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Corticosterone Induces Depressive-Like Behavior in Female Peri-Pubescent Rats, but Not in Pre-Pubescent Rats

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Abstract

Background: There are no data on the effect of exogenous corticosterone on depressive-like behavior in juvenile rats. Furthermore, it has not been tested whether the effects of corticosterone in female rats is different before or after puberty.
Objective: We tested the effect of corticosterone treatment on female pre- and peri-pubescent juvenile rats on depressive-like behavior.
Methods: Female juvenile rats were divided into pre-pubescent (post-natal day 7–27) or peri-pubescent (post-natal day 28–48) groups and administered daily corticosterone (40 mg kg^{-1} day^{-1}) for 21 days. Depressive-like behavior was assessed using a modified forced swim test and the sucrose preference test. After behavioral assessment, brains were analyzed to determine if there were changes in cell proliferation and newborn neuron survival in the dentate gyrus of the dorsal hippocampus.
Results: Chronic corticosterone treatment did not affect behavior or neurogenesis in female pre-pubescent juvenile rats. However, female peri-pubescent rats injected with corticosterone showed increased depressive-like behavior as well as a decrease in cell proliferation in the subgranular zone. Furthermore, there was an inverse correlation between time spent immobile in the forced swim test and cell proliferation in the granule cell layer in peri-pubescent rats.
Conclusions: Corticosterone induces depressive-like behavior in peri-pubescent, but not in pre-pubescent female rats. Finally, our results suggest that depressive-like behavior may be associated with a decrease in hippocampal cell proliferation in female peri-pubescent rats.

Keywords

depression, neurogenesis, puberty, female, juvenile

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Introduction

In the United States, the 12-month occurrence of major depressive disorder (MDD) is about 7%.[1] *The Diagnostics and Statistical Manual of Mental Disorders, Fifth Edition* (DSM-5) notes that "females experience 1.5- to 3-fold higher rates than males beginning in early adolescence."[1] Indeed, gender differences in MDD emerge at menarche, that is, the incidence of MDD increases after puberty in girls.[2]

Adult females are two to three times more likely than males to develop MDD.[3] This higher incidence of MDD in females has been attributed to phenotypic sex differences, rather than a general trend for women to report symptoms more.[4] Adolescent females are twice as likely to be depressed as boys, these sex differences in depression appear in adolescents only after the age of 14 years.[5] These differences could be due to differences in brain development between males and females during and after puberty.[5] Females rats exposed to stress have been shown to have reduced hippocampal volume.[6] This suggests that stress early in life forms a risk factor for the development of stress-related disorders in females. It is speculated that female juveniles and adolescents are more likely than males to carry risk factors for

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depression and that these risk factors may lead to depression after stress incidences that increase prevalence into adolescence.[5] Clearly, there is a need to understand the effect of puberty on stress and depression.

The exact etiology of MDD is not well understood; however, studies consistently find that stress can lead to the development of MDD.[7–9] It is proposed that the onset of depression is connected with alterations in the activity of the hypothalamic–pituitary–adrenal (HPA) axis, which is involved in the stress response.[10,11] Activation of the HPA axis results in the release of glucocorticoids from the adrenal gland. The HPA axis has profound effects on the brain.[12–14] For instance, glucocorticoids regulate neurogenesis, neuronal survival, and the acquisition of new memories and emotional appraisal of events.[15,16] Individuals with MDD have a decrease in hippocampal size,[12,17] although the changes in hippocampal volume being actually caused by decreased neurogenesis seems unlikely.[17]

Rodents have been used as models to study the etiology of depression. Exogenous corticosterone (CORT) exposure has been used to mimic hyperactivity of the HPA axis in response to stressful stimuli.[18] In adult rodents, exogenous CORT exposure increases depressive-like behavior assessed using modified forced swim tests (FSTs).[19–23] CORT treatment also induces alterations in hippocampal neurogenesis[24–26] in adult male and female rodents. Similarly, chronic restraint stress increased CORT levels in female rats and was associated with decreased neurogenesis.[27] In adult rats, antidepressant drugs increase hippocampal BDNF and TrkB expression. Whether decreased TrkB expression is involved in depressive-like behavior in rats has not been established, but CORT exposure decreased TrkB expression in adult rodents.[28] Also whether CORT induced decreased TrkB expression is causally related to a reduction in neurogenesis is not established. But interestingly, antidepressant drugs increase TrkB expresssion in juvenile rodents.[28] In female juvenile rodents, it is not known whether CORT treatment alters either depressive-like behavior or neurogenesis.

In this study, we test whether there are developmental differences in the effect of CORT on depressive-like behavior and neurogenesis in female juvenile rats. Specifically, we tested whether CORT alters depressive like-behavior in pre-pubescent and peri-pubescent female rats, because the incidence of MDD increases after puberty in girls.[2] To further test the hypothesis that adult hippocampal neurogenesis may be involved in the etiology of depression, we use assays for hippocampal cell proliferation and adult neurogenesis in rats treated chronically with CORT. In the cell proliferation paradigm (Figure 1), rats were treated with or without CORT, 5-Bromo-2′-deoxyuridine (BrdU) administration occurs at the end to determine if the basal rate of cell proliferation was altered. In the cell survival paradigm (Figure 1), BrdU was administered in the beginning to mark newborn cells, then colocalization of NeuN and BrdU was used to assess neurogenesis. Moreover, we tested whether CORT exposure alters hippocampal cell proliferation or adult neurogenesis in pre-pubescent and peri-pubescent female rats. This study sought to better define the role sexual maturity plays in the response of female rats to high levels of CORT, which may lay a foundation for addressing the developmental difference in the risk for depression in adolescent females.

