ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Research paper Acta Neurobiol Exp 2008, 68: 131–138

# The effects of chronic corticosterone on hippocampal astrocyte numbers: A comparison of male and female Wistar rats

**Nikola Bridges[1]*, Karel Šlais[2,3], and Eva Syková[2,4]**

[1]School of Psychology, University of Central Lancashire, Preston, Lancashire, UK, *Email: njbridges@uclan.ac.uk; [2]Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, Department of Neuroscience, Prague, Czech Republic; [3]Department of Pharmacology, Faculty of Medicine, Masaryk University, Brno, Czech Republic; [4]Department of Neuroscience and Center for Cell Therapy and Tissue Repair, Charles University, Second Medical Faculty, Prague, Czech Republic

Chronic stress and/or glucocorticoid administration produces atrophy of hippocampal neurons. However, evidence of the impact of glucocorticoids on glial cells, especially in both males and females, is limited. In the present study, we investigated the total percentage body weight, hippocampal volume and hippocampal astrocyte numbers following chronic corticosterone treatment in male and female Wistar rats. Males had greater left and right hippocampal volumes overall, but no effect on hippocampal volume was seen after corticosterone treatment. Total body weight was dose-dependently lower in both sexes, but the decrease was more prominent in male rats. Corticosterone treatment dose-dependently increased astrocyte numbers in the CA1 region, but not in the lateral and medial CA3 hippocampal regions. This increase was similar in both male and female rats. The astrogliosis observed following chronic corticosterone may have implications for extrasynaptic communication and neuron-glia interactions and is similar to changes in the astrocytic population observed in aged rats.

Key words: astrocyte, corticosterone, hippocampus, rat, sex differences

## INTRODUCTION

The hippocampus is an important site for the action of corticosteroids and the response to stress. It is also particularly vulnerable to damage in situations of excessive corticosterone concentrations. In addition, atrophy of the hippocampus and volume reduction are features associated with neuropsychiatric conditions such as post-traumatic stress disorder (PTSD) (Sapolsky 2001, Lindauer et al. 2004, Shin et al. 2004, Wignall et al. 2004) and are also observed in animal models of chronic stress (Lucassen et al. 2001).

High glucocorticoid levels produce atrophic changes in the hippocampus similar to those exhibited during aging, and it appears that the hippocampus becomes more vulnerable to the damaging effects of glucocorticoids with increased age (McEwen 1999, 2002, Sapolsky 1999, Miller and O'Callaghan 2003, Simon et al. 2005). In rats, prolonged corticosterone treatment produced aging-like morphological changes in the CA1 and CA3 hippocampal regions and produced atrophy of CA3 dendrites and CA1 pyramidal cells (Arbel et al. 1994, Sousa et al. 2000). Kadar and colleagues (1998) reported that CA1 pyramidal cells showed morphological changes after 9 weeks of corticosterone treatment, whilst no changes were evident in the CA3 region until 12 weeks of corticosterone exposure. Furthermore, is has been highlighted that glial cell alterations may be an important factor in the hippocampal changes that occur during chronic stress (Lucassen et al. 2001).

Glial cells play an important role in supporting neurons and influencing the movement of neuroactive substances throughout the extracellular space (ECS)

*Correspondence should be addressed to N. Bridges, Email: njbridges@uclan.ac.uk*

*Received 21 November 2006, accepted 15 March 2008*

(Syková 2004, 2005). Thus, if stress proves detrimental to glial cells within the CNS, this will subsequently affect neuronal functioning and signaling. However, evidence on the effects of stress and/or corticosterone treatment on hippocampal glia is limited. It has been shown that following six days of activity stress in rats, there is a 30% increase in glial fibrillary acidic protein (GFAP)-immunoreactive astrocytes within the hippocampus compared to controls (Lambert et al. 2000), which may be part of a neuronal protective mechanism or may subsequently produce greater neuronal damage by impeding the movement of substances to neurons *via* the ECS (Syková 2001, 2004, 2005). O'Callaghan and coauthors (1989) reported that chronic (12 day) corticosterone treatment reduced GFAP levels in adult male rats, whilst adrenalectomy conversely increased GFAP. Levels normalised within 7 days following the withdrawal of corticosterone, suggesting that glucocorticoid release subsequently alters glia–glia and neuron–glia interaction.

