Chronic **high** corticosterone reduces neurogenesis in the dentate gyrus of adult male and female rats

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9 **List of Abbreviations**

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11 **CORT:** corticosterone
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14 **BrdU:** bromodeoxyuridine
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16 **HPA axis:** hypothalamus pituitary adrenal axis
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19 **NGS:** normal goat serum
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21 **PBS:** phosphate buffered saline
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23 **DCX:** doublecortin
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26 **GCL:** granule cell layer
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28 **SGZ:** subgranular zone
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31 **GR:** glucocorticoid receptors
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33 **MR:** mineralocorticoid receptor
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Abstract

Adult neurogenesis in the dentate gyrus of the hippocampus is altered with stress exposure and has been implicated in depression. High levels of corticosterone (CORT) suppress neurogenesis in the dentate gyrus of male rats. However both acute and chronic stress do not consistently reduce adult hippocampal neurogenesis in female rats. Therefore, this study was conducted to investigate the effect of different doses of corticosterone on hippocampal neurogenesis in male and female rats. Rats received 21 days of s.c. injections of either oil, 10mg/kg or 40mg/kg CORT. Subjects were perfused 24h after the last CORT injection and brains were analyzed for cell proliferation (Ki67-labeling) or immature neurons (doublecortin-labeling). Results show that in both males and females high CORT, but not low CORT, reduced both cell proliferation and the density of immature neurons in the dentate gyrus. Furthermore, high CORT males had reduced density in immature neurons in both the ventral and dorsal regions while high CORT females only showed the reduced density of immature neurons in the ventral hippocampus. The high dose of CORT disrupted the estrous cycle of females. Further, the low dose of CORT significantly reduced weight gain and increased basal CORT levels in males but not females, suggesting a greater vulnerability in males with the lower dose of CORT. Thus we find subtle sex differences in the response to chronic CORT on both body weight and in the dorsal dentate gyrus that may play a role in understanding different vulnerabilities to stress-related neuropsychiatric disorders between the sexes.

Keywords: Glucocorticoids, sex difference, hippocampus, stress, cell proliferation, cell survival

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7 The ability of the dentate gyrus of the hippocampus to produce new neurons in adulthood
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9 has been a focus of neuroscience research for the last decade. Hippocampal neurogenesis is
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11 associated with learning and memory and may play a role in neurodegenerative and
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13 neuropsychiatric disorders (for review see: Barha et al., 2009). In addition, both acute and
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15 chronic stress suppresses cell proliferation in the dentate gyrus of adult males in a variety of
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17 species (e.g. rat, mouse, tree shrew and marmosets; for a review see: Mirescu and Gould, 2006).
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19 Furthermore stress reduces hippocampal neurogenesis via adrenal steroids, as adrenalectomy
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21 eliminates the suppression in cell proliferation induced by exposure to a predator odor (Tanapat
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23 et al., 2001). In addition, adrenalectomy alone increases the number of proliferating cells, further
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25 supporting the idea that the adrenal steroid environment plays an important role in mediating the
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27 level of adult neurogenesis in the hippocampus (Cameron and Gould, 1994).
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33 Stress likely involves more complex processes to alter neurogenesis than the direct
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35 application of glucocorticoids (Jamieson et al., 1997; Fuchs et al., 2001), but direct treatment
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37 with corticosterone (CORT; the major glucocorticoid in rodents) may give us a more precise idea
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39 of how the effects of stress on neurogenesis are mediated in males and females. Glucocorticoid
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41 administration to male rats reduces both cell proliferation and survival of new cells in the
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43 hippocampus (Wong and Herbert, 2004; Kim et al., 2004; Wong and Herbert, 2006). For
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45 example, Wong and Herbert (2004) found that treatment with high CORT (40mg/kg) within 18
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47 days after BrdU injection led to a decrease in cell survival, while treatment with high CORT after
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49 18 days did not decrease cell survival in male rats. However, to date no one has examined the
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51 effect of direct application of glucocorticoids on neurogenesis in the hippocampus of female rats.
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53 This is an important proof of principle because acute and chronic stress can differentially affect
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hippocampal neurogenesis and behavior in male and female rats (for review see: Pawluski et al., 2009).

