Published in final edited form as:

Behav Brain Res. 2014 July 15; 268: 104–110. doi:10.1016/j.bbr.2014.03.031.

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^{1,2,*}, Jennifer N. Pearson³, Mary E. Gasparini^{1,2}, Kayla F. Brooks^{1,2}, Chakeer Drake-Frazier^{1,2}, Megan E. Zajkowski^{1,2}, Alison D. Kreisler⁴, Catherine E. Adams^{1,2}, Sherry Leonard^{1,2,3}, and Karen E. Stevens^{1,2}

¹Veterans Affairs Medical Center, 1055 Clermont Street, Denver CO, 80220 USA

²Department of Psychiatry, University of Colorado Anschutz Medical Campus, Building 500, 1300 East 17th Place, Aurora CO, 80045 USA

³Neuroscience Program, University of Colorado Anschutz Medical Campus, RC-1, MS 8351, Rm 7107 12800 E. 19th Ave, Aurora CO, 80045 USA

⁴Neuroscience Department, University of Pittsburgh, A210 Langley Hall, Pittsburgh PA, 15260 USA

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-

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

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

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.

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 $\alpha 7^*$ and $\alpha 4 \beta 2^*$ 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 $\alpha 7^*$ -dependent brain inhibitory function in infants [21]. Similarly, rodent studies demonstrate that supplementing dams during pregnancy and lactation permanently increases offspring levels of hippocampal $\alpha 7^*$ 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 (5g/kg of choline chloride) through gestation and lactation, and half were fed a standard diet (1.1g/kg of choline chloride). Bedding (Tekfresh, Harlan Laboratories Inc., Indianapolis, IN), food (Dyets Inc., Bethlehem, Pennsylvania) and filtered water were changed weekly. One day prior to parturition, the females were transferred to larger cages (40.6×30.5×20.3) 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, offspring (n=12 overall n=96) were assigned to same-sex (male/female) groups based on stress condition [prenatally stressed (PS)/nonstressed (NS)] and diet condition (choline diet/control diet). All offspring were housed 2 per cage in same-sex, same-condition sibling groups. Offspring had *ad libitum* access to food (Dyets Inc., Bethlehem, Pennsylvania). All animals were maintained on a 12:12 light/dark cycle, and the room temperature was held constant at 21°C. All animals were treated in accordance with NIH guidelines and all protocols were approved by the IACUC of the University of Colorado Denver.

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 9am and 5pm 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 hours) 4) overnight fast, 5) exposure to loud radio static (80db, 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.

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.

2.3.1. Open Field—Indices of anxiety-related behavior were assessed early in adulthood at 79 days using an open field arena. The open field arena was constructed of mat black expanded PVC (70cm × 70 cm; wall height = 47.6 cm). In the behavior room, two arenas were oriented so that they were dimly lit by fluorescent light bulbs. At the beginning of each test, the animal was lowered into the arena alongside the wall and placed in the perimeter of the arena. Animals were tested two at a time in separate arenas and were allowed to explore the arena for a total of five minutes. Following exploration, animals were removed and the arenas were cleaned as described above. Specialized behavior recognition software (Topscan, Cleversys Inc), allowed the arenas to be visually split into multiple sections including a center zone, a perimeter area and corner zones. The center zone comprised 60% of the total area of the arena. The behavioral analysis software monitored animals for the number of visits, time spent, and distance traveled the center zone of the open field.

