ORIGINAL ARTICLE

Female Early Adult Depression Results in Detrimental Impacts on the Behavioral Performance and Brain Development in Offspring

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SUMMARY

Aims: The present study was to understand whether early adult depressive females implicated their offspring. **Methods:** Seven-week-old female mice were subject to chronic mild stress (CMS) to establish the animal model of depression. The behavioral performance of their offspring were tested via neonatal reflexes tests, hole-board test, and morris water maze test in different ages. Astrocyte number, hippocampal volume, and neurogenesis were analyzed via immunohistochemical blotting. Glucocorticoid receptor (GR) expression and serum cortisol levels were measured by western blotting and ELISA. **Results:** Female depressive mice had normal fertility, but their offspring had lowered neonatal survival rate and body weight from neonatal period to early adulthood. The offspring of female depressive mice exhibited the impairments of neonatal reflex attainment and memory, but had higher emotionality as adults. Furthermore, the astrocyte number, hippocampal volume, and neurogenesis were reduced in the offspring. However, the expressions of GR were increased in the hippocampus of offspring. **Conclusion:** This study reveals that female early adult depressive mice have normal reproductive ability, but make long-term detrimental impacts on the behavioral performance and brain development of their offspring.

Introduction

Depression is a common mental disorder that manifests as depressed mood, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, low energy, and poor concentration. At its worst, depression can lead to suicide. About 10–20% individuals develop depression over their lifetime [1]. It is also a risk factor for many diseases, such as obesity, cardiovascular disease, and neurodegenerative disease. It is notable that the lifetime prevalence in women is twice as much as that in men [2,3]. Women are more susceptible to depression in adolescence, pregnancy, and the menopausal period [4]. Adolescence is especially of high risk in young women [5] and the prevalence of adolescent depression for girls is reported at 5.6% [6].

Many studies have shown that stress during pregnancy or maternal depression has a negative impact on the behavior of offspring and increases their risk for affective disorders [7,8]. The risks for anxiety disorders, major depression, and substance dependence are approximately three times as high in the offspring

of depressed parents as in the offspring of non-depressed parents. Social impairment was also observed in the progeny of depressed parents [9,10]. In addition, studies on animals showed prenatal stress resulted in a reduced hippocampal volume and inhibition of neurogenesis in the dentate gyrus in monkey [11], and studies on rats demonstrated that prenatal stress induced a long-lasting astroglial reaction and a reduced dendritic arborization, with synaptic loss in the brain of adult offspring [12,26,32]. However, it is unclear whether early adult depressive females implicate their offspring.

In the present study, we used chronic mild stress (CMS) [13] to induce depression-like behaviors in female early adult mice before mating. We then examined the intergenerational impacts of depression on the offspring, focused on chronicity, timing and neurobehavioral development. We also examined the adult neurogenesis and astrocyte number in hippocampus, hippocampal volume, the levels of blood corticosterone as well as the expressions of glucocorticoid receptor (GR) of neonatal or adult offspring so as to explore the possible neurobiological mechanism.

Table 1 Chronic mild stress protocol

Sun	09.00—7 hours of strobe lighting with room lights off, until cages 17.00—switch off strobe light, food and water deprivation for all animals; soil bedding, 150 ml water.
Mon	08.00—make up test solution; weigh all mice and solutions 09.00—sucrose preference test
Tues	19.00—weigh bottles; restore food; commence 85dB white noise (intermittently) for 3 h; switch lights on overnight 10.00—offer empty water bottles for 1 h; 7 h of strobe lighting, room lights off 11.00—restore water
Wed	17.00—switch off strobe light; remove water and food 09.00—restore water and food; tilt cages for 7 h
Thurs	16.00—untilt cages; paired house overnight 10.00—weigh all mice; rehouse singly; give 3 h of restricted food, few 45 mg pellets 13.00—restore ad lib food; commence 85 dB white noise (intermittently) for 5 h 18.00—switch off white noise; expose to novel odour overnight
Fri	08.00—remove novel odour; place foreign object in cage 17.00—remove food and water; remove foreign object; switch lights to on overnight
Sat	10.00—weigh all mice; restore food and water; tilt cages 15.00—untilt cages, restraint the move of mice for 6 hours 19.00—paired house overnight

