



Prenatal Activation of Glucocorticoid Receptors Induces Memory Impairment in a Sex-Dependent Manner: Role of Cyclooxygenase-2

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Abstract

Prenatal exposure to dexamethasone (DEX) results in long-lasting effects on cognitive functions such as learning and memory impairment. However, the mechanisms underlying these DEX-induced deleterious effects are not well known. Here, we assessed whether cyclooxygenase-2 (COX-2) is involved in the impact of prenatal exposure to DEX on learning and memory during adulthood. Pregnant Sprague–Dawley rats received daily injections of either DEX (0.2 mg/kg; i.p.) or saline from gestation day (GD) 14 until GD21. Gene and protein expression of COX-2, as well as presynaptic (synaptophysin) and postsynaptic (postsynaptic density protein-95) proteins, were monitored in the dorsal and ventral hippocampi of adult male and female offspring. A different cohort of adult male and female rat offspring was given daily injections of either vehicle or a specific COX-2 inhibitor (celecoxib 10 mg/kg, i.p.) for 5 consecutive days and was subsequently subjected to Morris water maze memory test. Prenatal DEX enhanced the expression of COX-2 protein and *cox-2* mRNA in the dorsal hippocampus of adult female but not male rats. This enhanced COX-2 expression was associated with reduced expression in pre- and postsynaptic proteins and altered memory acquisition and retention. Administration of COX-2-specific inhibitor alleviated prenatal DEX-induced memory impairment in adult female rats. This study suggests that prenatal activation of glucocorticoid receptors stimulates COX-2 gene and protein expression and impairs hippocampal-dependent spatial memory in female but not male rat offspring. Furthermore, COX-2 selective inhibitors can be used to alleviate the long-lasting deleterious effects of corticosteroid medication during pregnancy.

Keywords Synaptic proteins · Dorsal hippocampus · Brain programming · Celecoxib

Introduction

Synthetic glucocorticoids such as dexamethasone (DEX) are prescribed to pregnant women at risk of preterm labor to induce lung maturation [1, 2] and to women bearing fetuses at risk of developing congenital adrenal hyperplasia to prevent virilization of female external genitalia [3]. However, exposure to DEX during critical developmental periods can lead to lifelong effects on offspring brain's plasticity and function [4]. Indeed, prenatal DEX induces learning and memory deficits during adulthood likely by altering the developmental trajectory of the hippocampus [5–7]. The

mechanism underlying the long-lasting deleterious effect of DEX on cognitive functions is still not clear.

Cyclooxygenases (COXs) are the rate-limiting enzymes in the production of prostaglandins and thromboxane. COXs convert arachidonic acid into an unstable prostaglandin called PGH₂. PGH₂ is further metabolized into prostaglandins of the E₂, I₂, D₂, F₂ series, and thromboxane (TXA₂) by specific enzymes [8]. There are two cyclogenases: COX-1 and COX-2. COX-1 is constitutively expressed under basal condition, while COX-2 is generally induced under pathological conditions such as inflammation and cancer [9, 10].

In addition to its induction by inflammatory insults, COX-2 is also expressed in several brain areas under basal conditions. For example, COX-2 is constitutively expressed in the CA3 region, the dentate gyrus, and to a lesser degree in the CA1 region of mouse and rat hippocampi [11, 12]. This constitutively expressed COX-2 is involved in learning and memory [13]. Indeed, specific inhibition of COX-2 alters learning and memory likely through alteration of

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synaptic transmission and long-term potentiation (LTP) [14, 15]. However, transgenic overexpression of COX-2 in cortical areas including hippocampal neurons results in spatial memory deficit [16]. We and others have shown that early-life adverse events such as immune or psychological stresses lead to a sustained increase in COX-2 protein expression in several adult brain areas including the hypothalamus [17], the spinal cord [18], and the prefrontal cortex [19]. The physiological significance of this enhanced expression of COX-2 is not known.

COX-2 is expressed in neuronal cells as early as GD15 in rats [20] and can be directly affected by DEX since this drug can cross the placental barrier and reach fetal brain because it is not metabolized by the placental enzyme 11 β -hydroxysteroid dehydrogenase type 2 [21, 22]. Thus, it is plausible that the long-lasting impact of prenatal DEX operates through a COX-2-dependent mechanism. Therefore, in the present study, we assessed whether prenatal exposure to DEX alters adult learning and memory through COX-2. We provide experimental evidence to show that prenatal exposure to DEX enhanced the expression levels of COX-2 protein and *cox-2* mRNA in the dorsal hippocampus of female, but not male, adult offspring. This enhanced COX-2 expression was associated with memory deficit in female offspring. More importantly specific inhibition of COX-2 alleviated this memory deficit.

Materials and Methods

Animals

All experiments were performed in accordance with the guidelines of the humane handling of experimental animals as established by the Kuwait University Health Sciences Center, Animal Research Ethics Committee. Male and female Sprague–Dawley (SD) rats were housed in the Animal Resources Center of Kuwait University. Animals were maintained on 12-h light/dark cycle (7:00 AM–7:00 PM) at a controlled temperature 22 °C. They were provided with chow food ad libitum and had free access to water. Adult female rats were mated with proven breeder male rats. Daily vaginal smears were taken to monitor the presence of sperms. The day of detection of vaginal sperm was considered as gestation day (GD) 0 of pregnancy. Pregnant dams received daily intraperitoneal (i.p.) injections of either dexamethasone phosphate (DEX: 0.2 mg/kg, Sigma-Aldrich) dissolved in pyrogen-free saline or an equivolume of pyrogen-free saline (control) from GD 14 to GD 21. This dose/regimen resulted in minimal side effects on dam-pups interaction such as neglect and reduced suckling. After birth, pups were kept with their dams until weaning on postnatal day 21. Offspring rats were then housed 4 per cage until