2.3.2. Elevated Zero—Additional testing for anxiety-related behaviors was carried out at 98 days of age using an elevated zero maze (San Diego Instruments, San Diego, CA). The elevated zero maze is a test of anxiety that is similar to the elevated plus maze, but instead of being a plus shape it has a circular shape which allows the rat to continuously investigate the maze without turning around, thereby reducing variability in the dataset. The elevated zero maze has been validated pharmacologically with anti-anxiety drugs [36] and generates anxiety levels comparable to the elevated plus maze [37]. Similar to the elevated plus maze, the elevated zero maze allows indices of anxiety to be determined based on the amount of time spent in the open wall sections versus the amount of time spent in the closed wall sections. Time spent in the closed or open sections of the apparatus indicates more or less anxiety-related behavior, respectively [38]. The elevated zero maze was constructed of black non-porous plastic, is circular in design (diameter of circle = 121.92 cm; width of runway = 20.32 cm) with adjacent, alternating, open and closed sections and was elevated 76.2 cm off the floor. Each animal was tested only once in the elevated zero. To begin each test, an animal was placed in the closed section of the maze and allowed to investigate for a total of five minutes. Following investigation, animals were placed back in their home cage and the arenas were cleaned following the procedure described above. The behavior analysis system (Topscan, Cleversys Inc) measured the duration of time spent in the open section as well as the number of entries into the open section. The risk-assessment behavior stretch-attend was also calculated when an animal sniffed the open section of the zero while situated in the closed section (front paws on open section with hind paws in the closed section).

2.3.3. Social Interaction—Social interaction testing was performed at 106 days of age in an open-field arena constructed of mat black expanded PVC (70 cm × 70 cm; wall height = 47.6 cm). Non-sibling, non-cage mates from the same stress and diet conditions were matched according to sex and weight and allowed to interact in the open-field arena for a total of ten minutes. Behaviors were recorded with video software and later scored by a single observer blind to experimental condition. Sniffing behaviors were recorded independently for each subject in the dyad. Only the sniffing contacts initiated by a given subject were included in that subject's total sniffing duration.

2.4 Statistics

2.4.1. Open Field—The effects of stress condition (PS/NS), and diet (choline supplemented/control) on behavior in the open field were analyzed separately for males and females by 2 factor between-subjects ANOVA.

2.4.2. Elevated Zero—The effects of stress condition (PS/NS), and diet (choline supplemented/control) on behavior in the elevated zero were analyzed separately for males and females by 2 factor between-subjects ANOVA. Pairwise comparisons were also conducted using t-tests tests.

2.4.3. Social Interaction—The effects of stress condition (PS/NS), and diet (choline supplemented/control) on social behavior were analyzed separately for males and females by mixed ANOVAs in which stress condition and diet were the between-subjects factors, and time spent sniffing the partner was the repeated measure.

2.4.4. Body Weight—The effects of stress condition (PS/NS), diet (choline supplemented/control), and sex on body weight were analyzed by mixed ANOVAs in which stress condition, diet, and sex were the between-subjects factors, and time was the repeated measure.

2.4.5. Experimental Attrition—Three males (2 PS choline, 1 NS choline) and two females (1 NS choline, 1 NS control) fell off the elevated zero maze during testing and were therefore excluded from statistical analysis. In addition, 8 animals (2 PS choline males, 2 NS choline females, 2 PS choline females, and 2 PS control females) were not utilized for elevated zero or social interaction testing because their social experience differed from all other animals in the study. Specifically, in the week prior to testing, these animals were mistakenly housed with an unfamiliar cagemate.

3. Results

3.1. Open Field: Choline decreases female but not male anxiety-related behaviors in the open field

In female offspring, choline significantly increased the number of center visits [Figure 1A; $F(1, 44) = 4.30, p = 0.0443$], time spent in the center zone [Figure 1B; $F(1, 44) = 8.33, p = 0.0060$], and distance traveled in the center zone [Figure 1C; $F(1, 44) = 4.62, p = 0.0372$]. No main effects of stress condition or interactions between diet and stress condition were found in females. In males, no effects of stress condition, diet, or interactions between these factors were found (Figures 1A, 1B, and 1C).