Materials and Methods

Animals

ICR female mice of 7 weeks (30–32 g) were obtained from Experimental Animal Center of Jiangsu. Mice were housed with free access to food and water in a room with an ambient temperature of $22 \pm 2^\circ\text{C}$ and a 12:12 h light/dark cycle. After 1 week of sucrose consumption training phase, 33 animals were divided into two matched groups on the basis of their baseline sucrose preference score (see supplement 1) and body weight: the chronic mild stress group (CMS group, $n = 18$) and the nonstressed control group (control group, $n = 15$). Mice in the CMS group were subjected to a 6-week CMS according to a previously reported protocol [13]. Mice in the control group were housed in separate individual rooms without any contact with CMS group. Anhedonia was evaluated by weekly monitoring of sucrose intake in both control and CMS groups during the 7 weeks of the stress procedure. One day (24 h) after the stress procedure both control and CMS groups were tested in the forced swimming test (FST) and tail suspension test (TST). All experiments were carried out in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

CMS procedure

The CMS procedure was performed as described [13]. One or two of these stressors were randomly scheduled for the mice every day for 6 weeks. Detailed experimental design was presented in Table 1. During the 10 hours of the sucrose intake test (from 09:00 to 19:00, once a week), no stressors were applied. Testing procedures for sucrose preference, FST, and TST can be found in supplement 1.

Pregnancy of the female mice mating

Two days after the 6-week CMS procedure, female mice were caged with sexually experienced males of the same strain (ratio

3:1). The day of delivery was designated as postnatal day 0 (PND0). Litters were culled to 8 pups (4 males, 4 females). The pups were weaned at the age of 21 days and then housed in groups of six to seven according to their sex. Several reproduction parameters recorded for CMS and control females were: the survival rates of pups at weaning (% of pups surviving to PND21), individual body masses, and the male/female ratio (% female pups per litter).

Behavioral tests on offspring

The behaviors of progeny were observed from neonatal period to adulthood (detailed procedure shown in Figure S1). The offspring were tested on PND 7 for neonatal reflexes (see supplement 1 for procedural details). They were tested in Morris Water Maze (MWM) test on PND 64, sucrose intake test on PND70, and the FST on PND72 (see supplement 1 for procedural details).

Procedural details on bromodeoxyuridine labeling, immunohistochemical blotting, hippocampal volume analysis, western blot analysis, measurement of serum corticosterone levels can be found in supplement 1.

Data Analysis

All values are reported as mean $\pm$ SEM. The significant difference between control and CMS group was determined by *t*-test or X^2 -square test. Prior to the use of parametric statistics, we ensured that data were normally distributed (Shapiro–Wilk test). Differences were considered significant at $P < 0.05$.

Results

Female early adult mice exposed to CMS exhibited depression-like behaviors

Stress-induced decrease in sucrose preference in rodents is regarded as an analog of anhedonia, a key symptom of

Figure 1. Effect of CMS on sucrose intake (A) and sucrose preference (B), TST immobility time (C), and body weight (D) of female adolescent mice. CMS procedure significantly decreased the sucrose preference and sucrose intake of female mice (A, B), and increased the immobility time in TST (C). Under normal condition, the body weight of females in week 6 was significantly heavier than that in week 0, but no significant difference of body weight was observed between week 0 and week 6 of CMS group (D). Data represent the mean $\pm$ SEM. ** $P < 0.01$, * $P < 0.05$ versus control group; ## $P < 0.01$ versus control in week 0.

depression. CMS female mice showed decreased sucrose intake (solution (g)/body weight) and sucrose preference from week 5 to the end of the CMS procedure ($P < 0.01$ and $P < 0.05$ respectively) (Figure 1A, B). Immobility is interpreted as a "behavioral despair" in TST. Accordingly, increased immobility time was observed in CMS exposed female mice in the TST, ($P < 0.05$) (Figure 1C). Besides the results above, CMS can change the body weight, too. Figure 1D showed the body weight of mice from beginning (week 0) to week 6 of the CMS procedure. Prior to onset of CMS, sucrose intakes, and body weight were similar in both control group and CMS group. The female mice exposed to CMS had a significant reduction ($P < 0.01$) of body weight from week 1 to week 6 while the control female mice has a weight gain of 7.9 g during the whole procedure.