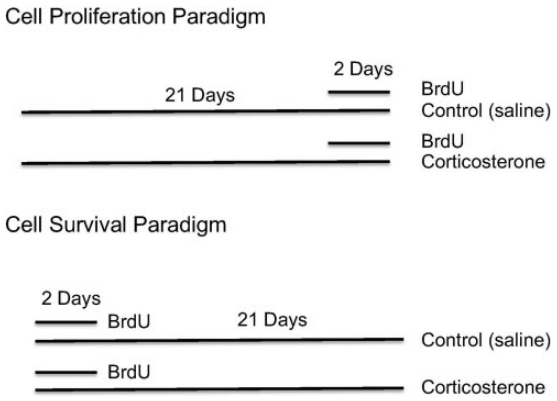

Figure 1. Corticosterone treatment timeline and experimental paradigms. Treatment of CORT or control was administered by IP injection for 21 days. Injections began on PND 7 for pre-pubescent rats and continued until PND 27. Peri-pubescent rats began treatment on PND 28 and continued until PND 48. CORT (16 mg ml^{-1} equivalent to 40 mg kg^{-1}) or vehicle was injected by IP injection once daily for 21 days at a volume of 2.5 $\mu l\ g^{-1}$ body weight. In the cell proliferation paradigm, BrdU was administered three times daily for two consecutive days prior to sacrifice. In the cell survival paradigm, BrdU was administered three times daily on the first two days of the 21-day treatment timeline.
BrdU: 5-Bromo-2′-deoxyuridine.

Materials and Methods

Animals

Female Sprague-Dawley rat pups were purchased from Hilltop Laboratories and were housed in cages under regulated temperature ($21 \pm 1°C$) and light conditions (12-h light/dark cycle). Pre-pubescent pups were housed with a mother until day 21. The rats had ad libitum access to commercial rat chow and water. Experiments were performed on female pre-pubescent (post-natal day (PND) 7) and peri-pubescent (PND 28) rats that were randomly divided into groups ($n = 6$), individual experiments had two groups, control and CORT. In this study, 48 rats were used. The onset of puberty was determined by vaginal opening, which occurs around PND 33.[29] Behavioral testing and treatments occurred during the dark cycle in a dimly lit room.

All procedures were approved by the KCOM IACUC, which is accredited by AAALAC and has an assurance with OLAW. Each animal was used only once, and all efforts were made to minimize animal suffering and to reduce the number of animals required for the experiments.

Drugs

CORT (11β,21-Dihydroxy-4-pregene-3,20-dione; Sigma, St. Louis, MO) was mixed in vehicle solution (1.5% Tween 80, 0.9% NaCl; Sigma), in distilled water, and sonicated.[30] CORT (16 mg ml^{-1} equivalent to 40 mg kg^{-1}) or vehicle was injected by intraperitoneal (IP) injection once daily for 21 days at a volume of 2.5 µl g^{-1} body weight.[10,20,21] BrdU (Sigma) dissolved in phosphate-buffered saline (PBS; 11.9 mM phosphates, 137 mM sodium chloride, 2.7 mM potassium chloride; Fisher Scientific, Houston, TX[31]) was used to label neurogenesis. BrdU typically labels cells that divide during a 2- to 4-h window following injection.[32]

Drug Administration

CORT or vehicle treatments began on PND 7 for pre-pubescent rats and continued until PND 27 in order to avoid the onset of puberty. Peri-pubescent rats began treatment on PND 28 and continued until PND 48 to allow pubertal development. Rats in the cell proliferation paradigm group received BrdU (20 mg ml^{-1} equivalent to 300 mg kg^{-1}) injections at a fixed volume of 5 µl g^{-1} three times daily for two consecutive days before sacrifice. Rats in the cell survival paradigm group received BrdU (20 mg ml^{-1} equivalent to 100 mg kg^{-1}) injections at a fixed volume of 2.5 µl g^{-1} three times daily for the first two days at the beginning of treatment (Figure 1).

Behavioral Tests

Modified FST. The modified FST was used to detect the presence of depressive-like behavior. In studies addressing the CORT model of depression, the modified version of the FST was given to avoid inducing a state of despair.[18] According to this model, an increase in time spent immobile indicates depressive-like behavior. In the present study, rats underwent the modified FST the final day of the 21-day treatment. Rats were placed in a clear cylinder of water and allowed to swim for ten minutes. During the modified FST, rats were observed for the following behaviors: (a) swimming, (b) climbing, and (c) immobile. Swimming behavior was characterized by active motions (i.e., animals moving around the container). Climbing behavior was characterized by rats making active movements with their forepaws in and out of the water, usually directed against the wall. Immobility behavior was characterized by floating without struggling and making only those movements necessary to keep the head above water. Depressive-like behavior was indicated by an increase in time spent immobile.

Sucrose Preference Test. The sucrose preference test (SPT) was used to measure anhedonia (a reduced preference for normally enjoyable activities), which is indicative of depressive-like behavior.[9,33] The SPT commenced three days before sacrifice. A two-bottle preference test was used, where all animals had access to one bottle of 2% sucrose solution and one bottle of water for a 48-h period.[34] To prevent potential location preference of drinking, the positions of the bottles were changed after 24 h. The amount of sucrose solution and water consumption was determined by weighing the bottles. Sucrose preference was calculated as the percentage of sucrose solution ingested relative to the total amount of liquid consumed (sucrose preference = [sucrose solution, g/sucrose solution, g + water, g] × 100).

