Coping style & stressor susceptibility
Veenema, Alexandra Hendrika

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2003

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Download date: 15-01-2019
Intraperitoneal injections were used in chapter 5 and chapter 6. In chapter 5, LAL and SAL mice were injected with BrdU, which is incorporated in the DNA of cells in S phase. In this way we were able to measure the cell proliferation rate in the hippocampus by visualizing BrdU-positive cells with immunocytochemistry. In chapter 6, LAL and SAL mice were injected with two different types of 5-HT$_{1A}$ receptor agonists, 8-OH-DPAT and S-15535, to measure the effect of activation of 5-HT$_{1A}$ receptors on changes in forced swimming behaviour.
Chapter 5

DIFFERENCES IN BASAL AND STRESS-INDUCED HIPPOCAMPAL CELL PROLIFERATION IN ADULT LOW AND HIGH AGGRESSIVE MICE

Neuroscience, submitted

A.H. Veenema\textsuperscript{a}, P.J. Lucassen\textsuperscript{b}, M.C. de Wilde\textsuperscript{a}, A.J. Roelofs\textsuperscript{a},
B. Buwalda\textsuperscript{a}, J.M. Koolhaas\textsuperscript{a}, E.R. de Kloet\textsuperscript{c}

\textsuperscript{a}Department of Animal Physiology, Center for Behavioral and Cognitive Neurosciences, University of Groningen, The Netherlands
\textsuperscript{b}Institute for Neurobiology, Swammerdam Institute for Life Sciences, University of Amsterdam, The Netherlands
\textsuperscript{c}Division of Medical Pharmacology, Leiden/Amsterdam Center for Drug Research, Leiden University Medical Center, The Netherlands
Abstract

Male wild house-mice, selected for long attack latency (LAL) and short attack latency (SAL), display distinctly different coping styles. This genetic trait in coping style was recently found to be associated with a differential regulation of the Hypothalamic-Pituitary-Adrenal (HPA) axis under basal and stress conditions, with LAL mice showing higher HPA reactivity. High glucocorticoid concentrations induced by stress suppress the proliferation rate of newborn cells in the dentate gyrus of adult rodents. Therefore, the hypothesis is tested that the differences in HPA activation between LAL and SAL mice are reflected in the rate of cell proliferation. Cell proliferation in the subgranular zone of the dentate gyrus, assessed by systemic application of bromodeoxyuridine (BrdU, one per day for three days), was almost two-fold lower in LAL mice than in SAL mice. This was, however, paralleled by significantly higher plasma corticosterone levels in LAL mice. To estimate basal cell proliferation in these mice, the endogenous proliferation marker Ki-67 was used. Under basal conditions, LAL mice had slightly lower (85%) numbers of Ki-67 positive cells in the SGZ than SAL mice. Forced swim stress for 5 min resulted 24 h later in a significant reduction in number of BrdU-positive cells in the subgranular zone of LAL but not of SAL mice. In conclusion, these results demonstrate that dentate gyrus cell proliferation rate reflects a genetic trait in coping style and stress responsiveness. Granule cell proliferation in SAL mice is resistant to stress, while it is further suppressed in LAL mice with high circulating corticosterone concentrations. This decreased granule proliferation displayed by the LAL mice may have implications for structure and function of the hippocampus during stress.
Introduction

Male wild house-mice selected for long attack latency (LAL) and short attack latency (SAL) show profound differences in coping style when exposed to environmental challenges. LAL mice display the ‘passive’ coping style whereas SAL mice show the ‘active’ coping style (Benus et al., 1989, 1991a,b; Sluyter et al., 1996).

This genetic difference in coping style was recently found to be associated with a differential regulation of the Hypothalamic-Pituitary-Adrenal (HPA) system under basal and acute stress conditions (Veenema et al., 2003a). LAL mice showed less day-night variation in corticosterone secretion, and a higher adrenocortical sensitivity to ACTH than SAL mice. When subjected to forced swimming for 5 min, LAL mice showed a higher and prolonged increase in plasma corticosterone compared to SAL mice (Veenema et al., 2003a).

Elevated circulating levels of glucocorticoids and stressful experiences have been shown to inhibit the ongoing granule cell proliferation in the hippocampal dentate gyrus of adult rats (Gould et al., 1997, 1998; Tanapat et al., 1998). In the present study, we hypothesized that the differential HPA (re)activity in LAL versus SAL mice is reflected in differences in hippocampal cell proliferation.

