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Original Research

Prenatal exposure of citalopram elicits depression-like and anxiety-like behaviors and alteration of morphology and protein expression of medial prefrontal cortex in young adult mice

Aqeela Zahra[1,†], Le Du[1,†], Meng Jia[2,3,4], Muhammad Umar Butt[5], Qun Wang[2,4], Yunfu Wang[6], Jianping Wu[1,2,3,4,*]

[1]School of Chemistry, Chemical Engineering and Life Sciences, Wuhan University of Technology, 430070 Wuhan, Hubei, China
[2]Beijing Tiantan Hospital, Capital Medical University, 100070 Beijing, China
[3]Advanced Innovation Center for Human Brain Protection, Capital Medical University, 100070 Beijing, China
[4]National Clinical Research Center for Neurological Disease, 100070 Beijing, China
[5]School of Medicine, Jianghan University, 430070 Wuhan, Hubei, China
[6]Taihe Hospital, Hubei University of Medicine, 442000 Shiyan, Hubei, China
*Correspondence: biojpwu@gmail.com (Jianping Wu)
[†]These authors contributed equally.

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Abstract

Background: Treatment of major depression disorder with Selective serotonin reuptake inhibitors (SSRIs), such as citalopram (CTM), during pregnancy effects on the neurological trajectory of the offspring and induces enduring consequences, notably emotional and cognitive impairment. The associations between prenatal exposure to SSRIs and neurological underpinnings of these atypical behaviors in offspring are contentious and poorly understood. **Methods**: We examined modifications in physiological, morphological, and biochemical characteristics in male and female offspring of C57BL/6 exposed to CTM during the third trimester of gestation. We utilized different behavior procedures to observe depression and anxiety-like behavior in 1~2 month old CTM-exposed mouse offspring. We employed Golgi-Cox staining to examine the neuronal structure of medial prefrontal cortex (mPFC) in CTM-exposed mice following protein expression levels by utilizing biochemical techniques. **Results**: Our results indicate an impaired behavior such as anxiety and altered locomotion along with the substantial reduction in dendritic length and the number of dendritic branches in CTM-exposed mice. We observed differentially increase c-Fos expression in the mPFC following altered protein expression levels relative to controls. **Conclusions**: Our finding supports the function of CTM as a prenatal modulator of susceptibility to depressive-like behavior in offspring. We indicate that prenatal CTM exposure elicits a negative impact on the central nervous system, especially those regions involved in cognition and drug reinforcement. Furthermore, genetic, chemo-genetic, and optogenetic methods should be used to explain the function of SSRIs such as CTM during pregnancy in the regulation of mood and emotion-related behaviors in children.

Keywords: depression; selective serotonin reuptake inhibitors; citalopram; medial prefrontal cortex

1. Introduction

Depression influences almost 300 million people and is a major source of disability worldwide (World Health Organization) [1,2]. Numerous investigations have revealed neuroendocrine, neurochemical, and neuroanatomical alterations in severe depressive individuals. Many of these alterations influence neural networks known to modulate stress and emotion [3]. Early life events affect the risk of neurodevelopmental disorders, notably during susceptible periods of brain development [4,5]. Epidemiologic studies have established the gestational period as a target factor for the development and persistence of mood disorders such as depression in early life [6,7].

Depression and the use of SSRIs during gestation are both prevalent and entail neurobiological problems. Prenatal SSRI exposure is related to an increased risk of premature birth, low birth weight, congenital abnormality, and persistent pulmonary hypertension in infants [8]. Several studies reported that prenatal exposure to SSRIs continues to influence the cognitive and social development of the child [9,10]. It has been linked to an increased risk of intellectual impairment, autism spectrum disorder (ASD) [11,12], and impaired motor and cognitive abilities [13,14]. The in-utero exposure to SSRIs during early infancy has been associated to adverse neurodevelopmental outcomes [15]. SSRI exposure during pregnancy is hypothesized to raise levels of 5-HT in the fetal brain, however over the period, negative feedback may inhibit the growth of the 5-HT circuitry, resulting in lowered serotonergic activity and the levels of 5-HT in the nervous system during development [16]. Intriguingly, prenatal alterations in 5-HT levels have also been associated with an increased risk of psychiatric conditions in young adults [17,18].

Researchers also determined that exposure to early-life SSRIs results in behavioral problems in both humans and animals [19,20]. Significant growth of the nervous system is associated to fetal developing period therefore SSRIs experiences during this time induce a number of neurocognitive, neurochemical, and immunological abnormalities later in life [21]. Parental SSRI use during pregnancy may disrupt microstructure and brain development in the fetus, as shown by magnetic resonance imaging [22].

On the other hand, long-term implications include recurrent modifications to intracellular signaling pathways that facilitates receptor and channel transport, genetic mutations, and neuronal outgrowth. PFC contains a diverse array of glutamatergic and GABA (Gamma-aminobutyric acid) interneurons that comprise excitation–inhibition (E:I) equilibrium, as many studies suggest this brain region in the etiology of the depressive condition [23,24].

Numerous researches will give us insight into the underlying neurological issues associated withdepression. However, the detailed mechanisms underlying SSRIs like CTM-induced depression in mPFC are not fully understood. Thus, the PFC may be a critical area for elucidating the underlying pathophysiology of depression as well as the mechanism of action of antidepressants. It is also possible that early experience of SSRIs causes additional molecular or cellular changes [25,26] that are not yet observed, but contribute more specifically to the occurrence of depressive episodes.