Tissue Collection

We performed transcardial perfusion as described by Wojtowicz and Kee.[31] The pre-pubescent group was sacrificed prior to vaginal opening (PND 27) and the peri-pubescent group was sacrificed after vaginal opening and regular estrous (PND 48).[29] Rats were completely anesthetized using IP injection of 50 mg kg^{-1} of sodium pentobarbital. Brains were flushed via transcardial perfusion with 35 ml of PBS. The brains were then perfused with 35 ml of 4% paraformaldehyde in PBS. Once removed, brains were weighed and then placed in 4% paraformaldehyde for 24 h. Brains were then transferred to 30% sucrose and PBS solution for three days or until they sank to the bottom of the vial.[35] Following this, brains were frozen by placing them on dry ice[36] and stored at −80°C.

Slicing

Slices of whole brains were prepared 30 microns in coronal sections at −22°C using a cryostat (Leica CM 1900). The brains were sliced through the dentate gyrus of the hippocampus. Slicing began at a region similar to 3.14 to 4.52 mm posterior to the bregma point in the adult rat brains (dorsal hippocampus). Pre- and peri-pubescent juvenile rat brains were correlated with adult rat brains using the Rat Brain in Stereotaxic Coordinates atlas.[37] A total of 72 slices were obtained for each rat brain. Slices were stored in phosphate buffer at 4°C.

Immunohistochemistry

Briefly, detection of proliferating cells ("newborn" cells that have undergone mitogenesis) were marked by

treating rats with the thymidine analog, bromodeoxyuridine (BrdU). Juvenile rats will be were treated with or without chronic CORT and BrdU. After varying treatment regimens, rat brains were fixed, sliced, and BrdU; and the neuronal marker protein, NeuN, was detected using fluorescent immunohistochemistry. Cell proliferation in the dentate gyrus was quantified using immunohistochemical detection of BrdU and counting BrdU-positive cells using epifluorescence microscopy. Neurogenesis (formation of newborn neurons) was assayed by immunohistochemical detection of colocalized BrdU and NeuN using confocal microscopy. Newborn cells and newborn neurons in the dentate gyrus of the hippocampus were identified by immunohistochemistry. Fluorescent antibodies allowed us to visualize cells containing BrdU and NeuN (a neuronal nuclear marker[31]). Brain slices were washed in PBS for 5 min, three times. The slices were then incubated in 1 M hydrochloric acid at 45°C for 1 h and washed six times with PBS and incubated in a blocking solution (PBS, 0.3% Triton X-100, 2% equine serum) for 1 h. Once blocking solution was removed, brain slices were placed in a 1:4000 solution of primary antibody (rat anti-BrdU; mouse anti-NeuN) in blocking solution. Slices were then incubated for 24 h at 4°C. Brain slices were then washed for five minutes in PBS three times and incubated with secondary antibodies (Alexa Fluor 594 donkey anti-rat, Invitrogen; Alexa Fluor 488 goat anti-mouse, Invitrogen) diluted at 1:1000 in PBS with 0.3% Triton-X 100 for 2 h. Slices were washed for 5 min in PBS three times and then mounted onto microscope slides.

Fluorescent Imaging

We identified cells undergoing cell proliferation by BrDU incorporation within the dentate gyrus of the hippocampus using fluorescent microscopy (Nikon ECLIPSE 80i). Excitation was achieved with a Nikon Intenslight C-HGFI mercury lamp with FitC and Texas Red filter sets. All single labeled BrdU-positive cells were counted in the subgranular zone (SGZ). BrdU-positive cells were also counted within the hilus. The number of labeled cells was calculated in eight coronal sections from each rat. Detection of BrdU (red) and NeuN (green) was with secondary antibodies labeled with Alexa 594 and Alexa 488, respectively.

Confocal Imaging

Newborn neuron survival was quantified using a Leica DMI 6000B confocal laser-scanning microscope. Excitation was achieved with a 488 nm Kr/Ar laser and a 594 nm He/Ne laser. Two separate photo multiplier tubes were used to scan at wavelengths 500 to 580 nm and 605 to 700 nm for the emissions from the Alexa 488 and the Alexa 594 fluorescent antibodies respectively. Eight fields of the dentate gyrus were imaged at 63× magnification for each rat brain. Colocalization of signal from BrdU-positive cells expressing NeuN neuronal marker was considered newborn neurons. Colocalized fluorescence was analyzed using the Leica LAS Advanced Fluorescence software tool, which identifies and plots pixels with colocalized emission above a specified intensity. The percentage of colocalization in the dentate gyrus was determined by the number of BrdU-/NeuN-positive cells divided by the number of total BrdU-positive cells (% colocalization = [(number of BrdU-/NeuN-positive cells)/(number of BrdU-positive cells)] × 100).

Data Analysis

Analysis of all experiments comparing control and CORT groups was performed using an unpaired two-tailed t test; p values ≤ 0.05 were considered significant. T tests were used because all of the experiments had only two groups (Control and CORT). Unpaired two tailed t tests were used because animals or measurements were not paired and the experiments were novel, so there was not a prediction of which means would be larger. The data used in the t tests had normal distributions using the Shapiro–Wilk test. To determine whether an increase in immobility time was correlated with a decrease in cell proliferation or cell survival, a two-tailed Pearson product–moment correlation coefficient was performed. Linear regression was used to provide a graphic visual aid. p values ≤ 0.05 were considered significant. Daily weights were compared using an analysis of variance (ANOVA) for repeated measures.

