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## Influence of gender and social environment in an animal model of affective disorders

Westenbroek, Christel

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## Differential effects of chronic stress on Fos-reactivity and phospho-CREB expression in isolated and mixed-gender pair-housed male and female rats

C. Westenbroek, G. J. Ter Horst, T. A. B. Snijders<sup>‡</sup>, D. S. Fokkema, M. Gerrits,  
J. A. Den Boer

*Department of Psychiatry, section Biological Psychiatry, Graduate school of Behavioral and Cognitive Neurosciences, <sup>‡</sup>Department of Sociology,  
University of Groningen, PO box 30.001, Groningen, the Netherlands*

**Submitted**

*Chronic stress is thought to be an important factor in the aetiology of depression, whereas social support has stress-reducing properties and a positive influence on the course of a depressive episode. 21 Days of chronic footshock stress was used as a model for affective disorders in rats and group housing of rats could provide a suitable model for social support to study the underlying neurobiological mechanisms. Male and female rats were either individually or socially housed in mixed-gender pairs. Limbic Fos expression and phospho-CREB in the dentate gyrus were used as markers of stress coping. In socially housed males most brain areas analysed showed a stress-induced increased Fos expression, whereas in isolated males stress-induced increases were lower and reached significance only in the PVN, prelimbic and anterior cingulate cortex, and median raphe nucleus. Stress exposure affected Fos-ir only in the SON and MPN of isolated females, whereas socially housed females showed increased Fos-ir in more regions. Phospho-CREB expression in the dentate gyrus was increased after stress in socially but not in individually housed males. In isolated females, pCREB expression was decreased, which could not be prevented by pair-housing. In conclusion: Social housing appeared to increase Fos reactivity to stress, but it augmented DG pCREB expression in males. In females pair-housing with a male only had slight ameliorating effects, but was superior to isolation.*

# Abstract

## Introduction

Stress, as well as lack of social support, plays an important role in the onset and/or relapse of a depressive episode.<sup>12,18,26</sup> Psychotherapy that could be viewed as formalised social support, improves symptoms<sup>13</sup> and normalises brain activity in depressed patients, similar to antidepressant treatment.<sup>5</sup> This suggests a neurobiological basis for the ameliorating effects of social support/psychotherapy.

Chronic stress has been associated with the onset of a depressive episode, and therefore chronic stress exposure paradigms are seen as valid animal models of affective disorders.<sup>47</sup> Symptoms of depression, like anhedonia, hypercortisolism, sleep disturbances, and memory dysfunctions, can be induced in rodents by chronic stress exposure, and prevented by antidepressant treatment.<sup>7,47</sup> In rats, like in humans, the social environment is an important determiner of the way animals cope with stress. In male rats social housing is able to counteract the effects of a social defeat.<sup>30,43</sup> Also gender differences are found in influence of housing conditions.<sup>2</sup> While social instability affects females more than males,<sup>17</sup> crowding is stressful for males but it appears to calm females.<sup>6</sup>

The occurrence of affective disorders has been associated with reduced synaptic plasticity in the brain.<sup>11</sup> A dysfunction of processes involved in neural plasticity could result in the inability to respond and/or adapt to aversive stimuli. Clinical and preclinical studies have reported plasticity related changes in the prefrontal cortex and hippocampus, and the effects of antidepressant hereon. Post mortem studies detected reduced levels of cAMP response-element binding protein (CREB) in the human temporal cortex and reduced brain derived neurotrophic factor (BDNF) levels in the hippocampus of untreated depressed patients vs. patients treated with antidepressants and healthy controls.<sup>8,10</sup> In rats stress has negative effects on brain plasticity, shown by a reduction in hippocampal neurogenesis and apical dendrite arbor complexity.<sup>20,25</sup> Also chronic stress reduces phosphorylated (p)CREB and BDNF expression in several brain regions,<sup>40</sup> whereas antidepressant have been found to increase pCREB levels in rodents.<sup>39</sup>

Previously we have shown that social housing of rats in unisex groups can improve stress coping in females but not in males.<sup>46</sup> In this follow up experiment we investigated whether mixed-gender pair housing could improve stress-coping in male and female rats. Phospho-CREB expression in the dentate gyrus was used as a measurement of neuronal plasticity changes in the hippocampus. Expression of limbic c-Fos was used as a marker of neuronal activity.<sup>32</sup> Since chronic stress affects limbic activity and Fos expression and treatment with antidepressants is able to modulate the Fos response of stress-exposed rats,<sup>1,21</sup> Fos-expression provides a marker for the modulatory effects of pair-housing.

## Material & Methods

### *Rats & housing conditions*

Male (n=30) and female (n=30) Wistar rats were either individually or pair-housed with a rat of the opposite sex (n=6 per group). Pair housing occurred in the following 3 combinations; control male with a control non-stressed female, control male with a stressed female and stressed male housed with a control non-stressed female (see table 1 for group names). Isolated rats were divided in a control and stress group (n=6 per group).

A plastic tube ( $\varnothing$  8 x 17 cm.) was placed in each cage as a shelter. To prevent pregnancy in pair-housed females, the male partner rats were vasectomised under halothane anaesthesia 10 days before the start of the experiment and 3 days before being housed with a female. The light-dark cycle was reversed (lights on 19.00-7.00 hr) and water and food was provided ad lib. At the start of the experiment rats were of the same age with males weighing  $287 \pm 3$  g. and females  $233 \pm 2$  g. All experimental procedures were approved by the Animals Ethics Committee of the University of Groningen (FDC: 2509). Efforts were made to minimise the number of animals used and their suffering.

### *Stress procedure*

Rats were subjected to a chronic inescapable stress protocol for 3 weeks. Daily, at random times, rats in the stress group were placed in a box with a metal grid floor and received 5 inescapable footshocks at different intervals during a 30-120 minute session (0.8 mA in intensity and 8 sec in duration). A light signal (10 sec) preceded each footshock adding a 'psychological' component to the noxious event. On the last day, the stress-exposed animals were placed in the box for 30 minutes and subjected to the light stimulus only, so Fos activation changes would reflect the 'psychological' aspect of stress exposure and not that of a foot shock-related pain response, that can activate the same or related brain circuitry.<sup>38</sup> Control rats were handled daily but were not exposed to the adverse environment. All rats were weighed daily.

### *Immunohistochemistry*

The rats were sacrificed on day 22, two hours after the start of the last stress session, by sodium pentobarbital anaesthesia (1 ml, 6%). The rats were transcardially perfused with 50 ml heparinised saline and 300 ml of a 4% paraformaldehyde solution in 0.1 M sodium phosphate buffer (pH 7.4), 2 hours after the start of the last exposure to the stress box. The brains were removed and postfixed in the same fixative overnight at 4°C. Adrenal weights, corrected for body weight, were used as indication of the amount of stress perceived.<sup>9</sup>

Following an overnight cryoprotection in a 30% sucrose solution, serial 40  $\mu$ m coronal sections were made with a cryostat microtome and collected in 0.02 M potassium phosphate saline buffer (KPBS). Fos and pCREB immunostaining was performed on free-floating sections. Sections were rinsed with 0.3% H<sub>2</sub>O<sub>2</sub> for 10 minutes to reduce endogenous peroxidase activity, thoroughly washed with KPBS and incubated with the rabbit anti-Fos antibody (1:10,000, Oncogene Research Products,

**Table 1.** Group names

|                    |               | <b>control male +<br/>control female</b> | <b>control male +<br/>stressed female</b> | <b>stressed male +<br/>control female</b> |
|--------------------|---------------|--|---|---|
| <i>group names</i> | <b>male</b>   | control(S♀)                              | control(C♀)                               | stress(C♀)                                |
|                    | <b>female</b> | control(C♂)                              | control(S♂)                               | stress(C♂)                                |

