

# Social Isolation Increases Number of Newly Proliferated Cells in Hippocampus in Female Flinders Sensitive Line Rats

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**ABSTRACT:** Genetic background influences the responsiveness to stress and plays a crucial role in the pathophysiology of depression. In an animal model of depression, Flinders Sensitive Line rats, and Sprague Dawley controls we analyzed if 7 weeks of social isolation of adult animals affect the number of newly proliferated cells in the dentate gyrus or mRNAs of Neuropeptide Y (NPY), the NPY-Y1 receptor, nociceptin, BDNF, and the serotonin 5HT1A and 5HT2A receptors, which are molecules involved in hippocampal plasticity. Since depressive illness more frequently affects women than men, and females seem to respond differently to stressful experiences than males, female rats were used in this study. Bromodeoxyuridine, which is a thymidin analogue that is incorporated into the DNA of newly formed cells, was administered during 9 days to even out the effects of hormonal fluctuations. Social isolation increased the number of newly proliferated Bromodeoxyuridine-immunoreactive cells in the Flinders Sensitive Line rats, whereas it had no impact on the number of cells in the Sprague Dawley strain. Group housed Sprague Dawley rats had a higher expression of BDNF, NPY, and the serotonin 5HT2A receptor mRNA than “depressed” Flinders Sensitive Line. Social isolation downregulated these molecules in Sprague Dawley but not in Flinders Sensitive Line rats thereby eliminating the differences between the two strains. We demonstrate strain and gender specific responses to stress induced regulation of factors important for hippocampal plasticity. © 2007 Wiley-Liss, Inc.

**KEY WORDS:** depression; stress; neurogenesis; neuropeptides; female

## INTRODUCTION

The hippocampus is a brain region with high functional and structural plasticity that is thought to be important for learning and memory processing (Gould et al., 1999; Lisman, 1999). It is one of the few brain structures in the adult brain where new neurons are formed (Altman, 1962). One hypothesis postulates that depressive symptoms are associated with reduced hippocampal plasticity and decreased neurogenesis (Duman et al., 1999). Hippocampal neurons are sensitive to stress, and preclinical studies indicate that stress may cause atrophy and death of pyramidal neurons in hippocampus as well as decreased neurogenesis in

the dentate gyrus (McEwen et al., 1992; McEwen and Magarinos, 1997; Gould and Tanapat, 1999). Moreover, hippocampal volume is decreased in depressed patients, and the degree of hippocampal volume reduction is correlated with the duration of the disorder (Sheline et al., 1996; Bremner et al., 2000; Campbell et al., 2004).

The social context influences the individuals' responsiveness to stressors, and social isolation is a mild stressor for rodents. Group housing in rats can buffer the influence of certain types of stress on the activity of the hypothalamic pituitary adrenal axis (Ruis et al., 1999; Bartolomucci et al., 2003; Weiss et al., 2004). Recently we demonstrated that social isolation differentially regulates dopamine D2 receptor mRNA levels in striatal dopamine pathways in the Flinders Sensitive Line rats (FSL) and Sprague Dawley (SD) rats, suggesting that strain specific traits may regulate adaptation of the striatal dopamine pathways to the chronic stress of social isolation (Bjørnebekk et al., 2007). Moreover, not only genetic background but also gender can profoundly modify the effect of stress on hippocampal neurogenesis (Fowler et al., 2002; Westebroek et al., 2004; Aberg et al., 2005; Shors et al., 2007). Male rats have been used in most studies that demonstrate a decrease in Bromodeoxyuridine (BrdU) labeling after social isolation and other stress regimes (Gould and Tanapat, 1999; Malberg and Duman, 2003). Lastly, stress plays a role in the development of affective disorders, and both preclinical and human research indicates that females react differently to stress than males (Bowman et al., 2001; Wolf et al., 2001; Westebroek et al., 2004). Interestingly, recent studies show that both acute and chronic stress differentially affect neurogenesis in male and female rats (Westebroek et al., 2004; Aberg et al., 2005; Shors et al., 2007).