they reached the age of 2 months, after which they were housed 2 per cage. Male and female rat offspring were randomly selected for memory testing, western blot, and real-time rtPCR. Each rat group consists of randomly selected rats born to different dams to minimize the litter bias. Prenatal DEX injection did not affect the litter size (data not shown) as previously shown [23]. Adult male and female offspring (PND70) born to dams given either DEX (Male-DEX and Female-DEX) or saline (Male-Saline and Female-Saline) were subjected to Morris water maze memory test as previously described [24]. Dorsal and ventral hippocampi were collected, snap frozen in liquid nitrogen, and stored at –80 °C until use in either western blot or real-time rtPCR as detailed below. Dorsal and ventral hippocampi were studied separately because they are preferentially involved in spatial memory and emotionally associated functions, respectively [25].

Real-Time PCR

Total RNA isolation, DNase treatment, and reverse transcription were performed as previously described [23, 26]. Forward and reverse primers (0.5 μ M each) were added to a PCR buffer (in mM: 20 Tris, 50 KCl, 3 MgCl₂, 0.5 dNTPs) containing template (1 μ l) and recombinant Taq DNA polymerase (1.25 U) in a final volume of 25 μ l. The RT-PCR was carried out in a programmable thermal cycler (PerkinElmer model 9700). The thermal cycles were as follows: 50 °C for 2 min, followed by 95 °C for 10 min (one cycle) and 95 °C for 15 s and 72 °C for 1 min for the required number of cycles. TaqMan rtPCR method was used to study *cox-2* and *gapdh* (*cox-2* assay: Rn RN01483828_m1 and *gapdh* assay: Rn01775763_g1). All data were normalized against the mRNA levels of *gapdh*, expressed relative to their saline-treated male controls using the “delta–delta threshold cycle” method [27] and analyzed as previously described [23].

Western Blot

Proteins were extracted from dorsal and ventral hippocampi of adult male and female offspring and separated using electrophoresis as previously described [28]. Briefly, proteins were transferred to a nitrocellulose membrane and incubated with a series of primary antibodies (see Table 1) followed by appropriate horse radish peroxidase-tagged secondary antibodies. Nitrocellulose membranes were stripped of antibodies and re-exposed to a polyclonal anti- β -actin to detect the house keeping protein. Immunoreactive bands were revealed using an enhanced chemiluminescent assay (Clarity Western ECL Substrate, BioRad, USA) followed by an exposure to an X-ray film (Kodak, USA). The area under the intensity profile curve of each immunoreactive band was evaluated using ImageJ software [29] and the ratios of protein of

Table 1 List of primary and secondary antibodies

Primary antibody	Dilution	Company	2 nd antibody	Dilution	Company
Anti-COX-2 (mouse)	1:1000	Cayman Chemical	HRP-conjugated anti-rabbit	1:2000	Santa Cruz Biotechnology
Anti-synaptophysin	1:1000	Cell Signaling Technology	HRP-conjugated anti-mouse	1:2000	Santa Cruz Biotechnology
Anti-PSD95	1:1000	Cell Signaling Technology	HRP-conjugated anti-rabbit	1:2000	Santa Cruz Biotechnology
Anti- β -actin	1:5000	Cell Signaling Technology	HRP-conjugated anti-rabbit	1:2000	Santa Cruz Biotechnology

interest/ β -actin are presented as a semi-quantitative measurement of protein levels as previously described [28].

Morris Water Maze (MWM)

Spatial memory was tested in adult (PND70) male and female offspring born to dams given either saline or DEX using Morris water maze as previously described [24]. Briefly, the maze consists of a water tank with a 2-m diameter, which is divided into 4 virtual quadrants. One quadrant (target quadrant) contains a circular platform submerged underwater. The rats were trained in the water maze on five consecutive days with a total of nine sessions: one session on the first day and two sessions each day on the following days. Each session consisted of three trials. Each trial was of 120-s in duration. The time taken to reach the hidden platform (escape latency) was measured and analyzed. Twenty-four hours after the last learning session, the rats were subjected to a memory retention test. During the memory retention test, the platform was removed from the target quadrant. Rats were placed in the water maze for 30 s, and the time spent in the target platform quadrant and latency to enter the target quadrant were monitored using the EzVideoTM 5.70 Digital Video Tracking system (Accuscan Instruments, Inc., Columbus, OH, USA). In a different cohort of rats, adult male and female offspring (PND65) born to dams given either DEX or saline received daily intraperitoneal injections of a specific COX-2 inhibitor celecoxib (Cbx) (10 mg/kg, Pfizer Germany) for 5 consecutive days and subjected to Morris water maze test on the 6th day as described above. This dose of celecoxib was previously shown to exert a neuroprotective effect [30].

Statistical Analysis

Developmental change in offspring's body weight and Morris water maze data were compared using repeated measure ANOVA followed by a Student–Newman–Keuls post hoc test. All remaining data were compared using two-way ANOVA followed by a Student–Newman–Keuls post hoc test. Statistical significance is declared when the p value was less than 0.05. Power analyses were performed on statistical tests performed on each experiment's data using G-Power

software. The power analyses showed that all studies had a power of more than 80%.