Research has focused primarily on the use of male animals, with little investigation of sex differences. Adult male rats possess more GFAP-immunoreactive astrocytes in the CA3 region, whilst females show a greater number (approximately 25–40%) within the CA1 region (Mouton et al. 2002, Conejo et al. 2003) Sex differences in GFAP-immunoreactivity are also present during rat hippocampal development, especially in the CA1 region (Catalani et al. 2002). With regard to sex differences in morphological changes following stress, dendritic atrophy of CA3 pyramidal neurons significantly altered in male rats following chronic restraint stress, whilst no alteration was observed in females (Galea et al. 1997). Similarly, an inhibition of cell proliferation and cell death following acute predator odor stress occurred in male rats but not in females (Falconer and Galea 2003). Distinct sex differences are also evident in neuronal activity and in some of the factors implicated in neuronal plasticity (e.g. extracellular-signal-regulated kinase ERK1 and ERK2) after repeated footshock stress (Trentani et al. 2003). Overall, these findings suggest that the vulnerability of the hippocampus to stress-induced damage is sex-dependent.

In this study, corticosterone was chronically administered to male and female rats over a period of 21 days, previously reported as being a putative animal model of PTSD (Levy et al. 2001). Changes in hippocampal volume and astrocyte numbers in the CA1 and CA3 regions were then determined following corticosterone treatment and, in addition, sex differences in these measures were assessed.

## METHODS

### Animals

Male ($n$=32) and female ($n$=32) Wistar rats weighed 300 g ± 44 g and 200 g ± 30 g, respectively, at the outset of the experiment. Animals were 3 months of age on arrival and were given one week to habituate to their surroundings before any procedures were performed. They were given free access to food and water and maintained at a temperature of 22°C ± 1°C on a 12:12 hour light–dark cycle (lights on at 08:00 AM).

### Drug treatment and tissue preparation

Rats received either 1 (150 mg) or 2 (together 300 mg) 21-day time-release corticosterone pellets (Innovative Research of America, USA), a placebo pellet or no treatment ($n$=8 in each group, together 32 male and 32 female rats). The dose range of corticosterone used has been previously reported to result in plasma corticosterone levels of 200–500 ng/ml (Arbel et al. 1994, Dachir et al. 1995, Streffel et al. 2001). To implant the pellets, animals were anaesthetized using isoflurane gas (initially 5% and then maintained at 2%) and pellets were inserted into a small incision in the scruff of the neck and sutured. The animals were then individually housed following implantation to prevent cagemates from removing the pellets. The weight of each animal was recorded daily. All animals survived surgery and three weeks of treatment without any complications or unnecessary pain or discomfort. On day 21 subsequent to implantation, animals were terminally anaesthetized using sodium pentobarbital (120 mg/kg) and intracardially perfused using 0.1 M phosphate buffer followed by 4% paraformaldehyde in phosphate buffer. Brains were then removed from the skull, post-fixed in 4% paraformaldehyde in phosphate buffer at 4°C for 2–3 hours and then placed in a 20% sucrose/phosphate buffer solution for at least 4 hours until required for staining.

### Hippocampal volume determination

Coronal sections of 40 μm thickness were sliced using a cryostat. Every eighth coronal section, starting

at bregma = −2.3 (Paxinos and Watson 1998), was mounted onto gelatinized slides and stained using 0.2% cresyl violet (6 coronal sections together, bregma = −2.3, −2.62, −2.94, −3.26, −3.58 and −3.9). Sections were then dipped into a series of ethanol dilutions (70–100%), placed in xylene and finally coverslipped. Images were taken using a Leica DM RXA microscope and were viewed on a computer monitor. To determine the volume, the boarders of the right and left hippocampal formations, including the dentate gyri, were traced for each of the 6 sections using Neurolucida (MicroBrightField, Inc.). The traced areas were computed, summed and multiplied by the thickness of the section (Lucassen et al. 2001, Heine et al. 2004).The volumes of right and left hippocampus were then calculated on the basis of the Cavalieri principle (Gundersen and Jensen 1987).

