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Gender-specific effects of social housing in rats after chronic mild stress exposure

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Stress plays an important role in the development of affective disorders. Women show a higher prevalence for these disorders then men. The course of a depression is thought to be positively influenced by social support. We have used a chronic mild stress model in which rats received foot-shocks daily for 3 weeks. Since rats are social animals we hypothesised that 'social support' might reduce the adverse effects of chronic stress. To test this hypothesis, male and female rats were housed individually or socially in unisex groups of 4 rats. An open field test was repeated 4 times during the 3 weeks of treatment. Neuronal activation in the paraventricular nucleus of the hypothalamus (PVN) and dorsal raphe nucleus (DRN) in response to stress was measured the last day with c-fos. Chronic stress exposure increased locomotor activity in the open field, especially during the first minute. This was most pronounced in the individually housed females. In females social housing prevented the stress-induced increase of locomotor activity, while in males social housing had no effect. Fos-ir in the PVN was increased in all stress-exposed groups, except for the socially housed females due to a higher Fos-ir in controls. Individually housed males and socially housed females showed increased Fos-ir in the DRN and the increase was almost significant in socially housed males. In conclusion: These results show that social housing can enhance coping with stress in female rats, whereas in male rats group housing did not have a positive influence on stress-sensitivity.

Abstract

Introduction

The onset of major depression is often preceded by chronic stress or stressful life events, indicating the importance of stress in the development of mood disorders. Stressful life events are associated with financial and employment problems in men and with network or interpersonal problems in women.[31,59] Recently gender-specific coping styles have been suggested to reduce the impact of stress. While men illustrate a 'classic' 'fight-flight' response, women seem to have adopted a 'tend-befriend' strategy, actively seeking social contact in times of stress.[57] Social support has been reported to have beneficial effects on the outcome of a depression, although clear gender differences have not been found.[16,28,34,41] It has been suggested however that social support, provided by women, but not men, reduces stress induced elevated blood pressure[22] and cortisol levels[32] in women as well as in men.

During recent years increased attention is being paid to the possible effects of housing conditions on rodent behaviour and their stress response.[5,16] Social housing can reduce the effect of a stressful experience, counteracting for example the behavioural and physiological effects of a social defeat.[50,63] Since only male rats are susceptible to social defeat however the latter model may not be suitable to represent mood disorders for which a higher prevalence is reported in women.[43] Gender differences in the effects of housing conditions have also been found. While social instability affects females more than males,[24] crowding is stressful for males but it actually calms females.[6]

Chronic mild stress, suitable for both male and female rats, has been proposed as a valid animal model for depression. Chronic stress impairs the ability to anticipate reward in some studies.[14,25,38,66] This condition resembles anhedonia and can be reversed by antidepressants.[65] In addition these rats also demonstrate various sleep disturbances, another characteristic of depression.[8,65,66]

We used an animal model of chronic footshock stress, to study effects of social housing on stress coping in male and female rats, using changes of behaviour and limbic activity. Social housing most likely is the best translation of 'human social support' to animal studies aiming to characterise the neurobiological mechanisms of social support in humans. In this model the stressor remains the same throughout the three weeks, but the duration, shock interval and application time of the stress exposure is varied, in order to make the stress as unpredictable as possible and prevent habituation to the stressor, which usually occurs when rats are repeatedly exposed to the same stimuli.[56] We investigated whether social housing could prevent stress induced changes in open field behaviour and possible gender difference in this response. As females have a higher sympathoadrenal reactivity to stress and higher baseline corticosterone levels,[47,48] it was hypothesised that females show stress

induced behavioural changes earlier in the stress protocol than to males subjected to the same procedure. In addition, it was assumed that social housing could prevent stress-induced behavioural and neurobiological changes in female rats while perhaps increasing adverse effects in males. Repeated open field tests, which are widely used to study anxiety and emotionality in rats,[46] were used to investigate behavioural changes during the 3 weeks of stress exposure. Using c-fos as a marker for neuronal activation,[52] brain activity was measured in the paraventricular nucleus (PVN), as an indication of the HPA (Hypothalamic-Pituitary-Adrenal)-axis response,[35] and the dorsal raphe nucleus (DRN), as indication of the serotonergic activity. A dysfunction of the latter system has often been implicated in the pathobiology of affective disorders.[20,54]

Animals, material and methods

Animals

Male (n=24) and female (n=24) Wistar rats were either individually (males: n=10, females n=10) or socially (males: n=14, females n=14) housed in unisex groups of 4 rats, in the same room. Of the individually housed rats, 5 rats were subjected to chronic stress and 5 rats to a control treatment. From each social group, two rats underwent stress exposure and two served as controls (n=7 per group). To have an equal number of 4 rats in each cage an extra rat was added to two cages of each gender. At the start of the experiment rats were of the same age with males weighing 298 ± 3 g. and females weighing 214 ± 1 g. The light-dark cycle was reversed (lights on 19.00-7.00 hr) and water and food was provided ad lib. All experimental procedures were approved by the Animals Ethics Committee of the University of Groningen (FDC: 2509). The oestrous cycle of the females was monitored by stroking them gently on the back, producing lordosis during oestrus and weight loss was observed on the day of oestrus.