Results

Impact of Prenatal DEX on Male and Female Offspring Body and Brain Weights

Repeated measure analysis of variance showed that there was a statistically significant effect of prenatal administration of DEX on body weight ($F = 3.94$, $p < 0.05$). Post hoc analysis showed the prenatal DEX significantly decreased male (Fig. 1a, Male-Saline vs. Male-DEX, $p < 0.001$) and female (Female-Saline vs. Female-DEX, $p < 0.001$) rat offspring body weight on PND5. DEX-induced reduction of body weight was short-lived as it was not apparent on either PND15, PND35, or PND70 ($p > 0.05$). Exposure to DEX during fetal period led to a significant reduction in brain weight at PND5 ($F = 10.43$, $p < 0.001$). Post hoc analysis showed that DEX reduced brain weights in both male ($p < 0.001$) and female ($p < 0.001$) rat offspring at PND5 (Fig. 1b). However, the brain/body ratio was rather enhanced by prenatal exposure to DEX ($F = 11.56$, $p < 0.001$). Post hoc analysis showed that the increase in brain/body ratio occurred in both male ($p < 0.001$) and female rat offspring ($p < 0.01$) indicating a brain-sparing effect of DEX treatment (Fig. 1c).

Impact of Prenatal DEX on *cox-2* (psts2) Gene Expression in the Hippocampus During Adulthood

Dorsal hippocampus is largely involved in spatial memory while the ventral hippocampus, along with its connection with the amygdala, is mainly involved in anxiety-related functions [25]. For this reason, we monitored *cox-2* gene expression in dorsal and ventral hippocampi separately. We observed that maternal administration of DEX did not significantly affect *cox-2* mRNA levels in either dorsal (Fig. 2a; $p > 0.05$) or ventral hippocampi (Fig. 2b; $p > 0.05$) of adult male offspring. Similar effect was seen in the ventral hippocampus of adult female offspring (Fig. 2b; $p > 0.05$). Interestingly, prenatal exposure to DEX led to a significant increase in the expression levels of *cox-2* mRNA in the

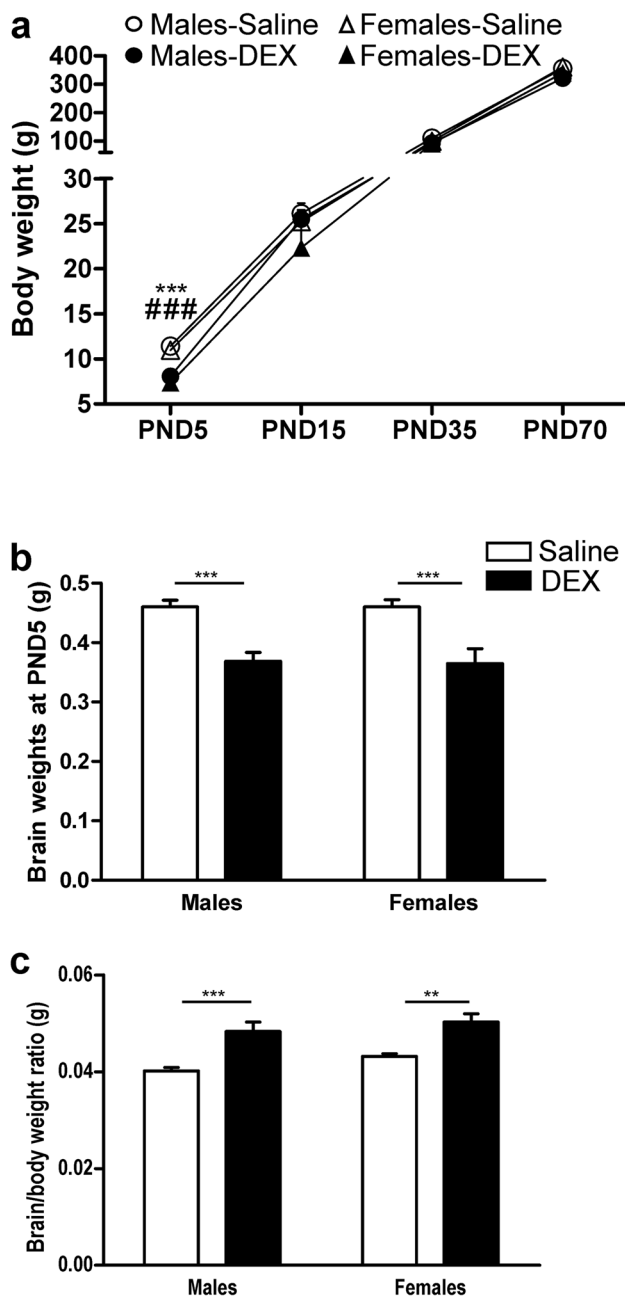


Fig. 1 Impact of maternal exposure to dexamethasone on offspring brain and body weights. Pregnant rats were given daily intraperitoneal injections of either saline or dexamethasone (DEX). **a** Body weights of male and female rat offspring were monitored from postnatal day 5 until postnatal day 70. Maternal exposure to DEX significantly reduced body weights in both male (Saline ($n=6$) vs. DEX ($n=6$); $p<0.001$) and female offspring (Saline ($n=6$) vs. DEX ($n=6$); $p<0.001$) at PND5. This effect was absent at PND15 and older. **b** Maternal exposure to DEX induced a reduction in brain weights of both male (Saline ($n=6$) vs. DEX ($n=6$); $p<0.001$) and female offspring (Saline ($n=6$) vs. DEX ($n=6$); $p<0.001$) at PND5. **c** Ratio of brain/body weights shows that DEX induced a brain-sparing effect. $**p<0.01$, $***, ###p<0.001$