In terms of development, adolescence is a crucial period for human and rodent neurological development. Thus, it is necessary to understand the underlying processes of adolescent depression if we are to enhance therapy and begin prevention [27]. As adolescence is a time of fluctuating positive and negative emotional experiences, it is a vulnerable time for depression. Although SSRIs have been used for years, the underlying mechanism in the brain remains unknown. The purpose of this study is to explain the structural and molecular alterations induced in the pyramidal neurons of layer II/III of the mPFC during the third trimester of gestation by prenatal CTM administration, as well as to investigate the transition among SSRI-induced toxicity during adolescence.

2. Materials and methods

2.1 Research animals and procedure

Two groups of pregnant female mice (C57/BL6) were used to study consequences of prenatal exposure to CTM. Females were placed separately to raise their own litters; and were prepared for the neurodevelopment and behavioral studies. There were 29 litters in the study: 14 treated with saline and 15 treated with CTM, at least 6–7 pups (3–4 per sex) each litter to be required. Because we had no preconceived idea which sex could be more susceptible, we conducted tests on both male and female offspring [28]. All the process of experimentation and animal housing was performed in accordance with the authorized protocols of Wuhan university of technology's Ethics Committee as well as the National Institute of Health's Guidelines for Animal Care.

Experimental design

All pregnant female mice (C57/BL6) individually housed in normal laboratory conditions: 12/12 h reversed light/dark period, 22 $\pm$ 1 °C, provided ad libitum food plus water and the cages were changed two times weekly. The first vaginal plug was observed on gestational day zero (G0), which was confirmed by the pregnant dams' normal weight gains. All pregnant mice were given intraperitoneal injection at dose of 20 mg/kg citalopram (CTM) (2 mg/mL) consistent with other studies [29,30] diluted with saline and Control (Ctrl) group were injected with physiological saline simultaneously in last trimester of gestation as shown in Fig. 1. The administration pathway in this investiga-tion corresponded with the previous studies [31]. During the experiment, 30 pregnant female mice were utilized, we required at least three male and three female pups from each litter to be enrolled in this study.

2.2 Behavior examination

All behavior experiments were conducted in a hygienic practical area. Mice were taken 1 h prior to the behavior room to get habituated with the environment. Mice in fixed sequence, i.e., similar litter sets were utilized for all behavior testing. Mice under went three days of rest between tests in order to reduce carryover effects from previous testing. The investigator was naïve to CTM treatment and all behavior analyses were carried out on the offspring.

2.2.1 Behavioral assessment of anxiety

The EPM (Elevated plus maze) is frequently utilized to evaluate anxiety-like activity in animal models [32]. The EPM consists of four elevated arms which radiate from a central platform, forming a plus shape. A plus maze with 30 cm long, 5 cm width, and 15 cm high arms was 50 cm directly above the floor and used to calculate the time spent open against closed arm (in seconds) and the number of crossings between the closed and open arms was recorded when whole body of animal entered in each arm (ZhengHua instruments, Anhui, China). Place the test animal in the intersection of the open and closed arms of the EPM so that the mouse is facing an open arm. Each mouse has been reviewed separately for 10 minutes [33]. After each mouse examination, the whole apparatus was washed with 75% ethanol.

2.2.2 Behavioral assessment of depression-like activity

The force swimming test (FST) is used to examine the depression-like behavior in both mice and rats [34]. The FST is regarded as a reliable test for interpreting, analyzing, and determining sensitivity to negative mood states such as

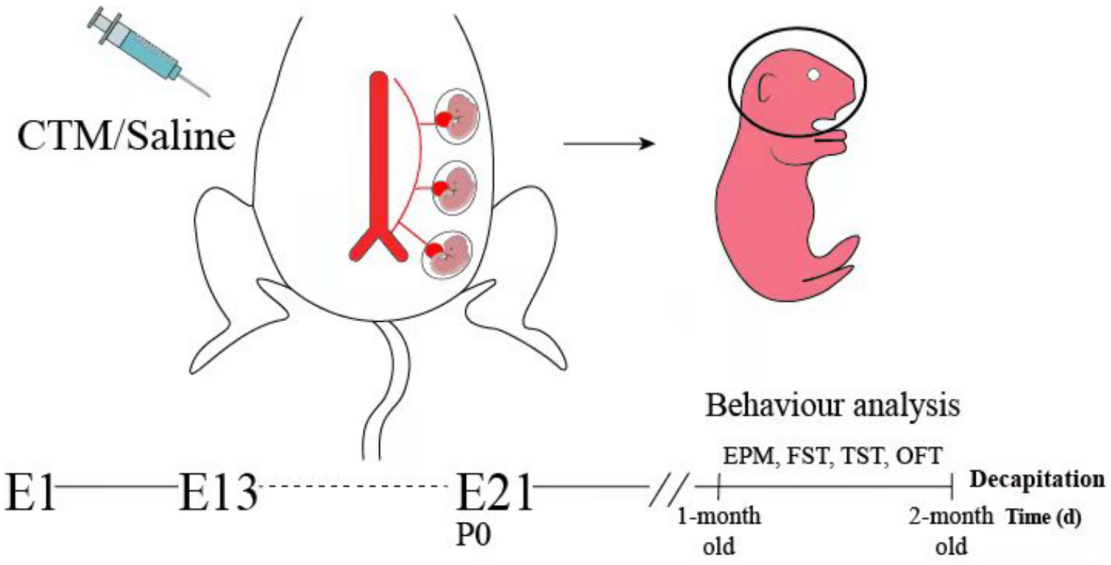

Fig. 1. Illustration of experimental design. Two groups of females C57BL/6 during last trimester embryonic day (E) 13 to 21 was intraperi-tonially injected with citalopram (CTM) and saline simultaneously. P0 is selected as day of birth. After three weeks weaning was done and offspring were subjected as 4–6 mice per cage. At the age of 1~2-month, mice were followed by behaviors analysis including elevated plus maze (EPM) test, force swimming test (FST), tail suspension test (TST) and open field test (OFT). After behavior experiments mice were decapitated for further biochemical analysis.

depression. As a result, it acts as a valid method for evaluating the antidepressant's effectiveness [14]. Mice were positioned separately in a Plexiglas cylinder (30 cm diameter) full of water (24 $\pm$ 1 °C) and behavior was video-recorded for 6 minutes (ZhengHua instruments, China). FST behaviors were scored, when mice are acting just to hold the head above the water. The animal was dried with a towel and the water was changed after each swimming test.