In the first experiment, proliferation of progenitor cells was determined in the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG) in LAL and SAL mice, by using systemic application of 5-bromo-2-deoxyuridine (BrdU) once per day for three consecutive days.

In the second experiment, immunocytochemistry for the endogenous proliferation marker Ki-67 was used to measure proliferation rate in the SGZ of naïve LAL and SAL mice. Ki-67 is a well known mitotic and proliferation marker present in all phases of the cell cycle, except G0 (Gerdes et al., 1984; Endl and Gerdes, 2000). Also, Ki-67 numbers are highly comparable to those obtained with BrdU when determined after short survival times (Kee et al., 2002).

In the third experiment, mice were injected with BrdU and immediately subjected to forced swimming for 5 min to study the effect of acute stress on cell proliferation. To investigate whether this forced swim stressor induced a line difference in HPA activation, plasma corticosterone concentrations were also measured in LAL and SAL mice decapitated 15 min after being subjected to forced swimming.
Methods

Mice

In this study, male LAL and SAL mice were used at the age of 18 weeks (±/− 2 weeks). These mice, genetically selected for attack latency, originated from a colony of wild house-mice (*Mus musculus domesticus*) maintained at the University of Groningen, The Netherlands, since 1971. The LAL males came from the 38-40th generation of selection and the SAL males came from the 66-68th generation. The mice were housed in perspex cages (17 x 11 x 13 cm) in a room with a 12:12 light/dark cycle (lights on from 0030 to 1230). Standard laboratory chow and water was available *ad libitum*. The mice were weaned at 3-4 weeks of age, and were paired male-female at the age of 6-8 weeks. At the age of 14 weeks male mice were subjected to the attack latency test (Van Oortmerssen and Bakker, 1981). Briefly, a male is confronted with a standard non-aggressive male opponent in a neutral cage on three consecutive days. The attack latency score is the mean of these daily scores. Neither LAL nor SAL mice experienced a social defeat. Only non-attacking LAL mice and SAL mice with an attack latency less than 50 s were used for the experiments. All experiments were in accordance with the regulations of the Committee for Use of Experimental Animals of the University of Groningen (DEC nr. 2326).

Experiment 1: Cell proliferation in LAL and SAL mice using BrdU

BrdU (5-bromo-2-deoxyuridine, Sigma, St. Louis, Missouri) is a thymidine analogue that will be incorporated in the DNA of cells in S phase. As such, it is a marker of proliferating cells and their progeny (Nowakowski et al., 1989). BrdU was dissolved in sterile 0.9% NaCl. LAL and SAL mice (*n* = 9 per line) received i.p. injections of 10mg/ml BrdU (daily dose: 50mg/kg body weight) once per day for three consecutive days. Twenty-four hours after the last injection mice were perfused as described below.

Experiment 2: Cell proliferation in LAL and SAL mice using Ki-67

LAL and SAL mice (*n* = 8 per line) were left undisturbed in their home cage for at least one week. Thereafter, mice were perfused and numbers of proliferating cells were determined with Ki-67 immunocytochemistry as described below. Ki-67 is a nuclear protein expressed in all phases of the cell cycle except G0 and used as an endogenous marker for adult proliferation, that is independent of differences in liver clearance or blood brain-barrier passage, that can affect BrdU incorporation in brain (Scholtzen and Gerdes, 2000).

Experiment 3: Effect of forced swimming on cell proliferation in LAL and SAL mice

All mice received a single i.p. injection of 20mg/ml BrdU (dose: 100mg/kg body weight, dissolved in sterile 0.9% NaCl). Control LAL and SAL mice (*n* = 8 per line) were then left undisturbed, whereas another 8 SAL mice and 10 LAL mice were, immediately after the BrdU injection, forced to swim in a narrow plexiglass cylinder (diameter of 10 cm) filled with water of 25°C for 5 min before they returned to their home cage. Twenty-four hours later, mice were perfused as described below. To further determine the role of forced swim stress-induced increases in plasma corticosterone on cell proliferation in this experimental design, LAL and SAL mice received a single i.p. injection with sterile 0.9% NaCl after which they were either left undisturbed in their home cage (control mice, *n* = 7 per line) or were immediately subjected to forced swimming for 5 min (stressed mice, *n* = 8...
per line). For measurement of plasma corticosterone, trunk blood was obtained by decapitation, 15 min after forced swimming.