Results

Chronic CORT Treatment Elicits Depressive-Like Behavioral Changes in Female Peri-Pubescent Juvenile Rats, but Not in Female Pre-Pubescent Juvenile Rats

Female peri-pubescent rats injected with CORT spent more time immobile ($p < 0.001$), and less time climbing ($p = 0.004$) and swimming ($p < 0.001$) compared to controls (Figure 2), which indicates CORT increase depressive-like behavior. In contrast, there was no difference in time spent immobile ($p = 0.57$), climbing ($p = 0.71$), or swimming ($p = 0.94$) in pre-pubescent rats injected with CORT compared to controls (Figure 2).

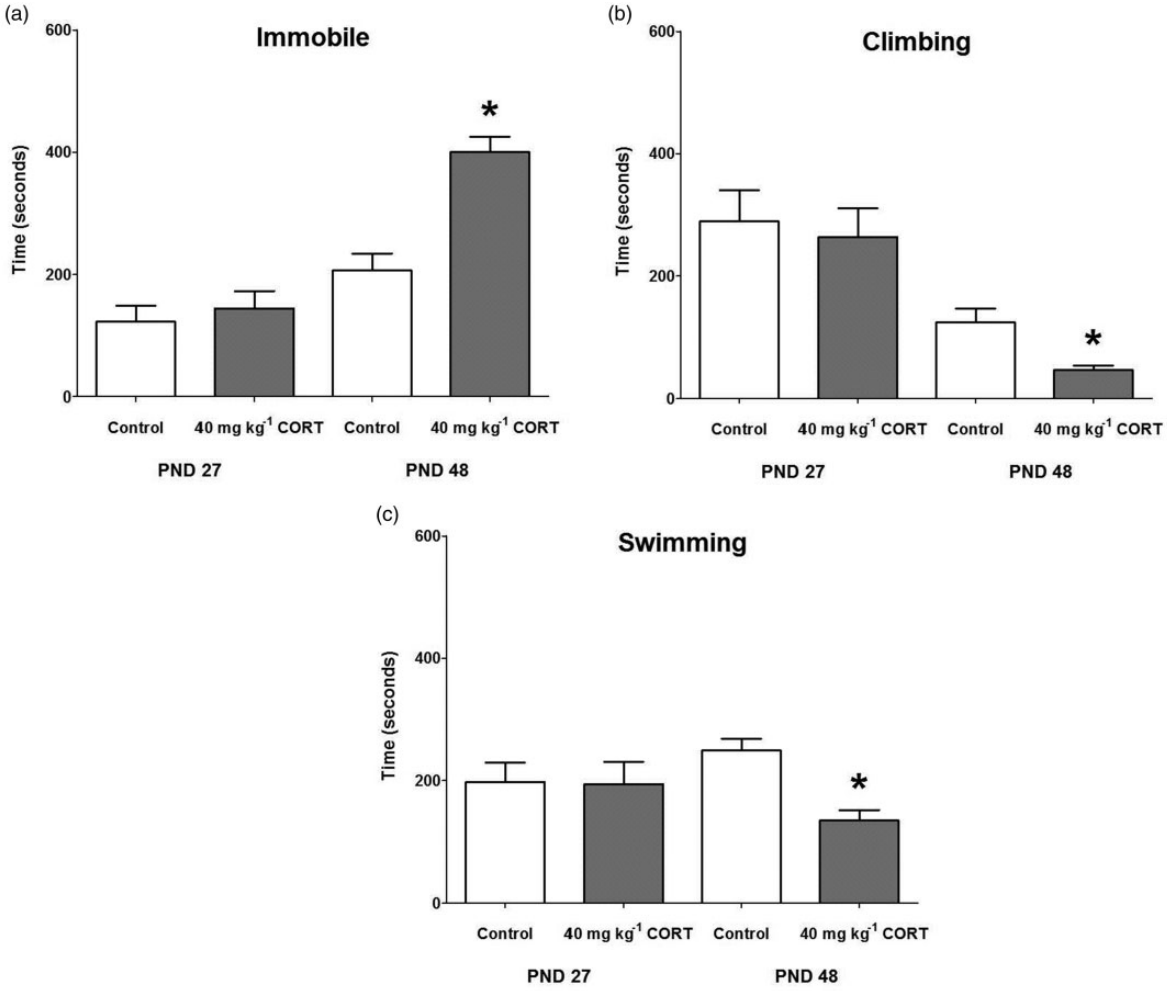

Figure 2. Modified forced swim test. Time spent immobile (a), climbing (b), or swimming (c) for pre-pubescent rats (PND 27) and peri-pubescent rats (PND 48) receiving CORT compared to control rats. PND 27 and PND 48 were independent experiments analyzed by unpaired two-tailed t tests. Values are expressed as mean ± SEM (* denotes $p \leq 0.05$, $n = 6$). PND: post-natal day.

Chronic CORT Treatment Does Not Induce Significant Behavioral Changes in Female Pre- and Peri-Pubescent Juvenile Rats as Measured by the SPT

There was no difference in sucrose preference in pre-pubescent ($p = 0.21$) and peri-pubescent ($p = 0.16$) juvenile rats injected with CORT compared to controls (Figure 3).