It is recognized that a combination of gender, genetics, and environmental factors plays a crucial role in the pathophysiology of depressive illness. Therefore, in this study we investigated the effect of social isolation in adulthood in a genetic rat model of depression, the FSL and SD control rats. The majority of the depressed population is females but most preclinical studies have investigated male animals, therefore female rats were used in this study. Rats were either group or single housed for 7 weeks, and 5-bromo-2deoxyuridine (BrdU) was administered in the drinking

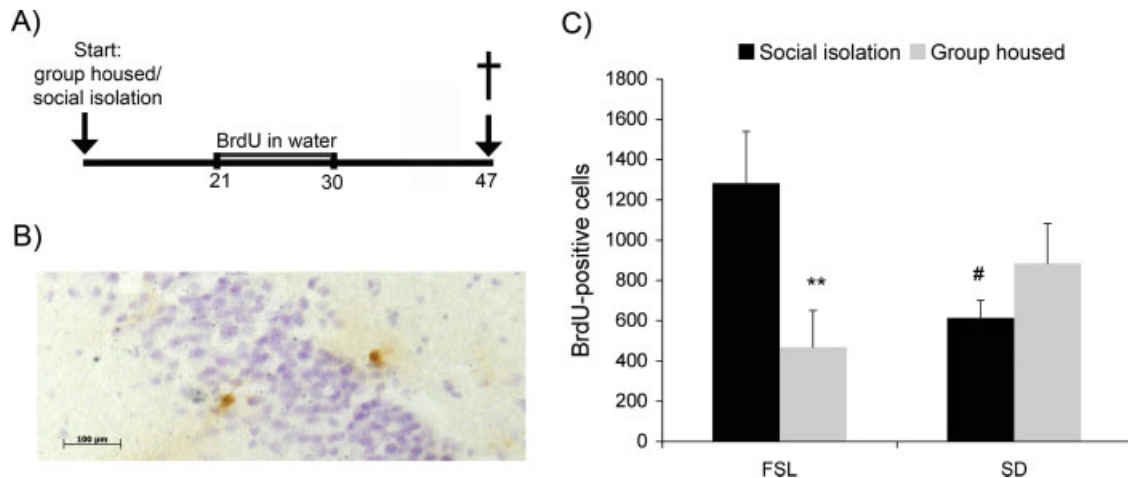
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**FIGURE 1.** Effect of social isolation on BrdU immunoreactive cells. (A) A schematic presentation of the experimental design. Half of the animals were assigned to standard group housing conditions (four/cage) and half were assigned to single housing conditions (one/cage). These housing conditions were maintained for 7 weeks. From experimental Day 21–30 the thymidin analogue BrdU was administered in the drinking water (1 mgBrdU/ml water) of all animals, to trace cells that proliferate during this time period. With this administration paradigm, the BrdU immunoreactive cells in this study will be between 17–26 days old. After 7 weeks of social isolation or standard group housing conditions the animals were sacrificed. (B) Micrograph of two BrdU immuno-

reactive cells in different locations of the granule cell layer of the dentate gyrus. Scale bar; 100  $\mu$ m. (C) Histograms illustrate that social isolation increases number of BrdU immunoreactive cells in female FSL rats, whereas it has no impact on the number of proliferated cells in the SD strain. Socially isolated FSL rats have more BrdU immunoreactive cells, 17–26 days old, than SD rats, whereas there is no strain difference between group housed animals. \*\*  $P < 0.01$  indicates a within strain difference in number of BrdU-positive cells between socially isolated and group housed animals. #  $P < 0.05$  indicates a difference in number of BrdU positive cells between the two strains. [Color figure can be viewed in the online issue, which is available at [www.interscience.wiley.com](http://www.interscience.wiley.com).]

water for 9 days starting on experimental day 21. We aimed to analyze how social isolation during 7 weeks in adult animals affects survival of proliferating cells (17–26 days old) in the dentate gyrus as well as levels of mRNAs for BDNF, nociceptin, Neuropeptide Y (NPY), NPY-Y1 receptor, serotonin 5HT-1A, and 5HT-2A receptor, factors that are involved in neuronal plasticity and mood disorder. Also, with this BrdU administration schedule new hippocampal cells are labeled during all phases of two full estrous cycles thus eliminating large variations related to the stage of the estrous cycle (Tanapat et al., 1999).