### Astrocyte counting in CA1 and CA3 regions

Astrocyte determination was performed using an antibody directed against glial fibrillary acidic protein (GFAP). Slices were incubated overnight at 4°C with a mouse monoclonal antibody directed against GFAP conjugated with Cy3 (Sigma), diluted 1:200 in phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA, Sigma) and 0.5% Triton X-100 (Sigma). The slices were subsequently mounted using Vectashield mounting medium (Vector Laboratories, Burlingame, CA, USA). Determination of astrocyte numbers was performed using four GFAP stained sections (bregma = −2.98, −3.14 −3.3, −3.46) from each rat. Images were taken using a Leica TCS SP confocal microscope (5× objective). Counting was conducted using Neurolucida (MicroBrightField, Inc.), with a counting frame of 400 μm × 400 μm. Counts were then made in three regions of the GFAP stained sections in the medial (next to the dentate gyrus) and lateral CA3 regions and in the CA1 region. Each stained astrocyte body was counted as one astrocyte (in the pyramidal cell layer in the centre of the counting square).

### Statistical analyses

A repeated measures ANOVA was used for the analysis of body weight, with the within factor of the time of weight measurement (1–21 days) and the between subjects factors of sex (male and female) and drug treatment group (vehicle, placebo pellet, 150 mg corticosterone pellet and 300 mg corticosterone pellet). ANOVA's were used to detect overall differences between drug groups on specific days. One-way ANOVA's were performed separately for each sex to determine significant effects for each drug treatment group for each day. *Post-hoc* analysis of significant effects comprised of Tukey's HSD tests.

For astrocyte number and hippocampal volume, analysis comprised of a 2 (sex: male and female) × 4 (drug treatment group: control, placebo pellet, 150 mg corticosterone and 300 mg corticosterone) ANOVA. *Post-hoc* analysis was conducted on significant effects using a least significant difference (LSD) test.

## RESULTS

### Total body weight

Statistical analysis revealed a significant interaction effect of Day × Sex on body weight ($F_{2.73,152.67}$=17.83, $P$<0.001) but no significant result was seen for Sex alone ($F_{1,56}$=0.026, $P$=0.87). A significant interaction effect was seen for Day × Drug ($F_{8.18,152.67}$=10.250, $P$<0.001) and also for Drug treatment alone ($F_{3,56}$=49.95, $P$<0.001). ANOVA's for each day (with $F$-values between 4.16 and 71.34) showed that overall, corticosterone treated rats (both dose 1 and dose 2) had a reduced percentage body weight compared to control and placebo rats. Tukey's HSD showed that 150 mg and 300 mg corticosterone treated rats had significantly lower percentage body weight than placebo treated rats over days 3–21. Additionally, 150 mg and 300 mg corticosterone treated rats showed reduced percentage body weight at days 3–13, 15–21 and 2–21 compared to controls. No differences between control and placebo rats were seen at any day.

The main effects of corticosterone treatment for each day were observed separately for each sex, showing a significant effect of treatment group for females on days 3–10 and 17–21. A significant effect of treatment was seen in males for days 2–21. *Post-hoc* tests of significant ANOVA effects (see Fig. 1) revealed that corticosterone dose dependently reduced percentage body weight in both female and male rats when compared to placebo treated rats. However, this effect was more prominent in males. Again, no significant differences were seen between control and placebo male and female rats, indicating that surgery and pellet implantation did not affect body weight.