Chronic CORT Treatment Decreases Cell Proliferation in the SGZ of Female Peri-Pubescent Rats, but Not in Female Pre-Pubescent Rats

Whether chronic CORT treatment alters the basal rate of cell proliferation in the SGZ was tested using a cell proliferation paradigm (Figure 1). There were fewer BrdU-positive cells in the SGZ of the dorsal hippocampal dentate gyrus of female peri-pubescent rats injected with CORT compared to controls in the cell proliferation paradigm

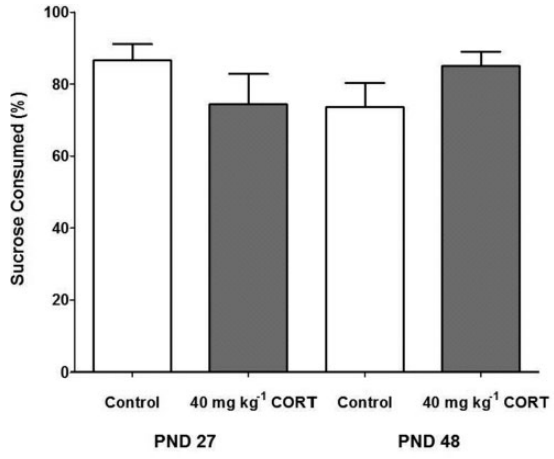

Figure 3. Sucrose preference test. Percent sucrose consumed for pre-pubescent rats (PND 27) and peri-pubescent rats (PND 48) receiving CORT compared to control rats. Data analysis: unpaired two-tailed t tests and values are expressed as mean ± SEM (* denotes $p \leq 0.05$, $n=6$). PND: post-natal day.

($p = 0.04$). Furthermore, there was no difference in the number of BrdU-positive cells in the hilus ($p = 0.23$) or total dentate gyrus ($p = 0.08$) of female peri-pubescent rat (Figure 4). There was no difference in BrdU-positive cells in the total dentate gyrus ($p = 0.23$) or the SGZ ($p = 0.69$) or the hilus ($p = 0.19$) of female pre-pubescent rats injected with CORT compared to controls in the cell proliferation paradigm (Figure 4). In conclusion, chronic CORT treatment decreases cell proliferation in the SGZ of peri-pubescent, but not in female pre-pubescent rats.

Chronic CORT Treatment Does Not Decrease Cell Survival in the Dentate Gyrus in Female Pre- or Peri-Pubescent Rats

Whether chronic CORT treatment alter neurogenesis in the dentate gyrus was tested using a survival paradigm where newborn cells are labeled up front with BrdU, then given two weeks to express the neuronal marker, NeuN (Figure 1). There was no difference in the number of BrdU-positive cells between CORT and vehicle treated female pre-pubescent ($p = 0.72$) or peri-pubescent ($p = 0.63$) rats. There was also no difference in the percentage of BrdU-/NeuN-positive cells in the granule cell layer (GCL) of the dentate gyrus of female pre-pubescent ($p = 0.68$) or peri-pubescent ($p = 0.64$) rats in the cell survival paradigm (Figure 5). This suggests that CORT does not affect the fate of neuronal precursor cells, which differentiate into neurons, oligodendrocytes, and astrocytes.

There Was an Inverse Correlation Between Immobility in the FST and Cell Proliferation in the SGZ in Peri-Pubescent Rats Only

There was an inverse correlation between the time spent immobile in the FST and BrdU-positive cells