Stress procedure

Rats were subjected to a daily footshock protocol for 3 weeks. Rats in the stress group were transferred to a footshock box and received 5 inescapable footshocks during a 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. This way we could also avoid the noxious stimulus on the last day of the procedure when only the light signal was given. The shock interval was varied in each session, as were the time of the day of the exposure and the duration of the session (30-120 min.). Control rats were placed in similar, non-electrified, cages. The rats were regularly weighed allowing calculations of weight gain changes from day one of the procedure.

Behaviour

Animals were tested in an open field for a period of 8 minutes. The open field test was performed under red-light conditions between 10 am.- 2 pm., during the active period of the animals, at least 16

hrs. after the last stress session and before the stress procedure of that day. The test was repeated 4 times, on day 2, 8, 14 and 21. Rats were placed in the centre of the open field at the start of the test. The open field consisted of a circular black arena with a diameter of 1 m. Locomotor behaviour was recorded with a videotracking system (EthoVison,1.96®, Noldus information Technology, Wageningen, the Netherlands). The distance covered per minute in cm. was analysed.

Immunohistochemistry

The rats were sacrificed on day 22 by deep anaesthetisement with sodium pentobarbital (1 ml, 6%). The rats were transcardial 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 control or stress box. On the last day, the stress-exposed animals were subjected to the light stimulus only so data of Fos activation changes would reflect the 'psychological' aspect of stress exposure and not of a foot shock related pain response. The brains were removed and postfixed in the same fixative overnight at 4°C. Adrenal and thymus weights, corrected for body weight, were calculated and used as indication of the amount of stress perceived.

Following an overnight cryoprotection in a 30% sucrose solution, serial 40 μm coronal sections were made with a cryostat microtome and collected in 0.02 M potassium phosphate saline buffer (KPBS). Fos immunostaining was performed on free-floating sections. Sections were rinsed with 0.3% H_2O_2 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) 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 (1:1000 in 0.02 M KPBS) and avidin-biotin-peroxidase complex (Vectastain ABC Elite Kit, Vector Laboratories). After thorough washing, the peroxidase reaction was developed with a DAB-nickel solution and 0.3% H_2O_2. 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.

Fos positive cells in the paraventricular nucleus (magno- and parvocellular part) of the hypothalamus (PVN) and the Dorsal Raphe Nucleus (DRN) were blindly quantified using a computerised imaging analysis system. 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. Regions of interest were outlined with a light pen, measured and the Fos positive nuclei were counted using a computer-based image analysis system LEICA (LEICA Imaging System Ltd., Cambridge, England). The resulted data was reported as number of positive cells/0.1mm^2. The PVN (magnocellular and parvocelluar) was quantified bilaterally (no left-right asymmetry of Fos immunoreactivity (ir) was found) and therefore the mean ± standard error (SEM) for both sides were calculated.

Statistical analysis

Statistical analyses were done with SPSS (version 10.0), and $p \leq 0.05$ was considered significant. Weight gain for each gender was analysed with a repeated measures ANOVA with days as within subject factors and treatment (control or stress) and housing (individual or social) as between subject variables. Locomotor activity was analysed with a repeated measures ANOVA, with minutes and days as within subject variables and treatment (control or stress), gender (male or female) and housing (individual or social) as between subject variables. Sphericity assumed modelling, with Greenhouse-Geisser and Huynh-Feldt adjustments, was applied.[45] Fos-ir was analysed with an ANOVA with treatment (control or stress), gender (male or female) and housing (individual or social) as between subject variables.

Results

Weights

Weight gain was significantly affected by chronic stress in male rats ($F_{1,20}$ = 39.369, $p \leq 0.001$), reducing the growth rate in both individually and socially housed males (resp. $F_{1,20}$= 27.627, $p \leq 0.001$ and $F_{1,20}$= 12.172, $p=0.002$). However no significant housing effect was observed (Fig. 1A). There was a significant day effect ($p \leq 0.001$) with weight steadily increasing over the days and an interaction effect between day and treatment ($p \leq 0.001$) (Greenhouse-Geisser correction). In contrast, in females chronic stress had no significant effects on the growth rate but here an effect of housing was observed ($F_{1,20}$= 8.070, $p=0.010$), socially housed control rats showing a reduced growth rate ($F_{1,20}$= 5.110, $p=0.035$) compared to individually housed control females (Fig. 1B). In females there was a significant effect of day ($p \leq 0.001$) and interaction effect between day and housing ($p=0.034$) (Greenhouse-Geisser correction).