Most studies to date have investigated the effects of stress on adult hippocampal neurogenesis in males and less is known about the relationship between stress, glucocorticoids and neurogenesis in females. However the few studies that have examined the effects of stress on hippocampal neurogenesis in males and females have found differential effects between the sexes (Falconer and Galea, 2003; Westenbroek et al., 2004; Shors et al., 2007). For instance, acute exposure to fox odor or foot shock stress suppresses cell proliferation in the hippocampus of male, but not female, rats (Falconer and Galea, 2003; Shors et al., 2007). Considering the fact that males and females show different sensitivities to stress and/or different strategies to handle stress (for review see: Ter Horst et al., 2009) this suggests that females will show a different modulation of neurogenesis after chronic stress or glucocorticoid exposure.

Female rodents can have a differential neurogenic response to chronic stress compared to male rodents (Westenbroek et al., 2004). Chronic foot shock stress for 21 days decreased cell survival in males, but increased cell survival in females, suggesting that females have a different neurogenic reaction towards the same chronic stressor. However, a later experiment from the same laboratory showed females had reduced cell survival with chronic foot shock when BrdU was administered later in the stress regimen (Kuipers et al., 2006), suggesting that the effect of stress to reduce neurogenesis in the female depends on a number of factors. Furthermore, elevations in adrenal steroids do suppress cell proliferation in the hippocampus of adult female rats, as adrenalectomy eliminates estradiol-induced and postpartum-induced suppression in cell proliferation in female rats (Ormerod et al., 2003; Leuner et al., 2007). [A recent study by McCormick et al. \(2010\) showed that chronic social instability stress during adolescence does not affect cell proliferation but decreases short-term cell survival in adolescent females.](#) These studies

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4 suggest that females do show neurogenic alterations in response to adrenal steroids. Thus it
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6 remains to be seen whether there will be sex differences in the glucocorticoid response to
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8 neurogenesis in the hippocampus.
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11 There are also sex differences in the behavioral effects of chronic administration of
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13 glucocorticoids. Chronic administration of high levels of CORT (40mg/kg) leads to greater
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15 depressive-like behavior, body weight reduction and anxiety-like behavior in males compared to
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17 females (Kalynchuk et al., 2004). In line with this, a lower dose of CORT (20mg/kg) per day for
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19 20 days did not increase immobility in the forced swim test in female rats while it did so in male
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21 rats (Hill et al., 2003). Taken together these findings suggests that males and females show
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23 different vulnerabilities for behavioral effects of high levels of glucocorticoids, which in turn
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25 implies that elevated levels of CORT might also effect adult hippocampal neurogenesis
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27 differently in males and females.
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33 There is evidence that the dorsal and ventral hippocampus are structurally and
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35 functionally different. The dorsal hippocampus is thought to be more important for spatial
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37 memory while the ventral hippocampus is thought to be more involved in emotional, social and
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39 endocrine processing (Moser and Moser, 1998; Bannerman et al., 2002). The ventral
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41 hippocampus is connected to subcortical areas such as the hypothalamus and may thus potentially
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43 be more vulnerable to the effects of glucocorticoids, than the dorsal hippocampus (Maggio and
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45 Segal, 2009). In addition, there are differences in neurogenesis rates between the ventral and
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47 dorsal dentate gyrus (Ambrogini et al., 2000; Ferland et al., 2002; Dawirs et al., 1998) and
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49 recently it has been reported that learning affects cells differently in the dorsal and ventral
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51 hippocampus (Snyder et al., 2009a). Furthermore there are sex differences in the effects of
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53 chronic stress on hippocampal dendritic morphology or receptor levels that are depending on the
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55 region of the hippocampus (Gresack et al., 2009; Galea et al., 1997; Reich et al., 2009). For
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instance, chronic unpredictable mild stress reduces CB1 receptors in males but increases them in females in a region-specific manner (Reich et al., 2009). Thus we hypothesized that there are sex differences and dorsal/ventral hippocampus differences in neurogenesis levels in response to corticosterone..

Therefore, the current study was conducted to investigate the effect of 21 days of a low (10mg/kg) or high dose (40mg/kg) of CORT given to male or female rats on hippocampal cell proliferation and neurogenesis. We hypothesized that both males and females would show a reduction in cell proliferation and in the survival of immature neurons after the high dose of CORT, but that the low dose would affect males more than females. Further we expected regional differences with a greater effect of chronic CORT on neurogenesis in the ventral region than the dorsal region of the dentate gyrus.

Experimental procedures

Animals

All protocols were in accordance with ethical guidelines set by the Canada Council for Animal Care and were approved by the University of British Columbia Animal Care Committee. Fifteen female and fifteen male Sprague–Dawley rats were obtained from Charles River (Saint Constant, QC, Canada) at approximately 3 months of age. After one week rats were housed individually in clear polyurethane bins (48 × 27 × 20 cm) with absorbent bedding and were given Purina rat chow and tap water ad libitum. Rats were maintained in a 12 h: 12 h light/dark cycle (lights on at 7:30 a.m.).