2.2.3 Behavioral assessment of depression-like activity

The tail suspension test (TST) is employed to evaluate immobility in mice [35]. It is used to evaluate possible antidepressant medications and other interventions that may impact depression-related behaviors. Mice were dangling by the tail with tape to a perpendicular aluminum bar. A 6-minute test session is recorded and scored by observer (ZhengHua instruments, China). As the session continued, mice displayed a variety of escape-oriented movements interspersed with periods of immobility. The period of "immobility", known as the time when mice were determined to have stopped escaping-motivated actions, was used to score behavior experiment.

2.2.4 Behavioral assessment of locomotors activity

The OFT is used to assess the locomotors activity in mice [36]. Locomotor changes may be an indicator of modified neurological processes and, as a result of aberrant brain functions. This apparatus consists of square Plexiglas boxes (45 cm long by 45 cm wide with 45 cm high walls) illumined at power of 800 Lux (ZhengHua instruments, China). Spray the box with 75% ethanol and wipe it off with a paper towel before testing. In a quiet room, a single mouse was sited individually in the midpoint of the field for 10 min of exploration, (i) the total distance travelled, and (ii) the number of entries into the central region of the field was recorded by a video-computerized tracking. After each mouse examination, the open-field box was wiped with 75% ethanol.

2.3 Immunohistochemistry

The mice were transcardially perfused with 4% PFA under anesthesia following behavioral tests. After 24 hours, brains were extracted and fixed in 30% sucrose in 0.01 M phosphate-buffered saline (PBS) around 4 °C for 3 days After that, the brains were sectioned into 30 μm slices using a freezing microtome adjusted to –22 °C. To stain the mPFC tissue sections, they were washed three times (5 minutes each) in 0.01 M PBS and blocked for 1 hour at room temperature with 5% BSA in 0.3 percent Triton X-100 (in PBS; PBS-T). Following that, sections were treated overnight at 4 °C with rabbit polyclonal anti-c-Fos or rabbit polyclonal anti-parvalbumin primary antibodies (Abcam, Cambridge, UK). Then, the slices were bathed three times, 5 minutes individually, with a 0.01 M PBS and incubated at room temperature of 0.3 percent PBST at 1 hour, with goats-anti-rabbit-packed secondary antibodies (Life Technologies, Waltham, USA) in 1% BSA. Since mounting the stained sections on glass slides and covering them with a custom-ized anti-fade solution, photographs were taken using a fluorescence microscope Eclipse Ni-U (LUKAS-

Microscope service-Nikon, Japan). Positive cell counting was conducted on individual sections via Image-Pro Plus 6.0 (Media Cybernetics, USA) and Image J (Java2HTML Version 1.5, National Institutes of Health, Bethesda, MD, USA).

2.4 Histological measures by Golgi staining

After the behavioral evaluations and c-Fos stainning, 2-month-old animals were perfused transcardially via 0.9% saline combined with 4% PFA. Their brains were separated and stained with improved Golgi stain solution for 14 days (change solution alternatively after 48 h to remove sediments) at room temperature (RT) in dark. Once the sedimentation exclusion period was complete, the brains were put in protecting solution for 7–8 days at 4 °C in dark [37]. The brains were sliced into 200 m pieces using a microtome (Leica, USA), and were placed on gelatin incrusted slides. This technique written by Gibb and Kolb is used to stain all brains in the final process [38]. According to previously available measures (i) total dendritic length of each neuron, (ii) number of branches of each neuron, (iii) comprehensive staining of basal and apical dendritic arbors (iv) no extensive dendrites overlapping of neighboring neurons is followed. PFCs were quickly identified for study by using Golgi-Cox staining methods. The distinctive soma form, pronounced dendrite length and branches of pyramidal neurons have been determined. Neuron morphology both basal and apical is assessed by Sholls analysis (dendritic length and branches along with intersection of neurons with concentrical circles set apart at a distance of 20 μm) and dendrite density depend on the number of branch joints.

2.5 Western blotting

As previously mentioned, mice were quickly sacrificed and the PFCs were mounted on ice [39]. Tissue section of PFC were homogenized in SDS buffer (50 mM Tris pH 7.5, 150 mM NaCl, and 5mM EDTA pH 8.0, 1% SDS). Cellular debris was detached at 4 °C, 14000 rpm in 10 min by centrifugation and supernatant was composed for exami-nation. The lysates of tissues were exposed to SDS-PAGE, shifted to nitrocellulose membranes. This membrane was blocked with 5% non-fat dry milk and then incubated with specific primary antibodies against NMDAR1 (Abcam, ab109182; diluted 1:5000 at use), CaMKII (Abcam, ab52476; diluted 1:5000 at use), and GABAb1 (Abcam, ab52476; diluted 1:5000 at use) (Neurolab, diluted 1: 3000). Anti-Tubulin antibody (Sigma, T6557; diluted 1:10000) was used as a positive control. Prior to inserting second antibody, membrane was washed by TBST for 2–3 times. Prepare second antibody (CWS) in 5 % non-fat dry milk by TBST and place membrane at RT for 1 h. Followed by few washes of TBST immobilon western system ECL was used to analyze bands.