Open field locomotor activity

The most pronounced locomotor effects were observed in the first minute after the animal was placed in the open field. First minute activity was compared as

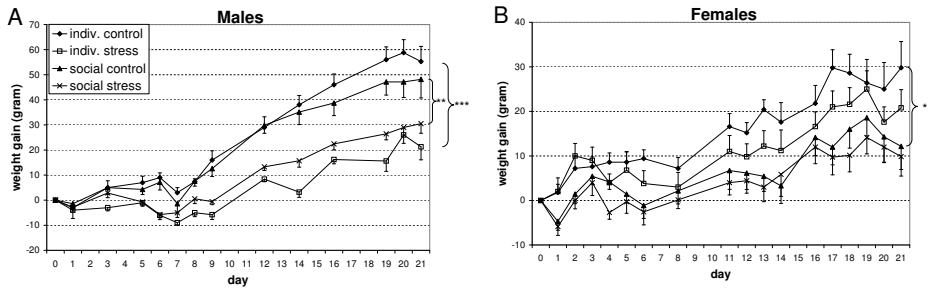

Figure 1. Weight gain in grams ± SEM. (A) Stress caused a significant reduction in weight gain of male rats (***, $p \leq 0.001$; **, $p \leq 0.01$). (B) Social housing reduced weight gain in control females (*, $p \leq 0.05$).

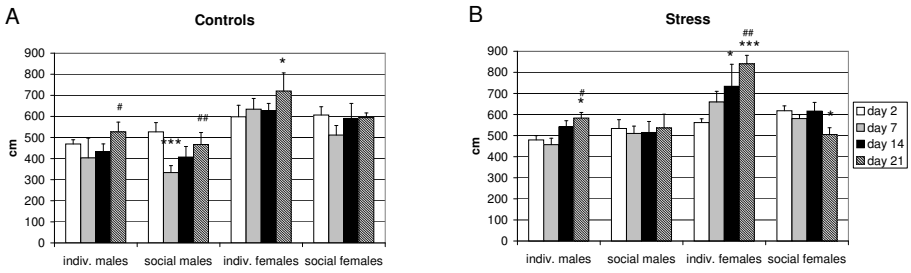

Figure 2 (A and B). Comparisons between open field tests of the mean distance walked per minute (cm). Results are expressed as group means ± SEM. Compared to day 2; * (*, p≤0.05; ***, p≤0.001), compare to day 7 (#, p≤0.05; ##, p≤0.01).

an indication of how rats reacted to a change in their environment, in addition to the mean locomotor activity per minute. Test of between subject effects showed a significant effect of gender ($F_{1,40}$= 34.232, p≤0.001), housing ($F_{1,40}$= 4.415, p=0.04), and stress ($F_{1,40}$= 4.465, (p= 0.04) on the locomotor activity in the open field, and a trend was visible for the interaction between gender and housing ($F_{1,40}$= 3.135, p=0.084), indicating that males and females react differently to housing conditions. Repeated measures ANOVA with Huynh-Feldt ε correction, showed a significant effect of day (p=0.001), an interaction between day and housing (p≤0.001), a minute effect (p≤0.001), and interactions between minute and housing (p ≤0.001), minute and housing and treatment (p=0.05) and day and minute (p≤0.001), minute and treatment showed a trend (p=0.082). This pattern of significances illustrates that housing conditions and treatment affect the way animals react during open field tests and how they respond to repeated open field exposures.

Effects of repeated open field exposures

Males: (Fig. 2) Chronic stress increased locomotor activity of individually housed males during the eight minutes of the open field exposure. The distance covered per minute was significantly increased after 3 weeks of chronic stress exposure compared to day 2 (p=0.05) and day 7 (p=0.014) (Figure 2B). First minute locomotor activity changes occurred earlier during the stress exposure, and were found in both the individually and socially housed males after 1, 2 and 3 weeks (individually housed: (resp. from 588 ± 60 on day 2 to 856 ± 47 on day 7 (p=0.047), to 948 ± 64 on day 14 (p = 0.029) and to 970 ± 77 cm on day 21 (p=0.010); socially housed: resp. from 831 ± 66 on day 2 to 1017 ± 75 on day 7 (p=0.015), to 1089 ± 96 on day 14 (p = 0.012) and to 1041 ± 116 cm on day 21 (p=0.015) (Figure 3A). Such locomotor changes were not found in control males, who did show a change in mean activity per minute after repeated open field exposures. Individually housed control males showed a significantly increased activity on day 21 compared to day 7 (p=0.016)