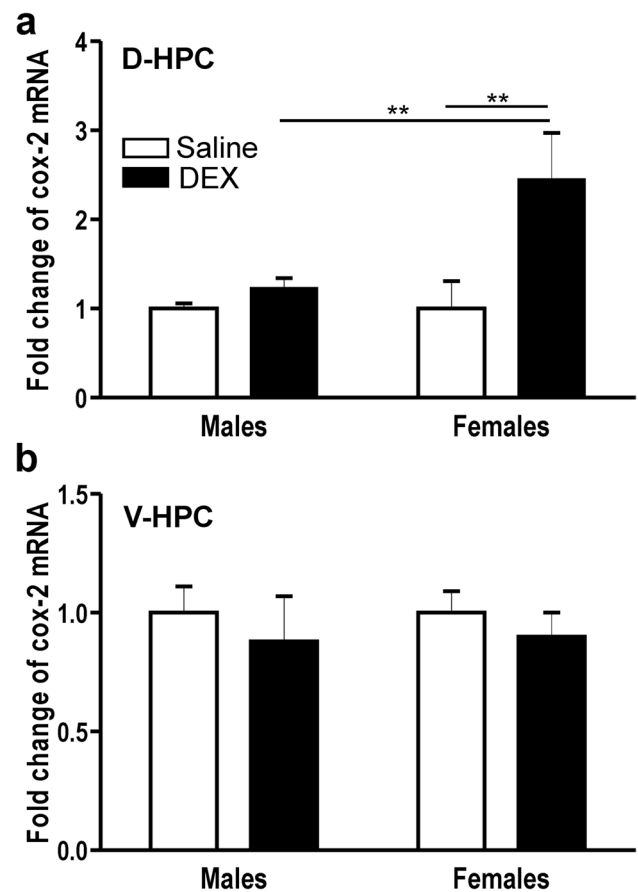


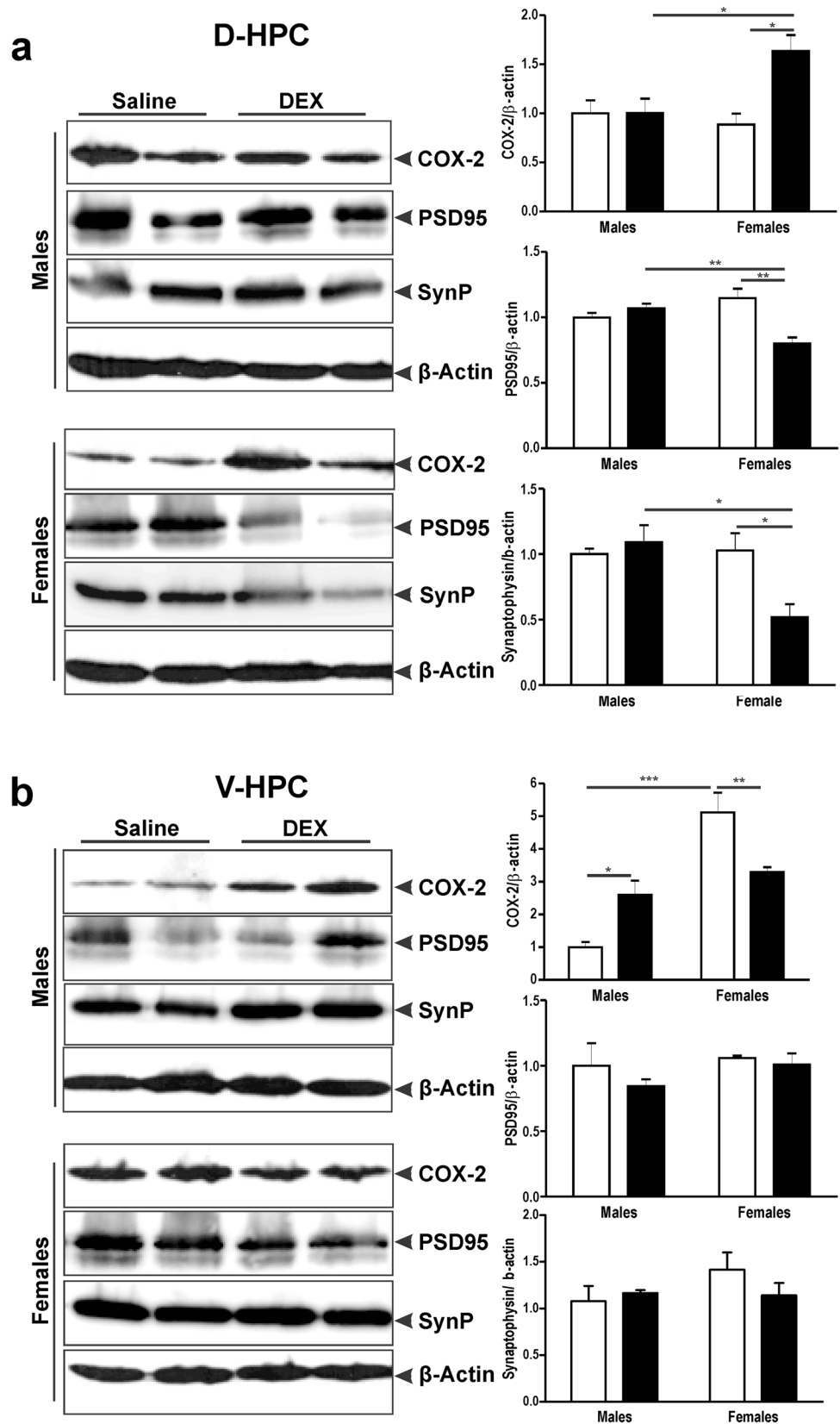
Fig. 2 Maternal DEX enhances *cox-2* mRNA in dorsal hippocampus of female rat offspring. **a** Maternal exposure to DEX led to an enhanced expression of *cox-2* mRNA in the dorsal hippocampus (D-HPC) of adult female rat offspring (Saline ($n=6$) vs. DEX ($n=5$); $p<0.01$). This effect was not seen in adult male rat offspring (Saline ($n=6$) vs. DEX ($n=6$); $p>0.05$). **b** Maternal exposure to DEX did not affect *cox-2* mRNA expression in the ventral hippocampus (V-HPC) of either male (Saline ($n=5$) vs. DEX ($n=5$); $p>0.05$) or female rat offspring (Saline ($n=5$) vs. DEX ($n=5$); $p>0.05$). $**p<0.01$

dorsal hippocampus of adult female rats (Fig. 2a; $F=5.49$, $p<0.01$).