3.2. Elevated Zero: Choline mitigates the anxiety-related behaviors of prenatally stressed females but not males in the elevated zero

In females, a significant effect of stress condition was found for time in the open areas of the elevated zero [$F(1, 36) = 4.30, p = 0.0462$]. This main effect was driven primarily by differences between PS and NS females under control as opposed to choline diet conditions. Specifically, PS females spent significantly less time in the open areas than did NS females

under control diet conditions [$t(1, 19) = 5.16, p = 0.0349$]. In contrast, differences in open area duration were mitigated under choline diet conditions, as no differences were found between PS and NS females. Similarly, ANOVA revealed trends toward effects of stress condition on both the number of open entries [$F(1, 36) = 3.67, p = 0.0635$] and stretch-attend number per open entry [$F(1, 36) = 2.88, p = 0.0985$]. These differences also appeared to be driven by PS-induced alterations in anxiety-related behaviors under control but not choline diet conditions. Specifically, under control diet conditions, PS females showed fewer open arm entries [Figure 2B; $t(1, 19) = 5.17, p = 0.0347$] and increased stretch-attend number per open area entry [Figure 2C; $t(1, 19) = 5.32, p = 0.0325$] relative to NS females. In contrast, differences between PS and NS females were mitigated under choline diet conditions, as no differences were found between PS and NS females for open entry number or stretch-attend number per open entry. In males, no effects of stress condition, diet, or interactions between these factors were found for any of the anxiety-related behaviors assessed (Figures 2A, 2B, and 2C).

3.3. Social Interaction: Choline mitigates the anxiety-related behaviors of prenatally stressed males but not females during social interactions

In males, choline treatment increased overall investigation of conspecifics, measured as sniffing duration [Figure 3, right panel; $F(1, 46) = 3.958, p = 0.0526$]. A significant interaction between stress condition and time was also detected, such that NS males maintained high sniffing behavior longer than PS males (Figure 3; $F(3, 138) = 3.176, p = 0.0262$). Specifically, PS males only displayed high levels of sniffing during the first two minutes of testing, and significantly decreased thereafter [$F(3, 138) = 6.40, p = 0.0006$; 0–2 min bin vs. 2–4 min, $p = 0.0006$; 0–2 min bin vs. 4–6 min, $p = 0.0003$; 0–2 min bin vs. 6–8 min $p = 0.0016$]. In contrast, NS males maintained high levels of sniffing behavior for a full four minutes before significantly decreasing sniffing [$F(3, 138) = 7.33, p = 0.0002$; 2–4 min bin vs. 4–6 min, $p = 0.0038$; 2–4 min bin vs. 6–8 min $p = 0.0040$].

For females, only a significant effect of time was detected for sniffing durations [$F(3, 108) = 14.111, p < 0.0001$], such that sniffing of conspecifics decreased after the first two minutes of testing [0–2 min bin vs. 2–4 min bin, $p = 0.0006$]. No significant effects of stress condition, choline, or interactions between stress condition, choline, or time were found in females (data not shown).

3.4. Body Weight: Prenatal stress decreases animal body weight irrespective of choline supplementation

A mixed ANOVA revealed a significant mean increase in body weight for all animals over the course of the study ($F(1.51, 138.55) = 8014.05, p < 0.001$). A significant main effect of sex was also detected ($F(1, 92) = 1164.67, p < 0.001$), such that males were significantly larger than females. Additionally, a significant main effect of stress condition was found, such that PS animals were slightly, yet significantly, smaller than NS animals ($F(1, 92) = 18.95, p < 0.001$; PS female, $\bar{x} = 204.92$; NS female, $\bar{x} = 219.11$; PS male, $\bar{x} = 338.20$; NS male, $\bar{x} = 355.35$). These main effects were qualified by significant interactions between time and sex ($F(1.51, 138.55) = 928.16, p < 0.001$) and between time and stress condition ($F(1.51, 138.55) = 6.35, p = 0.005$). Simple comparison analyses revealed the significant

interaction between time and stress condition was driven by a prenatal stress-induced decrease in body weight beginning at week 9 ($F(1, 92) = 5.42, p = 0.0221$) and remained at weeks 13 ($F(1, 92) = 6.57, p = 0.0120$) and 17 ($F(1, 92) = 10.44, p = 0.0017$). The significant interaction between time and sex was driven by the difference in body weight between males and females beginning at week 9, and remaining throughout the study (data not presented graphically).