2.6 Statistical analysis

Origin 9.0 software (OriginLab, Northampton, USA) was applied for creating graphs. Statistical studies were achieved with Prism6 (GraphPad Software). ImageJ (Java2HTML Version 1.5) was applied for generating morphological photomicrographs in Golgi staining technique. Statistical analyses were achieved by unpaired two-sample *t*-test, $*p < 0.05$, and $**p < 0.01$ was measured as statistically significant.

3. Results

3.1 CTM mice exhibit behavioral alternation in adolescence

The prenatal CTM exposure on behavior alteration in 1~2-month-old mice is shown in Fig. 2. The traces for anxiety-like behavior of the 1~2-month-old mice in the EPM is shown in Fig. 2A. CTM treated mice significantly increased the preference to stay in the closed arms, relative to control group, and this was significant at an amount of 20 mg/kg, indicated by the fraction of the time spent in the close arms versus the time spent in the open arms shown in Fig. 2B,C. Our data revealed that CTM mice spent a substantially less time in the open arms relative to control mice, $p = 0.0011$, unpaired two-sample *t*-test. Similarly, there is an increased time spent in the closed arms by CTM mice $p = 0.0337$, unpaired two-sample *t*-test) as comparison to control group shows significantly increased anxiety-like behavior. Similarly, the prenatal CTM exposure on depression like activity of the 1~2-month-old mice in the FST and TST is shown in Fig. 2D,E. It displays that mobility and swimming time was significantly decreased in CTM treated mice verses control mice. Results revealed significantly increase immobility time of CTM mice showing increased depressive like behavior as compared to controls (D and E: $p = 0.024$ and $p = 0.033$, unpaired two-sample *t*-test). The track paths of prenatal CTM and control mice of the 1~2-month-old mice in the OFT is shown in Fig. 2F. Our results revealed that CTM mice showed a significant decreased locomotors activity as well as less exploratory behavior compared with control mice (G and H: $p = 0.021$ and $p = 0.043$, unpaired two-sample *t*-test).

3.2 CTM mice exhibit amplified activation of c-Fos protein expression in PFC

We observed c-Fos activation in mPFC of control and CTM-treated mice as presented in Fig. 3A. Result shows substantial effect of CTM treatment as compared to controls on mPFC neurons. However, prenatal CTM treatment was found differentially increase c-Fos expression in the mPFC of mice during adolescence. c-Fos has been applied extensively as a measure of neuronal activation [40]. c-Fos staining is conducted to assess in what way behavioral modifications affect the activity of the neurons and interestingly, c-Fos-positive cells have been substantially increased. CTM mice, relative to controls, (B: $p = 0.0062$, unpaired two-