**Tissue processing**

For all experiments, mice received an overdose of CO₂ anaesthesia and were perfused transcardially with 4% paraformaldehyde in cold phosphate buffer (pH 7.4). Blood was obtained from the heart just prior to perfusion and collected in chilled tubes containing EDTA for determination of corticosterone levels. The brains were post-fixed overnight and then transferred into 30% sucrose and stored at 4°C. After an additional 24-48 h, coronal sections of 40 µm thickness were cut on a freezing microtome. The sections were stored at -20°C in cryoprotectant containing 25% ethylene glycol, 25% glycerin, and 0.5 M phosphate buffer.

**Immunocytochemistry**

BrdU immunocytochemistry was performed according to Kuhn et al. (1997). Briefly, free-floating sections were treated with 0.6% H₂O₂ in TBS (pH 7.6) for 30 min, following incubation in 50% formamidex2xSSC (0.3 M NaCl and 0.03 M sodium citrate) for 2 h at 65°C, acidification with 2M HCl for 30 min at 37°C and incubation in 0.1M boric acid pH 8.5 for 10 min at room temperature. Sections were then incubated overnight at 4°C with mouse anti-BrdU (Novocastra, New Castle, UK 1:200) in TBS-plus (TBS/0.1% Triton X-100/3% normal horse serum). After washing with TBS-plus for 30 min, sections were incubated with biotinylated horse anti-mouse (1:200) for 45 min, followed by the streptavidin-HRP and DAB (0.25 mg/ml DAB, 0.01% H₂O₂) visualization method.

The Ki-67 antigen is a 345 to 395 kDa non-histone protein complex present only in mitotic and proliferating cells and is a well-accepted proliferation marker in tumour biology. The numbers of Ki-67-positive cells are highly comparable to BrdU numbers after short survival times (Kee et al., 2002). Ki-67 immunocytochemistry was performed as described by Heine et al. (2003a). Briefly, mounted sections were rinsed with 0.1M Tris buffered saline pH 7.6 (TBS). Sections were placed in 2 plastic jars filled with citrate buffer (0.01 M, pH 6.0) and pretreated in a domestic microwave oven (Samsung M 6235, 800 W). Microwave treatment took 15 min in total, starting at 800 W for 5 min until boiling was reached, after which the setting was lowered to 260 W. Following 30 min of cooling at room temperature, a-specific binding was blocked by incubating in TBS + 2% milk powder (Campina Melkunie, Eindhoven, The Netherlands) for 30 min. Sections were then incubated overnight at 4°C with polyclonal anti-Ki-67 (Novocastra, New Castle, UK, 1:2000) in Supermix (TBS/0.25% gelatin/0.5% Triton X-100). Following rinsing in TBS, sections were incubated with biotinylated sheep anti-rabbit (Amersham Life Sciences, Den Bosch, The Netherlands, 1:200) in Supermix for 1.5 h, after which signal was amplified with ABC Elite (Vector, 1:800) in TBS/BSA 1% for 2 h. The ABC signal was further amplified with biotinylated tyramide (1:500, produced and kindly provided by Dr I. Huitinga, Netherlands Institute for Brain research, Amsterdam) and 0.01% H₂O₂ for 30 min followed by another 1.5 h incubation with ABC (1:1000). Colour development was performed with diaminobenzidine (0.50 mg/ml DAB, Sigma, 0.01% H₂O₂) for 10 min.

**Stereological quantification of proliferation**

Ki-67 and BrdU-labelled cells were counted using a light microscope (400 ×) by a researcher blinded to the study code. In experiment 1 and 3, the total number of BrdU-
positive cells in the SGZ (defined as a two cell-body-wide zone along the border of the granule cell layer with the hilus) was determined unilaterally (exp 1) or bilaterally (exp 3) using a stereological approach throughout the rostro-to-caudal extent of the entire DG in coronal 40-µm sections, 200 µm apart. As a control, in experiment 1, the surface area of the granule cell layer was estimated in the same sections in which BrdU cell number had been assessed using an automatic image analysis system (Quantimet 500, Leica, Cambridge). The surface areas of the brain sections were summed per mouse and expressed as mm². In experiment 2, numbers of Ki-67-positive nuclear profiles were scored in the SGZ unilaterally in coronal 40-µm sections, 400 µm apart.

