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Research report

The effects of prenatal stress on learning in adult offspring is dependent on the timing of the stressor

Amita Kapoor[a], Alice Kostaki[a], Christopher Janus[d], Stephen G. Matthews[a,b,c,*]

[a] Department of Physiology, Faculty of Medicine, University of Toronto, Medical Sciences Building, 1 King's College Circle, Toronto, Ontario, M5S 1A8 Canada
[b] Department of Obstetrics, Faculty of Medicine, University of Toronto, Medical Sciences Building, 1 King's College Circle, Toronto, Ontario, M5S 1A8 Canada
[c] Department of Gynaecology and Medicine, Faculty of Medicine, University of Toronto, Medical Sciences Building, 1 King's College Circle, Toronto, Ontario, M5S 1A8 Canada
[d] Mayo Clinic Jacksonville, Department of Neuroscience, 4500 San Pablo Road, Birdsall Building R215, Jacksonville, FL 32224, USA

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ABSTRACT

Impaired fetal development has been linked with deficits in behavioural and emotional development during postnatal life. In order to investigate the mechanisms underlying this relationship, we studied the effect of acute stress at two different critical phases of pregnancy on cognitive function in adult guinea pig offspring. Pregnant guinea pigs were exposed to a psychological stressor (2 h/day) on gestational days (gd) 50, 51, and 52 (PS50) or 60, 61, and 62 (PS60). Male offspring were grown to adulthood and tested in the Morris water maze (MWM) to assess spatial learning and memory. Latency, path length, swim speed and the strategy used to find the platform in each session of the MWM were measured. A reverse learning trial was performed where the platform was moved to a different area of the pool and the ability of the guinea pigs to learn a new platform position was assessed. There was no effect of stress at gd50 on latency to find the platform during any of the sessions in the MWM. PS60 male offspring exhibited enhanced development of a spatial strategy during sessions 3 and 4 of the MWM, but this was not associated with decreased latency. In the reversal task PS50 male offspring demonstrated use of non-spatial strategies to find the platform during the reversal task. This would suggest decreased retention of spatial memory in these animals. In contrast, control and PS60 male offspring demonstrated no bias to a particular strategy type. In conclusion, there are subtle effects of prenatal stress on spatial learning. PS60 offspring appear to exhibit enhanced spatial learning, while PS50 male offspring exhibit impaired spatial learning. These findings are consistent with those in humans, which indicate a strong effect of maternal anxiety during pregnancy on cognition in children, and that the timing of the maternal stress is critical to determining outcome. This model will allow us to determine the mechanisms that underlie the association between prenatal stress and altered learning strategy and ability.

1. Introduction

Prenatal stress is associated with a number of adult diseases, including cardiovascular and affective disorders [1,2]. The mechanism underlying this relationship is thought to be exposure of the fetus to components of the stress response: glucocorticoids, the end product of hypothalamic-pituitary-adrenal (HPA) axis activation or catecholamines, the product of sympathetic nervous system activation [3,4]. Indeed, a number of studies have demonstrated that prenatal stress results in alterations in HPA axis function and stress-related behaviour in animal models, including rats, guinea pigs and non-human primates [5–7]. More recently, human studies have demonstrated deficits in cognitive and behavioural development in children whose mothers were exposed to excess glucocorticoids during pregnancy [8,9]. Importantly, it is also emerging from these studies that the timing of the stress is critical as there are differential outcomes depending on the trimester of exposure [10,11]. One specific aspect of cognition is hippocampal-dependent spatial learning and memory [12,13]. Studies in non-human primates and rodents have demonstrated alterations in structure and function of the hippocampus as a consequence of prenatal stress [14–17].

The Morris water maze (MWM) is a validated tool used to measure hippocampal-dependent spatial memory and non-spatial discrimination learning in rodent models including guinea pigs [18,19]. Results from studies examining prenatal stress and spatial learning and memory using the MWM in rats and mice offspring have been variable, and have suggested significant interaction

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[*] Corresponding author at: Department of Physiology, Faculty of Medicine, University of Toronto, Medical Sciences Building, 1 King's College Circle, Toronto, Ontario, M5S 1A8 Canada. Tel.: +1 416 978 1974; fax: +1 416 978 4940.
E-mail address: stephen.matthews@utoronto.ca (S.G. Matthews).