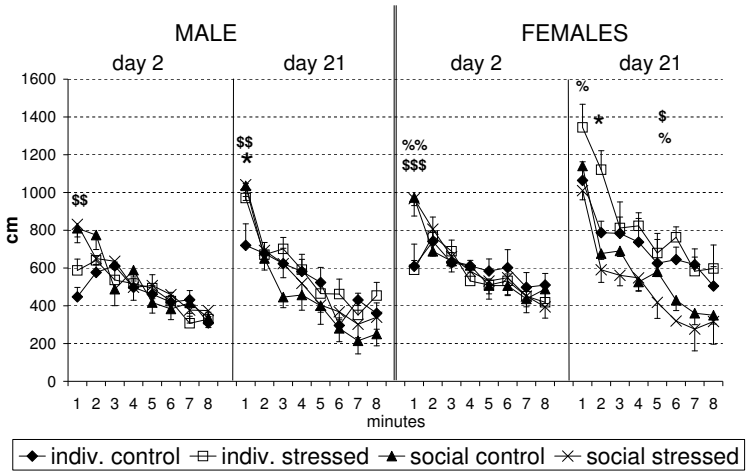

Figure 3. Distance walked per minute (cm) of the 4 open field tests. Results are expressed as group means ± SEM. Effects of housing conditions of control rats ($, p≤0.05; $$, p≤0.01; $$$, p≤0.001). Stress effects of individually housed rats (**, p≤0.01) and socially housed rats (&, p≤0.05). Housing effects of stressed rats (%%, p≤0.01).

whereas socially housed control males were less active on day 7 compared to day 2 (p≤0.001) and day 21 (p = 0.003).

Females: (Fig. 2) Individually housed control females showed, compared to day 2, an increased locomotor activity on day 21 (p=0.022). After chronic stress exposure, however activity was increased already after 14 days and remained elevated up to 21 days (resp. p=0.021 and p≤0.001). Also compared to day 7, on day 21 there was a significantly increase in activity (p=0.001). Whereas socially housed control females showed no changes in activity and socially housed stressed rats showed a decreased activity on day 21 compared to day 2 (p=0.013). First minute activity of individually housed control females was increased during the second and fourth exposure to the open field (resp. from 610 ± 117 on day 2 to 972 ± 73 on day 7 (p=0.020) and 1094 ± 99 cm on day 21 (p=0.003), but first minute activity of socially housed control females did not change. Stress exposure increased the first minute activity of individually housed females on day 7, day 14 and day 21 (resp. from 591 ± 49 to 1125 ±116 (p≤0.001), 1014 ± 119 (p=0.011) and 1345 ± 123 cm (p≤0.001). Also mean activity per minute was higher after 3 weeks of stress exposure compared to all previous tests (day 7: p=0.040, day 14: p=0.026). In socially housed females there was no effect of stress on first minute locomotor activity.

Effects of treatment, gender and housing within open field tests

Stress: When looking at locomotor activity over 8 minutes, a stress effect was only present during day 7 in socially housed males, when socially housed stressed males

were more active than controls ($F_{1,40}$= 8.821, p=0.005). Chronic stress did affect first minute locomotor activity, although only in females. While in socially housed females no effect of chronic stress was observed, individually housed females showed an increased activity during the first two minutes after 3 weeks of stress ($F_{1,40}$= 7.972, p =0.007)(Fig. 3).

Housing: (Fig. 3) Male rats showed no effect of housing conditions on mean locomotor activity in any of the open field tests. There was however a clear housing effect on the novelty response to the first open field test. Individually housed control males were less active during the first minute than the socially housed control males ($F_{1,40}$= 8.145, p=0.007). In stressed males housing conditions had no effect, but in females, first minute activity of individually housed control and stressed rats was lower than that of their socially housed counterparts (resp. $F_{1,40}$= 8.167, p=0.007 and $F_{1,40}$= 8.736, p= 0.005). During the second and third exposure to the open field no effects of housing conditions were found. After 3 weeks of stress, females demonstrated an effect of housing conditions on mean locomotor activity, when individually housed females had a higher locomotor activity than the socially housed rats ($F_{1,40}$=21.39, p≤0.001). First minute activity was only affected in stressed females, as individually housed females had a higher activity than socially housed rats ($F_{1,40}$= 5.564, p= 0.023). In male rats first minute activity was affected by the housing conditions only in control males, socially housed males being more active than isolated males ($F_{1,40}$= 4.943, p=0.032). Summarising, when differences between housing conditions were observed, individually housed females were more active in the open field than socially housed females and males showed the opposite response, with socially housed males showing higher activity.

Gender: During the first open field test, individually housed control female rats were more active than males ($F_{1,40}$= 5.123, p=0.029) (Fig. 3). Gender differences in first minute activity were only seen in socially housed stressed rats, females overall having a higher initial activity than males ($F_{1,40}$= 3.959, p=0.05). Females were more active on day 7 except the socially housed stressed animals (individually housed controls: $F_{1,40}$ = 10.750, p = 0.002; individually housed stressed: $F_{1,40}$ = 8.294, p = 0.006; socially housed controls: $F_{1,40}$=8.294, p=0.005). During the first minute of the second open field test no differences between males and females were observed. On day 14 all females, except socially housed