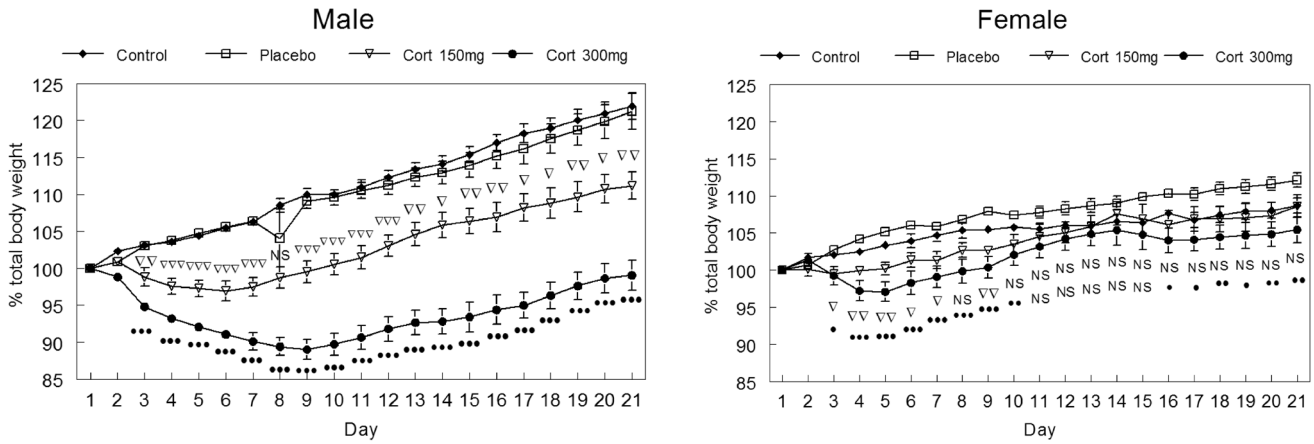


Fig. 1. Percentage total body weight (from measurement in grams) from day 1 in male (left) and female (right) rats following the 21-day corticosterone (Cort) treatment period. Data are presented as means ± SEM. *Post-hoc* comparisons were made against the placebo group. Where significant differences between placebo vs. Cort 150 mg groups were found, these are represented as $^{\nabla}P<0.05$, $^{\nabla\nabla}P<0.01$, $^{\nabla\nabla\nabla}P<0.001$. Where significant differences between placebo vs. Cort 300 mg groups were found, these are represented as $^{\bullet}P<0.05$, $^{\bullet\bullet}P<0.01$, $^{\bullet\bullet\bullet}P<0.001$. (NS) non-significant difference in comparison to placebo group.

**Hippocampal volume**

The mean hippocampal volumes for males and females following corticosterone are displayed in Table I. Statistical analysis revealed that there was a significant effect of the sex of the animal on left and right hippocampal volumes: left ($F_{1,56}$=45.21, $P$<0.001) and right ($F_{1,56}$=33.96, $P$<0.001). Observation of the means showed that both the left and right male hippocampal regions were, as expected, larger than the respective female hippocampal regions. No significant effect of corticosterone dose was seen for either left ($F_{3,56}$=1.55, $P$=0.21) or right hippocampi ($F_{3,56}$=0.55, $P$=0.65), thus the administration of corticosterone did not produce an alteration in hippocampal volumes. In addition, no significant sex × corticosterone dose interaction was revealed for the left ($F_{3,56}$=0.32, $P$=0.82) or right hippocampus ($F_{3,56}$=0.17, $P$=0.91), therefore the effects of corticosterone on hippocampal volume do not appear to be sex-dependent.

**Hippocamapal astrocyte numbers**

A significant main effect of sex on astrocyte number was found in the lateral CA3 region ($F_{1,56}$=14.57, $P$<0.001), regardless of treatment group. Inspection of

Table I

Left and right hippocampal volumes (mm$^3$) of female and male rats following chronic corticosterone (Cort) administration

| Treatment | Females | | Males | |
|---|---|---|---|---|
| | Left volume (mm$^3$) | Right volume (mm$^3$) | Left volume (mm$^3$) | Right volume (mm$^3$) |
| Control | 8.47 ± 0.24 | 8.34 ± 0.18 | 9.09 ± 0.18 | 9.62 ± 0.17 |
| Placebo | 8.13 ± 0.21 | 8.34 ± 0.20 | 9.10 ± 0.24 | 9.31 ± 0.11 |
| Cort 150 mg | 8.37 ± 0.13 | 8.49 ± 0.20 | 9.56 ± 0.16 | 9.26 ± 0.16 |
| Cort 300 mg | 8.40 ± 0.24 | 8.30 ± 0.19 | 9.22 ± 0.31 | 9.00 ± 0.28 |