Impact of Prenatal DEX on the Expression of COX-2 and Synaptic Proteins in the Hippocampus During Adulthood

We then explored whether prenatal exposure to DEX exerts a long-lasting impact on COX-2 protein expression in dorsal and ventral hippocampi. Similar to what have been seen at the gene expression level, prenatal exposure to DEX resulted in increased expression of COX-2 protein in the dorsal hippocampus of adult female but not male rats (Fig. 3a, top graph bar on the right; $F=6.47$, $p<0.05$). The impact of prenatal DEX on COX-2 expression in the ventral hippocampus

Fig. 3 Long-lasting impact of prenatal exposure to DEX on hippocampal expression of COX-2 and synaptic proteins. Micrographs on the left panel show representative immunoblots showing protein expression of COX-2, postsynaptic density protein 95 (PSD95), synaptophysin (SynP), and β -actin in the dorsal hippocampus (**a**, D-HPC) and the ventral hippocampus (**b**, V-HPC) of adult male (top micrograph) or female (bottom micrograph) rats born to dams given either saline or DEX during pregnancy. Semi-quantitative protein analyses are shown in the graph bars on the right panel. **a** Prenatal DEX led to a significant increase in COX-2 protein expression in D-HPC of adult female (Saline ($n=4$) vs. DEX ($n=4$); $p < 0.05$) but not male rats (Saline ($n=4$) vs. DEX ($n=4$); $p > 0.05$). Prenatal DEX resulted in decreased protein expression of PSD95 and SynP in the D-HPC of adult female (PSD95: Saline ($n=4$) vs. DEX ($n=4$); $p < 0.01$ and SynP: Saline ($n=4$) vs. DEX ($n=4$); $p < 0.05$) but not male rats (PSD95: Saline ($n=4$) vs. DEX ($n=4$); $p > 0.05$ and SynP: Saline ($n=4$) vs. DEX ($n=4$); $p > 0.05$). **b** Prenatal DEX led to a significant decrease in COX-2 protein expression in V-HPC of adult female (Saline ($n=4$) vs. DEX ($n=4$); $p < 0.01$). Prenatal DEX did not significantly affect the expression levels of either PSD95 (Saline ($n=4$) vs. DEX ($n=4$); $p > 0.05$) or SynP (Saline ($n=4$) vs. DEX ($n=4$); $p > 0.05$) in the V-HPC of adult female rats. Prenatal DEX led to an increased expression of COX-2 protein in the V-HPC of adult male rats (Saline ($n=4$) vs. DEX ($n=4$); $p < 0.05$). Prenatal DEX did not significantly affect the expression levels of either PSD95 (Saline ($n=4$) vs. DEX ($n=4$); $p > 0.05$) or SynP (Saline ($n=4$) vs. DEX ($n=4$); $p > 0.05$) in the V-HPC of adult male rats. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$



showed a peculiar pattern. Indeed, ANOVA analysis showed that prenatal exposure to DEX affected COX-2 expression during adulthood ($F = 19.72$, $p < 0.0001$). Post hoc analysis showed that prenatal DEX led to enhanced expression of COX-2 in ventral hippocampus of adult male rats ($p < 0.05$), while it decreased its expression levels in adult female rats (Fig. 3b, top graph bar on the right; $p < 0.01$). We have also noticed that basal expression of COX-2 in the ventral hippocampus was significantly higher in female rats when compared to that seen in male rats ($p < 0.001$).

Previous studies have shown that early-life experiences lead to persistent increase in the postsynaptic density protein-95 (PSD-95) and the presynaptic marker, synaptophysin (SynP) [31]. Therefore, we assessed whether maternal exposure to DEX affects the expression of these synaptic proteins in the hippocampi of male and female offspring. Maternal exposure to DEX did not have a significant effect on the expression levels of either SynP or PSD-95 in both dorsal and ventral hippocampi of adult male offspring (upper micrographs and graph bars on the right panels in Fig. 3a, b, $p > 0.05$). However, prenatal DEX led to a decreased expression of PSD-95 ($F = 6.97$, $p < 0.01$) and SynP ($F = 5.02$, $p < 0.05$) in the dorsal hippocampus of adult female rats (lower micrograph and graph bars on the right panel in Fig. 3a). This effect was not seen in the ventral hippocampus of adult female rat offspring (lower micrograph and graph bars on the right panel in Fig. 3b, $p > 0.05$).