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between the stress associated with swimming and cognitive ability. Recent studies using the MWM have developed approaches for analyzing swim patterns in order to classify the strategies that are being used to find the platform [20,21]. In this way, it can be determined whether spatial learning is occurring or whether other types of search strategies are being utilized.

In rats and mice, the phase of rapid brain growth (a period of established vulnerability) [22] occurs postnatally, unlike the human, the non-human primate and the guinea pig in which this crucial period of brain development is initiated prenatally [22]. Fetal brain growth in the guinea pig is well characterized; the period of rapid brain growth occurs around gestational day (gd) 50, whereas gd60 represents a period of rapid myelination [23]. We have previously shown that maternal plasma cortisol levels in pregnant guinea pigs rose by 20% and 40% in response to strobe light exposure on gd54 and gd60, respectively [6]. We have also shown that prenatal stress during the period of rapid brain growth (gd50) results in male offspring that exhibit increased basal HPA axis activity, increased anxiety behaviour and decreased plasma testosterone levels. In contrast, maternal stress late in gestation (gd60) results in male offspring that exhibit an increased plasma cortisol response to a stressor and decreased body weight from the time of weaning to adulthood [6].

Given that studies in humans are demonstrating alterations in cognitive function in children exposed to stress *in utero* and that there are differential effects depending on the timing of the prenatal stress, our aim was to determine the effect of prenatal stress on performance in the MWM and to assess the use of search strategies used in the MWM in a species that gives birth to neuroanatomically mature young. We hypothesized that prenatal stress would affect spatial learning and memory in male guinea pigs in the MWM and furthermore these changes will be dependent on the specific timing of the stressor.

2. Methods

2.1. Animals

Female guinea pigs (400–500 g) (Hartley strain, Charles River Canada, St. Constant, PQ, Canada) were mated in our animal facility as previously described [24]. This method produces accurately time-dated pregnant guinea pigs. Food (Guinea Pig Chow 5025, Ralston Purina International, Leis Pet Distributing Inc., Wellesley, ON, Canada) and water were available *ad libitum*. The animals were kept in a 12:12 h light–dark cycle, with lights off at 19:00 h. Room temperature was 23 °C. All the studies were performed according to protocols approved by the Animal Care Committee at the University of Toronto, in accordance with the Canadian Council for Animal Care.

Pregnant guinea pigs were exposed to a high frequency strobe light for 2 h, from 09:00 h to 11:00 h, on gd50, 51, and 52 (PS50) or gd60, 61, and 62 (PS60). A control group of pregnant guinea pigs was left undisturbed throughout gestation except for routine maintenance. All animals were allowed to deliver normally. Animals were weaned on postnatal day (pnd) 25, weighed and placed into individual clear polycarbonate cages. There was no significant effect of prenatal stress on body weight at pnd25 [6]. Animals were within visual, auditory and olfactory contact with at least two other animals at all times. Male offspring (control; $n = 11$, PS50; $n = 9$, PS60; $n = 9$) remained undisturbed except for biweekly cage maintenance and MWM testing around pnd70 (range pnd 70–76).

2.2. Morris water maze testing

2.2.1. Testing room and apparatus

The water maze apparatus consisted of a circular pool (1.54 m diameter) made of white plastic. The pool was filled with water (23 °C) that was made opaque by the addition of non-toxic white tempura paint. During MWM training, an escape platform (20 cm diameter) made of clear plastic with a grooved surface was submerged 5 cm under the water level. All tests were carried out in the same experimental room and the MWM testing area was isolated from the operator and computer by a white curtain. Dark boards of different shapes provided landmarks in the testing room. Three days prior to the commencement of the acquisition phase, guinea pigs underwent training to acclimatize them to the testing room and pool. The platform was placed in the centre of the pool and guinea pigs were placed on the platform three times, for 15 s.

