Behavioural Brain Research 268 (2014) 104–110

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Dietary choline supplementation to dams during pregnancy and lactation mitigates the effects of in utero stress exposure on adult anxiety-related behaviors

Kalynn M. Schulz[a,b,*], Jennifer N. Pearson[c], Mary E. Gasparrini[a,b], Kayla F. Brooks[a,b], Chakeer Drake-Frazier[a,b], Megan E. Zajkowski[a,b], Alison D. Kreisler[d], Catherine E. Adams[a,b], Sherry Leonard[a,b,c], Karen E. Stevens[a,b]

[a] Veterans Affairs Medical Center, 1055 Clermont Street, Denver, CO 80220, USA
[b] Department of Psychiatry, University of Colorado Anschutz Medical Campus, Building 500, 1300 East 17th Place, Aurora, CO 80045, USA
[c] Neuroscience Program, University of Colorado Anschutz Medical Campus, RC-1, MS 8351, Rm 7107 12800 E. 19th Ave, Aurora, CO 80045, USA
[d] Neuroscience Department, University of Pittsburgh, A210 Langley Hall, Pittsburgh, PA 15260, USA

HIGHLIGHTS

- We implemented a prenatal stress (PS) intervention in rodents by supplementing pregnant dams with dietary choline.
- Stressed and nonstressed dams received chow with normal or five times the normal level of choline during pregnancy and lactation, and offspring anxiety-related behaviors were assessed in adulthood.
- Perinatal choline supplementation mitigated the effects of PS on female anxiety-related behaviors in the elevated zero maze.
- Perinatal choline supplementation mitigated the effects of PS on male anxiety-related behaviors in the social interaction test.
- Perinatal choline supplementation diminishes the sex-specific effects of PS on anxiety-related behaviors in adulthood.

ARTICLE INFO

Article history:
Received 3 October 2013
Received in revised form 7 March 2014
Accepted 17 March 2014
Available online 24 March 2014

Keywords:
Choline
Diet
Supplementation
Prenatal stress
Hippocampus
Anxiety
Psychopathology
Maternal stress
Nicotinic acetylcholine receptor

ABSTRACT

Brain cholinergic dysfunction is associated with neuropsychiatric illnesses such as depression, anxiety, and schizophrenia. Maternal stress exposure is associated with these same illnesses in adult offspring, yet the relationship between prenatal stress and brain cholinergic function is largely unexplored. Thus, using a rodent model, the current study implemented an intervention aimed at buffering the potential effects of prenatal stress on the developing brain cholinergic system. Specifically, control and stressed dams were fed choline-supplemented or control chow during pregnancy and lactation, and the anxiety-related behaviors of adult offspring were assessed in the open field, elevated zero maze and social interaction tests. In the open field test, choline supplementation significantly increased center investigation in both stressed and nonstressed female offspring, suggesting that choline-supplementation decreases female anxiety-related behavior irrespective of prenatal stress exposure. In the elevated zero maze, prenatal stress increased anxiety-related behaviors of female offspring fed a control diet (normal choline levels). However, prenatal stress failed to increase anxiety-related behaviors in female offspring receiving supplemental choline during gestation and lactation, suggesting that dietary choline supplementation ameliorated the effects of prenatal stress on anxiety-related behaviors. For male rats, neither prenatal stress nor diet impacted anxiety-related behaviors in the open field or elevated zero maze. In contrast, perinatal choline supplementation mitigated prenatal stress-induced social behavioral deficits in males, whereas neither prenatal stress nor choline supplementation influenced female social behaviors. Taken together, these data suggest that perinatal choline supplementation ameliorates the sex-specific effects of prenatal stress.

Published by Elsevier B.V.

* Corresponding author at: University of Colorado Anschutz Medical Campus, RC-1, MS 8344, Rm 8106 12800 E. 19th Ave, Aurora, CO 80045, USA. Tel.: +1 303 724 0591.
E-mail addresses: Kalynn.Schulz@UCDenver.edu, kalynns@gmail.com (K.M. Schulz).

http://dx.doi.org/10.1016/j.bbr.2014.03.031
0166-4328/Published by Elsevier B.V.

1. Introduction

Maternal stress is associated with increased offspring anxiety and depressive-related behaviors in humans [1] and animals [2–6]. The mechanisms by which prenatal stress impacts anxiety-related behaviors are likely complex, but emerging evidence suggests that prenatal stress may alter adult anxiety via changes in hippocampal cholinergic function. Hippocampal nicotinic acetylcholine receptors (nAChRs) modulate anxiety- and depressive-related behaviors in adult animals [7–9], and are also sensitive to corticosterone and psychological stress in adulthood [10–12]. Furthermore, cholinergic abnormalities are associated with anxiety and depression in humans [13–18]. In rodents, prenatal stress alters levels of both alpha7* and alpha4 beta2* hippocampal nAChRs [19], and alters stress-dependent hippocampal cholinergic function in adulthood [20], suggesting that the effects of prenatal stress on anxiety-related behaviors may be driven by altered development of the hippocampal cholinergic system.