Corticosterone (CORT) preparation and injection

All animals were handled for 5 min per day 4 days prior to the start of the experiment. Male and female rats were each divided into three groups (n=5 per group) based on the type of injections they received (oil, low CORT, high CORT). Each rat received daily subcutaneous injections of either sesame oil (control (0.2ml)) or CORT (low: 10mg/kg or high: 40mg/kg, Sigma-Aldrich, St. Louis, MO, USA) for 21 consecutive days. Body weight was measured every 4 days and the dose adjusted accordingly. All injections were given between 9 a.m. and 11 a.m. daily. In addition, females were lavaged daily to determine their estrous cycle phase. In the early morning of the last day of injections, blood samples were taken from the tail to assess CORT levels. All blood samples were collected within 3 minutes of touching the animal's cage. Twenty-four hours after the last CORT injection animals were deeply anesthetized with sodium pentobarbital and then perfused with saline followed by 4% paraformaldehyde.

Hormone preparation

An emulsion of CORT was prepared every few days at a concentration of 10 or 40 mg/ml by mixing CORT with 10% ethanol in sesame oil. The high dose was chosen on the basis of previous findings showing that this dose of CORT led to reduced neurogenesis in the dentate gyrus of adult male rats (Wong and Herbert, 2004) and caused elevated blood levels of CORT over a 24h period (Hellsten et al., 2002; Sapolsky, 1985; Johnson et al., 2006). The lower dose was chosen as this dose suppresses HPA axis function after 21 days of exposure but does not increase depressive-like behavior in male rats (Johnson et al., 2006).

Corticosterone radioimmunoassay

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4 Blood samples were collected on the last day of injections and stored overnight at 4°C. Serum
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6 was collected after blood samples were centrifuged at $10,000 \times g$ for 10 min. Serum CORT was
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8 assayed using a commercial radioimmunoassay kit (MP Biomedicals, Orangeburg, NY) with an
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10 intra-assay coefficient of variation less than 7%. All samples were run in duplicates.
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15 16 **Estrous cycle determination** 17

18 Estrous cycle phase was determined by daily vaginal lavage. For this a pipette was inserted 1mm
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20 into the vagina and a few drops of sterile water were released into the vagina which were then
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22 immediately extracted. Contents of the pipette were caught on a plain microscope slide, and
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24 immediately stained with cresyl violet acetate. Slides were examined under 10x objective and
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26 estrous phase was estimated based on criteria of the cells in the vaginal mucus (Marcondes et al.,
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28 2002). Briefly, observation of mainly nucleated epithelial cells was considered proestrus, mainly
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30 unnucleated cornified cells was defined as estrus, mostly leukocytes identified diestrus and a
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32 mixture of cells or lack of a dominating cell type was considered metestrus. To account for the
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34 effect of the proestrus to increase the number of proliferating cells (Tanapat et al., 1999), the
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36 female estrous' cycle phase was investigated, however none of the females were in proestrus at
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38 that the time of perfusions so none were excluded from the analysis.
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48 **Immunohistochemistry** 49

50 After perfusion extracted brains were stored in 4% paraformaldehyde for 24 h, and then
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52 transferred into a solution of phosphate-buffered sucrose solution (30%) for a minimum 48 h.
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54 Brains were sliced through the entire extent of the dentate gyrus using a vibratome (Leica
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56 VT1000S) at 40 μm thickness. Sections were later stained for Ki67 (endogenous protein present
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58 in actively cycling cells) to label cells undergoing proliferation and for doublecortin (endogenous
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protein present in immature neurons) to label immature neurons of approximately 1-14d of age (Brown et al., 2003).

Ki67

If not otherwise stated sections were always rinsed three times for 10 min in PBS between each step of the protocol. Sections were pretreated with 0.6% H₂O₂ for 30 min and then transferred to a primary antibody solution containing a 1: 1000 Rabbit anti-Ki67 monoclonal antibody (Vector Laboratories), 1 % NGS and 0.5% Triton-X in 0.1M PBS for 24 hrs. The tissue was then incubated in a secondary antibody solution containing 1:200 Goat anti-rabbit (Vector Laboratories) in 0.1M PBS for 2 hours, followed by an incubation in an avidin–biotin horseradish peroxidase complex (ABC Elite Kit; 1:200; Vector Laboratories) for 40 min. The sections were developed with 0.02% diaminobenzidine (DAB; Sigma Aldrich Chemicals) with 0.0003% H₂O₂ for 5 min and then mounted on gel-coated glass slides and dried overnight. All sections were lightly counterstained with cresyl violet, dehydrated and coverslipped with Permount (Fisher Scientific, Nepean, CDA).