4.0 Discussion

Anxiety in rodents is multidimensional, and factor analysis studies demonstrate that behaviors observed in the open field, elevated plus, and social interaction tests load on distinct factors [34, 39, 40]. Thus, while each of these tests assesses anxiety-related behaviors, they appear to tap different aspects of anxiety. We report here that that prenatal stress and choline exposure influenced anxiety-related behaviors in a test- and sex-specific manner. In the open field, no effects of prenatal stress were observed for either sex. However, choline exposure increased exploratory behavior in females but not in males. For the elevated zero, the effects of prenatal stress and choline were also female-biased. Under control diet conditions, prenatally stressed females significantly decreased their frequency and duration of visits to the open arms. In contrast, under choline-supplemented conditions, no differences were observed between PS and NS females, suggesting that choline exposure ameliorates the anxiogenic effects of prenatal stress. For the social interaction test, the effects of prenatal stress and choline were male-biased. Specifically, choline exposure increased male social behavior durations, especially in prenatally stressed males. Thus, we demonstrate here that early developmental choline exposure mitigates the deleterious effects of prenatal stress on particular dimensions of anxiety-related behaviors in males and females.

A significant effect of prenatal stress but not choline diet on body weight was observed in the current study. This was surprising given that we previously found significant prenatal stress-induced increases in body weight in males [2]. Moreover, other studies demonstrate that postnatal dietary choline intake influences energy metabolism and lean body mass composition [41]. The differences in weight gain between these two studies may be due to differences in the fat content of the diets. In our previous study in which PS males gained weight, 17% of the calories in the chow were derived from fat (2018 Teklad Global 18% Protein Rodent Diet). In contrast, only 11% of calories were derived from fat in our current study (AIN-76A, Dyets Inc., Bethlehem, Pennsylvania). Given that prenatal stress and high fat diet interact to increase obesity in rats [42], it is possible that differences in chow fat content between these two studies caused differential effects of prenatal stress on weight gain. Thus, our future studies will carefully control the fat content in food or manipulate chow fat content as a variable of interest.

The mechanisms by which perinatal choline mitigates the effects of prenatal stress are not yet known, but many of the neurodevelopmental consequences of prenatal stress relevant to anxiety-related behaviors are also impacted by prenatal choline exposure. For example, prenatal stress significantly reduces hippocampal neurogenesis [43], whereas prenatal choline supplementation increases hippocampal neurogenesis in offspring [44, 45].

Similarly, alterations in neurotrophic factors such as BDNF have been implicated in the pathology and treatment of psychiatric illnesses including obsessive compulsive disorder, schizophrenia and depression [46–50], and are also reduced by prenatal stress [51]. Given that prenatal choline supplementation increases several brain neurotrophic factors including BDNF, NGF and IGF2R [26, 44, 52], perinatal choline may ameliorate the deleterious effects of prenatal stress on anxiety via increased neurotrophic factors. Interestingly, BDNF may also influence anxiety and depression via changes in nAChRs, given that BDNF up-regulates $\alpha 7^*$ nAChR receptor levels in hippocampal interneurons [53]. Therefore, further research is necessary to elucidate the potential interactions between choline supplementation, neurotrophic factors and $\alpha 7^*$ nAChR receptors.

In addition to possible indirect effects of perinatal choline on nAChRs, perinatal choline may also directly impact nAChR levels to influence anxiety related behaviors. For example, perinatal choline is capable of increasing nAChRs in mouse strains with low levels of hippocampal nAChRs [27, 54]. Given that we have recently found that prenatal stress alters hippocampal levels of both $\alpha 4 \beta 2^*$ and $\alpha 7^*$ nAChRs [19], it is plausible that prenatal choline counteracts the impact of prenatal stress by maintaining normative levels of nAChRs in the hippocampus. Studies are currently underway to compare the levels of hippocampal nAChRs in prenatally stressed animals gestated on control or choline supplemented diet.