Data presented as mean ± SEM

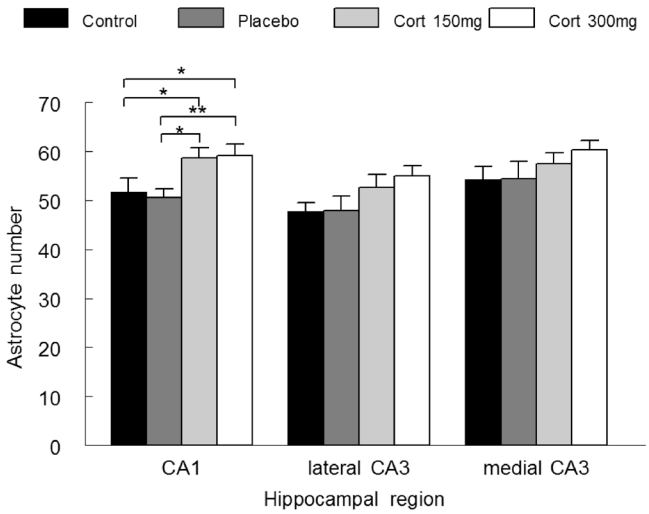


Fig. 2. The effects of chronic corticosterone (Cort) treatment on astrocyte numbers (in a 400 μm × 400 μm area) in three hippocampal regions, irrespective of sex. Data are presented as means ± SEM; **P*<0.05, ***P*<0.01.

the means revealed that overall, females had a greater number of astrocytes (55.1 ± 2.1) in this region than males (46.5 ± 0.9). ANOVA also revealed a significant main effect of corticosterone treatment, regardless of sex, in the CA1 hippocampal region ($F_{3,56}$=3.97, *P*<0.05), but not the medial and lateral CA3 regions. *Post-hoc* analysis (see Fig. 2) showed that 150 mg and 300 mg of corticosterone produced a significant increase in the number of CA1 astrocytes when compared to both controls [150 mg (*P*<0.05) and 300 mg (*P*<0.05)] and placebo treated animals [150 mg (*P*<0.05) and 300 mg (*P*=0.01)]. No significant sex × corticosterone dose interactions were found in any of the three hippocampal regions, suggesting that the effects of corticosterone were not sex-dependent.

## DISCUSSION

The current investigation examined the effects of chronic corticosterone treatment (at two doses, 150 mg and 300 mg) on astrocyte numbers; the results revealed that overall, females had a greater number of astrocytes in the lateral CA3 region. This result differs from previous findings, which found that male rats had more astrocytes in the CA3 region and that female rats and female mice had greater numbers in the CA1 region (Mouton et al. 2002, Conejo et al. 2003). There are a number of reasons why this may have been observed. Whilst no effect was seen in the CA3 regions, chronic corticosterone treatment at both doses (150 mg and 300 mg), compared to both controls and placebo treated rats, produced an increase in CA1 astrocytes. It has been also demonstrated by Lambert and others (2000) that stressor exposure produces an increase in hippocampal astrocyte number. This may therefore indicate that direct prolonged corticosterone treatment produces similar effects to chronic stress. In addition, it has previously been reported that changes to pyramidal cells in the CA3 region may not occur until 12 weeks of corticosterone exposure and may be a result of interaction with the CA1 region (Kadar et al. 1998). This may explain why in the current study, alterations in glial numbers were only seen in the CA1, but not CA3 region after 21-days of corticosterone exposure.

The significance of the astrogliosis observed following stress or corticosterone exposure is unknown. However, it may be possible that the increased glial reactivity plays a supportive function or, conversely, that it proves detrimental to the surrounding neurons in the hippocampus, which appears to be more likely when considering the neuronal loss and memory impairments seen in such conditions. Evidence has shown that excessive glucocorticoid exposure gives rise to hippocampal damage proximal with that occurring with progressive aging, especially in regard to the CA1 and CA3 regions (Arbel et al. 1994). In addition, the vulnerability of the hippocampus to damage by glucocorticoids during prolonged periods of stress appears to become more pronounced with increased age (McEwen 1999, 2002, Sapolsky 1999, Simon et al. 2005). Evidence has previously shown that changes within the ECS occur with age and that these show a relation to impaired learning (Syková et al. 2002). It may therefore be possible that the astrogliosis in the hippocampus following chronic corticosterone exposure may affect the composition of the ECS. This would have subsequent effects on extrasynaptic communication between glia and glia, and glia and neurons, as the movement of substances through the ECS becomes hindered (Syková 2001, 2004, 2005). This may provide some explanation for the deficits in memory and learning that are often observed during prolonged stress (Sousa et al. 2000, Kim and Diamond 2002).