Long-Lasting Impact of Prenatal DEX on Spatial Learning and Memory in Adult Offspring

The long-lasting impact of prenatal exposure on learning and memory was explored in adult male and female offspring. Adult rats were given daily intraperitoneal injections of either COX-2 inhibitor (Fig. 4b) or its vehicle (Fig. 4a) for a period of 5 days (from PND65 until PND69). Spatial memory testing started on PND70. The working memory data is presented in either the absence (Fig. 4a) or the presence of COX-2 inhibitor celecoxib (Fig. 4b) to allow for better visualization of DEX effect on memory acquisition and retention. In the absence of COX-2 inhibitor, female rats appear to have a slight but a significant delay in memory acquisition during training sessions (Fig. 4a, left line graph), when compared to their saline female counterpart. Repeated measure ANOVA had showed a significant effect of time ($F = 52.23$, $p < 0.0001$) and treatment (DEX vs. Saline/ $F = 5.99$, $p < 0.001$). Post hoc analysis showed that prenatal exposure to DEX reduced memory acquisition at session days 6 and 8 of adult females when compared to their female counterparts (Female-DEX vs. Female-Saline; $p < 0.05$) or to their DEX-male counterparts (Female-DEX vs. Male-DEX; $p < 0.05$). Analysis of variance of memory retention showed that prenatal DEX significantly affected

the time spent in target quadrant ($F = 7.33$, $p < 0.001$) and the entry latency ($F = 13.45$, $p < 0.0001$), 24 h after the last training session. Post hoc analysis showed male rat offspring was not significantly affected by prenatal exposure to DEX (Fig. 4a, right graph bars, $p > 0.05$). However, female rats born to DEX-dams spent significantly lesser time in target quadrant when compared to females born to saline-dams (Fig. 4a, right-top graph bars; $p < 0.05$). Furthermore, prenatal DEX led to increased entry latency to the target quadrant by female rats when compared to prenatal saline female rats ($p < 0.001$). These data indicate that maternal exposure to DEX affected spatial memory in female, but not in male adult offspring.

In the presence of a specific COX-2 inhibitor (celecoxib), we noticed that memory acquisition in adult Female-DEX rats was not significantly different from that of adult Female-Saline rats (Fig. 4b, line graph on the left). Indeed, female rat offspring spent similar time in target quadrant and has similar entry latency to target quadrant regardless of prenatal treatment (Fig. 4b, bar graphs on the right; $p > 0.05$), indicating that specific inhibition of COX-2 during adulthood alleviated DEX-induced learning and memory deficits in female rat offspring. In contrast, adult male rats born to DEX-dams and given COX-2 inhibitor during adulthood showed a significant increase in the entry latency to target quadrant when compared to those born to saline-dams and given COX-2 inhibitor during adulthood ($p < 0.05$). These data indicate that DEX-induced learning deficit in male offspring was apparent only when COX-2 was inhibited during adulthood.

Discussion

Prenatal activation of glucocorticoid receptors in key areas of the developing brain has long-lasting negative effects on cognitive functions such as learning and memory. Here we provide experimental evidence to show that administration of DEX during pregnancy induces impaired learning and memory in female, but not male, adult offspring. This altered cognitive function is likely due, at least in part, to an enhanced expression and activity of COX-2 in the hippocampus. This conclusion is supported by the following experimental evidences: (1) administration of DEX to pregnant rats led to enhanced expression levels of COX-2 gene and protein in the dorsal hippocampus of female, but not male, adult offspring; (2) this enhanced expression of COX-2 was associated with deficits in learning and retention of memory in female rat offspring; (3) administration of COX-2-specific inhibitor alleviated the memory impairment induced by prenatal DEX in female rat offspring. This sex-dependent effect of maternal DEX was also manifested by reduced expression of key pre- and postsynaptic proteins [31] in the dorsal hippocampus of female but not male adult offspring. To the