Female early adult depressive mice had normal fertility, but their offspring had lowered neonatal survival rate and body weight

The percentage of young pups from depressive group (of each litter) that survived from birth to weaning (88.75%) was lower than that in the control group (99.35%) ($\chi^2 = 13.3$, $P < 0.01$). But the number of pups (pups of control groups, 13.6 ± 0.7 ; pups of depressive groups, 14.2 ± 1.0), and the male/female ratio (pups of control, 48.6; pups of depressive groups, 51.9, $\chi^2 = 0.66$, $P >$

0.05) were similar in both groups. Chronic mild stress led to lowered birth weight of pups (pups of depressive group, 1.52 ± 0.02 g vs. pups of control group, 1.63 ± 0.01 g, $P < 0.01$). Figure 2A shows the body weight of mice offspring from the birth (PND0) to adulthood (PND75). Chronic stress in mothers decreased body weight gain of mice offspring from the birth to adulthood. The mean body mass of individual pups from depressive group was lower than that in pups from the control group from the birth to PND 63 ($P < 0.01$ or $P < 0.05$). But after PND 75, the body masses per pup were similar in both groups.

Offspring of the female depressive mice showed impaired the neonatal reflex attainment

Offspring of the female depressive mice slowed the attainment of the righting reflexes ($P < 0.05$) (Figure 2B), and shortened the times of hangwire compared to those in control group ($P < 0.05$) on postnatal day 7 to 10 (Figure 2C). But the time of cliff avoidance was similar in the two progeny groups (Figure 2B).

Offspring of the female depressive mice exhibited impaired behavior performance in the MWM test and the hole-board test but no depression-like behaviors

Figure 3A shows the learning curves for escape latency from the MWM over the course of 5-day training period. Mean escape latencies were similar for both offspring groups. From the second day to the fifth day, however, the escape latency of the adult offspring from depressive group was significantly shorter than that of the offspring from the control group ($P < 0.05$).

In the probe test, which was a trial without the platform, the adult offspring from the control group rapidly found the position where the platform had been, and their escape latency was significantly shorter than their previously acquired level. By contrast, the offspring of depressive group was impaired in their ability to adapt to the invisible escape position and their escape latency was much longer than that of control group ($P < 0.05$) (Figure 3C). In addition, during the probe trial, the number of times that mice crossed the removed hidden platform in the offspring from depressive groups was significantly fewer than that those from control groups ($P < 0.01$) (Figure 3D). But no significant differences in the swimming speed were identified in the two progeny groups (Figure 3B).

The effect of stress on the exploratory behavior of their adult offspring is shown in Figure 3E and 3F. In HB test for each group, the latency to head-dipping (Figure 3E), and ratio between head-dips and edge sniffs (Figure 3F) were evaluated. The latency to head-dipping was significantly longer and the head-dip/edge-sniffs ratio was significantly lower in offspring of depressive group than that in offspring of control group ($P < 0.05$), which indicated that chronic stress in mothers significantly lowered the levels of focused exploration and more anxiety in the offspring. No significant difference in sucrose preference or FST immobility times was found between the two progeny groups (Figure 3G, H).

Figure 2. The effect of female early adult depressive mice on the offspring's body weights (A), neonatal reflexes (B), and hangwire (C). Offspring of depressive mice showed significant decreases in body weights from PND 0 to PND 63. But no difference in body weights between the two offspring groups in PND75 (A) was observed. Offspring of depressive mice significantly increased the time of righting reflex (B) and decreased the time of hangwire (C). No significant difference was found between the two offspring groups in the time of cliff avoidance (B). Data represent the mean $\pm$ SEM. ** $P < 0.01$, * $P < 0.05$ versus offspring of control groups.