Doublecortin

For doublecortin staining sections were washed between each step with PBS for ten minutes each. Sections were treated with 0.3% hydrogen peroxidase for 30 min before transfer into the primary antibody solution (1:1000, goat anti-doublecortin (Santa Cruz Biotech) + 0.04% Triton-X in PBS). Twenty-four hours later sections were incubated in the secondary anti-body (1:500, rabbit anti-goat (Vector Laboratories) in PBS, followed by 4 hour incubation in ABC complex (ABC Elite Kit; 1:1000; Vector Laboratories). Then sections were reacted for approximately 5

min in 0.05% diaminobenzidine (DAB; Sigma Aldrich Chemicals) with 0.0003% H₂O₂ and 2.5% Nickel, mounted on slides, dried overnight, dehydrated and coverslipped with Permount.

Cell counting

Figure 1 shows an example of Ki67-labeled and DCX-labeled cells in the dorsal dentate gyrus.

[Figure 1 here] Ki67- and DCX-labeled cells were counted in every 10th section throughout the entire granule cell layer (GCL), including the subgranular zone (SGZ), separately for the dorsal and ventral part of the dentate gyrus using a Nikon E600 light microscope. Ki67-labeled cells were counted at 1000x magnification and DCX-labeled cells at 400x magnification. The area of the dentate gyrus was measured using an image analysis software (ImageJ, NIH) and the total volume for the dorsal and ventral dentate gyrus was estimated using Cavalieri's principle (Gundersen and Jensen, 1987), i.e. by multiplying the total area measured by the distance between measured sections (400 µm). The total number of Ki67- and DCX-labeled cells for each area (dorsal and ventral) was estimated by multiplying the total number counted by 10 as previously described (Kronenberg et al., 2003; Eadie et al., 2005) and densities were calculated by dividing the total number of cells by the volume of that area of the dentate gyrus. Counting was performed by an experimenter blind to the animal's history. Due to these potential functional differences between the dorsal and ventral hippocampus, we examined the density of cells in both the dorsal and ventral regions. For this, all slices on which the ventral portion of the dentate gyrus at the bottom of the slice was obviously present (-5.2 mm to -6.7mm below Bregma according to the dorso-ventral coordinates of Paxinos and Watson (2005)) were considered 'ventral', while all slices rostral to this section were considered 'dorsal' (-1.8mm to -5.2mm below Bregma) [resulting in 7-8 dorsal and 3-4 ventral slices.](#)

Data analyses

An analysis of variance (ANOVA) was used to analyze the change in body weight (day -1 to day 21 of CORT injections) in males and females over the 21 days with treatment (control, low CORT, high CORT) and sex (male, female) as the between-subjects factors. For serum CORT levels taken on the last day of injections, an ANOVA with sex and treatment used as between-subjects factor was conducted. The number of completed 4-5 day estrous cycles during the 21 day injection period was analyzed using a one-way ANOVA with treatment as between-subject factor. Volume of the dentate gyrus and density of Ki67- and DCX-labeled cells in the dentate gyrus were analyzed with a repeated-measures ANOVA with area (dorsal, ventral) as within-subjects factors and sex and treatment as between-subjects factors. All post hoc tests utilized Newman Keuls. *A priori comparisons were subjected to a Bonferroni correction.*

Results

High CORT significantly attenuated weight gain in males and females, while low CORT significantly attenuated weight gain in males only.

Due to individual and sex differences in body weight, we analyzed *percentage of* weight gain or loss. We calculated this by *setting the weight on the day before the 1st CORT injection as 100% and calculating the percentage gain or loss for body weight on day 22.* An ANOVA revealed a significant effect of treatment ($F(2, 24)=42.18$, $p<.001$) and sex ($F(1, 24)=6.55$, $p=.017$) as well as an interaction effect ($F(2, 24)=6.04$, $p=.007$). Subsequent post-hoc analysis revealed that the high CORT dose caused a weight loss of about ~2% in females ($p=0.002$) and ~5% in males ($p<0.001$), while controls gained ~10% and ~20%, respectively. The low dose reduced weight

gain non-significantly in females to ~6% gain between day 1 and day 21 ($p=.21$) and significantly in males (~13%, $p=.05$) (Fig 2). *[Figure 2 here]*

High CORT significantly increased CORT levels in both male and female rats, while low CORT increased CORT levels in males only

