze with same-sex cagemates twice daily for 5 min. A food reward was placed on top of a small plastic food dish at the distal end of each arm to allow animals to freely explore all arms of the maze for the entire 5 min habituation interval.

From PD 48 to PD 52, each Y-maze animal underwent trials in the rewarded alternation Y-maze task (Fig. 1B and C). Animals were fasted 4 h prior to commencing the task, which occurred between 12:00 h and 16:00 h. Each trial consisted of a sample arm and a choice arm. The trial was set up by baiting the sample and choice arms with a food reward, with access to the choice arm blocked by a door. First, an individual animal traveled from the start arm to the sample arm and was given