San Diego, CA) or polyclonal rabbit anti-phospho-CREB (pCREB) (1:1000 commercialized by Upstate Biotechnology, Charlottesville, VA, USA) diluted in 0.02 M KPBS with 0.25% Triton X-100 and 2% Normal Goat Serum for 72 hours at 4°C. After thorough washing, the sections were subsequently incubated for 2 hrs with biotinylated Goat-anti-Rabbit IgG (Vector Laboratories, Burlingame, CA) (1:1000 in 0.02 M KPBS) and avidin-biotin-peroxidase complex (Vectastain ABC Elite Kit, Vector Laboratories, Burlingame, CA). After thorough washing, the peroxidase reaction was developed with a DAB-nickel solution and 0.3% H<sub>2</sub>O<sub>2</sub>. Sections were washed for 15 minutes in buffer and mounted with a gelatine solution and air dried, dehydrated in graded alcohol and xylol solutions and then coverslipped with DePeX mounting medium (BDH). To reduce staining artefacts or intensity differences the sections from all groups were processed simultaneously. To control for cross-reactivity due to aspecific binding, immunostainings were performed by incubating the sections without the presence of one of the antibodies needed for the reaction (primary, secondary) thereby confirming the specificity of all the antibodies. All these reactions were negative and confirmed the specificity of the antibodies.

Fos positive cells were blindly quantified using a computerised imaging analysis system in the following regions:<sup>37</sup> nucleus accumbens; bregma +2.15 to 0.45 (core; NacC and shell; NAcS region), prefrontal cortex; bregma +3.20 to +2.15 (infralimbic; IL, prelimbic; PL, anterior cingulate, anterior part; ACa), anterior cingulate cortex caudal part; bregma +1.45 to -1.33 (ACc), PVN; bregma -1.08 to -1.78, SON; bregma -0.83 to -1.53, MPN; bregma -0.26 to -1.08, amygdala; bregma -2.00 to -2.85 (central; CeA, medial; MeA, lateral; LaA and basolateral; BLA part), DG: bregma -2.45 to -4.20, VTA; bregma -5.25 to -6.06, DRN; bregma -7.10 to -9.25, and MRN; bregma -7.60 to -8.85. The selected areas were digitised by using a Sony charge-coupled device digital camera mounted on a LEICA Leitz DMRB microscope (Leica, Wetzlar, Germany) at 100x magnification. The number of pCREB positive nuclei in the granule cell layer of the DG was analysed. Fos and pCREB positive nuclei were counted using a computer-based image analysis system LEICA (LEICA Imaging System Ltd., Cambridge, England). The resulted data were reported as number of positive cells/0.1mm<sup>2</sup>. No left-right asymmetry was found in Fos and pCREB immunoreactivity and therefore the mean ± standard error (SEM) of both sides was calculated.

### *Statistical analysis*

Main effects of housing (individual-social), treatment (control-stress) and treatment of the partner (control-stress) and the interaction effects were analysed by Multilevel (mixed model) analysis (MlwiN software, version 1.2),<sup>28</sup> with random effects for rats and cages, with rats (level 1) nested in cages (level 2). Weight gain was analysed with nested random effects for days (level 1), rats (level 2) and cages (level

3).<sup>34</sup> A natural log transformation was performed when the data showed a skewed distribution (Fos-ir in the ACc, MPN and PVN). For the multilevel analysis the number of rats was 60 (male + female), so the effective degrees of freedom is large enough for a Z test, and effects therefore were tested by Z tests. When the main effects were found to be significant further pairwise comparisons for the males were performed by using ANOVA in SPSS 10.0. Data are presented as group means  $\pm$  SEM.

## Results

### Weight gain

Rats in all groups continued to grow, as shown by a significant effect of day on weight gain ( $Z = 8,792$ ,  $p \leq 0.001$ ). Significant effects of treatment (stress or control), treatment by day, and treatment-partner by day on the growth rate were found (resp.  $Z = -4.243$ ,  $p \leq 0.001$ ;  $Z = -2.935$ ,  $p = 0.003$ ;  $Z = 2.162$ ,  $p = 0.03$ ). Housing conditions affected the growth rate in response to stress, as shown by a significant interaction effect of housing and treatment ( $Z = 2.660$ ,  $p = 0.008$ ). Chronically stressed rats, isolated ( $F_{1,10} = 40.614$ ,  $p \leq 0.001$ ), as well as socially housed males (compared to: control( $C_{\text{♀}}$ ):  $F_{1,10} = 25.288$ ,  $p \leq 0.001$ ; control( $S_{\text{♀}}$ ):  $F_{1,10} = 12.676$ ,  $p = 0.005$ ) showed a significant reduction in growth rate. Males housed together with a stressed female partner also showed a reduced weight gain compared to isolated controls ( $F_{1,10} = 5.008$ ,  $p = 0.049$ ). In females, only the socially housed females demonstrated a reduced growth rate when compared to control( $C_{\text{♂}}$ ) females ( $F_{1,10} = 5.846$ ,  $p = 0.036$ ) (Table 2).

**Table 2.** Mean body weight gain per day and relative adrenal weight.

|                | <b>BW gain/day (gr)</b>                           | <b>adrenal (mg/gr BW)</b>     |
|----------------|---|-------------------------------|
| <b>males</b>   | <b>indiv. controls</b>                            | 3,97(0,25)                    |
|                | <b>indiv. stressed</b>                            | 2,15(0,24) <sup>***</sup>     |
|                | <b>social controls(<math>C_{\text{♀}}</math>)</b> | 3,74(0,27)                    |
|                | <b>social controls(<math>S_{\text{♀}}</math>)</b> | 3,53(0,29) <sup>#</sup>       |
|                | <b>social stressed(<math>C_{\text{♀}}</math>)</b> | 2,24(0,21) <sup>***\$\$</sup> |
| <b>females</b> | <b>indiv. controls</b>                            | 0,91(0,17)                    |
|                | <b>indiv. stressed</b>                            | 1,19(0,34)                    |
|                | <b>social controls(<math>C_{\text{♂}}</math>)</b> | 1,43(0,17)                    |
|                | <b>social controls(<math>S_{\text{♂}}</math>)</b> | 1,25(0,32)                    |
|                | <b>social stressed(<math>C_{\text{♂}}</math>)</b> | 0,91(0,26) <sup>*</sup>       |

Significant stress effects within housing conditions (\*  $p \leq 0.05$ , \*\*\* $p \leq 0.001$ ), housing effect within treatment conditions (<sup>#</sup> $p \leq 0.05$ , <sup>##</sup> $p \leq 0.01$ ), and effects of stress compared to control( $S_{\text{♀}}$ ) (<sup>§</sup> $p \leq 0.005$ , <sup>\$\$</sup> $p \leq 0.01$ , <sup>\$\$\$</sup> $p \leq 0.001$ ).

*Relative adrenal weight*

Relative adrenal weight was significantly affected by treatment and housing conditions (resp.  $Z=5.366$ ,  $p\leq 0.001$  and  $Z=-2.232$ ,  $p= 0.026$ ). Main effects of gender and gender by housing were also found (resp.  $Z=16.883$ ,  $p\leq 0.001$  and  $Z=5.962$ ,  $p\leq 0.001$ ), the latter showing that housing conditions differently affected adrenal weight in males and females.