The ANOVA revealed a significant effect of treatment ($F(2, 20)=71.88$, $p<.001$) and an interaction of treatment by sex ($F(2, 20)=3.75$, $p=.04$), but no main effect of sex ($F(1, 20)=.27$, $p=.61$). Subsequent post-hoc tests showed that both males and females treated with the high dose of CORT had significantly higher serum levels of CORT than controls ($p's<.001$), while only low dose males ($p<.005$), but not females ($p=.31$) showed significantly higher levels of serum CORT than controls (Fig 3). *[Figure 3 here]*

High levels of CORT disrupt the female estrous cycle

An ANOVA on the number of completed estrous cycles during the 21 day injection period revealed a significant effect of treatment ($(F(2, 12)=6.85$, $p=.01$, Fig. 4). Subsequent post-hoc tests indicated that high CORT females underwent fewer cycles ($p=.008$) compared to controls, while low CORT females tended ($p=.09$) towards fewer cycles compared to controls. Figure 5 shows examples of individual cycling rats from each group. Qualitative observations showed that high CORT females were mainly in a met- or diestrous-like phase, but recovered a normal cycling pattern towards the end of the treatment. *[Figures 4 and 5 here]*

CORT treatment did not change the volume of the dentate gyrus, but males had a larger dentate gyrus than females

CORT treatment had no significant effect on the volume of the dentate gyrus (main effect of treatment: $p=0.27$), but there were main significant effects of sex ($F(1, 24)=7.34$, $p=0.01$) and area ($F(1, 24)=36.19$, $p<0.001$; Table 1). Post-hoc tests showed that males had larger dentate gyrus volumes than females and the dorsal region was larger than the ventral volume. There were no significant interaction effects (all p 's > 0.05). Due to the differences in volumes [between the sexes](#), we calculated the density of both Ki67-labeled and DCX-labeled cells.

High levels of CORT significantly suppressed cell proliferation in both males and females

There was a significant main effect of treatment on the density of Ki67-labeled cells ($F(2,23)=6.38$, $p=0.006$) and a main effect of area ($F(1,23)=13.56$, $p=0.001$), but no main effect of sex ($p=0.65$) or any significant interactions (all P 's >0.05). Post-hoc tests showed that the high CORT groups, regardless of sex, had a significantly lower density of Ki67-labeled cells in the dentate gyrus than controls ($p=0.005$), while the low CORT groups tended to have reduced cell proliferation density compared to controls ($p=0.08$; Fig. 6). As we expected to see sex differences in the dorsal and ventral regions we performed a priori tests to compare the effect of CORT on males and females. Results reveal that high CORT females have significantly lower Ki67-labeled cell density in the ventral region of the dentate gyrus than oil females ($p=0.01$) but not the dorsal region ($p= 0.12$). There were no significant differences between treatment groups in males (dorsal: $p=0.43$; ventral: $p=0.13$). *[Figure 6 here]*.

High levels of CORT significantly reduced number of DCX-labeled cells in both male and female rats

There was a significant effect of treatment ($F(2, 23)=18.74$, $p=.00001$), but no other main or interaction effects (all $p>0.05$). Post-hoc tests demonstrated that high CORT ($p=0.0001$), but not

low CORT ($p=0.22$) reduced the number of DCX-labeled cells in the dentate gyrus compared to controls (Fig 7). As we expected to see sex and region-specific differences in immature neuron survival we performed a priori tests. High CORT females had a significantly lower density of DCX-labeled cells in the ventral ($p<0.001$) but not dorsal part ($p=0.17$) of the dentate gyrus compared to oil females. High CORT males had a significantly lower density of DCX-labeled cells for both dorsal and ventral regions of the dentate gyrus compared to controls (ventral: $p<0.001$, dorsal: $p=0.006$). *[Figure 7 here]*

Discussion

The current study is the first to directly compare neurogenesis in the dorsal and ventral dentate gyrus in response to different doses of CORT in both male and female rats. Here we show that high levels of corticosterone suppress cell proliferation and immature neuron survival in both male and female rats. Interestingly, while the low dose of CORT did not significantly reduce hippocampal cell proliferation or neurogenesis in either sex, males showed significantly attenuated weight gain and increased baseline levels of CORT at the end of the 21 day period compared to females in response to low CORT. Furthermore we show evidence that there are subtle sex differences in the region of the dentate gyrus that was affected in response to high CORT, with female, but not male, rats showing a suppression in cell proliferation in the ventral region and male, but not female, rats showing fewer immature neurons surviving in the dorsal region of the dentate gyrus.