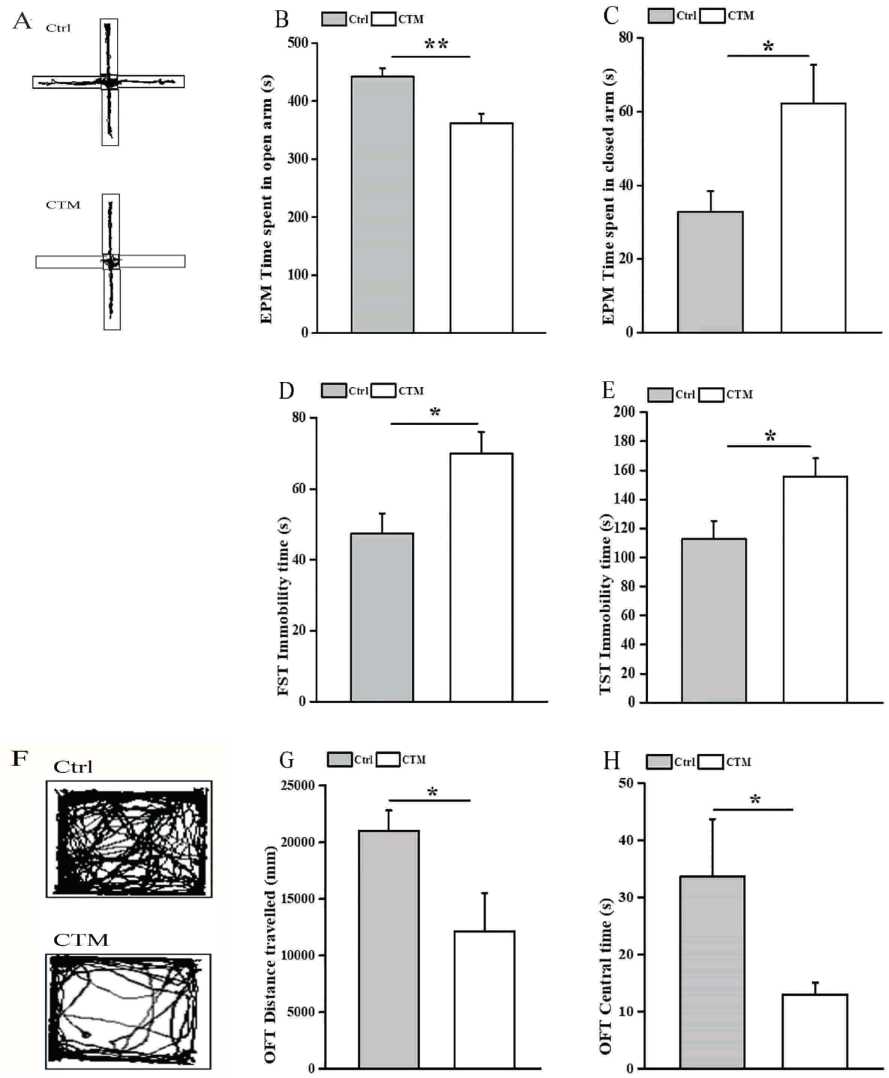

Fig. 2. CTM mice exhibit behavioral alternation in adolescence. Represents the impact of anxiety-like behavior by prenatal CTM of 1~2 month-old mice in EPM. (A) Shows route paths of control (n = 12) and CTM mice (n = 12). (B) Entry of mice into the EPM open arm and (C) entrance of mice in the closed arms of EPM test (432.80 ± 15.71 vs 263.55 ± 42.44, $p = 0.001$) (38.2 ± 4.60 vs 65.275 ± 11.03, $p = 0.033$) unpaired two-sample *t*-test. (D) Represents the impact of prenatal CTM exposure on immobility approach in 1~2 month CTM (n = 12) and control (n = 12) mice in FST (69.89 ± 6.22 vs 50.78 ± 4.93, $p = 0.024$) unpaired two-sample *t*-test. (E) Represents the impact of prenatal CTM immobilization exposure in 1~2 month-old CTM (n = 12) and control (n = 12) mice in TST (112.98 ± 12.43 vs 170.12 ± 21.93, $p = 0.033$) unpaired two-sample *t*-test. (F) Displays track routes of control (n = 12) and CTM (n = 12) mice in OFT. This represents the impact of prenatal CTM exposure on locomotory and exploratory activity of 1~2 month-old mice in OFT. (G,H) Shows distance traveled by control and CTM mice and central time of control and CTM mice (22916.50 ± 856.21 vs 18455.9 ± 1580.92, $p = 0.021$) and (34.59 ± 9.86 vs 12.96 ± 2.16, $p = 0.043$) unpaired two-sample *t*-test. Values represent mean ± SEM. $*p < 0.05$; $**p < 0.01$.

sample *t*-test) suggests that the fetal CTM-exposed animals have elevated neuronal activation in mPFC.

3.3 CTM mice exhibit altered PFC morphology

For Golgi-cox staining, together 12 cells from each animal were taken from structure for a final number of 36 cells per genotype. The camera lucida picture from pyramidal layer II/III of PFC in 2-month-old CTM and control mice is shown in Fig. 4A,B.

CTM treated mice shows reduction in dendritic length and sum of branches Fig. 5B,C. Our results revealed significantly decreased in dendritic length and branches of CTM mice as compared to controls (B and C: $p = 0.0191$ and $p = 0.0074$, unpaired two-sample *t*-test). Quantity of intersections in Fig. 5A (as the distance between the dendrites and the cell body of PFC neurons) demonstrate that CTM mice showed decreased number of intersections from 100–140 and 260–300 μm as compared to controls concerning

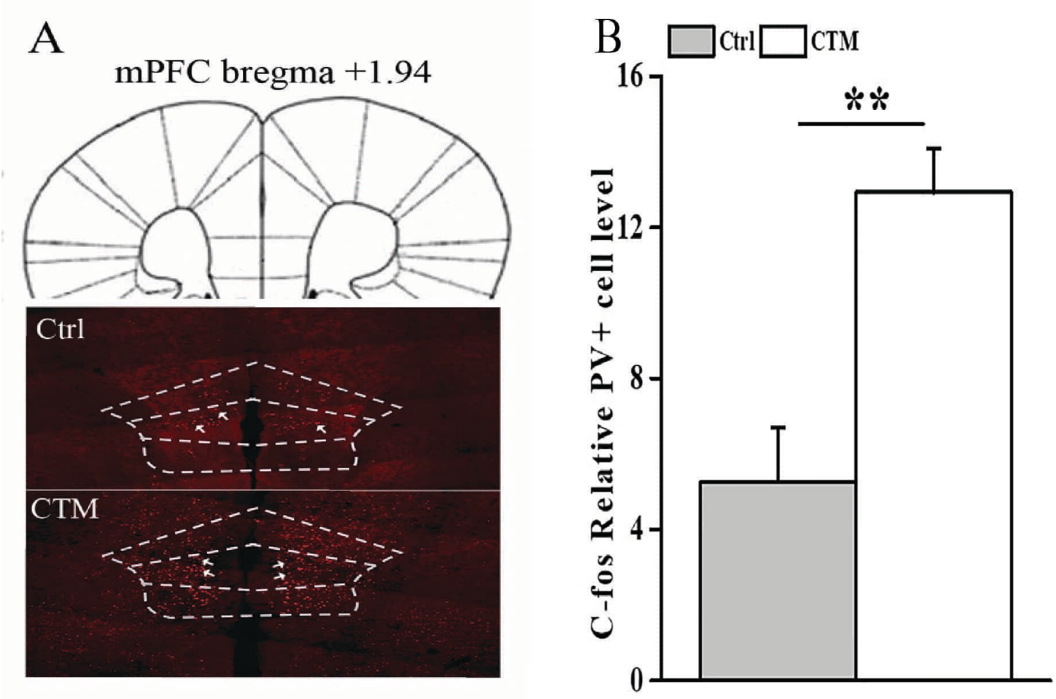

Fig. 3. CTM mice exhibit increased activation of c-Fos protein expression in PFC. Represents c-Fos activation in mPFC of control and CTM-treated mice. (A) Prenatal CTM treatment was found to differentially increase c-Fos protein expression in the mPFC of mice. (B) Shows significantly increased c-Fos activation in CTM mice as compared to control (n = 6 per group) (5.25 ± 1.445 vs 12.93116 ± 1.17658, $p = 0.0062$) unpaired two-sample *t*-test. Values represent mean ± SEM. *$p < 0.05$; **$p < 0.01$.