In male rats chronic stress exposure increased adrenal weight in isolated ( $F_{1,10} = 24.960$ ,  $p=0.001$ ) and socially housed animals (compared to control( $C\text{♀}$ ):  $F_{1,10} = 28.984$ ,  $p\leq 0.001$  and control( $S\text{♀}$ ) males:  $F_{1,10} = 41.739$ ,  $p\leq 0.001$ ). Compared to isolated control males, socially housed control( $C\text{♀}$ ) males developed higher adrenal weights ( $F_{1,10} = 4.992$ ,  $p=0.049$ ), but the difference with control( $S\text{♀}$ ) males did not reach significance. In addition, socially housed stressed males showed higher adrenal weights than isolated stressed males ( $F_{1,10} = 12.488$ ,  $p= 0.005$ ). In socially, but not individually housed females chronic stress exposure significantly increased the adrenal weight (compared to control( $C\text{♂}$ ):  $F_{1,20} = 4.541$ ,  $p=0.046$ ; control( $S\text{♂}$ ):  $F_{1,20} = 6.427$ ,  $p=0.02$ ). However isolated control females had higher adrenal weights than social control( $C\text{♂}$ ) and control( $S\text{♂}$ ) females (resp.  $F_{1,20} = 11.087$ ,  $p=0.003$  and  $F_{1,20} = 13.455$ ,  $p=0.002$ ). The difference between isolated and socially housed stressed animals was not significant ( $F_{1,20} = 3.609$ ,  $p=0.072$ ) (Table 2).

*Fos*

Data related to gender differences are only presented when main or interaction effects involving gender were significant. Group means per brain region of both females and males are listed in table 3.

**Nucleus accumbens: Core (NacC):** Treatment had a significant effect on Fos expression in the core of the accumbens ( $Z=2.659$ ,  $p=0.008$ ). The effects on NacC Fos-ir of housing conditions and the treatment-partner were almost significant (resp.  $Z=1.919$ ,  $p=0.056$  and  $Z=1.946$ ,  $p=0.051$ ). Stress exposed socially housed males showed increased Fos-ir in the NacC ( $F_{1,8} = 12.375$ ,  $p=0.008$ ) compared to control( $C\text{♀}$ ) males and they also expressed more Fos labelled cells in this area than the isolated stressed males ( $F_{1,7} = 7.304$ ,  $p=0.031$ ). Also the socially housed females showed a significantly increased Fos expression in the NAcC after stress exposure compared to control( $C\text{♂}$ ) females ( $F_{1,17} = 7.512$ ,  $p=0.048$ ).

**Shell (NacS):** In the shell region of the accumbens significant main effects of housing ( $Z=3.735$ ,  $p\leq 0.001$ ) and housing by treatment ( $Z=2.192$ ,  $p=0.029$ ) were found. Fos-ir in the NAcS of socially housed males was increased after stress exposure compared to control( $C\text{♀}$ ) males ( $F_{1,8} = 6.132$ ,  $p=0.038$ ) and was higher in socially housed stressed males than in isolated stressed males ( $F_{1,7} = 5.736$ ,  $p=0.048$ ). In females no effect of stress exposure on Fos-ir in the NAcS was found,

however the presence of a stressed male companion increased Fos-ir in this region ( $F_{1,9}=5.945$ ,  $p=0.037$ ). Also we observed a significant difference between socially housed and isolated control females (compared to control( $C\text{♂}$ )):  $F_{1,8}=5.645$ ,  $p=0.045$ ; control( $S\text{♂}$ ):  $F_{1,9}=13.480$ ,  $p=0.005$ ).

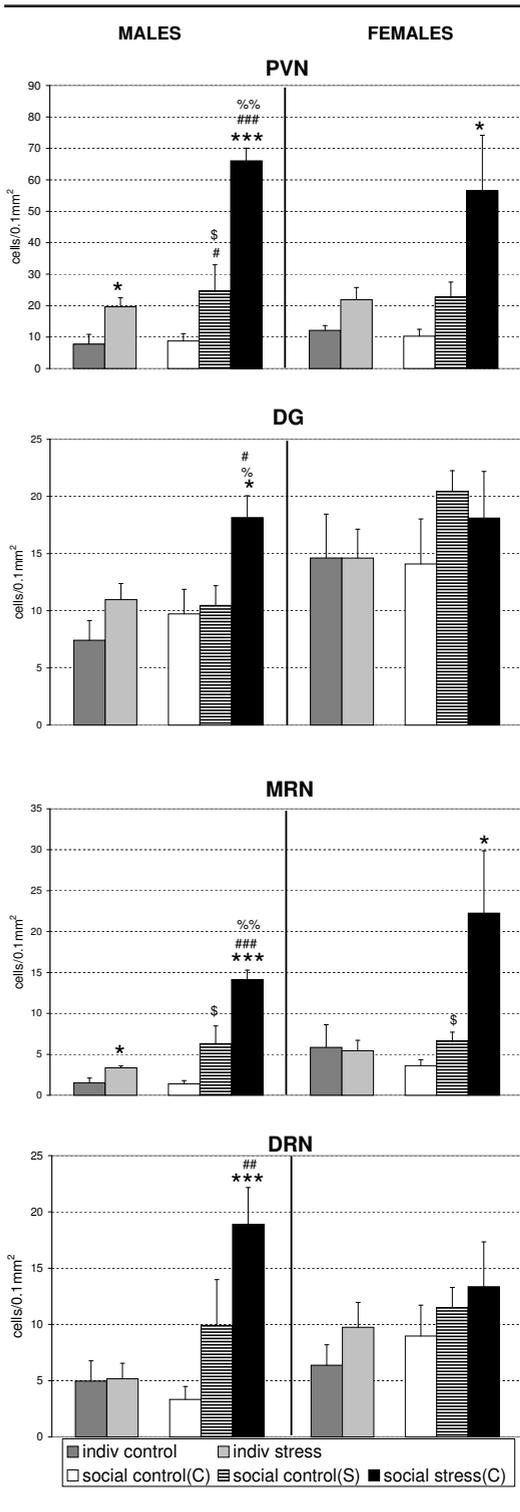
**Prefrontal cortex (PFC):** Treatment, housing and treatment-partner had significant main effects in all regions of the PFC (IL: resp.  $Z=4.954$ ,  $p\leq 0.001$ ;  $Z=4.437$ ,  $p\leq 0.001$  and  $Z=2.501$ ,  $p=0.012$ ). PL: resp.  $Z=4.858$ ,  $p\leq 0.001$ ;  $Z=4.671$ ,  $p\leq 0.001$ ;  $Z=2.619$ ,  $p=0.008$ ). Aca: resp.  $Z=4.014$ ,  $p\leq 0.001$ ;  $Z=5.215$ ,  $p\leq 0.001$  and  $Z=3.379$ ,  $p\leq 0.001$ ). ACc: (resp ( $Z=4.477$ ,  $p\leq 0.001$ ;  $Z=3.879$ ,  $p\leq 0.001$  and  $Z=3.616$ ,  $p\leq 0.001$ ). An interaction effect of housing by treatment was observed in the IL, PL and Aca (resp.  $Z=2.177$ ,  $p=0.03$ ;  $Z=2.642$ ,  $p=0.008$ ;  $Z=2.215$ ,  $p=0.028$ ).

**Infralimbic cortex (IL):** In socially housed males, stress increased Fos-ir in the IL compared to control( $C\text{♀}$ ) ( $F_{1,9}=15.644$ ,  $p=0.003$ ) and control( $S\text{♀}$ ) males ( $F_{1,9}=11.966$ ,  $p=0.007$ ). Socially housed stressed males also showed significantly more Fos-ir in the IL than isolated stressed males ( $F_{1,8}=11.653$ ,  $p=0.009$ ). Chronic stress and housing conditions did not affect Fos-ir in female rats.