Low levels of CORT attenuate weight gain and increase CORT levels only in males whereas high levels of CORT interrupt body weight gain in both sexes and estrous cyclicity in females

We found that low CORT significantly attenuated weight gain only in males, while high CORT significantly attenuated weight gain in both males and females. Our results are consistent with previous studies showing a attenuated body weight gain after chronic exposure to high levels corticosterone in males and females with a greater relative effect in males (Johnson et al., 2006; Kalynchuk et al., 2004; Bridges et al., 2008). Furthermore our study is consistent with previous studies showing that chronic low corticosterone reduced body weight gain in males (Johnson et al., 2006; Bridges et al., 2008). In humans weight loss is associated with depressive symptoms (Chaput et al., 2008; Faulconbridge et al., 2009) and weight change and appetite disturbances are listed as single compound symptoms of depression by the DSM IV.

In the present study only high CORT significantly disrupted the estrous cycle of females. High CORT female rats showed a pattern of a met- or diestrous-like phase continuously for the first 10 days after high CORT administration. These results are consistent with previous studies showing a stress-induced disruption in normal cycling (Herzog et al., 2009; Baker et al., 2006; Konkle et al., 2003). In addition, our data showing a recovery of a normal cycling pattern is in congruence with results from Kalynchuk et al. (2004), who found that female rats showed all phases of the estrous cycle during behavioral testing following 21 days of chronic CORT (40mg/kg) injections. The initial disruption of estrous cycling followed by a recovery in females suggests that females adapted quickly to high CORT levels.

Chronic treatment with CORT does not change the volume of dentate gyrus

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4 Due to the differences in body weight between vehicle and CORT-treated animals and the fact
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6 that chronic stress can result in hippocampal atrophy and volume reduction (McEwen, 1999), we
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8 measured the volume of the dentate gyrus to account for potential differences between the groups
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10 and the sexes. However, in the present study we did not see a reduction in dentate gyrus volume
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12 in CORT-treated animals. This finding is in line with previous studies using CORT pellets or
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14 injections which also failed to find volumetric changes or changes in overall cell number of the
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16 hippocampus or dentate gyrus (Bodnoff et al., 1995; Bridges et al., 2008; Sousa et al., 1998).
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18 However, very prolonged exposure to chronic stress (6 weeks) induces a reduction in overall
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20 hippocampal volume and cell number, which is not seen after 3 weeks of stress (Pham et al.,
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22 2003), suggesting that under more prolonged exposure to elevated CORT levels one will see a
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24 reduction in dentate gyrus volume. In addition, a meta-analysis by McKinnon and colleagues
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26 (2009) showed that depression is associated with decreased hippocampal volume in young and
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28 older patients who have suffered from depression for at least 2 years, but not prior to 2 years.
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38 **Chronic high corticosterone reduces hippocampal cell proliferation in females and cell** 39 40 **survival in both males and females**