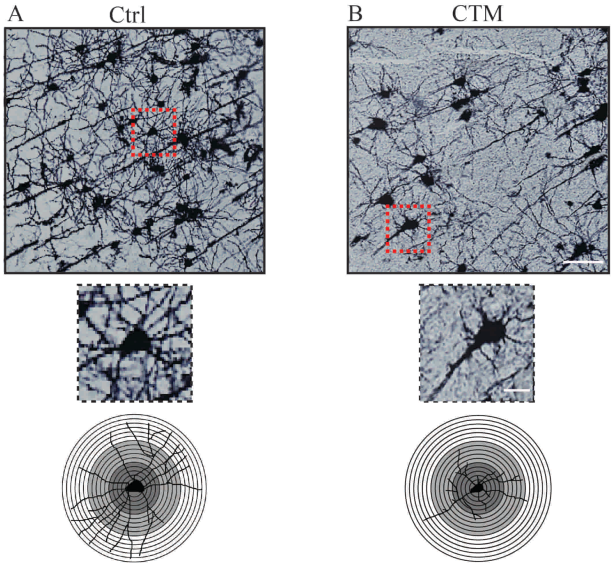

Fig. 4. CTM mice exhibit altered PFC morphology. Represents the impact of maternal CTM exposure on mPFC neuron. (A,B) Show photomicrographs of PFC neurons from 2-month-old CTM and control mice along with dendrites allocation between repeat concentration rings of 20 μm-spaced (n = 3 per group).

the Sholl's analysis. (A: $p = 0.0069$, unpaired two-sample *t*-test), which suggests reduced dendritic density in a radius to the soma.

3.4 CTM mice exhibit altered protein expression in PFC

Based on western blotting technique using tissue lysate of PFC from control and prenatally CTM exposed mice as shown in Fig. 6A, we observed significantly decrease level of CaMKIIα (C: $p = 0.0050$, unpaired two-sample *t*-test), in CTM mice as compared to controls. Similarly, level NMDAr1 and GABAb1 was drastically decreased (B and D: $p = 0.018$ and $p = 0.042$, unpaired two-sample *t*-test), in PFC of CTM mice in comparison to controls. Decreased NMDA receptor and GABA level makes neurons more susceptible to neurotoxicity, thus down regulation in expression of NMDA receptors possibly will make the PFC extra vulnerable to neurotoxicity during progression.

4. Discussion

Maternal exposure to SSRIs during pregnancy is a well-known contributor to the development of mood disorders, particularly depression, later in life [41]. SSRI exposure during pregnancy was associated with an increased risk for poor neonatal adaption syndrome, including respiratory distress, temperature instability, feeding difficulties, irritability, sleep problems, and tremors. Our research establishes that fetal CTM exposure results in compromised behavior and neuromorphological alterations specifically in the mPFC of adolescent mice. Current methodologies have led to substantial advancements in the knowledge of the