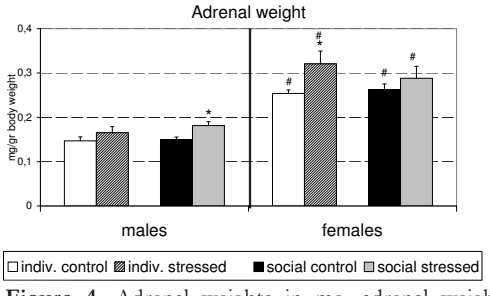

Figure 4. Adrenal weights in mg. adrenal weight per gr. body weight ± SEM. Significant difference between stress and control treatment within housing conditions (*, p<0.05), significant gender different with male counterparts (#, p<0.05).

stressed females, were more active than the males (individually housed controls: $F_{1,40}$= 4.690, p=0.036; individually housed stressed: $F_{1,40}$= 4.679, p=0.037; socially housed controls: $F_{1,40}$=.914, p=0.02), while no gender differences were observed in first minute activity. On day 21 only isolated females, both stressed and controls, were more active than males, both on the mean locomotor (resp. $F_{1,40}$= 10.788, p=0.002 and $F_{1,40}$= 6.131, p=0.018) and the first minute activity (resp. $F_{1,40}$= 5.962, p=0.019 and $F_{1,40}$= 5.033, p=0.030) (Fig. 3). Generally, females showed a higher locomotor activity in the open field than males, whether or not they were exposed to a stressor.

Adrenal and thymus weights

Chronic stress had a significant effect on adrenal weight ($F_{1,32}$ = 10.263, p = 0.003) (Fig. 4), socially housed but not isolated male rats, showing adrenal hypertrophy after chronic stress exposure ($F_{1,16}$= 6.854, p=0.019). In females, stress induced a significant increase in adrenal weight in individually housed rats ($F_{1,16}$= 4.603, p= 0.048) whereas in the socially housed females the adrenal weight was not increased. Besides stress effects, we found a gender difference in the relative adrenal weight. The female adrenal was significantly larger than the male adrenal ($F_{1,32}$=116.434, p≤0.001). Housing conditions alone had no significant effect on adrenal weight. Thymus weights were neither affected by stress nor by housing conditions.

c-Fos

PVN: The PVN controls HPA-axis activity, and accordingly showed increased Fos-ir after stress (Table 3). Since magno- and parvocellular nuclei showed similar responses, no further distinction was made between the two nuclei. There was a significant treatment ($F_{1,31}$= 13.310, p = 0.001) and gender effect ($F_{1,31}$= 11.885, p =0.002) with respect to Fos expression in the PVN. Pair wise comparisons showed that stress increased Fos-ir in the PVN in all groups except in the socially housed females (indiv. males: $F_{1,12}$= 16.566, p=0.002; social males: p=0.047, indiv. females: $F_{1,12}$ = 15.351, p = 0.002). A gender difference in PVN Fos-ir was only observed in the individually housed rats, males expressing more Fos-ir than the females (controls; $F_{1,12}$= 11.038, p=0.006; stressed: $F_{1,12}$= 12.072, p=0.005).

Dorsal Raphe: (Table 3) Stress had significant effects on Fos-ir in the DRN ($F_{1,27}$ = 19.748, p≤ 0.001), significant interaction between treatment, gender and housing ($F_{1,27}$= 4.808, p=0.037) was observed, indicating that housing conditions and gender influence the way the DRN reacts to stress. Stress exposure led to increased Fos-ir in the DRN of individually housed males ($F_{1,14}$=13.592, p=0.002) and socially housed females ($F_{1,13}$ = 9.708, p = 0.008), but failed to increase Fos-ir in individually housed females. Chronic foot-shock stress also did not significantly affect the Fos-ir of the

Table 1. Fos expression in the PVN and dorsal raphe nucleus (DRN)

	PVN		DRN	
	Males	**Females**	**Males**	**Females**
indiv. control	26.7 (6.4)	5.7 (2.3)$^{\#\#}$	8.9 (1.3)	13.7 (3.2)
indiv. stress	52.4 (3.0)**	30.4 (5.0)**$^{\#\#}$	41.2 (9.8)***	17.0 (4.3)$^{\#\#}$
social control	30.3 (4.5)	17.0 (4.6)	17.9 (2.2)	12.6 (3.0)
social stress	57.7 (13.5)*	29 (10.5)	30.9 (5.6)	28.4 (4.5)*

Mean number of Fos-positive cells per 0.1 mm^2. Significant stress effects: *, p≤0.05; **, p≤0.01; ***, p≤0.001, and significant gender differences: $, p≤0.05; $$,, p≤0.01

DRN in socially housed males ($F_{1,16}$= 4,087, p=0.060). An effect of gender was found in the individually housed stressed rats, males expressing more Fos-ir in the DRN than females ($F_{1,11}$= 7,941, p=0.017).