To our knowledge, this is the first report to demonstrate that perinatal choline counteracts the effects of prenatal stress on adult anxiety-related behaviors. Interestingly, a much earlier report from Tonjes and colleagues [55] found that postnatal injections of choline chloride counteracted the effects of neonatal maternal deprivation on memory function in males in adulthood. Choline chloride treatment was most effective when administered during the same timeframe as neonatal stress on postnatal days 1–14, but also mitigated the effects of neonatal stress when administered after neonatal stress treatments on days 15–28 [55]. More recently, Corriveau & Glenn [29] employed a ‘two hit’ rodent model of schizophrenia and found that adolescent dietary choline supplementation mitigated the memory deficits in males resulting from combined exposure to prenatal stress and the NMDA receptor antagonist MK801 in adulthood. Thus, our findings extend this budding literature by showing that perinatal choline counteracts the detrimental effects of prenatal stress on anxiety-related behaviors in both males and females.

References

1. Van den Bergh BRH, Marcoen A. High antenatal maternal anxiety is related to ADHD symptoms, externalizing problems, and anxiety in 8-and 9-year-olds. *Child Development*. 2004; 75(4):1085–1097. [PubMed: 15260866]
2. Schulz KM, et al. Maternal stress during pregnancy causes sex-specific alterations in offspring memory performance, social interactions, indices of anxiety, and body mass. *Physiology & Behavior*. 2011; 104(2):340–347. [PubMed: 21334352]
3. Markham JA, Koenig JI. Prenatal stress: role in psychotic and depressive diseases. *Psychopharmacology (Berl)*. 2011; 214(1):89–106. [PubMed: 20949351]
4. Richardson HN, et al. Exposure to repetitive versus varied stress during prenatal development generates two distinct anxiogenic and neuroendocrine profiles in adulthood. *Endocrinology*. 2006; 147(5):2506–2517. [PubMed: 16455779]