Cell proliferation in the hippocampus were inhibited in the offspring of female depressive mice

BrdU positive cells exhibited fusiform or irregular phenotypes and were clustered or aggregated in the SGZ (Figure 4A). The number of BrdU positive cells in the SGZ was similar in the two progeny groups on PND3 (Figure 4B). CMS-induced depression significantly inhibited cell proliferation in the SGZ of progeny on PND7 (51%) ($P < 0.01$), PND28 (39%) ($P < 0.01$), and PND75 (33%) ($P < 0.05$). In addition, cell proliferation of progeny from control groups on PND 7 was higher than that on PND3 (2.1-fold) ($P < 0.01$), which was abolished in the adult offspring from depressive groups.

Astrocyte number and hippocampal volume were decreased in the offspring of female depressive mice

As shown in Figure 5, CMS in females significantly decreased the number of GFAP positive cells in the hippocampus by 29% ($P < 0.01$) (Figure 5A, B) and the hippocampal volume by 10% ($P < 0.05$) (Figure 5C) in the adult offspring (PND75). But there was no significant difference in the number of GFAP positive cells or the hippocampal volume before adulthood (PND3, 7, 28).

The levels of serum corticosterone and expressions of GR in hippocampus were increased in the offspring of female depressive mice

As shown in Figure 6A, exposure to CMS in female mice resulted in 2.4-fold elevation in the concentration of plasma corticosterone ($P < 0.05$), but no significant difference was found in expression of GR in maternal hippocampus between CMS groups and control groups (Figure 6C).

As shown in Figure 6D, CMS in females increased the expression of hippocampal GR on PND7, 28, and 75 in their offspring. No significant difference was found on PND3. In addition, chronic stress in female mice resulted in a 31% decrease in the level of serum corticosterone in their offspring ($P < 0.05$) on PND28. But no significant difference in the levels of corticosterone was found in the two adult offspring groups on PND75 (Figure 6B).

Discussion

In this study, we used CMS to successfully establish an early adult depressive mouse model of depression. The sucrose intake test is an objective measure of depression-like behavior in rodent [14]. As expected, control mice increased their relative

Figure 3. Offspring's performance in Morris water maze (MWM) test on the spatial memory acquisition and retention (A–D), hole-board (HB) test (E, F), sucrose preference and forced swimming test (FST) (G, H). MWM learning curves showed that, in the 5 days of training, all offspring mice were capable of accomplishing the escape task, with the escape latency of offspring from depressive groups shorter than that of offspring from control groups (A). No difference was observed in the two progeny groups in swimming speed (B). In addition, figure C and D showed the effect of maternal depression on

the performance of the offspring in probe test of MWM test, which showed significant increased times of escape latency (C) and decreased times of crossed platform (D) in offspring of depressive groups. Besides, offspring of the depressive mice showed the increases of latency to headdipping (E) and decreases of head dip/head sniff ratio (F) in HB test. No significant difference of the immobility time in FST (G) and the sucrose preference (H) was found between the two offspring groups. Data represent the mean $\pm$ SEM. ** $P < 0.01$, * $P < 0.05$ versus offspring of control groups.

sucrose intake (sucrose intake/body weight) over time. On the contrary, CMS mice appeared to have no stable consumption of sucrose solution over all 6 weeks, and sucrose intake was lower than that of control mice. Similar findings were also indicated by analysis of sucrose preference. In addition, CMS increased the immobility time in tail-suspension test. Overall, CMS decreased the rewarding value of sucrose and caused depression-like behaviors in females, indicating the success of this approach in this study.