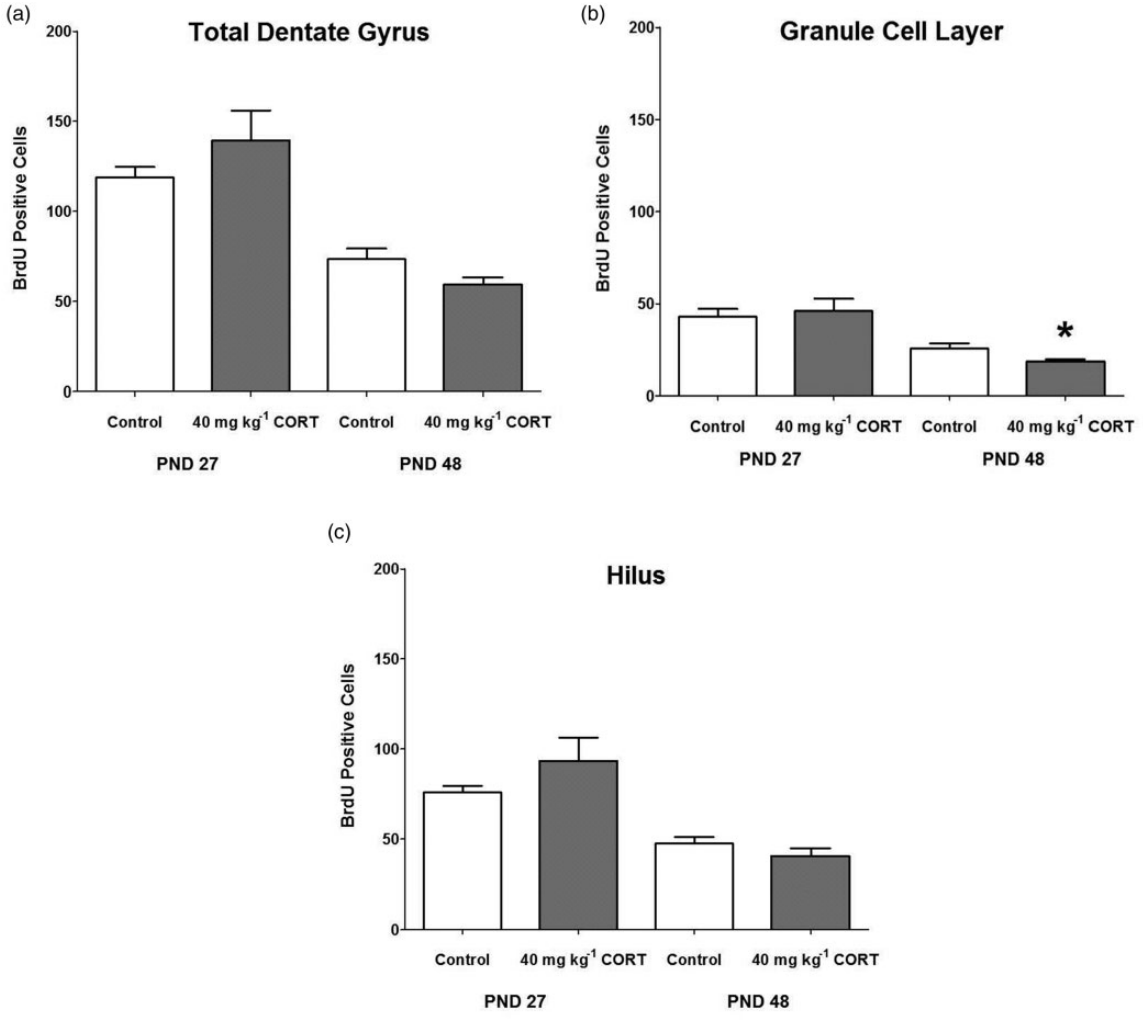

Figure 4. Cell proliferation. Number of BrdU-positive cells in the total dentate gyrus (a), GCL (b), or hilus (c) for pre-pubescent rats (PND 27) and peri-pubescent rats (PND 48) receiving CORT compared to control rats. Data analysis: unpaired two-tailed t tests and values are expressed as mean ± SEM (* denotes $p \leq 0.05$, n=6). PND: post-natal day; BrdU: 5-Bromo-2′-deoxyuridine.

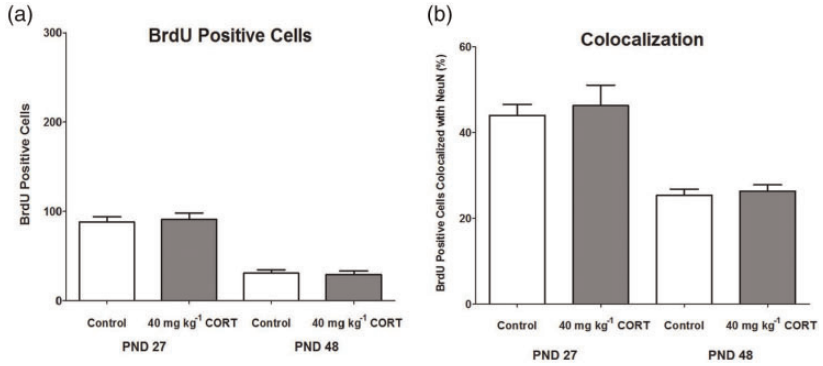

Figure 5. Cell survival. Number of BrdU-positive cells in the GCL (a) and percentage of colocalized cells in the GCL (b) for pre-pubescent rats (PND 27) and peri-pubescent rats (PND 48) receiving CORT compared to control rats. Data analysis: unpaired two-tailed t tests and values are expressed as mean ± SEM (* denotes $p \leq 0.05$, n=6). PND: post-natal day; BrdU: 5-Bromo-2′-deoxyuridine.

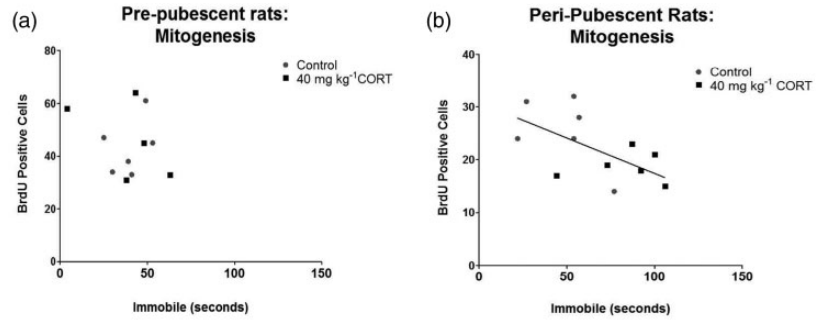

Figure 6. Immobility versus mitogenesis. Pearson product moment correlation coefficient comparing time spent immobile in the modified FST and BrdU-positive cells in the cell proliferation paradigm of female pre-pubescent rats (a) and peri-pubescent rats (b) ($p \leq 0.05$). There was a negative correlation between time spent immobile in the modified FST and the number of BrdU-positive cells in the GCL of female peri-pubescent rats. Data analysis: two-tailed Pearson product–moment correlation coefficient. BrdU: 5-Bromo-2′-deoxyuridine.

in the SGZ of peri-pubescent rats in the cell proliferation paradigm ($p = 0.05$; Figure 6). There was no correlation between time spent immobile in the FST and BrdU-positive cells in the dentate gyrus of peri-pubescent rats in the cell survival paradigm ($p = 0.84$) or in pre-pubescent rats in either the cell proliferation paradigm ($p = 0.49$) or the cell survival paradigm ($p = 0.19$; Figure 7).

Chronic CORT Administration Resulted in Limited Reductions in Rat Weight and Brain Weight

CORT administration resulted in limited reductions in weight gain, more so in the pre-pubescent rats (Figure 8(a)), making it seemly unlikely that changes in overall growth underlie the behavioral effects of CORT on peri-pubescent rats. Also, CORT administration was associated with a small, but significant, reduction in brain weight, in the pre-pubescent ups, but not in the peri-pubescent pups (Figure 8(b)).

Discussion

In this study, we found that CORT treatment induces depressive-like behavior as measured by the FST as well as decreases cell proliferation in the dorsal hippocampal SGZ in female peri-pubescent rats, but not pre-pubescent rats. Furthermore, our data revealed a negative correlation between the time spent immobile in the FST and cell proliferation in peri-pubescent rats. In pre-pubescent rats, CORT treatment did not induce depressive-like behavior or alter cell proliferation or newborn cell survival in the dentate gyrus. CORT was associated with limited reduction in growth, the pre-pubescent rats being more affected. It would be interesting to examine whether CORT affected sexual maturation in the pre-pubescent cohort. The results of this study suggest that the changes that occur during puberty may play a role in the effect of CORT in female rats, and further, that depressive-like behavior is correlated with a decrease in cell proliferation in the dentate gyrus of female juvenile rats.