## MATERIAL AND METHODS

### Animals and Treatment

The experiment was performed in female FSL ( $n = 16$ ), bred at the Karolinska Institute and age matched female SD rats ( $n = 16$ ) (Møllegaard breeding centre, Denmark). At the start of the experiment the animals were 29–30-weeks-old. Prior to the experiment all animals were housed under standard housing conditions (4/cage) and with access to food and water ad libitum. The animals were randomly assigned into two groups, one with standard group housing conditions (4/cage) and one housed in individual cages. These housing conditions were maintained for 7 weeks (Fig. 1A). The animals were only handled when weighed once a week. All animals were subjected to a controlled 12-h light: 12-h dark-schedule (lights on at 07.00 h). After 7 experimental weeks the animals were sacri-

ficed. The brains were rapidly removed and stored in  $-80^{\circ}\text{C}$  for analyses of mRNAs encoding neuropeptides, the NPY Y1 receptor, BDNF, and serotonin receptors as well as BrdU-immunoreactive cells. Experiments were approved by the local Ethical Committee for Animal Research in Stockholm.

### BrdU Administration

To evaluate number of proliferated cells in hippocampus, BrdU was administered in the drinking water (1 mg BrdU/ml water, Sigma) of all animals during 9 days starting on Day 21 of the experiment (Fig. 1A). This administration schedule labels new hippocampal cells during all the different stages of the estrous cycle. The estrous cycle of rat lasts for 4–5 days, and by administering BrdU for 9 days most of the potential variability caused by the estrous phase is eliminated. The intake of BrdU containing water was measured three times during the 9-day-period and average BrdU dose was calculated to  $51.8 \pm 1.5$  mg/BrdU/kg/day. Animals were sacrificed 17 days after the last day of BrdU administration (Fig. 1A). Thus, BrdU immunoreactive cells were 17–26 days old.

### BrdU Immunohistochemistry

For the immunohistochemistry, coronal 30- $\mu$ m sections were collected with a cryostat throughout the hippocampal formation. Antibodies and dilutions used were: mouse  $\alpha$ -BrdU (1:100 DAKO A/S, Denmark), horse  $\alpha$ -mouse-biotin (1:200 Vector, Burlingame, CA). Immunohistochemistry for BrdU was performed as follows: sections were taken out of freezer ( $-20^{\circ}\text{C}$ ) and post fixed for 10 min in 4% formaldehyde,

rinsed in PBS  $4 \times 5$  min, incubated 30 min in 2 M HCl at  $37^\circ\text{C}$ , rinsed  $3 \times 5$  min in PBS, and incubated for 1 h in blocking solution (horse serum 10%, 0.1% tween in PBS) at room temperature. This 1-h incubation was followed by overnight incubation with mouse  $\alpha$ -BrdU at  $4^\circ\text{C}$ . On Day 2, the samples were rinsed  $3 \times 30$  min in 0.1% tween PBS, incubated with horse  $\alpha$ -mouse-biotin for 60 min at room temperature, rinsed again for 90 min in PBS 0.1% tween followed by 30 min in PBS only. The sections were then incubated for 40 min at room temperature with avidin-biotin-peroxidase complex (1:100 in PBS, Vectastain Elite, Vector, Burlingame, CA), then rinsed in PBS for 1 h, followed by peroxidase detection (0.7 mg/ml, DAB dissolved in  $\text{H}_2\text{O}$ ) (DAB Peroxidase Substrate, Sigma) for about 25 s per section. The sections were rinsed in PBS and stained with a hematoxylin solution (Vector).