The results of this study did not show the effects of corticosterone to be sex dependent. Catalani and colleagues (2002) have previously shown that sex differences in glial reactivity are exhibited throughout hippocampal development; therefore, further investigation

is warranted to gain a more detailed understanding of the effect of chronic corticosterone in male and female rodents. It would also be interesting to determine whether the corticosterone-induced astrogliosis occurs in conjunction with neuronal impairment.

Volumetric measurement revealed that male rats had a larger hippocampal volume in general compared to females, which may be due to males being larger in general or as a possible consequence of testosterone exposure (Galea et al. 1999). Chronic treatment with corticosterone did not produce any significant reduction in hippocampal volume in either males or females. Our data does not follow the previously observed findings in which stress-related disorders typically produce a reduction in hippocampal volume (Lindauer et al. 2004, Shin et al. 2004, Wignall et al. 2004). In the chronic psychosocial stress model in the tree shrew, a reduction of hippocampal volume is also observed (Czéh et al. 2001). The reduction of volume following stressor exposure is associated with neuronal atrophy in this region and also neuronal impairment, especially in the CA3 hippocampal region (Uno et al. 1989, Magariños and McEwen 1995, Galea et al. 1997). In addition, similar effects have been reported following corticosterone administration (Sapolsky et al. 1990, Arbel et al. 1994, Bisagno et al. 2000). However, as no alteration in hippocampal volume was seen in the current study, it may suggest that stress factors other than corticosterone are involved in volume reduction or that the corticosterone administration may not have been sufficient to produce the expected volume reduction of the hippocampus.

With regard to percentage body weight, a dose-dependent reduction was seen in both male and female rats following chronic corticosterone treatment. However, this effect was more pronounced in males and was consistent throughout the 21-day period, whereas in females, the reduction occurred predominantly between days 3–10 and 17–21, suggesting greater fluctuation over the treatment period, possibly influenced by the oestrous cycle. The percentage body weight of females receiving 150 mg corticosterone did not drop below the starting weight (100%), except at day 3, whereas in females receiving 300 mg corticosterone the percentage body weight dropped below 100% at days 3–8. In males receiving 150 mg corticosterone, percentage body weight stayed below 100% at days 2–9, whilst those males receiving 300 mg corticosterone never regained their original body weight over the 21-day period. These results show that whilst corticosterone affects body weight, male rats appear to be more sensitive to the effects, or conversely that females are protected to a certain degree by factors such as ovarian hormones (Bowman et al. 2002, Beck and Servatius 2005). The current investigation did not, however, determine the oestrous stage of the female rats. It would therefore be of value in the future to assess whether the results seen here are dependent on hormonal status.

## CONCLUSION

To conclude, the slow release of corticosterone over a prolonged period may provide a useful way of mimicking the effects of chronic stress. However, as no reduction in hippocampal volume was observed in the current investigation, further study would be warranted to confirm this. Nonetheless, chronic corticosterone appears to dose-dependently increase astrocyte numbers in the CA1 hippocampal region. This may have implications in the understanding of both glial cell and neuronal functioning and the damaging effects of glucocorticoids in the hippocampus.

### Acknowledgements

We thank Hanka Hronova for technical assistance with histological procedures. This study was supported by grants from the Grant Agency of the Czech Republic 309/04/0753, the Academy of Sciences of the Czech Republic AV0Z50390512, the Ministry of Education, Youth and Sports of the Czech Republic 1M0021620803 and MSM0021622404, and the European Commission Marie Curie Training Site Programme HPMT-CT-2000-00187.