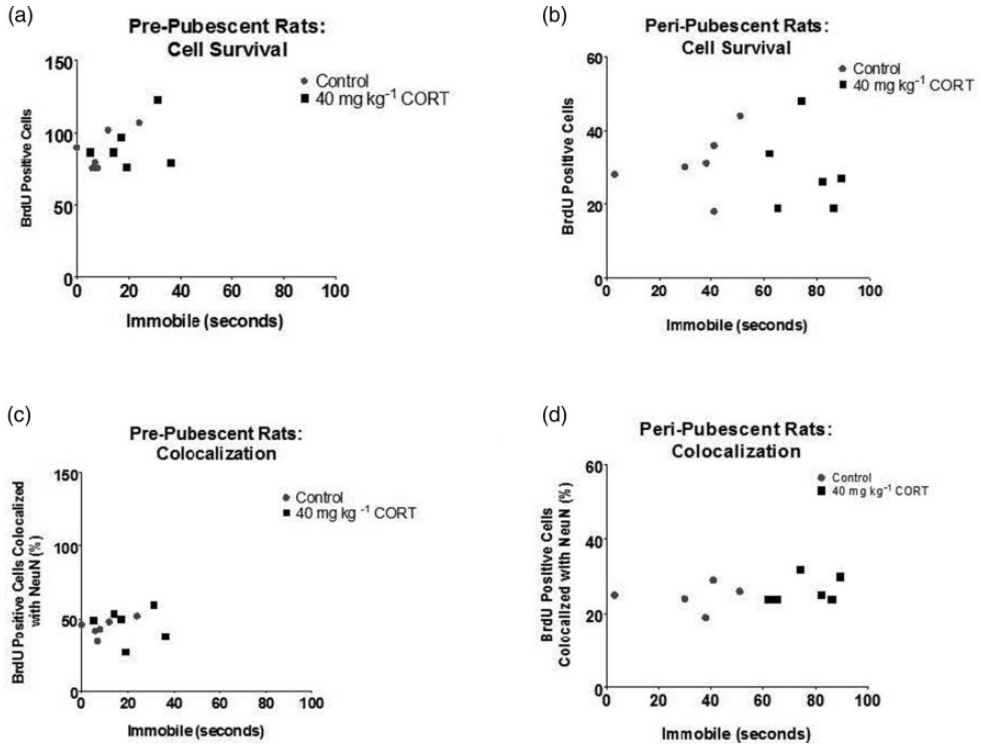

Figure 7. Immobility versus cell survival. Pearson product moment correlation coefficient comparing time spent immobile in the modified FST and BrdU-positive cells in the cell survival paradigm of female pre-pubescent rats (a) and peri-pubescent rats (b). Pearson product moment correlation coefficient comparing time spent immobile in the modified FST and percent colocalization in the dentate gyrus of female pre-pubescent rats (c) and peri-pubescent rats (d). Values are expressed as mean ± SEM (* denotes $p \leq 0.05$). Data analysis: two-tailed Pearson product–moment correlation coefficient. BrdU: 5-Bromo-2′-deoxyuridine.

Studies performed with female adult rats show that CORT treatment for 21 days, ranging from 10 to 40 mg kg^{-1} day^{-1}, increases the time spent immobile in the FST and decreases sucrose consumption in the SPT.[18,25,26,38] Conversely, our results demonstrate that female pre-pubescent rats treated with CORT did not have behavioral changes measured by the FST or the SPT. However, peri-pubescent rats injected with CORT spent more time immobile in the FST. It has been hypothesized there may be hypo-responsive period to circulating glucocorticoids during early post-natal development, which may serve as a protective mechanism.[39] The different results measured in pre-pubescent rats compared to peri-pubescent rats may demonstrate developmental differences in the effect of CORT. Puberty appears to be a critical time when CORT begins to exert effects on depressive-like behavior.

We found that chronic CORT treatment in peri-pubescent rats induced depressive-like behavior in the FST, but not the SPT. It is hypothesized that an increase in immobility in the FST measures depressive-like behaviors of motivation and despair, whereas a decrease in sucrose preference may indicate desensitization to the brain reward mechanism leading to anhedonic behavior.[9,18] Experiments using the Open Field Test and the Elevated Plus Maze Test indicated that there was no difference between control and CORT treated juvenile rats (data not shown). Therefore, the results from with the FST are likely not due to locomotor abnormalities or anxiolytic effects, but rather, depressive-like behavior. Therefore, the results of our study suggest that while chronic CORT treatment does induce depressive-like behavior as measured by the FST in peri-pubescent rats, but CORT does not alter anhedonic behavior and the brain reward mechanism in female pre- and peri-pubescent juvenile rats.