### Stereology of BrdU Positive Cells

For quantification of BrdU positive cells in the dentate gyrus the unbiased optical fractionator counting procedure was performed (West et al., 1991). Coronal 30- $\mu\text{m}$  sections were taken throughout the hippocampus and every 15th section (450- $\mu\text{m}$  apart) was selected for analysis of the right dentate gyrus. An unbiased counting frame with known area was superimposed on the field of view by appropriate software (Stereologer<sup>TM</sup>, SPA). The counting frames were systematically distributed with known  $x$  and  $y$  steps throughout the marked region from a random starting point. The area of the counting frame relative to the area associated with the  $x$  and  $y$  step gives the second fraction (area sampling fraction [asf]). The height of the optical dissector relative to the thickness of the section results in the third fraction (height [h]/thickness [h]). The total number of neurons is given by  $N_{\text{total}} = \sum Q - \times \frac{1}{\text{ssf}} \times \frac{1}{\text{asf}} \times \frac{t}{b}$  where  $\sum Q -$  is the number of neurons counted in the dissectors. The dentate gyrus was manually outlined using a  $10\times$  lens. Cell counts were performed with a  $60\times$  lens (numerical aperture = 1.4). Positive cells were counted if they were within the dissectors. Cells situated further than two cell body widths away from the base of the granular cell layer were defined as belonging to hilus, and thus not counted. Also, cells were excluded if they were situated in the lowermost focal plane. To estimate total number of BrdU cells per individual, a representative sample of BrdU immunoreactive cells in the dentate gyrus of the left hemispheres was compared with that of the right hemispheres.  $t$ -tests showed that there were no differences in number of BrdU immunoreactive cells between the two hemispheres, and the total number of cells per individual was calculated.

### In Situ Hybridization

Coronal brain sections (30  $\mu\text{m}$ ) were cut on a cryostat at  $-20^\circ\text{C}$ , and sections were thawed onto glass slides. Single-stranded oligonucleotide 48-mer DNA probes specific for NPY (nt 1671–1714) (Larhammar et al., 1987), Y1 receptor (nt 141–188, in rat NPY R sequence in genebank under accession no NM001013032.1), 5-HT<sub>1A</sub> receptor mRNA (nt 546–584)

(Albert et al., 1990), 5-HT<sub>2A</sub> receptor mRNA (nt 575–623) (Pritchett et al., 1988), nociceptin (nt 320–368) (Meunier et al., 1995), and BDNF (250–298) (Leibrock et al., 1989) were used. The probes were 3'-end labeled with  $\alpha$ -<sup>33</sup>P-dATP (Perkin-Elmer, Boston, MA) to a specific activity of  $\sim 1 \times 10^9$  cpm/mg using terminal deoxynucleotidyl transferase (Gibco) and hybridized according to a standardized protocol (Bjørnebekk et al., 2005). Autoradiogram films were scanned and optical density values quantified using appropriate software (ImageJ, NIH). A <sup>14</sup>C step standard (Amersham, Buckinghamshire, UK) was included to calibrate optical density readings and convert measured values into nCi/g.

### Statistics

Two-way ANOVAS (strain  $\times$  housing) with planned comparison and Tukey HSD post hoc tests were used to analyze the number of BrdU positive cells and the different mRNAs within each region in FSL and SD rats that were group housed or socially isolated (Statistica, StatSoft, Tulsa).

## RESULTS

Weight differences between the SD and the FSL strain and the changes in socially isolated and group housed rats have previously been described (Bjørnebekk et al., 2007). Briefly, at the start of the experiment there was an expected weight difference between the two strains, SD rats weighing in average 40% more than FSL. Whereas, SD strain gained more weight when socially isolated than group housed, the opposite was true in the FSL strain; only group housed animals increased their weight during the course of the experiment.

### Number of BrdU Cells in the Dentate Gyrus in Group Housed and Socially Isolated FSL and SD Rats

The BrdU protocol used in this study allowed quantitative analysis of proliferated BrdU-immunoreactive cells that had survived 17–26 days after they were generated (Fig. 1B). There was trend to lower level of BrdU immunoreactive cells, 17–26 day old, in group housed FSL compared with SD, albeit not statistically significant ( $P = 0.13$ ). Social isolation differentially affected BrdU-labeled cells in the two strains. While the number of BrdU labeled cells was markedly increased in socially isolated FSL rats no change was observed in SD rats. In fact, isolated FSL rats had more BrdU labeled cells than isolated SD rats (Fig. 1C).