**Prelimbic cortex (PL):** Chronic stress increased prelimbic Fos-ir in socially and isolated males (resp. versus control( $C\text{♀}$ ):  $F_{1,9}=17.848$ ,  $p=0.002$ ; versus control( $S\text{♀}$ ):  $F_{1,9}=5.224$ ,  $p=0.048$ ; isolated males:  $F_{1,8}=5.561$ ,  $p=0.046$ ). Prelimbic Fos-ir in socially housed stressed rats was higher than in isolated stressed rats ( $F_{1,8}=13.278$ ,  $p=0.007$ ). Compared to isolated control males, social housing with a control female did not affect Fos expression but housing with a stressed female partner increased prelimbic Fos-ir ( $F_{1,9}=7.609$ ,  $p=0.022$ ). In the PL of socially housed stressed females Fos-ir was significantly increased compared to control( $C\text{♂}$ ) females ( $F_{1,10}=6.197$ ,  $p=0.032$ ).

**Anterior cingulate (anterior part) (ACa):** Socially housed stressed males showed significantly more Fos-ir in the ACa than control( $C\text{♀}$ ) males ( $F_{1,9}=12.609$ ,  $p=0.006$ ). Also in isolated males stress exposure increased Fos expression in the ACa ( $F_{1,8}=5.306$ ,  $p=0.05$ ). Males housed with a stressed female and socially housed stressed males demonstrated more Fos-positive cells in the ACa than isolated counterparts (resp.  $F_{1,9}=7.832$ ,  $p=0.021$  and  $F_{1,8}=13.462$ ,  $p=0.006$ ). In socially housed females the presence of a stressed partner and exposure to stress increased Fos-ir in the ACa (resp.  $F_{1,10}=8.583$ ,  $p=0.015$  and  $F_{1,10}=6.978$ ,  $p=0.025$ ). Moreover control females with a stressed partner also showed more Fos labelling in the cortex than isolated controls ( $F_{1,10}=8.834$ ,  $p=0.014$ ).

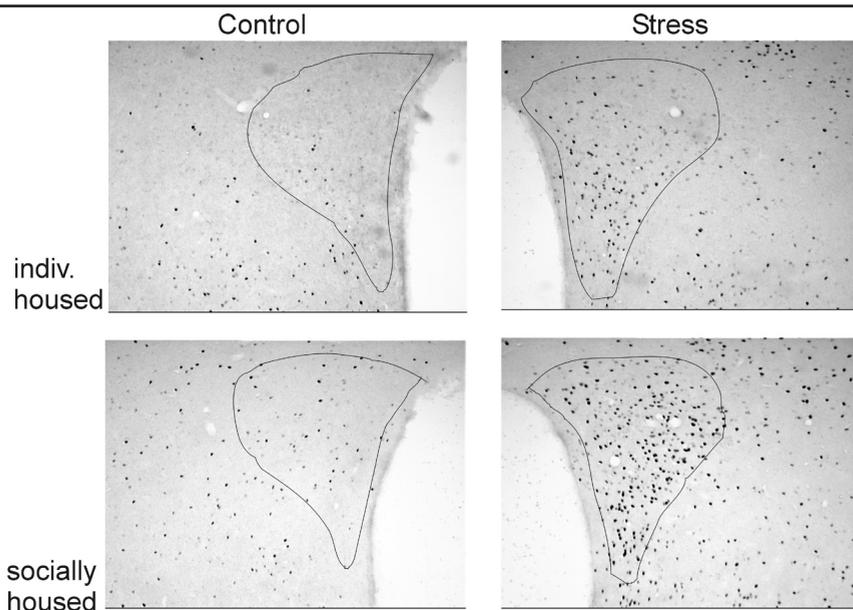
**Anterior cingulate (caudal part)(ACc):** Chronic stress increased Fos-ir in socially housed males (control( $C\text{♀}$ ):  $F_{1,10}=15.645$ ,  $p=0.003$ ; control( $S\text{♀}$ ):  $F_{1,10}=9.180$ ,  $p=0.013$ ). Socially housed control( $S\text{♀}$ ) and chronically stressed males had more Fos-ir in the ACc than their isolated counterparts (resp.  $F_{1,9}=6.674$ ,  $p=0.03$  and



$F_{1,10}=24.608, p=0.001$ ). In females stress increased Fos expression in the socially housed animals compared to the control( $C\sigma$ ) group ( $F_{1,10}=5.305, p=0.044$ ). Females with a stressed male companion showed more Fos labelling in the ACc than isolated controls ( $F_{1,10}=5.802, p=0.037$ ), and also socially housed stressed females demonstrated more Fos-ir than their isolated counterparts ( $F_{1,10}=5.075, p=0.048$ ).

**Medial pre-optic nucleus (MPN):** Treatment, housing and the treatment-partner had significant effects on Fos expression in the MPN (resp.  $Z=4.908, p\leq 0.001$ ;  $Z=3,540, p\leq 0.001$ ;  $Z=2.130, p=0.033$ ). Also a significant effect of gender ( $Z=3.876, p\leq 0.001$ ) and an interaction effect gender by housing ( $Z=2.986, p=0.003$ ) was observed, indicating that gender influenced the effect of housing conditions on Fos-ir. Compared to control( $C\sigma$ ) males, the socially housed stressed males showed significantly more Fos-ir in the MPN ( $F_{1,10}=15.814, p=0.003$ ). No effect of stress was observed in isolated males. Males with a stressed partner and socially housed

**Figure 1.** Number of Fos-positive cells per 0.1 mm<sup>2</sup>. Stress-effect indiv. control vs. indiv. stress and control( $C\sigma$ ) vs. stress( $C\sigma$ ): \* $p\leq 0.05$ , \*\* $p\leq 0.01$ , \*\*\* $p\leq 0.001$ , stress-effect control( $S\sigma$ ) vs. stress( $C\sigma$ ): %  $p\leq 0.05$ , %%  $p\leq 0.01$ , difference between control( $C\sigma$ ) and control( $S\sigma$ ): \$  $p\leq 0.05$ , \$\$  $p\leq 0.01$ , Differences between individually and socially housed counterpart: # $p\leq 0.05$ , ## $p\leq 0.01$ , ### $p\leq 0.001$ .



**Figure 2.** Representative photomicrographs of Fos-expression in the PVN of isolated and socially housed control(C♀) and stressed(C♀) males.

stressed males showed more Fos-ir than isolated animals (resp.  $F_{1,9}=6.842$ ,  $p=0.028$ ;  $F_{1,9}=12.435$ ,  $p=0.006$ ). Stress exposed socially housed females demonstrated more Fos-positive cells in the MPN than control(C♂) females ( $F_{1,8}=5.322$ ,  $p=0.05$ ), and isolated females ( $F_{1,8}=5.914$ ,  $p=0.041$ ).