Radioimmunoassay for corticosterone

Blood samples were centrifuged at 2600g for 10 min at 4°C. Plasma samples were stored at −20°C until assayed. Plasma corticosterone was determined in duplo using a commercially available radioimmunoassay kit (Mouse Corticosterone RIA Kit, ICN Biomedicals, Costa Mesa, CA, USA). The detection limit of the assay was 3 ng corticosterone/ml with an intra-assay variance of 4.4% and inter-assay variance 6.5%.

Statistical analysis

An unpaired Student’s t-test was used to determine line differences in BrdU-labelled cells, surface area and corticosterone concentrations in experiment 1, and Ki-67 positive cells in experiment 2. Univariate analysis of variance (ANOVA) was used to determine line and treatment effects of forced swimming on BrdU-labelled cells and corticosterone concentrations in experiment 3. When a significance was revealed in experiment 3, appropriate pairwise comparisons (LSD test) were done based on the estimated marginal means. For all tests the software package SPSS (version 11) was used. Data are presented as mean ± S.E.M. Significance was taken at $P < 0.05$.

Results

Experiment 1: Cell proliferation in LAL and SAL mice using BrdU

Twenty-four hours after three daily BrdU injections, LAL mice showed an almost two-fold lower total number of BrdU-positive cells in the SGZ compared to SAL mice ($P < 0.001$, Fig. 1A). No line difference was found for surface area of the granule cell layer (LAL: $976 \pm 35$ mm²; SAL $1013 \pm 16$ mm²). Plasma corticosterone concentrations, obtained 24 h after the last injection, were significantly higher in LAL mice than in SAL mice ($P < 0.05$, Fig. 1B). A typical example of BrdU labelling in the hippocampal DG of a LAL and SAL mouse is depicted in Fig. 2.
Fig. 1. Number of BrdU-positive cells in the subgranular zone of the dentate gyrus (A) and plasma corticosterone concentrations (B) of LAL and SAL mice, 24 h after the third i.p. injection with BrdU (one injection on three consecutive days). * $P < 0.05$, ** $P < 0.001$, Student’s $t$-test.

Fig. 2. BrdU labelling in tissue sections of the hippocampal dentate gyrus of a LAL mouse (A) and a SAL mouse (B). BrdU-positive nuclei are clearly present in the subgranular zone (arrowheads). GCL, granule cell layer of the dentate gyrus. H, hilus region of the dentate gyrus.

Experiment 2: Cell proliferation in LAL and SAL mice using Ki-67

Ki-67 immunocytochemistry revealed cells with an obvious nuclear staining pattern, mainly in the SGZ and DG hilus (Fig. 3A) but also in other hippocampal subregions like the CA1 stratum radiatum, generally at lower frequencies (Fig. 3A). At higher magnifications, obvious clusters and doublets of cells were
observed, clearly representing dividing cells (Fig. 3B,C). Quantification revealed significantly lower number of cells in the subgranular zone in LAL mice compared to SAL mice ($P < 0.05$, Fig 3D). Quantification of other hippocampal regions revealed that LAL mice had significantly more Ki-67-positive cells in the CA1 stratum radiatum than SAL mice ($P < 0.05$, Table 1).

![Fig. 3. Ki-67 labelling in tissue sections of the hippocampus revealed clearly cells mainly in the dentate gyrus hilus (H) and subgranular zone (SGZ) (arrowheads) (A). Also in the subventricular wall, proliferating cells are abundantly present (arrows). Only occasionally were positive cells found in the cornu ammonis (CA) stratum radiatum layers. Details of Ki-67-positive clusters (B) or doublets (C) clearly represent dividing cells. The number of Ki-67 cells was significantly lower in LAL mice than in SAL mice (D). * $P < 0.05$, Student’s $t$-test.](image-url)
Table 1. Number of Ki-67-positive cells in several regions of the hippocampus of naïve LAL and SAL mice. Ki-67-positive cells are counted in the molecular cell layer of the dentate gyrus (Mol cell layer), in dentate gyrus (including subgranular zone, granule cell layer, hilus and molecular cell layer), in CA1 stratum radiatum (Radiatum) and in the hippocampus (including dentate gyrus, pyramidal cell layer of CA1-2, CA1 stratum radiatum).