2.2.2. Acquisition phase and reversal task

All animals were tested over five consecutive days. On each day, animals received two sessions of testing, at 09:00 h and 13:00 h. Each session consisted of four trials with an inter-trial period of 10 min. For each trial, the animal was released from 1 of the 4 cardinal compass points (N, S, E, W). The animal was allowed a maximum of 60 s to locate and mount the escape platform with a post-trial timeout of 15 s on the platform. Animals that failed to locate the platform within the 60 s were placed on the platform. Acquisition was tested during sessions 1–6 and the platform was submerged in quadrant 1. On session 7 the platform was moved to quadrant 3 for the reversal task. The platform remained in quadrant 3 for sessions 8–10 for another round of acquisition testing. Trial 1, session 7 was considered as a probe trial. In probe trials, the time spent swimming in the quadrant where the platform had been previously, was recorded. This is considered to be the most specific test for spatial memory [25]. The swim speed and path of the guinea pig during each trial was recorded by a video camera suspended above the centre of the pool and connected to a video tracking system (HVS Image Advanced Tracker VP200, HVS Image, Buckingham, UK).

2.2.3. Analysis of strategy

The swim path for each trial during MWM testing was automatically plotted in the HVS image system. A single investigator blinded to prenatal treatment status assigned a predominant search strategy to the first trial of each session using a categorization scheme based on those previously developed [20,21]. Briefly, the strategy that best described the majority of the swim path was assigned. In a reanalysis of the categorization of search strategy by the same investigator 2 weeks later, the intra-observer agreement on strategy classification was 98%. For each session strategies were classified into three broad categories: spatial, systematic but non-spatial and repetitive looping path strategies. Spatial strategies included 'spatial direct' (swimming directly to the platform), 'spatial indirect' (swimming to the platform with at most one loop), and 'focal: correct target quadrant' (swimming directly to and searching intently in the quadrant containing the platform). Systematic but non-spatial strategies included 'scanning' (searching the interior portion of the tank without spatial bias), 'random' (searching the entire tank without bias towards any portion), and 'focal: incorrect target quadrant' (searching intently a quadrant of the tank that does not contain the platform). Strategies involving repetitive looping paths included 'chaining' (circular swimming at an approximately fixed distance greater than 15 cm from the wall), 'peripheral looping' (persistent swimming around the outer 15 cm of the pool), and 'circling' (swimming in tight circles).

2.3. Statistical analysis

All data were expressed as mean ± standard error of the mean (S.E.M.). All statistical comparisons were analyzed using Graphpad Prism (Graphpad Software Inc., San Diego, CA, USA). MWM performance data was analyzed by two-way analysis of variance (ANOVA) with repeated measures and one-way ANOVA followed by Newman–Keuls method of post hoc comparison. The difference in latency between sessions 6 and 7 was analyzed by Wilcoxon matched paired-test. Strategy data was analyzed by Chi square tests. Significance was set at $p < 0.05$.

3. Results

3.1. Acquisition and reverse learning

Analysis of latency by two-way repeated measures ANOVA revealed a significant effect of session, indicating the animals were learning the platform position ($p < 0.0001$; Fig. 1A), however, there was no effect of prenatal stress nor was there a significant interaction between time and latency to find the platform. Wilcoxon matched pair-test for comparison of the latencies between sessions 6 and 7 revealed that both control ($p < 0.01$) and PS50 ($p < 0.02$) demonstrated a significant increase in latency from session 6 to 7 as the platform position was changed from quadrant 1 to 3. However, this was not observed in PS50 male offspring.

Two-way repeated measures ANOVA of the path length to find the platform revealed a significant effect of session, as the animals exhibited a shorter path length over time ($p < 0.0001$; Fig. 1B). There was no significant interaction between session and prenatal stress, nor was there a significant effect of prenatal stress alone on path length. Wilcoxon matched pair-test for comparison of the path length between sessions 6 and 7 revealed that both control ($p < 0.01$) and PS50 ($p < 0.01$) demonstrated a significant increase in latency from session 6 to 7 as the platform position was changed from quadrant 1 to 3, however, this was not observed in PS50 male