**Paraventricular nucleus of the hypothalamus (PVN):** Significant treatment ( $Z=6.07$ ,  $p\leq 0.001$ ), housing ( $Z=3.47$ ,  $p\leq 0.001$ ) and treatment-partner effects ( $Z=3.456$ ,  $p\leq 0.001$ ) were found in PVN Fos expression. In both socially (control(C♀):  $F_{1,10}=30.071$ ,  $p\leq 0.001$ ; control(S♀):  $F_{1,10}$ ,  $p=15.532$ ,  $p=0.003$ ) and individually ( $F_{1,8}=9.349$ ,  $p=0.016$ ) housed stressed males PVN Fos-ir was increased. Socially housed control(S♀) and stressed males showed more Fos-ir in the PVN than their isolated counterparts (resp.  $F_{1,8}=5.436$ ,  $p=0.048$ ;  $F_{1,10}=68.138$ ,  $p\leq 0.001$ ) (Figure 2). Socially housed control(S♂) females showed significantly more Fos-ir than the control(C♂) females ( $F_{1,8}=6.337$ ,  $p=0.036$ ). Stress non-significantly increased Fos expression in the PVN of isolated females ( $F_{1,9}=4.157$ ,  $p=0.072$ ), whereas in socially housed females it was significantly increased compared to control(C♂) females ( $F_{1,9}=7.644$ ,  $p=0.013$ ) (Figure 1).

**Supra-optic nucleus (SON):** A significant effect of housing ( $Z=4.788$ ,  $p\leq 0.001$ ) and an interaction effect of housing by treatment ( $Z=2.923$ ,  $p=0.004$ ) were found. Socially housed stressed males demonstrated more Fos-ir in the SON than isolated counterparts ( $F_{1,10}=19.269$ ,  $p=0.001$ ). In isolated females stress

Table 4. Mean number of Fos-positive cells per 0.1 mm<sup>2</sup>

|      | MALES      |                        |                          |                              | FEMALES     |                          |                          |                          |
|------|------------|------------------------|--------------------------|------------------------------|-------------|--------------------------|--------------------------|--------------------------|
|      | individual |                        | social                   |                              | individual  |                          | social                   |                          |
|      | control    | stress                 | control(C <sub>♀</sub> ) | stress(C <sub>♀</sub> )      | control     | stress                   | control(C <sub>♂</sub> ) | stress(C <sub>♂</sub> )  |
| NAcC | 17.9(3.9)  | 23.2(2.8)              | 20.0(2.8)                | 41.4(5.4) <sup>**</sup>      | 23.8(4.8)   | 28.0(8.2)                | 17.7(4.7)                | 33.3(9.0)                |
| NAcS | 5.7(1.4)   | 4.0(0.6)               | 14.5(1.3)                | 14.4(3.8) <sup>#</sup>       | 3.6(0.9)    | 3.7(0.8)                 | 16.2(0.6) <sup>#</sup>   | 10.6(1.5) <sup>###</sup> |
| IL   | 9.8(2.2)   | 19.1(4.3)              | 13.9(4.9)                | 43.1(5.6) <sup>***%###</sup> | 15.3(2.6)   | 19.7(2.7)                | 14.7(3.9)                | 26.6(4.6)                |
| PL   | 7.4(1.6)   | 15.9(3.2) <sup>*</sup> | 11.1(4.0)                | 41.6(6.3) <sup>***###</sup>  | 13.4(3.0)   | 16.6(2.8)                | 12.0(3.1)                | 23.7(5.2)                |
| ACa  | 3.8(1.0)   | 9.3(2.1) <sup>*</sup>  | 7.4(3.9)                 | 30.5(5.4) <sup>***###</sup>  | 5.7(1.2)    | 8.7(2.2)                 | 5.6(1.7)                 | 22.3(5.5) <sup>##</sup>  |
| ACC  | 8.3(2.9)   | 23.8(6.8)              | 16.5(3.2)                | 38.7(8.6) <sup>***###</sup>  | 6.9(1.6)    | 10.2(1.8)                | 8.3(2.9)                 | 23.8(6.8) <sup>#</sup>   |
| PVN  | 7.7(3.1)   | 19.6(2.9) <sup>*</sup> | 8.8(2.3)                 | 66.1(4.0) <sup>***%###</sup> | 12.1(1.5)   | 21.9(3.9)                | 10.3(2.2)                | 22.7(4.8) <sup>§</sup>   |
| SON  | 90.9(21.4) | 68.4(21.6)             | 139.5(22.4)              | 196.1(19.5) <sup>###</sup>   | 103.5(14.5) | 39.9(10.6) <sup>**</sup> | 101.3(35.3)              | 118.8(31.6)              |
| MPN  | 5.7(0.8)   | 9.6(3.0)               | 9.6(2.2)                 | 37.7(7.6) <sup>***###</sup>  | 13.0(1.6)   | 30.1(6.5) <sup>*</sup>   | 13.6(3.1)                | 24.2(4.0)                |
| CeA  | 12.1(3.1)  | 14.8(2.6)              | 18.2(5.7)                | 26.8(2.1) <sup>##</sup>      | 12.2(2.9)   | 9.1(2.4)                 | 13.7(4.1)                | 17.9(3.5)                |
| MeA  | 9.2(3.3)   | 10.5(2.8)              | 9.7(2.6)                 | 32.0(3.2) <sup>***%###</sup> | 13.1(2.4)   | 15.1(3.3)                | 12.3(2.9)                | 22.5(2.5) <sup>##</sup>  |
| LaA  | 2.4(0.4)   | 3.7(0.8)               | 5.1(1.6)                 | 15.6(4.6) <sup>#</sup>       | 3.9(0.7)    | 4.1(1.6)                 | 5.0(1.2)                 | 9.8(1.6) <sup>###</sup>  |
| BLA  | 4.1(1.0)   | 7.8(1.8)               | 14.9(1.3)                | 17.2(2.3) <sup>***%###</sup> | 5.9(1.2)    | 6.6(1.8)                 | 16.2(1.5)                | 10.4(2.8)                |
| DG   | 7.4(1.7)   | 11.0(1.4)              | 9.7(2.1)                 | 18.2(1.9) <sup>***%#</sup>   | 14.6(3.8)   | 14.6(2.5)                | 14.1(3.9)                | 20.4(1.8)                |
| VTA  | 2.4(0.6)   | 3.6(0.6)               | 2.2(0.4)                 | 6.9(0.5) <sup>***###</sup>   | 6.1(2.1)    | 5.3(0.9)                 | 4.1(1.0)                 | 7.1(0.7)                 |
| DRN  | 5.0(1.8)   | 5.2(1.4)               | 3.3(1.2)                 | 18.9(3.3) <sup>***###</sup>  | 6.4(1.8)    | 9.8(2.2)                 | 9.0(2.7)                 | 11.5(1.8)                |
| MRN  | 1.5(0.6)   | 3.4(0.2) <sup>*</sup>  | 1.4(0.4)                 | 14.1(1.2) <sup>***%###</sup> | 5.9(2.8)    | 5.4(1.3)                 | 13.6(0.7)                | 6.6(1.1) <sup>§</sup>    |

Stress-effects: indiv. control vs. indiv. stress and control(C) vs. stress(C); \*p≤0.05, \*\*p≤0.01, \*\*\*p≤0.001, stress effects control(S) vs. stress(C); %p≤0.05, ##p≤0.01, Differences between control(C) and control(S); †p≤0.05, ‡p≤0.01. Differences between individually and socially housed counterparts; #p≤0.05, ###p≤0.01, ###p≤0.001.

reduced the number of Fos-positive cells ( $F_{1,10}=12.498$ ,  $p=0.005$ ) and they also showed significant less Fos-labelled cells in the SON than the socially housed stressed females ( $F_{1,10}=11.060$ ,  $p=0.008$ ).