<table>
<thead>
<tr>
<th></th>
<th>Mol cell layer</th>
<th>Dentate gyrus</th>
<th>Radiatum</th>
<th>Hippocampus</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAL (n=8)</td>
<td>27.5 ± 3.3</td>
<td>88.5 ± 2.9</td>
<td>49.5 ± 4.7*</td>
<td>138.4 ± 6.0</td>
</tr>
<tr>
<td>SAL (n=8)</td>
<td>24.6 ± 2.4</td>
<td>95.4 ± 5.8</td>
<td>36.6 ± 3.3</td>
<td>133.1 ± 6.8</td>
</tr>
</tbody>
</table>

* P < 0.05 vs. SAL, Student’s t-test

Experiment 3: Effect of forced swimming on cell proliferation in LAL and SAL mice

Univariate ANOVA revealed a significant line effect (F_{1,30} = 7.834, P < 0.01) and line * treatment interaction (F_{1,30} = 5.130, P < 0.05) for BrdU-positive cells in the subgranular zone of the dentate gyrus. LAL mice subjected to forced swim stress showed a significant decrease in BrdU-positive cells compared to LAL control (P < 0.05) and compared to SAL mice subjected to forced swimming (P < 0.005, Fig. 4A). Plasma corticosterone concentrations 24 h post-stress, revealed a significant treatment effect (F_{1,29} = 4.442, P < 0.05).

![Fig. 4](image-url) Effect of forced swim stress in LAL and SAL mice on number of BrdU-positive cells in the subgranular zone of the dentate gyrus (A) and plasma corticosterone concentrations (B) 24 h post-stress. Effect of forced swimming following a single i.p. injection on plasma corticosterone concentrations in LAL and SAL mice 15 min after forced swimming (C). * P < 0.05; ** P < 0.005, # P = 0.053, pairwise comparisons (LSD test) following univariate ANOVA.
Corticosterone concentrations were higher in LAL mice subjected to forced swimming compared to LAL control \( (P < 0.05) \), but failed to reach a significant difference compared to SAL mice subjected to forced swimming \( (P = 0.064, \text{Fig. 2B}) \). A treatment effect was also found for plasma corticosterone when measured 15 min after forced swimming \( (F_{1,20} = 10.811, P < 0.005) \). Here, corticosterone concentrations were significantly higher in LAL mice subjected to forced swim stress compared to control LAL mice \( (P < 0.05, \text{Fig. 2C}) \). SAL mice subjected to forced swim stress showed also higher levels compared to control SAL mice, but this difference just failed to reach significance \( (P = 0.053, \text{Fig. 2C}) \).

**Discussion**

The present study shows that a genetic trait in coping style is associated with a difference in hippocampal cell proliferation rate. LAL mice showed less proliferating cells in the SGZ of the DG than SAL mice, as revealed both by BrdU and Ki-67 immunolabelling. In response to forced swim stress, a significantly reduced number of new granule cells was observed in LAL but not SAL mice. This lower cell proliferation was found to be accompanied by higher post-stress corticosterone levels in LAL mice.

In order to study a line difference in hippocampal cell proliferation, LAL and SAL mice received three daily injections with BrdU. This resulted in an almost two-fold lower number of proliferating cells in the SGZ of the DG in LAL mice compared to SAL mice. However, LAL mice showed significantly higher corticosterone concentrations 24 h after the last injection than SAL mice. This difference in response may have affected cell proliferation rate. Therefore, a subsequent experiment was performed in naïve LAL and SAL mice using the endogenous marker Ki-67, to establish whether this difference in cell proliferation was a trait or a state characteristic. The total number of Ki-67 positive cells was slightly lower (85%) in the SGZ of the DG in LAL compared to SAL mice. In the CA1 stratum radiatum more Ki-67 cells were found in LAL mice than in SAL mice, but these cells most likely represent proliferating glia. Together, these data indicate that the difference in basal hippocampal cell proliferation between LAL and SAL mice was further amplified after repeated injections.

Exposure of animals to a repeated stressor can lead to habituation and subsequent suppression of the stress response (De Boer et al., 1990; Pitman et al., 1990). In contrast, the higher corticosterone concentrations in LAL mice observed 24 h after the third but not after a single injection suggests that rather an increased sensitivity to injection stress has developed. This line difference in stress habituation is, however, consistent with previous findings in which LAL mice showed long-lasting susceptibility to a sensory contact stressor, whereas SAL mice showed habituation to this stressor (Veenema et al., 2003b,c). This is consistent with another study, in which two inbred mouse strains showed a difference in their
ability to adapt to injection stress. In C57BL/6J mice, repeated injection stress led to habituation in the response of Fos immunoreactivity, which effect was not present in DBA/2J mice (Ryabinin et al., 1999). Another study in rats showed that strain differences in responsivity of the HPA axis to acute stress is paralleled by differences in habituation to repeated stress (Dhabhar et al., 1997). These studies, including the present one, demonstrate that differences in stress habituation between certain rodent lines and strains may have unintended effects, in particular when using injections.