Several studies have provided evidence that maternal stress during pregnancy can cause a decreased birth weight and survival rate in pups [15]. In the present study, we found that depression in early adult female mice had no effect in their reproductive ability, but resulted in lower body weight and survival rate in the offspring. Maternal symptoms such as daily hassles as well as depression and anxiety appear to be associated with both premature delivery and low-weight birth, which, in turn, are risk factors for impaired cognitive and social developmental outcomes [9,11]. Because prenatal event can disrupt fetal brain development at a critical period, risks for several neural illnesses and psychiatric disorders will be increased later in adulthood [9,11]. Many studies on animals also found that the offspring of animals exposed to CMS during pregnancy showed lower levels of focused exploration [11], and more anxiety in adulthood [16]. For the first time, our study revealed that the CMS-induced depression in females

affects the physiological and behavioral developments of their offspring: including reduced body weight gain, slowed attainment of the righting reflexes and significantly shortened times of hang-wire. Many clinical studies have also shown that maternal depression or anxiety during pregnancy leads to progeny substantially more likely to have emotional or cognitive problems, including an increased risk of attention deficit, hyperactivity, anxiety, and language delay. Studies on rats found that the adult offspring of female rats subject to CMS had a lower exploratory activity in the plus-maze test [17] and impaired retention in the Morris water maze [18]. Consistent with these clinical and preclinical studies, we found that adult offspring of depressive mice exhibited significant lower levels of focused exploration and much more anxiety in HB test. In addition, CMS to the mothers not only slowed neonatal reflex attainment but also influenced several psychological behaviors in the adult offspring. Haihong Li in 2010 reported that the adult rat offspring of female mice exposed to chronic unpredictable stress showed decreased sucrose intake and consumption percentage (the core symptom of depression) [18]. But in our study, sucrose test and TST did not reveal an increased likelihood of depression in the adult progeny from depressive mice. We think it may be due to different procedures or mouse strains. Meanwhile, there are also many reports of CMS causing significant effects in the opposite direction, termed as an 'anomalous' behavioural profile [19]. Studies concerning the effects of CMS on mice behavior

Figure 4. Effect of female early adult depressive mice on the cell proliferation in SGZ of offspring. Offspring of depressive mice showed significant inhibition of cell proliferation in SGZ from PND 7 to PND 75, and showed a decreased about 51%, 39%, and 13% in offspring compared to the progeny of control groups. But no difference was found in the two offspring groups on

PND 3. The number of Brdu positive cells of control progeny on PND 7 was higher than that on PND3, which wasn't be shown in offspring of depressive group. Brdu: 5-bromo-2-deoxyuridine. Data represent the mean $\pm$ SEM. ** $P < 0.01$, * $P < 0.05$ versus offspring of control groups; ### $P < 0.01$, versus offspring of control groups on PND 3. Scale bar, 55 μ m.

agreed on the importance of the animal strains used in this model [20–23].

Moreover, the offspring of depressive mice showed improved acquisition but impaired retention in the spatial memory in MWM. Two published studies on rats showed impaired and normal acquisition in the MWM [18,24], both of which were different from our results. There was no difference in swimming velocity between the two groups, which suggested that impaired retention of progeny from depressive mice was not related to the decreased locomotor activity in the MWM. So, the reason for the improved acquisition in spatial memory test in our research was not known. There were reports that entorhinal–hippocampal connections might be limited to maintaining some types of information (e.g., single object discriminations) for retention only, but not for acquisition [25]. Early studies have shown that lesions of the rat entorhinal cortex resulted in impaired retention but normal acquisition [26]. So, may be the dysfunction of hippocampus led to the impaired retention, but compensatory increased acquisition.

Our findings on adult neurogenesis are in support of the above conclusion. The hippocampus is the most active area of neurogenesis in the rodent brain. Previous studies provide evidence for the role of the hippocampal formation in learning and memory [27,28]. It has been reported that prenatal stress produces learning and memory deficits, associated with an inhibition of neurogenesis, and impaired long-term potentiation (LTP) in the hippocampus [29,30]. We analyzed the cell proliferation in SGZ in hippocampus through Brdu immunohistochemistry, which showed an age-related decrease of neurogenesis in the two groups. With an increase in age, there was a decline in precursor cell proliferation and net neurogenesis, which have been shown in previous studies [31,32]. Here, we reported that pregestational depression significantly decreased cell proliferation in progeny mice from neonatal period to adulthood, especially on postnatal day 7. Granule cell progenitors were numerous during the first postnatal week, and from the second postnatal week to adulthood, proliferation declined [33]. We inferred that pregestational