5. Vallee M, et al. Prenatal stress induces high anxiety and postnatal handling induces low anxiety in adult offspring: Correlation with stress-induced corticosterone secretion. *Journal of Neuroscience*. 1997; 17(7):2626–2636. [PubMed: 9065522]
6. Weinstock M. Sex-dependent changes induced by prenatal stress in cortical and hippocampal morphology and behaviour in rats: An update. *Stress-the International Journal on the Biology of Stress*. 2011; 14(6):604–613.
7. File SE, Cheeta S, Kenny PJ. Neurobiological mechanisms by which nicotine mediates different types of anxiety. *European Journal of Pharmacology*. 2000; 393(1–3):231–236. [PubMed: 10771018]
8. File SE, Kenny PJ, Cheeta S. The role of the dorsal hippocampal serotonergic and cholinergic systems in the modulation of anxiety. *Pharmacol Biochem Behav*. 2000; 66(1):65–72. [PubMed: 10837844]
9. File SE, Kenny PJ, Ouagazzal AM. Bimodal modulation by nicotine of anxiety in the social interaction test: Role of the dorsal hippocampus. *Behavioral Neuroscience*. 1998; 112(6):1423–1429. [PubMed: 9926824]
10. Hunter RG, et al. Regulation of the nicotinic receptor alpha7 subunit by chronic stress and corticosteroids. *Brain Research*. 2010; 1325:141–146. [PubMed: 20153739]
11. Stitzel JA, Farnham DA, Collins AC. Chronic corticosterone treatment elicits dose-dependent changes in mouse brain alpha-bungarotoxin binding. *Neuroscience*. 1996; 72(3):791–799. [PubMed: 9157324]
12. Pauly JR, Collins AC. An autoradiographic analysis of alterations in nicotinic cholinergic receptors following 1 week of corticosterone supplementation. *Neuroendocrinology*. 1993; 57(2):262–271. [PubMed: 8510802]
13. Bjelland I, et al. Choline in anxiety and depression: the Hordaland Health Study. *American Journal of Clinical Nutrition*. 2009; 90(4):1056–1060. [PubMed: 19656836]
14. Atmaca M, et al. Neurochemistry of the hippocampus in patients with obsessive-compulsive disorder. *Psychiatry and Clinical Neurosciences*. 2009; 63(4):486–490. [PubMed: 19531109]
15. Hong ST, et al. Specific hippocampal choline decrease in an animal model of depression. *British Journal of Radiology*. 2009; 82(979):549–553. [PubMed: 19221187]
16. Huang YY, et al. Effects of antidepressant treatment on N-acetyl aspartate and choline levels in the hippocampus and thalami of post-stroke depression patients: A study using H-1 magnetic resonance spectroscopy. *Psychiatry Research-Neuroimaging*. 2010; 182(1):48–52.
17. Steingard RJ, et al. Increased orbitofrontal cortex levels of choline in depressed adolescents as detected by in vivo proton magnetic resonance spectroscopy. *Biological Psychiatry*. 2000; 48(11):1053–1061. [PubMed: 11094138]
18. Hunter SK, et al. Antidepressants may mitigate the effects of prenatal maternal anxiety on infant auditory sensory gating. *The American journal of psychiatry*. 2012; 169(6):616–624. [PubMed: 22581104]
19. Schulz KM, et al. The effects of prenatal stress on alpha4 beta2 and alpha7 hippocampal nicotinic acetylcholine receptor levels in adult offspring. *Developmental neurobiology*. 2013
20. Day JC, et al. Corticotropin-releasing factor administered centrally, but not peripherally, stimulates hippocampal acetylcholine release. *Journal of Neurochemistry*. 1998; 71(2):622–629. [PubMed: 9681452]
21. Ross RG, et al. Perinatal Choline Effects on Neonatal Pathophysiology Related to Later Schizophrenia Risk. *American Journal of Psychiatry*. 2013; 170(3):290–298. [PubMed: 23318559]
22. Li Q, et al. Dietary prenatal choline supplementation alters postnatal hippocampal structure and function. *Journal of Neurophysiology*. 2004; 91(4):1545–1555. [PubMed: 14645379]
23. Mellott TJ, et al. Prenatal choline supplementation advances hippocampal development and enhances MAPK and CREB activation. *Faseb Journal*. 2004; 18(1):545–+. [PubMed: 14715695]
24. Montoya DA, et al. Prenatal choline exposure alters hippocampal responsiveness to cholinergic stimulation in adulthood. *Brain Res Dev Brain Res*. 2000; 123(1):25–32.
25. Sparks TA, et al. Maternal parenting stress and mothers' reports of their infants' mastery motivation. *Infant behavior & development*. 2012; 35(1):167–173. [PubMed: 21890208]