Chronic CORT treatment did not alter cell proliferation or newborn neuron survival in the dentate gyrus of female pre-pubescent juvenile rats. In contrast to our findings, studies performed on adult rodents found a strong correlation between chronic CORT treatment and significant hippocampal neurogenic changes.[25,26,28] These findings suggest that CORT has different effects on hippocampal neurogenesis during different stages of female rat development. They also further support the idea that juvenile rats may be hypo-responsive to increased levels of circulating CORT. In peri-pubescent rats, we found no difference in cell proliferation in the total dentate gyrus, but CORT treatment does cause a decrease in cell proliferation in the SGZ. There was no

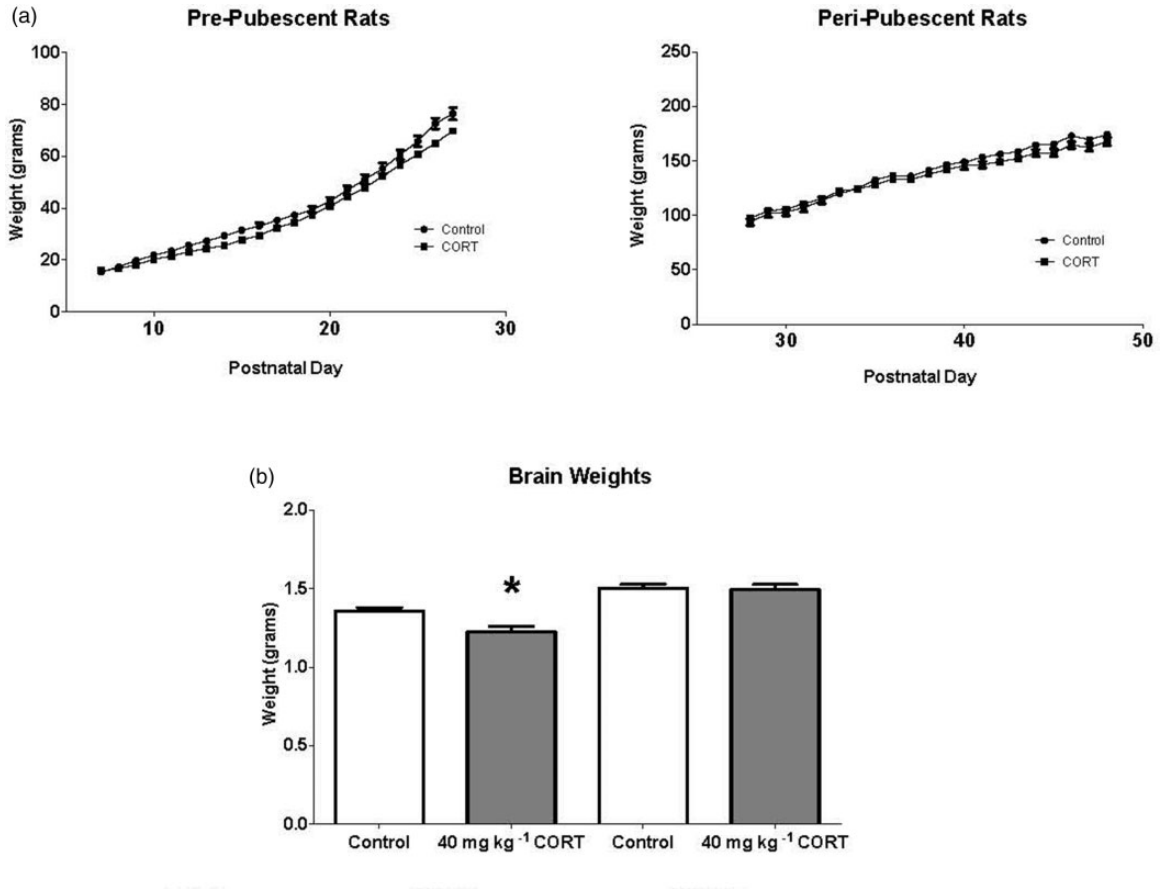

Figure 8. Rat and brain weights. Daily rat weights (a) and brain weights at PND 27 for pre-pubescent pups and PND 48 for peri-pubescent pups (b). Data analysis: ANOVA for repeated measures (a), unpaired two-tailed t tests, and values are expressed as mean ± SEM (* denotes $p \leq 0.05$, n=6) (b). PND: post-natal day.

difference in the hilus of the dentate gyrus between CORT and vehicle treated rats. The difference seen in cell proliferation in the SGZ reflects the effect of CORT treatment on cell proliferation in this brain region in female peri-pubescent, but not in pre-pubescent rats.

We found an inverse correlation between time spent immobile in the FST and cell proliferation in the SGZ in peri-pubescent rats. These results suggest that depressive-like behavior is associated with a decrease in cell proliferation in the dentate gyrus. Previous research has found correlations between depressive-like behavior and changes in hippocampal neurogenesis in adult rodents. Also, antidepressants have been found to stimulate hippocampal neurogenesis[25,26,28] and that blocking neurogenesis prevent effects of antidepressants in adult rodents.[40,41]

In summary, the results of this study suggest that CORT treatment has different physiological effects that induce depressive-like behavior and decrease hippocampal neurogenesis during different stages of female rat development. In adult rats, CORT treatment induces depressive-like behavior as measured by the FST and the SPT as well as alters neurogenesis in the hippocampus.[18,25,26,28] In contrast, the findings from this study demonstrate that CORT treatment does not have an effect on depressive-like behavior or neurogenesis in the dentate gyrus in female rats before puberty. This suggests that rats in this age group may be hyporesponsive to high levels of circulating glucocorticoids. However, chronic CORT treatment did induce depressive-like behavior and decrease cell proliferation in the SGZ in female peri-pubescent rats. It would appear that the changes that occur during puberty seem to increase susceptibility of female juvenile rats to high levels of CORT. Finally, our results suggest that a decrease in hippocampal cell proliferation may be associated with depressive-like behavior seen in the FST in female peri-pubescent rats. These findings suggest that stress-induced glucocorticoids may be involved in the etiology of depression during and after puberty in females. Importantly, chronic CORT treatment in female peri-pubescent rats may serve as a model of MDD in juvenile rodents.

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Declaration of Conflicting Interests

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