Figure 5. Effect of female early adult depressive mice on the glial fibrillary acidic protein (GFAP) positive cells number in hippocampus (A, B) and the hippocampal volume (C) of offspring. Hippocampal volumetry revealed that a significant reduce in GFAP positive cells in offspring of depressive mice on

PND 75 (29%) (A, B), and in hippocampal volume on PND 75 (10%) (C). But no difference was detected between the two offspring groups on PND 3, PND 7, and PND 28. Data represent the mean $\pm$ SEM. ** $P < 0.01$, * $P < 0.05$ versus offspring of control groups. Scale bar, 55 μ m.

depression resulted in inhibition of neurogenesis, and abolished the peak of neurogenesis on PND7. The hippocampus neurogenesis is implicated in emotional behavior [34]. Accumulating evidence has also suggested that chronic stress induces depressive behavior and reduces the proliferation of new neurons [35,36]. Similarly, the effects of the antidepressant drugs on rodent model were blocked, with x-radiation yielding an 85% decrease of BrdU-positive stained cells in the subgranular zone of the dentate gyrus [36]. However, it is still inconclusive whether neurogenesis is a cause or a result of depression [37]. Just as in our study, we found a decreased neurogenesis but not depression-like behaviors. Increased neurogenesis also correlates with increased network activity and improved cognition, and the ablation of adult neurogenesis in rats results in deficits in hippocampus-dependent trace conditioning tasks [38,39].

Simultaneously, we found the pregestational depression significantly decreased the numbers of GFAP-positive cells as well as reduced the hippocampal volume in adult offspring. Reduced glial cell density is also implicated in emotional disease [40] and poor memory performance [41]. Recent research has been focusing on the participation of astrocytes in cognitive functions, com-

mon to human and other mammalian species, such as learning, perception, conscious integration, memory formation/retrieval, and the control of voluntary behavior [42]. Glial-cell loss and neuronal-size reduction were found in postmortem brain tissue of subjects with MDD, schizophrenia, and bipolar disorder (BPD) [43,44]. Moreover, reduction of astroglia number and size induced by stress could contribute to the hippocampal volume changes [45]. This was consistent with our results that CMS-induced pregestational depression decreased hippocampal volume of adult progeny. It has been of great concern that volume change may precede rather than follow depression and it has been found that variance in hippocampal volume in children is as great as in adults, and greater than that reported between controls and patients with depression. So, it was suggested that individual differences in hippocampal volumes in humans may determine the vulnerability for psychopathology throughout the lifetime [46]. Although our study did not detect depressive behavior in adult progeny of depressive mice, the decreased hippocampal volume, the numbers of GFAP positive cells, and neurogenesis of hippocampus may denote the vulnerability for depression in progeny of depressive mice in future.

Figure 6. Effect of early adult depression on the serum corticosterone level in maternal and offspring blood (A, B) and on the expressions of GR in offspring and offspring hippocampus (C, D). CMS procedure caused a 2.4-fold rise in serum corticosterone in CMS group maternal mice (A), but no difference was detected between CMS and control group on the relative level of GR/β-actin (C). C: Control, M: CMS, * $P < 0.05$ versus control group. Serum corticosterone levels in offspring of depressive mice was around 68% of that

in offspring of control groups on PND28 but no difference was detected between the two offspring groups on PND75 (B). Relative level of GR/β-actin in hippocampus of offspring from depressive groups was higher than that in offspring from control groups on PND7, 28, and 75, but no difference was detected between the two offspring groups on PND3 (D). C: offspring of control groups, M: offspring of depressive groups, * $P < 0.05$ versus offspring of control groups. Data represent the mean $\pm$ SEM.