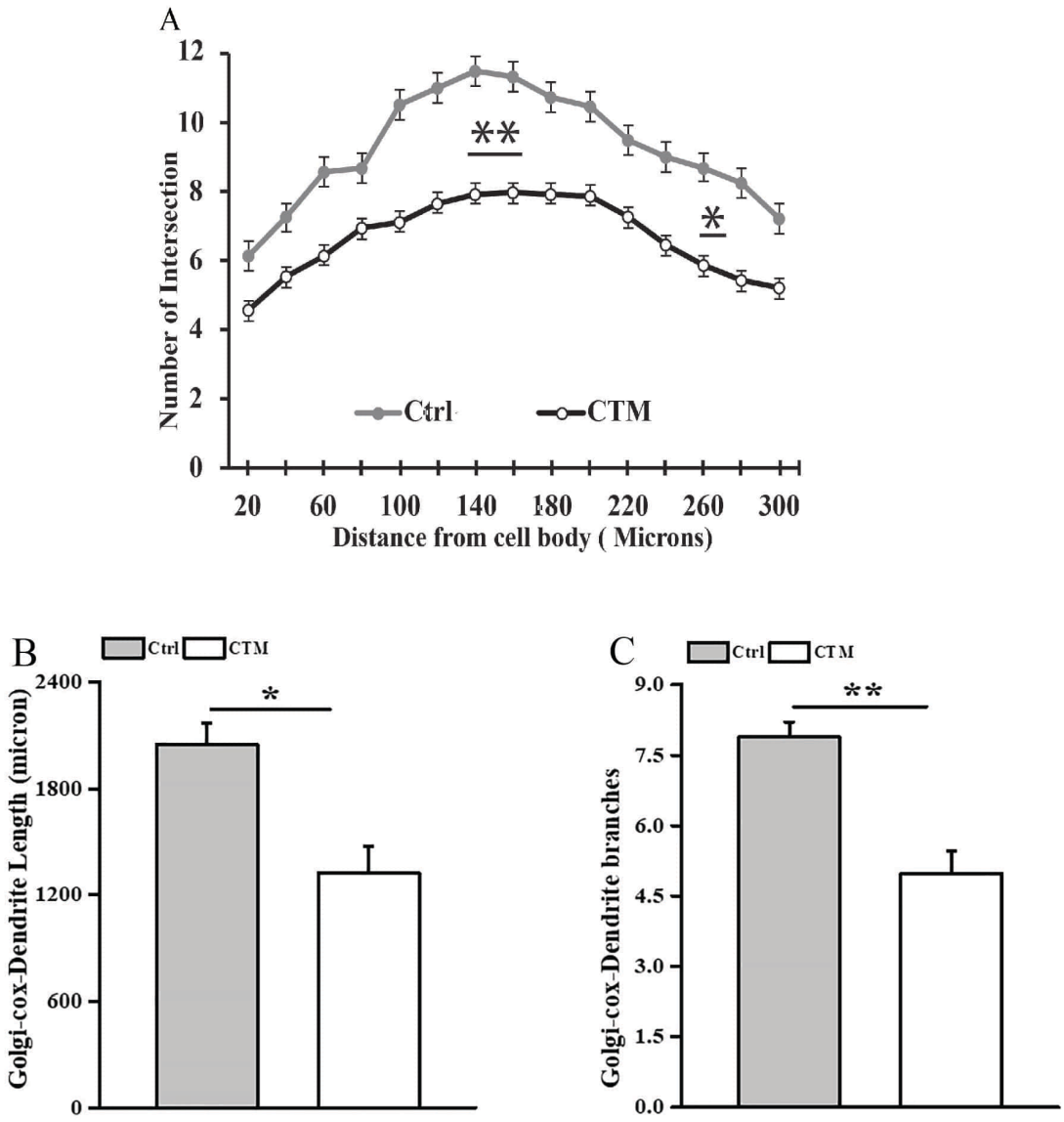

Fig. 5. CTM mice exhibit reduced dendritic morphology of PFC. Represents the impact of maternal CTM exposure on dendritic structure of mPFC neuron (A) Shows CTM mice has significantly lower points of intersection relative to controls (at 100–140 μm) to soma (8.97 $\pm$ 0.37 vs 7.28 $\pm$ 0.44, $p = 0.0069$) (n = 3 per group) unpaired two-sample t-test. (B,C) Shows decreased dendritic lengths, and branches of PFC neurons (2050 $\pm$ 118.14 vs 1325 $\pm$ 150, $p = 0.019$) and (7.88 $\pm$ 0.32 vs 4.97 $\pm$ 0.48, $p = 0.0074$) unpaired two-sample t-test. Values represent mean $\pm$ SEM. *$p < 0.05$; **$p < 0.01$.

neurobiological processes of adolescent emotional life [42]. It is found that adolescents are more sensitive to peer interactions than adults because they have increased activation in brain areas associated with socialization, such as the mPFC [43].

CTM treatment during the prenatal phase increases vulnerability to behavior changes [44], such as long-term anxiety, ASD, vulnerability to positive rewards, and depression-like activity [45]. We found that CTM animals with behavioral distress in FST and TST are correlated with an increase in anxiety-like behavior in EPM, which is consistent with prior studies in which adult mice exposed to prenatal stress displayed hyper anxiety in the EPM [46]. Additionally, we utilized the OFT to investigate depressive-like behaviors, in which decreased exploratory and locomotor activity is indicative of behavioral distress to cope with stressful conditions. Moreover, these developing exposures may increase immobility and alter PFC brain morphology dramatically among offspring.

The expression of c-Fos has been extensively utilized as a quantitative indicator of neuronal and circuit activity [47]. In current study c-Fos expression data showed significant increases in the mPFC in CTM mice compared with control mice. Interestingly, c-Fos-positive cells have been

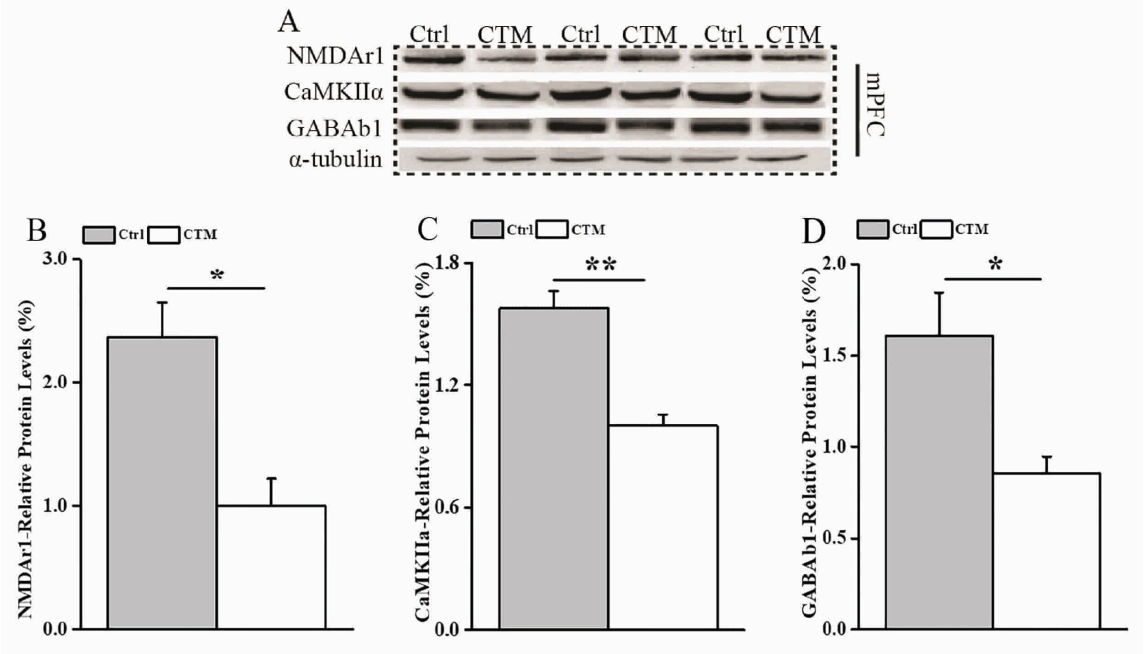

Fig. 6. CTM mice exhibit altered protein expression in PFC. Representative Immunoblots of PFC extracts from controls and CTM treated mice (n = 3, per group). It displays a substantial reduction in NMDAR1, CaMKII and GABAb1 in PFC of maternally CTM-treated mice relative to controls from immunoblot quantification. (A) Represents immunoblots of western blotting from a single procedure utilizing 3 pairs of PFC lysates, with two repeats. (B–D) Shows decreased protein expression level of NMDAR1 (1 ± 0.22 vs 2.36 ± 0.28, $p = 0.018$) CaMKII (1 ± 0.05 vs 1.57 ± 0.08, $p = 0.0050$) and GABAb1 (0.03 ± 0.002 vs 0.01 ± 0.004, $p = 0.042$) unpaired two-sample *t*-test. Values represent mean ± SEM. *$p < 0.05$; **$p < 0.01$.