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42 In the present study a chronic high dose of CORT reduces hippocampal cell proliferation (Ki67-
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44 labeled cells) and immature neuron survival (DCX-labeled cells) in both sexes, while low CORT
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46 did not significantly alter cell proliferation or survival in either sex. These results are consistent
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48 with previous findings showing that chronic stress suppresses cell survival in male (Westenbroek
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50 et al., 2004; Oomen et al., 2007) and female (Kuipers et al., 2006) rodents. Furthermore our
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52 findings are consistent with elegant studies from Herbert and colleagues documenting that the
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54 same high dose of corticosterone decreases cell proliferation and survival in the dentate gyrus of
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56 adult male rats (Wong and Herbert, 2004; Wong and Herbert, 2006). Westenbroek et al. (2004)
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4 reported that isolated male rats showed reduced, while female rats showed enhanced, cell survival
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6 in the dentate gyrus after 21 days of foot shock, suggesting sex differences in the effect of stress
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8 on hippocampal neurogenesis. However, in a later study using the same stress protocol female
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10 rats showed a decrease in BrdU-labeled cells (Kuipers (2006). One difference between the
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12 studies was that BrdU was administered at different times after the start of the stress regimen
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14 (day 13-16 of the chronic stressor (Kuipers et al., 2006) vs. day 3-7 (Westenbroek et al., 2004).
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16 Thus demonstrating that the effect of chronic stress on hippocampal neurogenesis in the female
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18 depends on the timing of the BrdU labeling compared to the treatment.
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24 Our findings that low CORT does not affect cell proliferation are consistent with previous
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26 studies in males (Nacher et al., 2004; Mayer et al., 2006). However, while in the present study we
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28 found that low CORT did not significantly influence survival of immature neurons, one study
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30 showed that the same dose of low CORT did reduce both BrdU-labeled and DCX-labeled cell
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32 survival in young male Wistar rats (Mayer et al., 2006). This inconsistency between the present
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34 study and that of Mayer's study (2006) may be due to strain or age differences, as in the present
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36 study our rats were approximately 3 months old and thus adults, at the initiation of treatment,
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38 while in the Mayer study the rats were about 1.5 months old, and thus in late adolescence, at the
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40 initiation of treatment.
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46 In the present study we found suggestions of regional specific sex-related differences in
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48 the effects of CORT, with high CORT females, but not in high CORT males, showing a reduced
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50 density of proliferating cells in the ventral area of the dentate gyrus relative to controls (see
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52 Fig.6). In addition, chronic high CORT treatment reduced the density of DCX-labeled cells in
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54 the dorsal dentate gyrus in male, but not in female, rats (see Fig. 7). Intriguingly, the recovery of
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56 estrous cycling towards the end of the treatment with high CORT suggests that females adapt to
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58 the high CORT levels, which might in turn explain why high CORT did not reduce the density of
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4 immature neurons in the dorsal dentate gyrus. This is in line with previous studies showing
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6 different stress sensitivities for males and females. For instance, chronic restraint stress causes
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8 apical dendritic atrophy in male, but not female, rats and basal dendritic atrophy in female, but
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10 not male, rats (Galea et al., 1997), while acute inescapable stress impairs eyeblink conditioning in
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12 females but not in males (Waddell et al., 2008). Taken together, these results suggest that sex,
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14 age, the timing of the stressor and timing of the stressor relative to labeling the production of new
15
16 neurons are very important to determine the effects of stress or elevated glucocorticoids on
17
18 neurogenesis. Noteworthy, many of the newly produced cells die within a short period of time,
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20 which explains why we see almost as many Ki67-labeled cells as DCX-labeled cells and further
21
22 underlines the importance of investigating and understanding the different phases of neurogenesis
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24 (cell proliferation, differentiation, migration and survival) as each phase may be differently
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26 affected by stress.
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36 **Regional dependent differences in hippocampal function and relation to neurogenesis**

37
38 Until recently the dissociation between dorsal and ventral function of the hippocampus has not
39
40 received much attention and only a few studies have reported basal differences in the number or
41
42 density of new cells in dorsal vs. ventral areas (Ambrogini et al., 2000; Dawirs et al., 1998;
43
44 Ferland et al., 2002; Snyder et al., 2009a; 2009b; Eadie et al., 2009). Considering that the ventral
45
46 part is connected to subcortical structures such as the hypothalamus and is involved in
47
48 neuroendocrine processing (for review see: Moser and Moser, 1998; Bannerman et al., 2002) it is
49
50 conceivable that this area is more sensitive to the effects of elevated cerebral glucocorticoid
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52 levels on neurogenesis.
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58 Our results show that the ventral portion of the dentate gyrus may be more sensitive to the
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60 effects of chronic glucocorticoids than the dorsal portion in female rats. We found a significant
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4 reduction in ventral Ki67-labeled cell density in females and ventral DCX-labeled cell density in
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6 males and females after high CORT exposure compared to oil controls, while the DCX-labeled
7
8 cell density was reduced in the dorsal region only in males (Fig 6 and 7). This is in line with
9
10 previous studies showing that chronic mild stress decreased the number of DCX-labeled cells and
11
12 the total number of granule cells in the ventral dentate gyrus in male rats (Jayatissa et al., 2008).
13
14 Only a few studies have examined dorsal/ventral differences and have found that the ventral
15
16 portion of the dentate gyrus is more vulnerable to changes in neurogenesis after particular
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18 external manipulations (Snyder et al., 2009a; Banasr et al., 2006; Dalla et al., 2009). For instance,
19
20 chronic treatment with agomelatine, an antidepressant, for 3 weeks increased cell proliferation
21
22 and survival only in the ventral dentate gyrus, while treatment for 6 weeks increased survival in
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24 the entire dentate gyrus in males (Banasr et al., 2006).
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31 Interestingly, the two types of corticosterone receptors differ in their distribution pattern
32
33 between the dorsal and the ventral region with more glucocorticoid receptors (GR) found in the
34
35 dorsal region and more mineralocorticoid receptors (MR) found in the ventral region.
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37 Furthermore the ventral and dorsal density of GR and MR are differently affected by chronic
38
39 stress in males (Robertson et al., 2005). Studies have reported sex differences in the overall
40
41 expression of glucocorticoid receptors in the hippocampus (e.g. Mashoodh et al., 2008;
42
43 Karandrea et al., 2002), and acute stress differently regulates hippocampal mRNA levels for GR
44
45 and MR in males and females (Karandrea et al., 2002). However, to our knowledge, it is not
46
47 known whether there is a sex difference in the GR/MR receptor distribution or sensitivity
48
49 between the ventral and dorsal dentate gyrus. Sex differences in the regional effects of stress have
50
51 also been reported for serotonin receptors (Van den Hove et al., 2006). For instance, prenatal
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53 stress significantly decreased 5HT1A receptor levels in the ventral hippocampus of 4 week old
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55 male rats but not of female rats or in the dorsal hippocampus of either sex (Van den Hove et al.,
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2006). A few studies, including the present one, suggest that there are sex-differences in the effect of stress or learning on neurogenesis in the dorsal versus ventral dentate gyrus (Dalla et al., 2009; Zuena et al., 2008). For instance, Dalla et al. (2009) showed that females had more surviving cells after a particular learning paradigm than males which was most prominent in the ventral dentate gyrus.