26. Napoli I, Blusztajn JK, Mellott TJ. Prenatal choline supplementation in rats increases the expression of IGF2 and its receptor IGF2R and enhances IGF2-induced acetylcholine release in hippocampus and frontal cortex. *Brain Res.* 2008; 1237:124–135. [PubMed: 18786520]
27. Stevens KE, et al. Permanent improvement in deficient sensory inhibition in DBA/2 mice with increased perinatal choline. *Psychopharmacology.* 2008; 198(3):413–420. [PubMed: 18446322]
28. Thomas JD, Garrison M, O'Neill TM. Perinatal choline supplementation attenuates behavioral alterations associated with neonatal alcohol exposure in rats. *Neurotoxicology and Teratology.* 2004; 26(1):35–45. [PubMed: 15001212]
29. Corriveau JA, Glenn MJ. Postnatal choline levels mediate cognitive deficits in a rat model of schizophrenia. *Pharmacology Biochemistry and Behavior.* 2012; 103(1):60–68.
30. Moon J, et al. Perinatal Choline Supplementation Improves Cognitive Functioning and Emotion Regulation in the Ts65Dn Mouse Model of Down Syndrome. *Behavioral Neuroscience.* 2010; 124(3):346–361. [PubMed: 20528079]
31. Wong-Goodrich SJ, et al. Water maze experience and prenatal choline supplementation differentially promote long-term hippocampal recovery from seizures in adulthood. *Hippocampus.* 2011; 21(6):584–608. [PubMed: 20232399]
32. Wong-Goodrich SJ, et al. Prenatal choline supplementation attenuates neuropathological response to status epilepticus in the adult rat hippocampus. *Neurobiol Dis.* 2008; 30(2):255–269. [PubMed: 18353663]
33. Glenn MJ, Adams RS, McClurg L. Supplemental dietary choline during development exerts antidepressant-like effects in adult female rats. *Brain Research.* 2012; 1443:52–63. [PubMed: 22305146]
34. Ramos A. Animal models of anxiety: do I need multiple tests? *Trends in Pharmacological Sciences.* 2008; 29(10):493–498. [PubMed: 18755516]
35. Koenig JI, et al. Prenatal exposure to a repeated variable stress paradigm elicits behavioral and neuroendocrinological changes in the adult offspring: potential relevance to schizophrenia. *Behavioural Brain Research.* 2005; 156(2):251–261. [PubMed: 15582111]
36. Kulkarni SK, Singh K, Bishnoi M. Elevated zero maze: A paradigm to evaluate antianxiety effects of drugs. *Methods and Findings in Experimental and Clinical Pharmacology.* 2007; 29(5):343–348. [PubMed: 17805436]
37. Kulkarni SK, Singh K, Bishnoi M. Comparative behavioural profile of newer antianxiety drugs on different mazes. *Indian Journal of Experimental Biology.* 2008; 46(9):633–638. [PubMed: 18949892]
38. Rodgers RJ, Dalvi A. Anxiety, defence and the elevated plus-maze. *Neuroscience & Biobehavioral Reviews.* 1997; 21(6):801–810. [PubMed: 9415905]
39. Ramos A, et al. A genetic and multifactorial analysis of anxiety-related behaviours in Lewis and SHR intercrosses. *Behav Brain Res.* 1998; 96(1–2):195–205. [PubMed: 9821556]
40. Trullas R, Skolnick P. Differences in fear motivated behaviors among inbred mouse strains. *Psychopharmacology (Berl).* 1993; 111(3):323–331. [PubMed: 7870970]
41. Zeisel SH. Metabolic crosstalk between choline/1-carbon metabolism and energy homeostasis. *Clinical Chemistry and Laboratory Medicine.* 2013; 51(3):467–475. [PubMed: 23072856]
42. Tamashiro K, et al. Prenatal Stress or High-Fat Diet Increases Susceptibility to Diet-Induced Obesity in Rat Offspring. *Diabetes.* 2009; 58(5):1116–1125. [PubMed: 19188431]
43. Lemaire V, et al. Prenatal stress produces learning deficits associated with an inhibition of neurogenesis in the hippocampus. *Proceedings of the National Academy of Sciences of the United States of America.* 2000; 97(20):11032–11037. [PubMed: 11005874]
44. Glenn MJ, et al. Prenatal choline availability modulates hippocampal neurogenesis and neurogenic responses to enriching experiences in adult female rats. *Eur J Neurosci.* 2007; 25(8):2473–2482. [PubMed: 17445242]
45. Glenn MJ, et al. Age-related declines in exploratory behavior and markers of hippocampal plasticity are attenuated by prenatal choline supplementation in rats. *Brain Research.* 2008; 1237:110–123. [PubMed: 18786518]
46. Fontenelle LF, et al. Neurotrophic factors in obsessive-compulsive disorder. *Psychiatry Research.* 2012; 199(3):195–200. [PubMed: 22494702]