### Serotonin Receptors, BDNF, Neuropeptide, and NPY Y1 Receptor mRNA Levels in Hippocampal Subregions in Group Housed and Socially Isolated FSL and SD

Levels of mRNAs encoding the serotonin 5HT<sub>1A</sub> and 5HT<sub>2A</sub> receptors, BDNF, and the neuropeptides NPY and

TABLE 1.

**Brain Derived Neurotrophic Factor (BDNF), Neuropeptide Y (NPY) and Serotonin 5HT<sub>2A</sub> Receptor mRNA in Group Housed and Socially Isolated SD and FSL Rats. Levels of BDNF, NPY and the Serotonin 5HT<sub>2A</sub> Receptor mRNA in Hippocampal Subregions were Quantified and are Shown as Mean nCilg ± S.E.M. SD and FSL Rats were Group Housed or Socially Isolated During Seven Weeks**

	SD		FSL	
	Group housed	Socially isolated	Group housed	Socially isolated
<b>BDNF</b>				
CA1	29.4 ± 2.7	23.2 ± 0.7*	26.6 ± 2.2	24.3 ± 1.0
CA3	74.8 ± 7.2	62.8 ± 1.7	57.9 ± 3.0#	56.9 ± 3.3
CA4	68.9 ± 6.8	58.9 ± 1.3	49.3 ± 2.3##	49.5 ± 2.6
DG	71.4 ± 8.3	57.4 ± 0.8	59.1 ± 3.2	58.4 ± 3.5
<b>NPY</b>				
CA1	40.0 ± 1.8	34.9 ± 1.2**	30.5 ± 0.9###	28.8 ± 0.8##
CA3	38.4 ± 1.6	32.7 ± 0.7***	28.8 ± 0.7###	28.0 ± 0.9##
DG	56.0 ± 2.8	44.6 ± 2.3***	43.9 ± 1.0###	41.8 ± 2.2
<b>5HT<sub>2A</sub>rec</b>				
CA3	4.1 ± 0.8	3.7 ± 0.3	3.1 ± 0.1##	3.2 ± 0.1
CA4	5.3 ± 0.3	4.7 ± 0.3	4.8 ± 0.9	4.8 ± 0.9

Decreased mRNA levels due to social isolation = \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . Lower mRNA levels in FSL compared to SD = # $P < 0.05$ , ## $P < 0.01$ , ### $P < 0.001$ . Note that social isolation selectively decreased mRNA levels in the SD strain, which tended to diminish the differences between the two strains.

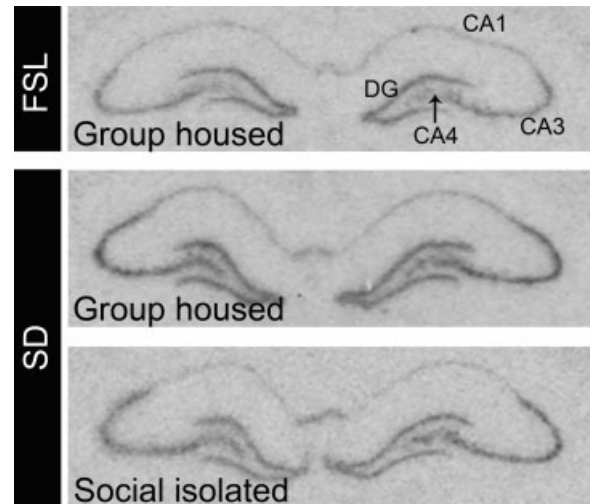
nociceptin, were analyzed in hippocampal subregions (Table 1). Analysis of mRNAs encoding BDNF revealed differences between the two strains, however, the difference was restricted to group housed rats. Group housed FSL rats had lower levels of BDNF mRNAs than group housed SD rats in CA3 and CA4 ( $P < 0.05$ , 0.01). In the SD strain, BDNF mRNA was lower in socially isolated compared with group housed animals in the CA1 region ( $P < 0.05$ ) (Fig. 2 and Table 1).

NPY mRNA levels were lower in FSL compared with SD rats in all analyzed regions in group housed and in socially isolated animals in the CA1 and CA3 ( $P < 0.01$ – $0.001$ ) (Fig. 3 and Table 1). In the SD strain, social isolation decreased NPY mRNA levels in all analyzed subregions ( $P < 0.01$ – $0.001$ ), whereas it did not have any impact on NPY mRNA regulation in the FSL strain.