Forced swim stress for 5 min induced a significant decrease in hippocampal cell proliferation in LAL mice when assessed 24 h later. Acute stress-induced inhibition of cell proliferation has been found by others as well (Gould et al., 1997, 1998, Tanapat et al., 1998; Heine et al., 2003b) and is likely mediated by elevated glucocorticoid levels (Gould et al., 1992; Tanapat et al., 2001; Ambrogini et al., 2002). Indeed, in the present study, swim stress-induced suppression of cell proliferation was associated with higher plasma corticosterone concentrations 15 min and 24 h post-stress in LAL mice compared to control LAL mice. This suggests a higher and prolonged swim stress-induced HPA activation in LAL mice which may underly the down-regulation of cell proliferation in response to forced swimming.

In contrast to LAL, SAL mice failed to show a swim stress-induced decrease in cell proliferation. However, corticosterone concentrations between LAL and SAL mice were not different shortly after they were subjected to forced swimming. This suggests a decreased sensitivity of the proliferating hippocampal precursor cells, or the structures they reside in, to stress and corticosterone in SAL mice. Although no line difference was found for GR or MR mRNA expression in hippocampus (Veenema et al., 2003a,b), it would be interesting to study possible differences in particular in GR protein levels or binding in these mice. Direct effects of corticosterone on cell proliferation rate are, however, rather unlikely due to the absence of MR and GR in most granule cell progenitors (Cameron et al., 1993). Alternatively, corticosterone can affect cell proliferation by acting through other factors, such as an NMDA receptor-mediated excitatory pathway (Cameron et al., 1998). A difference between LAL and SAL mice in, for example, NMDA receptor binding could possibly clarify the differential sensitivity of proliferating cells to corticosterone. However, binding to NMDA receptors was found to be similar in naïve LAL and SAL mice (Veenema, unpublished). Alternatively, it is proposed that the negative effects of stress and corticosteroids on cell proliferation can be prevented by simultaneous activation of positive regulators of adult neurogenesis. It is known, for example, that activation of the 5-HT system through 5-HT\textsubscript{1A} receptors is associated with an up-regulation of adult hippocampal cell proliferation (Brezun and Daszuta, 1999; Gould, 1999; Jacobs et al., 2000; Jacobs, 2002; Radley and Jacobs, 2002). Furthermore, adult hippocampal cell proliferation was increased after administration of fenfluramine, which causes the release of 5-HT, and after 8-OH-DPAT, which stimulates 5-HT\textsubscript{1A} receptors (Jacobs et al.,
In addition, the 5-HT₁A receptor antagonist, WAY-100635, could block the fenfluramine-induced increase in neurogenesis (Jacobs et al., 1998). WAY-100635 as well as two other 5-HT₁A receptor antagonists (NAN-190, p-MPPI) could also reduce basal cell proliferation rate (Radley and Jacobs, 2002). In this respect, it is of interest that SAL mice have higher hippocampal 5-HT₁A receptor expression and binding capacity (Korte et al., 1996, Veenema et al., 2003b) as well as higher hippocampal 5-HT responsiveness (Van Riel et al., 2002) than LAL mice. Although further research is required, a potential role of the hippocampal 5-HT₁A receptor in preventing the stress-induced decrease in cell proliferation in SAL mice is assumed.

In conclusion, cell proliferation in the dentate gyrus of adult LAL mice was suppressed by stressful stimuli probably through an action mediated by corticosterone, while cell proliferation in SAL was resistant to stress-induced down-regulation. This differential susceptibility in cell proliferation suggests that hippocampal plasticity reflects line differences in coping style during stress.

Acknowledgements
The authors thank Gerardus Zuidema and Auke Meinema for excellent animal care, and Vivi Heine, Suharti Maslam (IN, SILS, Amsterdam) and Jan Bruggink (AP, BCN, Groningen) for technical assistance.
References


Dhabhar FS, McEwen BS, Spencer RL. Adaptation to prolonged or repeated stress-comparison between rat strains showing intrinsic differences in reactivity to acute stress. *Neuroendocrinology* 1997, 65:360-368.


Radley JJ, Jacobs BL. 5-HT(1A) receptor agonist administration decreases cell proliferation in the dentate gyrus. *Brain Res* 2002, 955:264-267.