Discussion

In this study the authors have found that housing conditions affect the way rats react to chronic stress exposure. Isolation by itself appears to be a stressor for female rats and social housing appears to reduce these adverse effects. Male rats, in contrast, do not seem to benefit from social housing, as it actually appears to augment the negative effects of stress exposure.

In accordance with previous studies, chronic stress decreased the growth rate of male rats, but had no effect on weight gain in females.[4,14] Housing conditions had no effect on weight gain in males but reduced the growth of control females. A similar, but non-significant effect was found in the stressed females. This reduced growth rate is most likely attributable to increased activity in the home cage resulting from higher activity during estrus rather than elevated stress levels due to social housing. Concordant with previous findings, a synchronisation of the oestrous cycle among females was not observed.[51] In the current study we did not specifically investigated the effects of the oestrous cycle on the stress response.[62]. We hypothesised that since females were exposed to the stressor during all stages of the cycle this would overrule sex hormone related stress sensitivity differences. In addition open field activity did not seem to be affected by the oestrous cycle, since activity of females in oestrus during an open field test was similar to that of the non-oestrus females (not shown).

General effects of gender, housing conditions and chronic stress

In this study we found that stress increased activity most profoundly in the first minute of the open field test. Stress apparently affects the way in which rats react to a novel or change in environment, even after repeated exposures. Increased first

minute activity during the stress procedure was most pronounced in individually housed females, supporting a higher stress-sensitivity of females.[64] Social housing neither attenuated nor aggravated the effects of stress on first minute activity in males, but social housing was able to prevent this stress effect in females. For females, individual housing alone most likely already acts as a stressor, and chronic foot-shocks augment the behavioural and neuroendocrine changes in these isolated females. Stress exposure increased adrenal weight in this study, corroborating previous findings.[3,14,24,26,27] Also the magnitude of increases in adrenal weight were found to be dependent of the severity of the stress or stress-sensitivity of the rat strain.[12,68] In the current study adrenal hypertrophy was found in socially housed males and isolated females, another indication that social housing for males may be a stressor as isolation is for females. Hypertrophy of adrenal glands has also been found in depressed patients.[39,49] So adrenal weight could be a reliable measure of the amount of stress experienced during chronic exposure as an alternative for plasma corticosterone measurements which are more a measurement of an acute stress response. Gender specific effects of housing conditions have also been found in other studies. Basal corticosterone levels were higher in isolated than in socially housed females, while the opposite effect was found in males.[6] A positive influence of social housing on male rats has also been reported however. Social housing was able to reduce the adverse effects of a social defeat.[50,63] Although aggression, related to the development of a hierarchy, is more likely in a group of non-related males, and most likely provides additional stress for unrelated socially housed males as used in the current experiment.

Locomotor activity in the open field

The most commonly observed effect of chronic stress is decreased open field activity. Different designs and circumstances of test performance, like testing in the light period or shortly after stress exposure, as in other studies,[10,18,65] might explain why in this study we found a stress-induced increase in locomotor activity after chronic exposure. Duncko and co-workers have also reported increased first minute open field activity but found no change in total activity after chronic mild variable stress.[14] The use of one week intervals between open field tests in the current experiment was apparently too long for the animals to habituate, which would have resulted in a reduced activity.[11] However, the open field was probably not completely unfamiliar after repeated exposures, since the inhibition of first minute locomotor activity of individually housed rats, as seen in the first open field test, disappeared with subsequent exposures. The stress-induced increase of first minute open field activity could reflect a higher behavioural reactivity to a mild stress like the transfer to the open field. Non-stressed rats apparently do adapt to repeated open field tests

and do not show changes in locomotor activity.

After acute stress, on day 2, no locomotor effect was found, but socially and individually housed rats showed a different behavioural response, irrespective of gender. Locomotor activity was inhibited in isolated rats, implying that social housing can make rats more flexible and less anxious in a novel environment, since they start exploring immediately. A similar result has been reported by Zimmermann and coworkers[69] who showed that the latency to enter an open field from a start box was shorter in group-housed rats. This effect of housing conditions could be mediated by CRH, which is involved in regulating autonomic and behavioural responses to stress.[33,44]