Housing did not affect 5HT<sub>2A</sub> receptor mRNA levels within the strains.

However, there was a strain difference in mRNA encoding the 5HT<sub>2A</sub> receptor, which was restricted to group housed animals. 5HT<sub>2A</sub> receptor mRNA levels were lower in group housed FSL compared with group housed SD in CA3 ( $P < 0.01$ ) (Fig. 4 and Table 1).

No differences were found between the two strains independent of housing condition in mRNAs encoding the 5HT<sub>1A</sub> receptor, nociceptin, and the NPY Y1 receptor (data not shown).

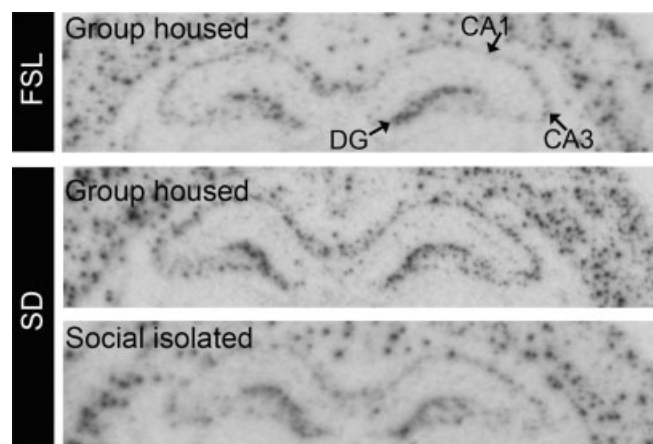


**FIGURE 2.** Hippocampal BDNF mRNA expression after social isolation. The autoradiogram is a representative illustration of BDNF mRNA in group housed FSL and SD rats, and socially isolated SD rats. In group housed rats, BDNF mRNA levels were higher in SD rats compared with FSL in the CA3 and CA4 region. In socially isolated SD rats BDNF mRNA was lower than in group housed SD in the CA1 region.

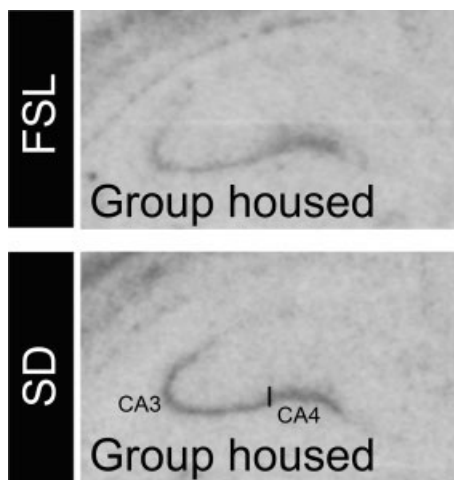
Correlative statistics were performed between all the different mRNAs and BrdU-counts, and the only factor measured that was correlated to the BrdU immunoreactive cells at this time period was 5HT<sub>1A</sub> receptor mRNA in the dentate gyrus ( $r = 0.42$ ,  $P < 0.05$ ).

## DISCUSSION

Stress, genetics, and reduced hippocampal plasticity have been implicated in the pathogenesis of depressive illness.



**FIGURE 3.** Neuropeptide Y (NPY) mRNA expression in hippocampus after social isolation. In situ autoradiogram illustrates NPY mRNA expression in group housed FSL and SD rats, and socially isolated SD rats. Note the higher expression in group housed SD compared with FSL in all hippocampal subregions analyzed. Social isolation decreased NPY mRNA selectively in the SD strain in all analyzed regions.



**FIGURE 4.** In situ autoradiogram of 5HT2A receptor mRNA expression in the hippocampus. The expression of 5HT2A receptor mRNA was higher in group housed SD rats compared with FSL. No strain differences were found between socially isolated SD and FSL rats.

Moreover, there are gender differences in stress response and depressive illness more frequently affects women than men. In the present study we analyzed in female “depressed” FSL and SD rats the effect of 7-week social isolation on the number of 17–26 days old hippocampal cells and mRNAs encoding putative key molecules for control of plasticity and mood disorders.