**Amygdala: Central amygdala (CeA):** Housing conditions and gender had an effect on Fos-ir in the Central nucleus of the amygdala (resp.  $Z=3.550$ ,  $p\leq 0.001$  and  $Z=-2.044$ ,  $p=0.021$ ). Also an interaction effect for gender by treatment-partner was found ( $Z=-2.014$ ,  $p=0.044$ ). The socially housed stressed males showed significantly more Fos labelling than the isolated stressed males ( $F_{1,10}=13.291$ ,  $p=0.004$ ). Like males, only isolated stressed and socially housed stressed females differed in Fos-ir, with the socially housed rats expressing more Fos-positive cells in the CeA ( $F_{1,10}=6.690$ ,  $p=0.027$ ).

**Medial amygdala (MeA):** In the medial amygdala treatment, housing and treatment-partner had significant effects on Fos-ir (resp.  $Z=4.418$ ,  $p\leq 0.001$ ,  $Z=4.923$ ,  $p\leq 0.001$  and  $Z=2.643$ ,  $p=0.008$ ). The interaction between housing by treatment was also significant ( $Z=3.529$ ,  $p\leq 0.001$ ), showing that housing conditions differently affected MeA Fos-ir in response to stress. Chronic stress increased Fos-ir of socially housed males (vs. control(C♀):  $F_{1,10}=29.006$ ,  $p\leq 0.001$ ; vs. control(S♀):  $F_{1,10}=8.938$ ,  $p=0.014$ ), and stress(C♀) males had a higher Fos expression than their isolated counterparts ( $F_{1,10}=24.892$ ,  $p=0.001$ ). In females both stress and the presence of a stressed male partner significantly increased Fos-ir (resp.  $F_{1,10}=7.255$ ,  $p=0.023$  and  $F_{1,10}=7.166$ ,  $p=0.023$ ). No differences were found between isolated controls and control(C♂) females, but control(S♂) females had more Fos labelling in the MeA than their isolated counterparts ( $F_{1,10}=7.422$ ,  $p=0.021$ ).

**Basolateral amygdala (BLA):** Treatment, housing and treatment-partner had significant effects on Fos-ir in the basolateral amygdala (resp.  $Z=4.579$ ,  $p\leq 0.001$ ,  $Z=4.733$ ,  $p\leq 0.001$  and  $Z=2.624$ ,  $p=0.008$ ). Males with a stressed female partner showed significantly more Fos-ir in this region than males with a control partner ( $F_{1,10}=7.897$ ,  $p=0.018$ ) and the isolated controls ( $F_{1,9}=10.670$ ,  $p=0.010$ ). Stress increased Fos expression of socially housed males (compared to control(C♀):  $F_{1,10}=22.369$ ,  $p=0.001$ ; compared to control(S♀):  $F_{1,10}=5.066$ ,  $p=0.048$ ). Moreover, Fos-ir in the BLA of socially housed stressed males was higher than that of isolated males ( $F_{1,10}=10.793$ ,  $p=0.008$ ). In females, stress increased Fos expression in the BLA of socially housed rats compared to control(C♂) females ( $F_{1,10}=6.494$ ,  $p=0.029$ ). Socially housed stressed females also showed more Fos labelling than isolated stressed females ( $F_{1,10}=5.410$ ,  $p=0.042$ ).

**Lateral amygdala (LaA):** Treatment, housing conditions and treatment of the partner had significant effects on Fos-ir in the lateral amygdala (resp.  $Z=2.373$ ,  $p=0.018$ ,  $Z=4.715$ ,  $p\leq 0.001$  and  $Z=2.158$ ,  $p=0.04$ ). Socially housed stressed males demonstrated more Fos-ir in the LaA than isolated counterparts ( $F_{1,9}=5.494$ ,

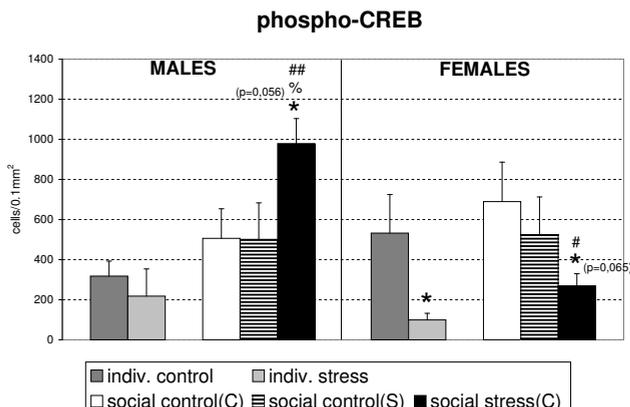
$p=0.044$ ). Whereas the stress-effect was not significant in socially housed females ( $F_{1,10}=3.502$ ,  $p=0.091$ ), the presence of a stressed male partner increased Fos-ir in the LaA ( $F_{1,10}=5.681$ ,  $p=0.038$ ). More Fos-labelled cells were observed in the control ( $S_{\text{♂}}^{\wedge}$ ) females than in the isolated controls ( $F_{1,10}=10.764$ ,  $p=0.008$ ).

**Dentate gyrus (DG):** Treatment ( $Z=3.372$ ,  $p\leq 0.001$ ) and housing ( $Z=2.705$ ,  $p=0.007$ ) had a significant effect of Fos-ir in the DG. In socially housed stressed males the Fos expression was increased compared to control ( $C_{\text{♀}}$ ) and control ( $S_{\text{♀}}$ ) males (resp.  $F_{1,10}=8.623$ ,  $p=0.015$  and  $F_{1,10}=8.930$ ,  $p=0.014$ ). Isolated males showed no effect of stress exposure on Fos-ir in the DG but the expression was significantly reduced compared to socially housed stressed rats ( $F_{1,10}=9.180$ ,  $p=0.013$ ). Even though significant main effects were found, there were no significant expression differences between the female groups, indicating that in the DG differences between the male groups account for the reported significant main effects (Fig. 1).

**Ventral tegmental area (VTA):** Fos expression in the VTA showed a significant effect of treatment ( $Z=2.574$ ,  $p=0.01$ ), housing ( $Z=2.226$ ,  $p=0.026$ ), and treatment-partner ( $Z=2.097$ ,  $p=0.036$ ). Chronic stress increased Fos-ir in the VTA of socially housed males compared to control ( $C_{\text{♀}}$ ) males ( $F_{1,9}=40.362$ ,  $p\leq 0.001$ ), as did the presence of a stressed female partner ( $F_{1,10}=10.407$ ,  $p=0.009$ ). Socially housed males with a stressed female partner showed significantly more Fos-ir than the isolated control males ( $F_{1,9}=6.762$ ,  $p=0.029$ ). Housing conditions also had an effect in stressed males; with social housing leading to a higher Fos expression ( $F_{1,9}=11.658$ ,  $p=0.008$ ). Since no significant differences were found between female groups, the reported main effects therefore are due to male group differences.

**Median raphe nucleus (MRN):** Treatment, housing and gender showed a significant main effect on Fos-ir in the MRN (resp.  $Z=4.204$ ,  $p\leq 0.001$ ;  $Z=4.146$ ,  $p\leq 0.001$ ;  $Z=2.322$ ,  $p=0.001$ ). Also the interaction treatment by housing was significant ( $Z=3.792$ ,  $p\leq 0.001$ ). Stress increased Fos expression in socially housed males compared to control ( $C_{\text{♀}}$ ) and control ( $S_{\text{♀}}$ ) (resp.  $F_{1,10}=106.710$ ,  $p\leq 0.001$  and  $F_{1,10}=10.128$ ,  $p=0.010$ ). A stressed female partner also led to increased Fos-ir in the MRN ( $F_{1,10}=5.023$ ,  $p=0.049$ ). Also in isolated males stress exposure increased the Fos expression ( $F_{1,8}=8.767$ ,  $p=0.018$ ), and in socially housed stressed males more Fos-positive cells were found in the MRN than in the isolated stressed males ( $F_{1,9}=97.733$ ,  $p\leq 0.001$ ). In females, a stressed partner, as well as stress exposure, significantly increased Fos-ir (resp.  $F_{1,10}=5.174$ ,  $p=0.046$  and  $F_{1,10}=5.951$ ,  $p=0.035$ ). Fos-ir in the MRN of socially housed stressed females was almost significantly higher than in isolated stressed females ( $F_{1,10}=4.755$ ,  $p=0.054$ ) (Figure 1).