Conclusions

In conclusion our results further support the notion that males and females exhibit different glucocorticoid/stress sensitivities and/or perhaps coping strategies. It is conceivable that ovarian steroids play a role in mediating the effect of chronic high levels of corticosterone on the different stages of neurogenesis and might explain the different effects of stress and/or glucocorticoids on cell proliferation and survival reported here and in other studies. Further our results suggest that neurogenesis in the dorsal and ventral portion of the dentate gyrus is differentially modulated by corticosterone but further studies need to be undertaken to investigate the effect of corticosterone administered directly into each of these regions in males and females. Taken together, more studies are needed to identify the unique function of gonadal hormones in modulating the stress-effect on neurogenesis and more attention needs to be paid to the functional heterogeneity of the hippocampal structure.

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Table 1: Volume of the dentate gyrus

	Area	Control	low CORT (10mg/kg)	high CORT (40mg/kg)
Males	dorsal	2.14 ± 0.10	2.05 ± 0.10	2.38 ± 0.18
	ventral	1.70 ± 0.12	1.67 ± 0.14	1.67 ± 0.15
Females	dorsal	1.91 ± 0.12	1.93 ± 0.06	2.05 ± 0.18
	ventral	1.22 ± 0.08	1.69 ± 0.24	1.52 ± 0.17

There was no significant effect of treatment in the volume of the dentate gyrus but males had a significantly larger volume than females. The dorsal region of the dentate gyrus was larger than the ventral region.

Figure legends

Fig. 1: Representative pictures of (A) Ki67-labeled and (B and C) Doublecortin-labeled cells in the dorsal dentate gyrus.

Fig. 2: Percent of weight gain or loss during 21 days of treatment. High doses of CORT caused both males and females to show attenuated weight gain over the 21 days. Low doses of CORT caused significantly attenuated weight gain in males only. * $p=0.5$, *** $p<.001$ compared to same sex controls.

Fig. 3: Levels of corticosterone (CORT) were measured in the early morning of the last day of treatment (i.e. before the daily CORT injection). Males showed higher serum CORT levels after the low dose (10mg/kg) as well as after the high dose (40mg/kg) administration, while females only showed significantly higher levels of CORT after the high dose. ** $p<0.01$, *** $p<0.001$ compared to same sex controls.

Fig. 4: Number of completed estrous cycles during 21 days of corticosterone injections. High CORT group completed significantly fewer cycles (** = $p<.01$) compared to controls.

Fig. 5: Examples of estrous cycles of individual rats from each treatment group. Females from the high CORT group were mainly in a met- or diestrous-like phase for the first 10 days after starting the CORT injections, but recovered a normal cycling pattern towards the end of the treatment.

Fig. 6: Density of Ki67-labeled cells in the dorsal and ventral dentate gyrus. High CORT significantly reduced density of Ki67-labeled cells compared to controls (** $p=0.005$). Females had lower Ki67-labeled cell density in the ventral portion of the dentate gyrus compared to controls (α : $p=0.01$).

Fig. 7: Density of Doublecortin (DCX)-labeled cells in the dorsal and ventral dentate gyrus. High CORT treatment resulted in fewer immature neurons compared to oil controls (***) $p<0.001$). Males had significantly fewer DCX-labeled cells in the dorsal and ventral regions, while females had significantly fewer DCX-labeled cells in the ventral region only (α : $p<0.001$, β : $p<0.01$ compared to same sex controls of same area).

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