## REFERENCES

Arbel I, Kadar T, Silbermann M, Levy A (1994) The effects of long-term corticosterone administration on hippocampal morphology and cognitive performance of middle-aged rats. Brain Res 657: 227–235.

Beck KD, Servatius RJ (2005) Stress-induced reductions of sensory reactivity in female rats depend on ovarian hormones and application of a painful stressor. Horm Behav 47: 532–539.

Bisagno V, Ferrini M, Ríos H, Zieher LM, Wikinski SI (2000) Chronic corticosterone impairs inhibitory avoid-

ance in rats: Possible link with atrophy in hippocampal CA3 neurons. Pharmacol Biochem Behav 66: 235–240.

Bowman RE, Ferguson D, Luine VN (2002) Effects of chronic restraint stress and estradiol on open field activity, spatial memory, and monoaminergic neurotransmitters in ovariectomized rats. Neuroscience 113: 401–410.

Catalani A, Sabbatini M, Consoli C, Cinque C, Tomassoni D, Azmitia E, Angelucci L, Amenta F (2002) Glial fibrillary acidic protein immunoreactive astrocytes in developing rat hippocampus. Mech Aging Dev 123: 481–490.

Conejo NM, González-Pardo H, Pedraza C, Navarro FF, Vallejo G, Arias JL (2003) Evidence for sexual difference in astrocytes of adult rat hippocampus. Neurosci Lett 339: 119–122.

Czéh B, Michaelis T, Watanabe T, Frahm J, de Biurrun G, van Kampen M, Bartolomucci A, Fuchs E (2001) Stress-induced changes in cerebral metabolites, hippocampal volume, and cell proliferation are prevented by antidepressant treatment with tianeptine. Proc Natl Acad Sci U S A 98: 12796–12801.

Dachir S, Robinzon B, Grauer E, Levy A (1995) Nimodipine counteracts corticosterone-habituated impairments. Neurobiol Learn Mem 63: 241–245.

Falconer EM, Galea LAM (2003) Sex differences in cell proliferation, cell death and defensive behavior following acute predator odor stress in adult rats. Brain Res 975: 22–36.

Galea LAM, McEwen BS, Tanapat P, Deak T, Spencer RL, Dhabhar FS (1997) Sex differences in dendritic atrophy of CA3 pyramidal neurons in response to chronic restraint stress. Neuroscience 81: 689–697.

Galea LAM, Perrot-Sinal TS, Kavaliers M, Ossenkopp KP (1999) Relations of hippocampal volume and dentate gyrus width to gonadal hormone levels in male and female meadow voles. Brain Res 821: 383–391.

Gundersen HJ, Jensen EB (1987) The efficiency of systematic sampling in stereology and its prediction. J Microsc 147: 229–263.

Heine VM, Maslam S, Zareno J, Joëls M, Lucassen PJ (2004) Suppressed proliferation and apoptotic changes in the rat dentate gyrus after acute and chronic stress are reversible. Eur J Neurosci 19: 131–144.

Kadar T, Dachir S, Shukitt-Hale B, Levy A (1998) Sub-regional hippocampal vulnerability in various animal models leading to cognitive dysfunction. J Neural Transm 105: 987–1004.

Kim JJ, Diamond DM (2002) The stressed hippocampus, synaptic plasticity and lost memories. Nat Rev Neurosci 3: 453–262.

Lambert KG, Gerecke KM, Quadros PS, Doudera E, Jasnow AM, Kinsley CH (2000) Activity-stress increases density of GFAP-immunoreactive astrocytes in the rat hippocampus. Stress 3: 275–284.

Levy A, Kadar T, Dachir S (2001) An animal model for studying therapeutic drugs against post-traumatic stress disorder. Mil Med 166: 74–75.

Lindauer RJL, Vlieger EJ, Jalink M, Olff M, Carlier IVE, Majoie CBLM, den Heeten GJ, Gersons BPR (2004) Smaller hippocampal volume in dutch police officers with posttraumatic stress disorder. Biol Psychiatry 56: 356–363.

Lucassen PJ, Vollmann-Honsdorf GK, Gleisberg M, Czéh B, Ron De Kloet E, Fuchs E (2001) Chronic psychosocial stress differentially affects apoptosis in hippocampal subregions and cortex of the adult tree shrew. Eur J Neurosci 14: 161–166.