Neurobiological changes after chronic stress

It has been found that central administration of CRH leads to decreased locomotor activity in a novel environment.[33] If individually housed rats are more sensitive to a change in their environment, exposure to a completely novel environment could be even more stressful leading to increased central CRH-levels which in turn could lead to reduced locomotor activity during the initial open field exposure. However elevated CRH levels in the brain could also lead to increased locomotor activity. Stress has been found to increase CRH-mRNA levels in the PVN,[1,14] and central CRH administration[53] and CRH-like peptides have been shown to increase locomotor activity in a familiar environment.[30] Moreover it has been found that chronic variable stress leads to increased CRH mRNA in the PVN in male but not female rats, although female baseline expression levels were higher.[14] Since the behavioural effects of chronic stress exposure in the current experiment were more pronounced in females than in males, this implies that females might be more susceptible to chronic inescapable footshock stress than to chronic variable stress. Increased CRH mRNA expression in the PVN was not observed after repeated exposure to the same stressor, due likely to habituation.[36] Fos data of the current experiment shows that rats do not habituate to the chronic footshock stress. Three weeks of exposure to the stress box still increased Fos expression in the PVN,[58] and the DRN, in most groups. Habituation and the associated loss of Fos expression in the PVN has been found after two weeks of chronic restrain stress.[56] The DRN showed a similar habituation, with acute restrain stress inducing Fos-ir in the DRN,[9] while expression was lost after repeated exposures.[55] Inescapable stress, but not escapable stress activates DRN 5HT neurons,[23,37] and rats undergoing 'psychological' stress have shown an increased 5HT release in the DRN.[19] The increased Fos expression in the DRN probably implies that serotonergic raphe neurons were activated in response to the 'psychological' stress of exposure to the stress box. While Fos-ir in the PVN of isolated females was increased after stress

exposure, the DRN of these animals was non-responsive. This suggests a lack of activation of the serotonergic system in isolated females whereas social housing can prevent this dysfunction and act as a 'natural antidepressant', maintaining serotonergic activity during chronic stress. The absence of stress-induced FOS expression in the DRN of individually housed females most likely is not due to habituation since the PVN Fos expression in the same animals was increased and socially housed females showed no habituation of DRN activity.

Oxytocin: A possible mechanism for the beneficial effects of social housing?

A possible mechanism underlying the social housing-induced alleviation of the stress response could be an oxytocin-mediated reduction of HPA-axis activity. Oxytocin is released by the supraoptic nucleus and the PVN [21] and has a well-known role in reproductive behaviour, labour and lactation.[15] However, several studies report that oxytocin can also modulate the stress response.[40,60,67] A single oxytocin injection in males can increase plasma corticosterone levels but repeated administration causes decreased levels lasting as long as 10 days after cessation of this treatment.[42] In addition, centrally administered oxytocin also decreases stress-induced corticosterone levels in female rats.[67] Besides the aforementioned functions oxytocin has also been shown to be involved in several aspects of social behaviour [7,29] and social recognition.[13,17] Social contact increases oxytocin release in rats,[61] and has been associated with the beneficial effects of social support during stress exposure. In humans, social contact is able to reduce the plasma cortisol levels. This effect most likely involves oxytocin.[7,15] Female rats, and women, may seek social contact during stress, which could increase oxytocin levels, acting as an endogenous anti-stress response. In the present study the higher Fos expression in the PVN of socially housed control females compared to isolated control females could be an indication of increased oxytocin release in socially housed females.

Conclusions

In summary, social housing can reduce adverse effects of chronic footshock stress in female rats, preventing the stress-induced adrenal hypertrophy and locomotor hyperactivity in the open field test. Moreover social housing of females prevented the chronic stress-induced non-responsiveness of Fos-ir in the raphe nucleus seen in isolated females. In male rats social housing augmented the stress-induced adrenal hypertrophy and could not prevent the increase in locomotor activity. Overall, the behavioural changes were quite small in stress-exposed males. Lack of stress-induced adrenal hypertrophy in individually housed males and a normal stress-induced increase of Fos expression in the DRN suggests that males are resistant to this type of stressor or may need longer exposure times to develop

stress-related pathology. Our results are consistent with findings that the female gender has higher stress vulnerability and that social housing of female rats might be a good representation of social support in humans. This creates an opportunity to explore biological mechanisms underlying the effects of social support in humans, which is able to effectively reduce the stress response,[2,16,22,64] especially in females.