### Social Isolation Increases Number of BrdU Immunoreactive Cells in the Dentate Gyrus

The effect of chronic mild stress on BrdU immunoreactive cells was different in female FSL and SD rats. Whereas 7 weeks of social isolation did not affect the number of BrdU labeled cells in SD rats, it increased their number in the FSL. This finding was contrainuitive and in contrast to numerous reports where adult cell proliferation is reduced in response to acute or chronic stress (Gould et al., 1997; Tanapat et al., 1998; Jacobs et al., 2000; Czeh et al., 2002; Malberg and Duman, 2003). However, most studies relating stress to reduced neurogenesis and depression have used only male animals. This is important as accumulated evidence shows that females respond differently to stressful experiences than males (Bowman et al., 2001; Wolf et al., 2001; Bale, 2006; Cahill, 2006) and that stress exposure has no impact or even increases proliferation and survival of proliferated cells in females (Fowler et al., 2002; Falconer and Galea, 2003; Westenbroek et al., 2004; Aberg et al., 2005; Shors et al., 2007). In addition, group housed FSL rats gained weight during the experiment whereas socially isolated FSL did not. In contrast, in the SD strain socially isolated rats gained more weight during the experiment than group housed animals. These observations, in view of the reduced appetite and

reduced body weight frequently observed in depressed patients, further support the notion that the increased number of newly proliferated cells in socially isolated FSL probably is not associated with a relief of depressive-like symptoms.

In the present study, mRNA levels of the 5HT1A receptor were positively correlated to BrdU labeled cells. The dentate gyrus is enriched with 5HT1A receptors (Azmitia et al., 1996), and our data are in line with the suggestion that activation of the 5HT1A receptor located on granule cell precursors may stimulate the production of neurons in the dentate gyrus (Gould, 1999).

Previously we have found that running decreases depressive-like behavior in the Porsolt swim test and increases cell proliferation and NPY mRNA in hippocampal regions (Bjørnebekk et al., 2005; Bjørnebekk et al., 2006) in single housed male FSL rats. NPY mRNAs in the dentate gyrus and hilus correlate positively to cell proliferation and also to survival of proliferated cells induced by treatments with antidepressive-like effects. These results support the notion that cell proliferation can be induced by NPY and other data indicate that activation of the NPY Y1 receptor is involved. The absence of a correlation between NPY and cell proliferation in this study implies that cell proliferation and cell survival after social isolation could be regulated by several factors. NPY and other neuropeptides are released by high-frequency stimulation or bursting activity (Edwards et al., 1982). Social isolation over a prolonged period suggests involvement of other mechanisms, as compared with those in physical activity in stimulating neuronal progenitor cells to divide and migrate.

The present results of increased number of 17–26 days old hippocampal cells after social isolation in an animal model of depression do not support the neurogenesis hypothesis of depression. Hypothetically, the newly formed hippocampal cells form functional connections, which are involved in spatial memory formation (Gould et al., 1999). Interestingly, chronic stress improves spatial memory, which is a hippocampal dependent learning task in females (Bowman et al., 2001), whereas chronic stress impairs spatial memory and decreases neurogenesis in males (Krugers et al., 1997; Conrad et al., 2003). It is possible that sex differences in hippocampal plasticity and spatial memory in response to chronic stress have evolved in response to different evolutionary demands on males and females regarding reproduction and taking care of offspring.

### Social Isolation Decreases BDNF and NPY mRNA Selectively in SD Rats

Reduced hippocampal BDNF and NPY have been suggested to be related to depressive illness, and both factors are elevated by antidepressant treatments (Stenfors et al., 1989; Nibuya et al., 1995; Nibuya et al., 1996; Husum et al., 2000; Bjørnebekk et al., 2006) and suggested to have antidepressant potential (Siuciak et al., 1997; Redrobe et al., 2002; Shirayama et al., 2002; Mathé and Gruber, 2004). Therefore, we analyzed whether there were any differences between the “depressed”

FSL strain and SD in hippocampal mRNA expression of BDNF and NPY and if social isolation would influence the expression of these factors.