**Dorsal raphe nucleus (DRN):** Fos-ir in the DRN demonstrated a treatment ( $Z=3.108$ ,  $p\leq 0.001$ ), housing ( $Z=3.324$ ,  $p\leq 0.001$ ) and treatment-partner effect ( $Z=1.743$ ,  $p=0.04$ ). Treatment by housing was nearly significant ( $Z=1.935$ ,



**Figure 3.** Number of pCREB positive cells per 0.1mm<sup>2</sup> in the dentate gyrus of the hippocampus. \* $p \leq 0.05$ : stress effect compared to control(C♀), # $p \leq 0.05$  compared to control(S♀),  $^{\$}p < 0.05$  compared to indiv. housed counterparts.

$p=0.055$ ). Stress increased Fos expression in socially housed males compared to control(C♀) ( $F_{1,10}=20.266$ ,  $p=0.001$ ) but not to control(S♀) males. Stress did not affect Fos-ir in isolated males, but isolated stressed males had less Fos than socially housed stressed males in the DRN ( $F_{1,9}=12.966$ ,  $p=0.006$ ). Even though significant main effects were found, none of the female groups showed significant differences, indicating that in the DRN group differences in male rats account for the reported main effects (Figure 1).

### pCREB

Housing conditions had a significant effect on pCREB levels in the dentate gyrus ( $Z=3.336$ ,  $p=0.008$ ). Treatment by itself and the treatment of the partner of socially housed rats had no effects on pCREB levels, but a significant interaction effect was observed for housing by treatment ( $Z=2.415$ ,  $p=0.016$ ), indicating that housing conditions influenced the pCREB response to stress. Also significant interaction effects were found for gender by treatment ( $Z=2.286$ ,  $p=0.011$ ) and gender by housing ( $Z=2.517$ ,  $p=0.012$ ), showing that hippocampal pCREB expression in male and female rats reacted differently to stress and housing conditions (Figure 3 and 4). Stress exposure increased pCREB labelling in the DG of socially housed males compared to control(C♀) ( $F_{1,10}=5.982$ ,  $p=0.034$ ). The difference between socially housed stressed males and control males housed with a stressed partner was almost significant ( $F_{1,10}=4.678$ ,  $p=0.056$ ). Stress exposure had no effect on pCREB expression in isolated males. Socially housed stressed males however showed significant more pCREB labelling than isolated stressed males ( $F_{1,9}=17.018$ ,  $p=0.003$ ). In females stress decreased pCREB labelling in both isolated ( $F_{1,19}=4.505$ ,  $p=0.047$ ) and socially housed rats, albeit the latter as not significant ( $F_{1,19}=3.838$ ,  $p=0.065$ ). Socially housed stressed females however showed more pCREB staining than isolated stressed females ( $F_{1,9}=6.807$ ,  $p=0.028$ ).

## Discussion

Male and female rats were differentially affected by chronic stress exposure and social housing in mixed gender pairs. While pCREB expression in the DG of isolated males was not changed after stress exposure, socially housed males showed an increased pCREB expression. In females social housing had an ameliorating effect, but was not able to prevent the stress-induced reduction of pCREB completely. In socially housed males most brain areas analysed showed a stress-induced increased Fos expression, whereas in isolated males stress-induced increases were lower and reached significance only in the PVN, prelimbic and anterior cingulate cortex, and median raphe nucleus. Also in females, stress-induced increases in Fos expression were most pronounced in socially housed rats. Individually housed females only showed a stress response in Fos expression in two brain regions, namely the MPN and SON. However, stress responses in Fos-ir of socially housed females were less pronounced than in males. Also the differences found between socially and individually housed rats were less evident in females.

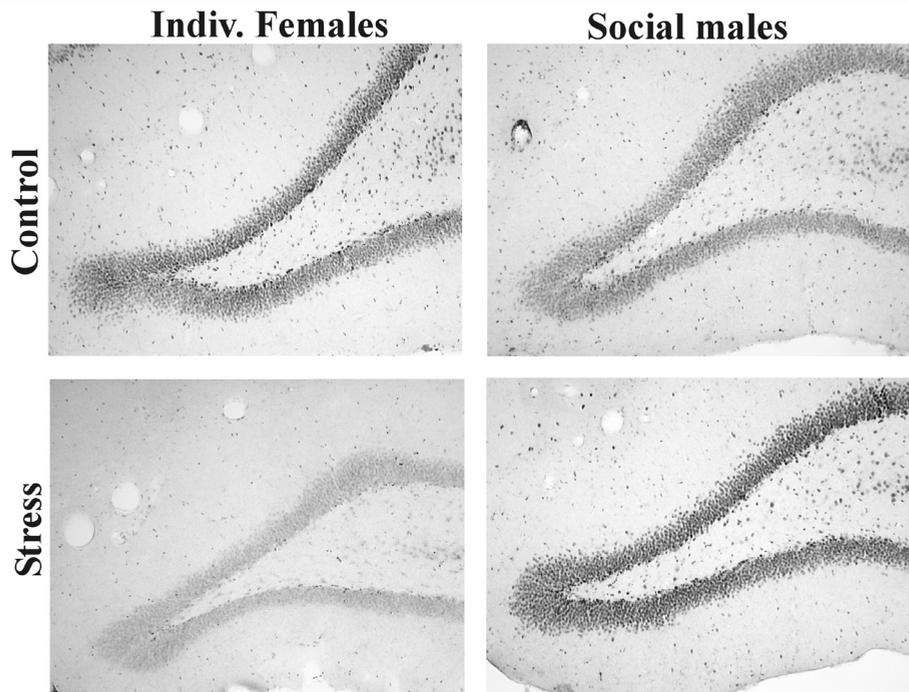
Fos expression is maximal between 1 and 3 hrs. after a stimulus,<sup>21</sup> therefore social behaviour occurring among cage mates in the period before the sacrifice could have generated additional Fos expression in the socially housed rats. Due to reintroduction into the home cage after stress exposure this could have occurred especially in control(S) and stress(C) rats. The presence of a stressed female partner increased Fos-ir in several brain regions of the control male, like in the BLA, VTA and MRN. Of these 3 regions only the VTA expression did not show an additional stress effect, so likely Fos expression in the VTA of control(S♀) and stress(C♀) rats was mostly due to increased social interactions. Control(S♀) and stress(C♀) males likely were subjected to a similar amount of social stimulation, so differences in Fos expression between these groups, as found in the PFC, PVN, MeA, BLA, DG and MRN, can most likely be contributed to stress exposure and not to increased social interactions in the home cage.