Given the relationships between prenatal stress, hippocampal nAChRs, and adult anxiety, here we tested whether an intervention aimed at the cholinergic system could counteract the deleterious effects of prenatal stress on adult anxiety. Specifically, we chose perinatal dietary choline exposure as a stress intervention for several reasons. First, perinatal choline supplementation facilitates alpha7*-dependent brain inhibitory function in infants [21]. Similarly, rodent studies demonstrate that supplementing dams during pregnancy and lactation permanently increases offspring levels of hippocampal alpha7* nAChRs and facilitates hippocampal function [22–27]. In addition, perinatal choline protects the nervous system against a host of developmental insults [28–32]. Finally, in normally developing female rats (i.e. not prenatally stressed) prenatal choline supplementation exerts antidepressant-like effects in adulthood [33]. Thus, perinatal choline supplementation enhances many brain and behavioral parameters that are typically compromised by prenatal stress, suggesting perinatal choline may be capable of counteracting the effects of prenatal stress on adult anxiety-related behavior. The current study tested this hypothesis by feeding stressed and nonstressed dams a choline-supplemented or control diet during pregnancy and lactation. The anxiety-related behaviors of offspring were assessed in adult male and female offspring by three different tests: (1) open field, (2) elevated zero maze, and (3) social interaction. These tests were chosen because they measure distinct but partially overlapping emotional constructs [34].

2. Materials and methods

2.1. Subjects

Twenty four timed pregnant female Sprague Dawley rats were ordered from Charles Rivers laboratories (Portage, MI) in two cohorts ($n = 12$ each), spaced one month apart and were 2 days pregnant upon arrival. Pregnant females were singly housed in static clear polycarbonate cages with wire bar lids and filtrated microisolator covers. All females had *ad libitum* access to food and water. Half of all pregnant females were fed a choline-supplemented chow (5 g/kg of choline chloride) through gestation and lactation, and half were fed a standard diet (1.1 g/kg of choline chloride). Bedding (Tekfresh, Harlan Laboratories Inc., Indianapolis, IN), food (Dyets Inc., Bethlehem, PA) and filtered water were changed weekly. One day prior to parturition, the females were transferred to larger cages ($40.6 \times 30.5 \times 20.3$) and extra bedding was provided as nesting material. At parturition, food and water continued to be replaced weekly, but the bedding and nests were left undisturbed until weaning at 21 days of age. Cage cleanliness was closely monitored during this time and additional bedding was provided when necessary. Upon weaning, all offspring ($n = 12$ overall $n = 96$) were fed a standard 2018 Teklad Global 18% Protein Rodent Diet (Harlan Laboratories Inc. Indianapolis, IN), assigned to same-sex groups based on stress condition [prenatally stressed (PS)/nonstressed (NS)] and diet condition (choline diet/control diet), and housed two per cage. All animals were maintained on a 12-h light:12-h dark cycle, and the room temperature was held constant at 21 °C. Subjects were treated in accordance with NIH guidelines and all protocols were approved by the IACUC of the University of Colorado Denver.

Table 1
Stress schedule.

Gestation day	Time of day		
	a.m.	Mid-day	p.m.
14	9 h social stress		
15		Radio static***	Overnight fast
16		Restraint ***	Cart transport**
17	Cart transport**	Swim*	Radio static***
18	Swim*	Radio static**	Restraint***
19	Radio Static***	Restraint***	Swim*
20	Restraint***	Swim*	Restraint***
21		Cart transport**	Radio static***

* 15 min.
** 30 min.
*** 60 min.

2.2. Prenatal stress procedures

Half ($n = 12$) of the pregnant female dams were randomly selected to experience unpredictable variable stress 2–3 times daily between 9 a.m. and 5 p.m. during the last week of gestation (prenatal days 14–21). The stressors were mild in nature and included (1) restraint in cylindrical restrainers (30 min), (2) swim in water at room temperature (15 min), (3) social stress (5 rats/cage for 8–9 h), (4) overnight fast, (5) exposure to loud radio static (80 db, 60 min), and (6) transport on a noisy cart (30 min). Our procedures were similar to Koenig [35], but cold room exposure and reverse light schedule were replaced with cart transport and radio static in our schedule due to facility constraints. All stressed animals were subjected to the same schedule of stressors. The remaining pregnant females ($n = 12$) served as controls and were exposed to only routine animal husbandry (Table 1).

2.3. Behavioral testing

The offspring of control and prenatally stressed dams underwent anxiety-related behavioral testing in adulthood beginning at 79 days of age. All testing occurred during the light phase of the light/dark cycle and consisted of open field, elevated zero and social interaction tests, in this order. In order to prevent potential carryover effects between tests, at least one week separated open field, elevated zero, and social interaction testing, and tests were conducted in the order of least to most stressful. Observers were blind to group assignment and remained out of sight of the animals during each test. On each behavioral testing day, the order in which individual animals were tested was randomized using a random sequence generator (random.org). Prior to the introduction of a new animal, each apparatus was cleaned with Simple Green Pro HD deodorizer (Huntington Harbour, CA). Behaviors were recorded and analyzed using Topscan behavioral analysis software (Clever Sys. Inc., Reston, VA). Animals were subjected to learning and memory testing earlier on in this study, and the results of which will be reported elsewhere.