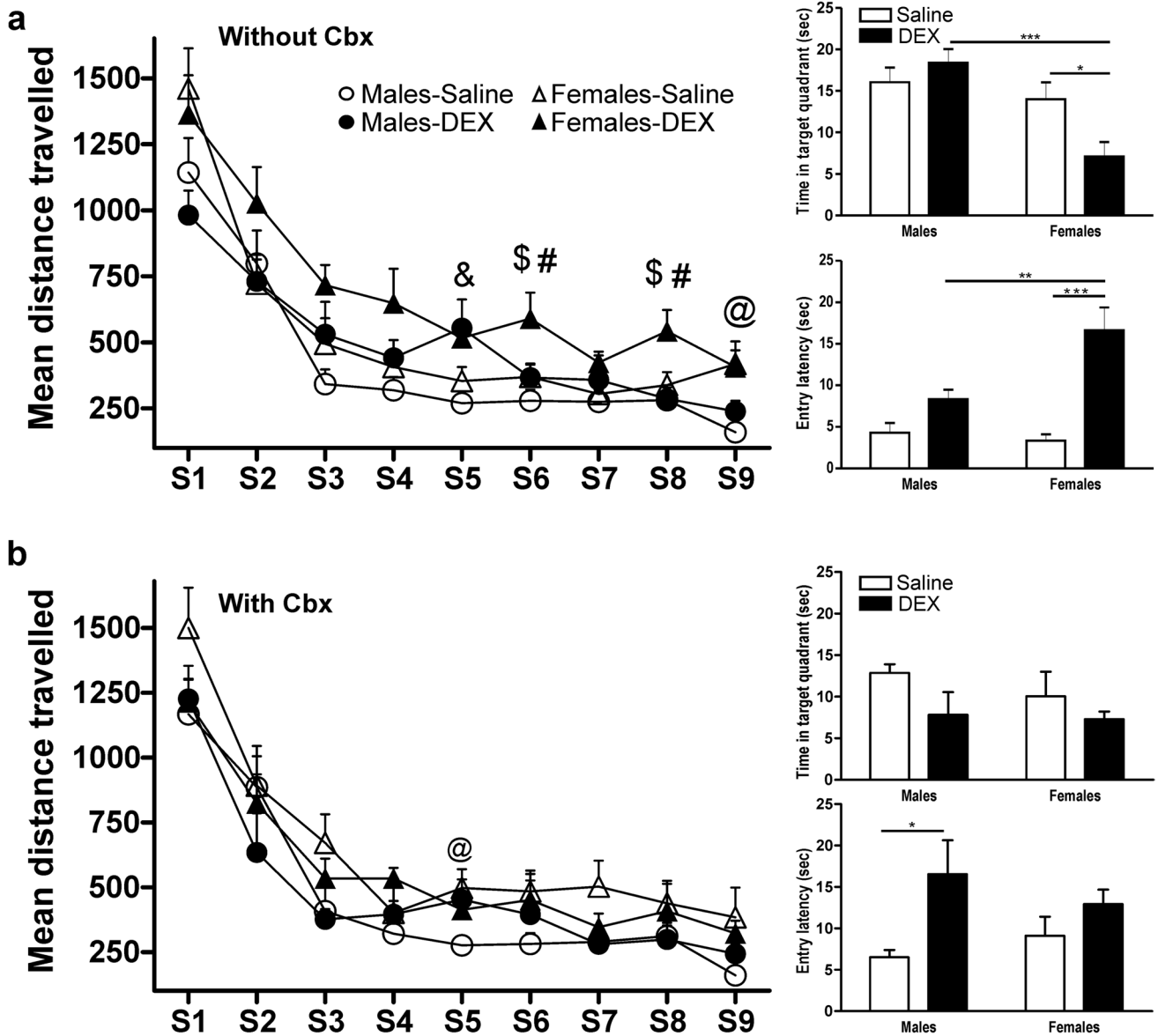


Fig. 4 Role of DEX-induced COX-2 in memory acquisition and retention. Line graphs in **a** and **b** show the mean distance travelled by adult male and female rats in 9 training sessions in Morris water maze in the absence (**a**) or the presence (**b**) of a selective COX-2 inhibitor (celecoxib: Cbx). **a** In the absence of COX-2 selective inhibitor, prenatal DEX resulted in relatively increased distance travelled by adult female offspring at sessions 6 and 8 (Saline ($n=9$) vs. DEX ($n=9$); $p < 0.05$). Prenatal DEX led to a slight but significant increase in the distance travelled by male offspring at session 5 (Saline ($n=9$) vs. DEX ($n=9$); $*p < 0.05$). There was a significant difference between Male-DEX and Female-DEX at sessions 6 and 8 ($^{\$}p < 0.05$); Male-Saline and Male-DEX at session 5 (($n=9$) vs. DEX ($n=9$); $^{\&}p < 0.05$); and Male-Saline and Female-Saline at session 9 ($^{\textcircled{a}}p < 0.05$). Bar graphs in the right panel illustrate the memory retention 1 day after the training session 9. The entry latency (Saline ($n=9$) vs. DEX ($n=9$); $p > 0.05$) and the time spent in target quadrant

(Saline ($n=9$) vs. DEX ($n=9$); $p > 0.05$) were not affected by prenatal DEX in adult male offspring. However, prenatal DEX led to a significant reduction in the time spent in target quadrant (Saline ($n=9$) vs. DEX ($n=9$); $p < 0.05$) and increased latency to enter the target quadrant (Saline ($n=9$) vs. DEX ($n=9$); $p < 0.001$) in adult female rats. **b** In the presence of selective COX-2 inhibitor, prenatal DEX did not affect the distance travelled by either adult male or female rat offspring during the 9 training sessions. Neither the time spent in target quadrant nor the latency to enter target quadrant (Saline ($n=10$) vs. DEX ($n=9$); $p > 0.05$) were affected by prenatal DEX in adult female rats. Prenatal DEX increased the entry latency of adult male rats to target quadrant (Saline ($n=9$) vs. DEX ($n=9$); $p < 0.05$), but it did not significantly affect the time they spent in target quadrant (Saline ($n=9$) vs. DEX ($n=9$); $p > 0.05$). $^{\textcircled{a}}$ Male-Saline vs. Female-Saline; $^{\#}$ Female-Saline vs. Female-DEX; $^{\&}$ Male-Saline vs. Male-DEX; $^{\$}$ Male-DEX vs. Female-DEX

best of our knowledge, this is the first report to document the importance of COX-2 in the long-lasting impact of prenatal exposure to DEX on learning and memory.

Maternal exposure to DEX led to reduced body and brain weights of both male and female offspring at PND5. However, maternal DEX led to enhanced brain/body ratios suggesting a brain-sparing effect of DEX. These data are in line with the intrauterine growth restriction effect of maternal DEX seen in this and other laboratories [23, 32–34]. DEX-induced body weight reduction was short lived as the DEX-induced reduction in body weights were not seen during either juvenile period or adulthood. Similar transient effect of DEX on body weights was also seen in rats [34], mice [5], and humans [35].

In the present paper, we have shown that prenatal activation of glucocorticoid receptors led to an enhanced expression of COX-2 in the dorsal but not the ventral hippocampus of adult female rats. Because dorsal hippocampus is preferentially involved in spatial memory while the ventral hippocampus contributes to anxiety-like behavior [25], it appears that DEX-induced COX-2 expression plays a role in spatial memory but not in anxiety-like behavior.