Decreased levels of NPY have been found in the cerebrospinal fluid (Heilig et al., 2004) and plasma (Nilsson et al., 1996) of depressed patients, and in post mortem brain tissue from suicide victims (Widdowson et al., 1992). NPY mRNA levels are decreased in the dentate gyrus of male FSL rats compared with FRL rats (Caberlotto et al., 1998; Bjørnebekk et al., 2006; Jimenez-Vasquez et al., 2007). Now we also demonstrate that NPY mRNA levels are decreased in group housed female FSL compared with SD controls in all fields of Ammon's horn and the dentate gyrus, indicating that decreased NPY mRNA levels are a robust feature of the "depressed" FSL phenotype. Various forms of stress reduce hippocampal NPY protein (Jiménez-Vasquez et al., 2001; Husum and Mathé, 2002; Husum et al., 2002) and mRNA expression (Sergeyev et al., 2005) and the number of NPY positive cells (Ishida et al., 2005).

Social isolation decreased NPY in all regions in the SD strain, whereas housing had no additional effect on NPY mRNA regulation in the FSL. This result is in line with the recent finding that the stress of maternal separation reduces NPY in the control FRL but has no additional effect in the genetically "depressed" FSL strain (Wörtwein et al., 2006).

BDNF immunoreactivity is higher in postmortem brains of depressed subjects treated with antidepressant medication compared with untreated subjects (Chen et al., 2001). Moreover, serum levels of BDNF are decreased in antidepressant-free patients with major depression compared with patients medicated with antidepressants and healthy controls. BDNF serum levels were also negatively correlated with severity of depression (Karege et al., 2002; Shimizu et al., 2003). Group housed FSL rats had lower levels of BDNF mRNA in the CA3 and CA4 region compared with SD rats. However, when comparing single housed male FSL to FRL rats, no difference in hippocampal BDNF mRNA was found (Bjørnebekk et al., 2005). Social isolation selectively decreased BDNF mRNA expression in SD rats in the CA1 region, but had no effect in the FSL strain. Moreover, in socially isolated animals there were no strain differences in BDNF mRNA expression indicating differential responses to social isolation between the two strains. Published data support the involvement of BDNF in response to the stress of social isolation. In male SD rats social isolation reduced hippocampal BDNF mRNA expression (Barrientos et al., 2003) and protein concentration but did not affect plasma corticosterone (Scaccianoce et al., 2006). The presented mRNA BDNF data are parallel to the NPY results indicating that the genetic changes in FSL are predominant and the added stress of social isolation has no longer an effect. Consistently with these findings, our previous data show effects of antidepressant treatment only in "depressed" FSL and not control rat strains (Bjørnebekk et al., 2005; El Khoury et al., 2006).

The role of postsynaptic 5HT2A receptors in depressive illness has not been elucidated. A significant reduction in 5HT2A receptors in the hippocampus of antidepressant-free

suicide victims with a diagnosis of major depression has been reported (Rosel et al., 2000). In preclinical studies stress decreases 5HT2A receptor protein and mRNA levels in hippocampus of learned helplessness rats (Dwivedi et al., 2005), and antisense oligodeoxynucleotide studies suggest that the 5HT2A receptor is implicated in anxiety and learned helplessness behavior (Papolos et al., 1996; Cohen, 2005). In the present study, group housed FSL rats had a lower expression of 5HT2A receptors mRNA in the CA3 subregion compared with SD. Thus, BDNF, NPY and 5HT2A receptor mRNA levels in hippocampal subregions in group housed FSL were lower compared with SD controls, supporting the depressive endophenotype of the FSL. Social isolation affected the gene regulation of these factors selectively in the SD strain. This is consistent with our interpretation of the genetic loading in FSL being so prominent that some systems are no longer influenced by a mild environmental stressor, in this instance social isolation.

## CONCLUSION

Our results support the notion that gender and genetic background modify the effect of mild stress on hippocampal cell proliferation. Moreover, we demonstrated strain specific responses to social isolation on mRNAs encoding the BDNF, NPY, and the 5HT2A receptor. Thus, gene, gender, and environmental interactions are important for regulations of key factors for hippocampal plasticity that are suggested to have a role in depressive illness.

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