To clarify the involved mechanisms, we further determined alterations in hypothalamic-pituitary-adrenal (HPA) axis including GRs expression and the serum corticosterone level in maternal mice at the end of lactation and in progeny from neonatal period to adulthood. HPA-axis dysregulation is observed in many mental disorders, such as posttraumatic stress disorder, social anxiety, and depression [47,48]. And glucocorticoids acting on GRs play feedback regulation of the HPA-axis, which is tightly related with the depression [49]. Our results showed that CMS-induced pregestational depression significantly increased the expression of GR in hippocampus of progeny and decreased the level of blood corti-

costerone in progeny before adulthood. In response to a stressful event, GR is considered both as a sensor of stress and as a breaking mechanism that limits the stress response once it has taken place [50]. Glucocorticoids function via the GR, which regulates gene transcription [such as brain-derived neurotrophic factor (BDNF), activity regulated cytoskeletal protein (Arc), and so on], and contribute to glucose homeostasis and cell differentiation [51]. On one hand, an elevated level of forebrain GR leads to increased environmental reactivity, emotional lability, and cognitive dysfunction [52]. On the other hand, loss of GR interferes with the negative feedback exerted by the hippocampus and the

frontal cortex. As a result, there is increased activity of corticosteroids, resulting in a "depression-like" syndrome [53]. Wei et al. found even in the absence of chronic stress, elevation of GR gene expression can accelerate hippocampal dysregulation and result in endocrine and cognitive sequelae [54]. In addition, previous research showed overactivated GR is associated with functional impairments in the depressive brain, especially in the hippocampus, where it results in reduced neurogenesis and impaired neuroplasticity [55]. High levels of GR in the brain would promote anxiety-like behavior [56], and the blockade of GR leads to improvements in cognition and mood in patients with bipolar disorder [57]. Our study also showed a decreased neurogenesis in subgranular zone (SGZ) in the progeny of depressive mice. So, the increased expression of GR and decreased levels of corticosterone might be one of the reasons for the impaired memory and exploratory ability in adult progeny of depressive mice. Hence, we proposed that innate differences in the neural expression of a stress-related gene GR could impair cognitive function with or without environmental stressors.

Previous study reported that pregnancy stress could cause an increase in maternal corticosterone in rodents, which reprogrammed the function of the HPA axis in the offspring and had a range of long-term effects on fetal development [8]. Prenatal stress and maternal exposure to exogenous glucocorticoids also could lead to permanent modification of HPA-axis function and stress-related behavior [58]. In order to detect whether CMS before pregnancy can change the level of maternal blood corticosterone until the end of lactation, we measured the plasma level of corticosterone and the expression of GR in hippocampus in maternal mice after lactation. We found CMS had no effect on the expression of GR in hippocampus but led to an elevated plasma corticosterone. So, we supposed that CMS before pregnancy would influence the HPA-axis function of progeny through the change of corticosterone levels in maternal blood. Thus, although basal levels of corticosterone in adult progeny are normal, the abnormal levels of GR render the progeny with an abnormal stress system and neural sequelae. Our study demonstrated that CMS-induced early adult female depression resulted in neurobehavioral disorders in the offspring from neonatal period to adulthood. Further, adult offspring of depressive mice showed decreases in astrocyte number and hippocampal volume, and inhibition of neurogenesis in hippocampus. Moreover, we found that maternal depression increased the GR expression of the offspring from juvenile period to adulthood, but decreased the levels of corticosterone in juvenile progeny. Our findings reveal

for the first time that depression in early adult female mice can have long-term detrimental impacts on the behavioral and brain development of their offspring. Thus, it is crucial for the healthy development of progeny to prevent and treat early adult depression. Further studies should be proceeded to explore the therapeutic strategies and to reveal the impacts of male depression on the offspring.

Author Contributions

Gong Y., Sun XL., conception and design, performing the experiments; acquisition, analysis, and interpretation of data; drafting and revising the article; final approval of the version to be published. Wu FF., Su CJ., Ding JH., perform the experiments; acquisition, analysis and interpretation of data; final approval of the version to be published. Hu G., conception and design, acquisition, analysis and interpretation of data; revising the article critically for important intellectual content; and final approval of the version to be published.

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Conflict of Interest

The authors declare no conflict of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. The behavioral tests on different postnatal days.

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