47. Gaughran F, et al. Hippocampal FGF-2 and FGFR1 mRNA expression in major depression, schizophrenia and bipolar disorder. *Brain Research Bulletin*. 2006; 70(3):221–227. [PubMed: 16861106]
48. Hoshaw BA, Malberg JE, Lucki I. Central administration of IGF-I and BDNF leads to long-lasting antidepressant-like effects. *Brain Research*. 2005; 1037(1–2):204–208. [PubMed: 15777771]
49. Gonul AS, et al. Effect of treatment on serum brain-derived neurotrophic factor levels in depressed patients. *European Archives of Psychiatry and Clinical Neuroscience*. 2005; 255(6):381–386. [PubMed: 15809771]
50. Siuciak JA, et al. Antidepressant-like effect of brain-derived neurotrophic factor (BDNF). *Pharmacology Biochemistry and Behavior*. 1997; 56(1):131–137.
51. Neeley EW, et al. Strain dependent effects of prenatal stress on gene expression in the rat hippocampus. *Physiology & Behavior*. 2011; 104(2):334–339. [PubMed: 21382392]
52. Sandstrom NJ, Loy R, Williams CL. Prenatal choline supplementation increases NGF levels in the hippocampus and frontal cortex of young and adult rats. *Brain Res*. 2002; 947(1):9–16. [PubMed: 12144847]
53. Massey KA, Zago WM, Berg DK. BDNF up-regulates alpha 7 nicotinic acetylcholine receptor levels on subpopulations of hippocampal interneurons. *Molecular and Cellular Neuroscience*. 2006; 33(4):381–388. [PubMed: 17029981]
54. Bouvrais-Veret C, et al. Sustained increase of alpha7 nicotinic receptors and choline-induced improvement of learning deficit in STOP knock-out mice. *Neuropharmacology*. 2007; 52(8):1691–1700. [PubMed: 17512560]
55. Tonjes R, et al. BEHAVIORAL-CHANGES IN ADULT-RATS PRODUCED BY EARLY POSTNATAL MATERNAL-DEPRIVATION AND TREATMENT WITH CHOLINE CHLORIDE. *Experimental and Clinical Endocrinology*. 1986; 88(2):151–157. [PubMed: 3556403]

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.

Figure 1. Anxiety-related behavior in the open field paradigm

Females exposed to perinatal choline (A) exhibited a greater number of visits to the center field of the arena, (B) spent more time in the center field, and (C) traveled a greater distance in the center field than control diet females, irrespective of stress condition. For males, no differences were observed between control and choline diet conditions under either stress condition. NS choline females, n=12; NS control females, n=12; PS choline females, n=12; PS control females, n=12; NS choline males, n=14; NS control males, n=12; PS choline

males, n=14; PS control males, n =12. Data expressed as mean $\pm$ SEM. Asterisk (*) indicates a significant difference ($p \leq 0.05$) between groups.

Figure 2. Anxiety-related behavior on the elevated zero maze

Under control diet conditions, prenatally stressed females (A) spent less time in the open areas, (B) made fewer entries into the open areas, and (C) exhibited greater risk-assessment behavior (stretch-attend number per open entry) than nonstressed (NS) females. In contrast, under perinatal choline diet conditions, PS failed to increase anxiety-related behaviors in the elevated zero. NS choline females, $n=9$; NS control females, $n=11$; PS choline females, $n=10$; PS control females, $n=10$; NS choline males, $n=13$; NS control males, $n=12$; PS

choline males, n=10; PS control males, n =11. Data expressed as mean $\pm$ SEM. Asterisk (*) indicates a significant difference ($p < 0.05$) between groups.

Figure 3. Male sniffing behavior during the social interaction test

Perinatal choline increased sniffing durations relative to control diet (histograms, right panel). A significant interaction between stress condition and time was also detected such that nonstressed (NS) males maintained high sniffing behavior longer than prenatally stressed (PS) males. No effects of diet or stress condition were found in females (data not shown). NS control males, $n=12$; NS choline males, $n=14$; PS control males, $n=12$; PS choline males, $n=12$. Data expressed as mean $\pm$ SEM. Asterisk (*) indicates a significant difference ($p < 0.05$) between groups.

Table 1

Stress Schedule

Gestation Day	Time of Day		
	A.M.	Mid-day	P.M.
14	9 hour 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