substantially increased followed by large decreases in excitation postsynaptic receptors and downstream CaMKIIα. However, a number of other psychoactive compounds also elevate c-Fos in many brain regions [48]. Neuroimaging research indicates that frontal cortex, amygdala, central and medial amygdaloid nuclei, and hippocampus may play a critical role in the dysregulation of emotional process [49]. Therefore, consistent to previous studies our result indicates that maternal CTM treatment dramatically improves c-Fos expression in the mPFC. Since fetal exposure to SSRIs increases neural stimulation and cortical interneuron projections in rats [50].

Adolescence is a complex phase characterized by biological, social, and cognitive-behavioral changes. SSRI exposure during gestation increases fetal brain weight and decreases neuron number in the frontal lobe [51]. Thus, it appears that gross brain development is differentially affected by SSRIs. The current study carried out a morphometric examination of dendritic morphology in Golgi-Cox-stained samples to observe how stress could alter the structure of the mPFC during adolescence. A pioneering finding of the study displays prenatal CTM-induced dendritic atrophy of layer II/III pyramidal neurons in mPFC along with a distinct decline in the quantity of branches and dendritic length. This limitation may be attributed to differential atrophy of terminal branches, as average branch number and length decreased.

CTM-induced changes in the dendritic morphology of the mPFC neurons can endow to cognitive changes associated with CTM. Our findings suggest that the fetal CTM experience may be associated with the behavioral and morphological characteristics of these neurodevelopmental animal groups, including a pubertal rise in depression-like activity associated with modifications in the morphology of the PFC's pyramidal neurons. Similarly, early developmental experience has been related with subsequent rises in anxiolytic behavior and substantial alterations in dendritic bifurcation, spine densities and structure in the hippocampus and mPFC [52]. Numerous lines of evidence indicate that depression is associated with a dysregulation of the PFC circuitry. These include structural abnormalities, glutamatergic and GABAergic neurotransmission markers, and connectivity with downstream pathways. Dysregulated glutamatergic and GABAergic transmission within the PFC would be expected to negatively impact cognitive function and emotion [53].

Presently, we have investigated changes in the GABAergic and glutamatergic mechanism along with associated second-messenger pathways in CTM mice. CTM exposure caused downregulation in expression of NMDA receptor subunit NMDAR1, as well as CaMKIIα and reduction in GABAb1 receptor function in the PFC. CaMKII dysfunction is a recurrent process underlying alteration in glutamatergic structural and functional synaptic plasticity

that may lead directly to neuropsychiatric disorders [54]. Importantly, our results emphasize the significance of preclinical research in providing a better knowledge of prenatal SSRIs exposure on PFC network function and organization for potential improvements in targeted neuroplasticity.

Collectively, these findings corroborate our hypothesis that changes in brain connectivity could be associated with the abnormalities of major excitatory and inhibitory circuits [55], resulting in impaired cognitive and synaptic functions, together with dendritic reorganization [56] and inadequate production of pyramidal neurons of mPFC. Significantly decreased NMDAR1, CaMKII and GABA levels, as well as an increased number of PV-positive interneurons, might reasonably be predicted to result in decreased neuronal activity and, therefore, decreased c-Fos expression. It is possible that decreased expression of NMDAR1, CaMKII, and GABAb1 accompanied with an elevated number of PV-positive interneurons, is a compensatory mechanism for prenatal CTM-induced prefrontal neuronal activation.

5. Conclusions

In conclusion, both Clinical and preclinical evidence demonstrates the effects of prenatal SSRI exposure on neural plasticity and brain development [57]. Additionally, SSRIs such as CTM probably have significant long-term implications on neurobehavioral outcomes. Present findings support the function of CTM as a prenatal modulator of susceptibility to depressive-like behavior in offspring. Our study indicates the critical role of mPFC circuitry in regulation of depression and anxiety-like behavior and also highlight CTM induces modifications to cognitive and neural architecture as well as alterations in NMDA, CaMKII and GABA levels in mPFC. We indicate that prenatal CTM exposure elicits a negative impact on the central nervous system, especially those regions involved in cognition and drug reinforcement. Furthermore, genetic, chemogenetic, and optogenetic methods should be used to explain the function of SSRIs such as CTM during pregnancy in the regulation of mood and emotion-related behaviors in children to develop more selective and effective antidepressant therapies.

Author contributions

AZ and LD—Conceptualization, Writing - original draft, Methodology, Investigation; MJ, UB—Formal analysis, Software; QW and YW—Reviewing and Editing, Visualization; JW—Supervision and approved the final version. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The whole procedure of testing and animal housing was carried out in accordance with the ap-proved protocols of the Ethics Committee of Wuhan University of Technology (SYXK2017-0092) as well as the National Institute of Health's Guidelines for the Care and Use of Laboratory Animals (NIH).

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Conflict of interest

The authors declare no conflict of interest.

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