Magariños AM, McEwen BS (1995) Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: Comparison of stressors. Neuroscience 69: 83–88.

McEwen BS (1999) Stress and the aging brain. Front Neuroendocrinol 20: 49–70.

McEwen BS (2002) Sex, stress and the hippocampus: allostasis, allostatic load and the aging process. Neurobiol Aging 23: 921–939.

Miller DB, O'Callaghan JP (2003) Effects of aging and stress on hippocampal function. Metab Clin Exp 52: 17–21.

Mouton PR, Long JM, Lei D-L, Howard V, Jucker M, Calhoun ME, Ingram DK (2002) Age and gender effects on microglia and astrocyte numbers in brains of mice. Brain Res 956: 30–35.

O'Callaghan JP, Brinton RE, McEwen BS (1989) Glucocorticoids regulate the concentration of glial fibrillary acidic protein throughout the brain. Brain Res 494: 159–161.

Paxinos G, Watson C (1998) The Rat Brain in Stereotaxic Coordinates (4th ed.). Academic Press Inc., San Diego, CA.

Sapolsky RM, Uno H, Rebert CS, Finch CE (1990) Hippocampal damage associated with prolonged glucocorticoid exposure in primates. J Neurosci 10: 2897–2902.

Sapolsky RM (1999) Glucocortocoids, stress, and their adverse neurological effects: relevance to aging. Exp Gerontol 34: 721–732.

Sapolsky RM (2001) Atrophy of the hippocampus in posttraumatic stress disorder: How and when? Hippocampus 11: 90–91.

Shin LM, Shin PS, Heckers S, Krangel TS, Macklin ML, Orr SP, Lasko N, Segal E, Makris N, Richert K, Levering J, Schacter DL, Alpert NM, Fischman AJ, Pitman RK, Rauch SL (2004) Hippocampal function in posttraumatic stress disorder. Hippocampus 14: 292–300.

Simon M, Czéh B, Fuchs E (2005) Age-dependent susceptibility of adult hippocampal cell proliferation to chronic psychosocial stress. Brain Res 1049: 244–248.

Sousa N, Lukoyanov NV, Madeira MD, Almeida OFX, Paula-Barbosa MM (2000) Reorganization of the morphology of hippocampal neurites and synapses after stress-induced damage correlates with behavioral improvement. Neuroscience 97: 253–266.

Streffel A, Storch MK, Linington C, Stadelmann C, Lassmann H, Pohl T, Holsboer F, Tilders FJH, Ruel JMHM (2001) Disease progression in chronic relapsing experimental allergic encephalomyelitis is associated with reduced inflammation-driven production of corticosterone. Endocrinology 142: 3616–3624.

Syková E (2001) Glial diffusion barriers during aging and pathological states. Prog Brain Res 132: 339–363.

Syková E. Mazel T, Hasenohrl RU, Harvey AR, Simonova Z, Mulders WHAM, Huston JP (2002) Learning deficits in aged rats related to decrease in extracellular volume and loss of diffusion anisotropy in hippocampus. Hippocampus 12: 469–479.

Syková E (2004) Extrasynaptic volume transmission and diffusion parameters of the extracellular space. Neuroscience 129: 861–876.

Syková E (2005) Glia and volume transmission during physiological and pathological states. J Neural Transm 112: 137–147.

Trentani A, Kuipers SD, te Meerman GJ, Beekman J, ter Horst GJ, den Boer JA (2003) Immunohistochemical changes induced by repeated footshock stress: revelations of gender-based differences. Neurobiol Dis 14: 602–618.

Uno H, Tarara R, Else JG, Suleman MA, Sapolsky RM (1989) Hippocampal damage associated with prolonged and fatal stress in primates. J Neurosci 9: 1705–1711.

Wignall EL, Dickson JM, Vaughan P, Farrow TFD, Wilkinson ID, Hunter MD, Woodruff PWR (2004) Smaller hippocampal volumes in patients with recent-onset posttraumatic stress disorder. Biol Psychiatry 56: 832–836.