In unisex groups of male rats,<sup>45</sup> the presence of stressed male cage mates resulted in similar Fos levels between controls and their stressed cage mates. In the current study we found that the presence of a stressed female did not produce a level of Fos expression that was comparable to the level in stressed counterparts. This illustrates that male rats better tolerate a stressed female partner than the presence of male cage mates. This in contrast to females with a stressed partner, who showed an increase in Fos-ir in more brain regions than males with a stressed partner, while the presence of other stressed females in unisex groups<sup>45</sup> did not result in an increased Fos expression. Although, when comparing Fos expression between individually and paired-housed females, the individually housed females appeared to be the ones least

stressed. One could argue that isolated stressed females habituated to the chronic stress exposure, since they showed no effect of stress on adrenal weight, and Fos-ir was increased significantly only in the SON and MPN. Open field behaviour of isolated control and stressed females also did hardly show a stress effect, although the behaviour of both groups differed from socially housed females.<sup>44</sup> Both isolated control and stressed females however showed adrenal hypertrophy compared to socially housed counterparts and pCREB expression in the DG was reduced in chronically stressed isolated females. This indicates that isolated females did not habituate to the stressor but rather that both, controls and stressed animals, were exposed to a stressful adverse environment, namely isolation.

Stress exposure results in a release of corticotropin releasing hormone (CRH) from the PVN.<sup>36</sup> In females, but not in males, CRH positive neurons are located in the MPN as well.<sup>24</sup> Possibly these neurons also release CRH during stress, which might explain the stress-induced increase in Fos reactivity in the MPN found in isolated females, but not in isolated males. The MPN is mainly associated with reproductive behaviours,<sup>19</sup> and it has been suggested that CRH neurons in the MPN are involved in the stress-induced suppression of reproductive function.<sup>16</sup> We did not investigate the effect of stress on sexual functioning, all females when in estrus, were observed to accept the sexual advances of the male, also all isolated females showed lordosis behaviour when stroked on the back during estrus. Although no detailed study was made of sexual functioning, it cannot be excluded that, in the present study, sexual behaviour was affected by chronic stress.

The higher stress-induced Fos expression in the brain of socially housed rats could be a consequence of the occurrence of frequent sexual interactions. Sexual experience has lasting effects on the brain<sup>19,23,29</sup> and limbic metabolic capacity has found to be increased after repeated copulations.<sup>31</sup> This was, however, investigated in male rats only, but it is quite possible that the same is true for female rats. Sexual experience could make the brain more sensitive to other stimuli as well, resulting in an increased Fos reactivity in socially housed females, even without affecting basal Fos expression. It is however also possible that pair-housed stressed rats do not show an increased Fos response to stress compared to isolated counterparts, but that isolated rats have an attenuated Fos reactivity to stress. Fos expression is lower in individually housed rats of the present study compared to previously reported results.<sup>45</sup> The difference between isolated rats in these experiments is the presence of the tube in the home cage. Placing kong toys and nestlets in the cage of singly housed rats has been found to be sufficient for reducing baseline levels of corticosterone and ACTH, and the stress response to the acute stressor of a *ip.* saline injection in females.<sup>3</sup> Isolated rats in the current experiment, as the pair-housed rats, had a tube at their disposal, which functioned, besides being a hiding



**Figure 4.** Photomicrographs of pCREB labelling in the dentate gyrus of isolated control and stressed females, and socially housed control(C♂) and stressed(C♂) males.

place, as a chewing object. If the presence of simple objects the rat can chew on, can reduce HPA-axis activity,<sup>3</sup> a tube likely has an even more pronounced suppressive effects on the HPA-axis. This could provide an explanation for the low Fos reactivity of isolated rats in the current experiment. Since rats show thigmotaxic behaviour, the chance to quietly hide in the tube after stress exposure instead being in the open “exposed” home cage, might attenuate the stress-response. This in contrast to socially housed rats, who were denied this opportunity of hiding, because of “required” social interactions with their cage mate. On the last day rats are exposed to the stress box for 30 minutes which allows the Fos-mRNA response to be maximal.<sup>48</sup> However, the isolated rats housed with a tube only showed a limited increase in Fos-ir, compared to rats without a tube.<sup>45</sup> Hypothetically, taking shelter in the home tube could signal “safety” and subsequently suppress the translation of Fos mRNA to protein. Although apparently without reducing the impact of chronic stress, since adrenal hypertrophy still occurred.

In the rat brain BDNF expression was found to be reduced after chronic stress exposure,<sup>41</sup> whereas chronic treatment of rats with antidepressants increased pCREB, and CREB mRNA levels, parallel with mRNA levels of BDNF, in the hippocampus,<sup>27,39</sup> suggesting pCREB can be used as a marker for neuronal plasticity.

In the current study chronically stressed isolated females had significantly less pCREB labelling in the dentate gyrus of the hippocampus, implying a stress-induced reduction in synaptic plasticity. In socially housed females a reduction was also observed, although this did not reach significance and these animals showed a higher level of pCREB expression than isolated stressed females. This suggests social housing decreases the impact of adverse events in the brain, but is not able to forestall the stress-induced plasticity decrease completely. Previously we have shown that that social housing in unisex female groups is able to prevent the occurrence of a blunted response of the DRN Fos-ir to stress, which occurred in isolated females.<sup>45</sup> It was suggested that this non-response is associated with a dysfunctional serotonergic system, as observed in depressed subjects. In the current experiment both isolated and socially housed females failed to show a stress-induced Fos response in the DRN, implying a disturbed serotonergic response to stress exposure in both isolated and socially housed females.<sup>15</sup> However, the MRN of socially housed, but not isolated females, did show a stress-induced increase in Fos-ir. The MRN sends serotonergic projections to the hippocampus<sup>42</sup> and is associated with improved stress-resistance.<sup>14</sup> Possibly the maintenance of serotonergic reactivity of the MRN can compensate the non-response of the DRN to some extent, and improve synaptic plasticity in the hippocampus as shown by the slightly higher pCREB expression in the dentate gyrus of socially housed females compared to isolated counterparts after chronic stress exposure. Isolated males showed no change in pCREB expression in the DG after chronic stress exposure.<sup>22</sup> This in contrast to pair-housed males, where chronically stressed rats showed an increased pCREB expression, suggesting an antidepressive effect of the presence of a female cage mate. The pCREB expression, and possibly increased hippocampal plasticity, corroborates with behavioural effects in the open field test,<sup>44</sup> where in males social housing with a female prevented the behavioural effects of stress, and in females, social housing with a male only had a small beneficial effect.

c-Fos is one of the target genes of pCREB<sup>33</sup> and therefore one would expect a similar expression pattern of Fos and pCREB. Although this was observed for males, pCREB expression in the DG of females deviated from the Fos expression. However, Fos and pCREB have a different time course of expression. Fos expression is maximal between 1 and 3 hrs. after a stimulus,<sup>21</sup> whereas pCREB has a biphasic response pattern.<sup>4,35</sup> pCREB shows a fast expression after a stimulus (5-15 min.) and dips at 1-2 hours and peaks again 6-8 hours later. Therefore the observed pCREB expression in the DG is not necessarily a consequence of exposure to the stress box, since rats were sacrificed at a time point after the stress at which Fos expression is maximal but pCREB expression is not. Intriguingly, socially housed stressed males still showed elevated pCREB levels 2 hours after the stress exposure,

indicating either a prolonged stress-induced expression or an elevated baseline level of pCREB.

Summarising, gender specific responses were found especially for pCREB expression in the dentate gyrus, whereas Fos expression showed generally responses in the same direction in male and female rats, although the changes were most pronounced in male rats. In both isolated male and female rats, Fos expression showed only few stress-induced changes, while weight gain, adrenal weight and pCREB expression showed that these rats did suffer from chronic stress exposure. Possibly the presence of a tube in the home cage, reduced Fos-reactivity without having long-term effects.

Concluding that, in male rats, social housing is able to modulate several of the stress-induced behavioural and neurobiological effects, whereas in females, social housing only has slight positive effects, but still is better than isolation for coping with chronic stress.

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