We show that the level of this basally expressed hippocampal COX-2 is enhanced by prenatal exposure to DEX, namely in female rat offspring. If COX-2 expression is involved in learning and memory, one would expect an enhanced memory acquisition and retention in female rats born to DEX-exposed dams. This was not the case. In fact, female-DEX rats performed worse than their male (Male-DEX) and female (Female-Saline) counterparts in learning and memory retention. This deleterious effect of prenatal exposure to DEX was halted when female rat offspring received COX-2 inhibitor. To the best of our knowledge, this is the first report to document the potential role of prenatal DEX-induced COX-2 in memory deficit in females.

These data are in apparent discrepancy with previous observation where systemic administration of selective COX-2 inhibitor disrupted spatial memory retention in female mice [36] or intrahippocampal injection of celecoxib rats [37]. It is noteworthy that these experiments were performed in “naive” animals while in our study, we used rats prenatally exposed to DEX. This prenatal DEX might have changed the developmental trajectory of the hippocampus including an induction of a sustained increase in COX-2 expression. Furthermore, transgenic mice with sustained overexpression of COX-2 also showed spatial memory deficit [16]. Similarly, lentivirus-induced overexpression of COX-2 worsens rats’ learning performance which was alleviated by specific inhibition of COX-2 [38].

Prenatal exposure to DEX did not affect the expression of COX-2 in male dorsal hippocampus, nor did it result in spatial memory deficit in male rats as has been seen by others [39]. However, we observed a slight but a significant

reduction in entry latency to target quadrant in DEX prenatally exposed males which were given COX-2 inhibitor during adulthood. This observation suggests that the long-lasting impact of DEX on memory retention in males requires the contribution of COX-2. The mechanism underlying this sex-dependent effect of prenatal exposure to DEX is not clear yet. It is plausible that fetal and neonatal increase in estradiol levels in male brains dampened DEX-induced programming effects on COX-2 through estradiol-induced methylation of *cox-2* gene [40]. Alternatively, male androgens may negatively affect the transcriptional effects of glucocorticoid receptors [41].

There was a discrepancy between the expression levels of COX-2 protein and *cox-2* mRNA. There are several mechanisms that could underlie the mismatch between protein and mRNA expressions, chiefly among which is the difference in regulatory processes that govern mRNA transcription and degradation and protein degradation and accumulation [42, 43]. It is noteworthy that 3'-untranslated region of *cox-2* mRNA promotes mRNA instability and degradation [44, 45]. Such *cox-2* mRNA instability and degradation might contribute to differential expression of *cox-2* mRNA and COX-2 protein.

Prenatal DEX did not only enhance COX-2 expression, but it also reduced the expression of key synaptic proteins in the dorsal hippocampus in female rats during adulthood. This effect was not seen in adult male rats. Owing to the role of synaptic proteins in memory formation and retention [46], it is possible that altered expression of synaptic proteins contributes to the memory deficit seen in female rats. It is striking that the lack of DEX effect in male offspring was associated with unaltered expression of either COX-2 or synaptic proteins. This observation strengthens the conclusion that the enhanced expression of COX-2 and reduced expression of synaptic proteins contribute, at least in part, to the memory deficit in female offspring.

The sex-specific and hippocampal area-specific effects of prenatal DEX have not been extensively explored. Recent studies have shown that dorsal and ventral areas of the hippocampus differ in their transcriptional properties [47–49]. Furthermore, experimental evidence showed that the expression levels of glucocorticoid receptors (GRs) are much higher in the dorsal hippocampus of non-stressed female than that of male rodents [50, 51]. It is plausible that the selective programming effects of DEX on female dorsal hippocampus are facilitated by the differential expression levels of GRs.

Owing to its anti-inflammatory properties, DEX effect on COX-2 expression was largely explored under inflammatory condition. Indeed, DEX blunts inflammation-induced COX-2 expression by either suppressing the function of transcription factors such as nuclear factor (NF)-IL-6, nuclear factor kappa B (NF- κ B), cAMP

response element, the activating protein-1 (AP1) [52, 53], or by destabilizing *cox-2* mRNA through an inhibition of the mitogen-activated protein kinase p38 [54]. In the present study, DEX was administered in the absence of inflammation during prenatal period. The mechanism underlying the long-lasting impact of DEX on basal COX-2 expression is not well-understood. It might involve epigenetic alteration of *cox-2* gene [55] in a sex- and brain area-dependent manner. It is noteworthy that similar enhanced basal COX-2 expression was observed in several brain areas of adult rats subjected to early-life adverse events that activate glucocorticoid receptors [17–19]. Further studies are required to delineate the underlying mechanism of this consistently observed alteration in basal COX-2 expression in response to early-life challenges.

Conclusion

Prenatal exposure to DEX has been associated with a myriad of cognitive and behavioral alterations. The mechanism underlying these long-lasting effects has not been extensively explored. Maternal exposure to DEX induces a lasting enhanced expression of COX-2 gene and protein in the dorsal hippocampus of female but not male rat offspring. This altered COX-2 expression was associated with spatial memory deficit. Selective inhibition of COX-2 reverted DEX-induced memory deficit in female offspring. These data pave the way for investigating the therapeutic use of COX-2 selective inhibitor to alleviate the deleterious effects of corticosteroid medication during pregnancy on offspring's cognitive functions.

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Author Contribution A.M. conceived, designed, and supervised the research project. M.D.A. supervised the work related to the RT-PCR. M.A. conducted the experiments. A.M., M.A., and M.D.A. analyzed the data. A.M and M.A co-wrote the manuscript. All authors have approved the final version of this manuscript.

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Data Availability All data related to this paper is presented in the “Results” section.

Declarations

Ethics Approval All experiments were performed in accordance with the guidelines of the humane handling of experimental animals as established by the Kuwait University Health Sciences Center, Animal Research Ethics Committee.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Conflict of Interest The authors declare no competing interests.

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