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References

1. **Bakshi VP, Kalin NH**. Corticotropin-releasing hormone and animal models of anxiety: gene-environment interactions. *Biol Psychiatry* 2000;48:1175-1198
2. **Barefoot JC, Brummett BH, Clapp-Channing NE, et al.** Moderators of the effect of social support on depressive symptoms in cardiac patients. *Am J Cardiol* 2000;86:438-442
3. **Blanchard RJ, Nikulina JN, Sakai RR, McKittrick C, McEwen B, Blanchard DC**. Behavioral and endocrine change following chronic predatory stress. *Physiol Behav* 1998;63:561-569
4. **Bowman RE, Zrull MC, Luine VN**. Chronic restraint stress enhances radial arm maze performance in female rats. *Brain Res* 2001;904:279-289
5. **Brotto LA, Gorzalka BB, Hanson LA**. Effects of housing conditions and 5-HT2A activation on male rat sexual behavior. *Physiol Behav* 1998;63:475-479
6. **Brown KJ, Grunberg NE**. Effects of housing on male and female rats: crowding stresses male but calm females. *Physiol Behav* 1995;58:1085-1089
7. **Carter CS**. Neuroendocrine perspectives on social attachment and love. *Psychoneuroendocrinology* 1998;23:779-818
8. **Cheeta S, Ruigt G, van Proosdij J, Willner P**. Changes in sleep architecture following chronic mild stress. *Biol Psychiatry* 1997;41:419-427
9. **Cullinan WE, Herman JP, Battaglia DF, Akil H, Watson SJ**. Pattern and time course of immediate early gene expression in rat brain following acute stress. *Neuroscience* 1995;64:477-505
10. **D'Aquila PS, Peana AT, Carboni V, Serra G**. Exploratory behaviour and grooming after repeated restraint and chronic mild stress: effect of desipramine. *Eur J Pharmacol* 2000;399:43-47
11. **Daenen EW, Van der Heyden JA, Kruse CG, Wolterink G, Van Ree JM**. Adaptation and habituation to an open field and responses to various stressful events in animals with neonatal lesions in the amygdala or ventral hippocampus. *Brain Res* 2001;918:153-165
12. **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
13. **Dluzen DE, Muraoka S, Engelmann M, Landgraf R**. The effects of infusion of arginine vasopressin, oxytocin, or their antagonists into the olfactory bulb upon social recognition responses in male rats. *Peptides* 1998;19:999-1005
14. **Duncko R, Kiss A, Skultetyova I, Rusnak M, Jezova D**. Corticotropin-releasing hormone mRNA levels in response to chronic mild stress rise in male but not in female rats while tyrosine hydroxylase mRNA levels decrease in both sexes. *Psychoneuroendocrinology* 2001;26:77-89
15. **Evans JJ**. Oxytocin in the human--regulation of derivations and destinations. *Eur J Endocrinol* 1997;137:559-571
16. **Ezquiaga E, Garcia A, Pallares T, Bravo MF**. Psychosocial predictors of outcome in major depression: a prospective 12-month study. *J Affect Disord* 1999;52:209-216
17. **Ferguson JN, Young LJ, Hearn EF, Matzuk MM, Insel TR, Winslow JT**. Social amnesia in mice lacking the oxytocin gene. *Nat Genet* 2000;25:284-288
18. **Ferretti C, Blengio M, Gamalero SR, Ghi P**. Biochemical and behaviour changes induced by acute stress in a chronic variate stress model of depression: the effect of amitriptyline. *Eur J Pharmacol* 1995;280:19-26

19. **Funada M, Hara C**. Differential effects of psychological stress on activation of the 5-hydroxytryptamine- and dopamine-containing neurons in the brain of freely moving rats. *Brain Res* 2001;901:247-251
20. **Garlow SJ, Musselman DL, Nemeroff CB**. *The neurochemistry of mood disorders: clinical studies.* In:Charney DS, Nestler E J, Bunney B S, eds. Neurobiology of Mental Illness. New York: Oxford university press, 1999:348-364
21. **Gimpl G, Fahrenholz F**. The oxytocin receptor system: structure, function, and regulation. *Physiol Rev* 2001;81:629-683
22. **Glynn LM, Christenfeld N, Gerin W**. Gender, social support, and cardiovascular responses to stress. *Psychosom Med* 1999;61:234-242
23. **Grahn RE, Will MJ, Hammack SE, et al.** Activation of serotonin-immunoreactive cells in the dorsal raphe nucleus in rats exposed to an uncontrollable stressor. *Brain Res* 1999;826:35-43
24. **Haller J, Fuchs E, Halasz J, Makara GB**. Defeat is a major stressor in males while social instability is stressful mainly in females: towards the development of a social stress model in female rats. *Brain Res Bull* 1999;50:33-39
25. **Harris RB, Zhou J, Youngblood BD, Smagin GN, Ryan DH**. Failure to change exploration or saccharin preference in rats exposed to chronic mild stress. *Physiol Behav* 1997;63:91-100
26. **Harro J, Haidkind R, Harro M, et al.** Chronic mild unpredictable stress after noradrenergic denervation: attenuation of behavioural and biochemical effects of DSP-4 treatment. *Eur Neuropsychopharmacol* 1999;10:5-16
27. **Harro J, Tonissaar M, Eller M, Kask A, Oreland L**. Chronic variable stress and partial 5-HT denervation by parachloroamphetamine treatment in the rat: effects on behavior and monoamine neurochemistry. *Brain Res* 2001;899:227-239
28. **Hogan BE, Linden W, Najarian B**. Social support interventions Do they work? *Clinical Psychology Review* 2002;22:381-440
29. **Insel TR, Winslow JT**. Serotonin and neuropeptides in affiliative behaviors. *Biol Psychiatry* 1998;44:207-219
30. **Jones DN, Kortekaas R, Slade PD, Middlemiss DN, Hagan JJ**. The behavioural effects of corticotropin-releasing factor-related peptides in rats. *Psychopharmacology (Berl)* 1998;138:124-132
31. **Kendler KS, Thornton LM, Prescott CA**. Gender differences in the rates of exposure to stressful life events and sensitivity to their depressogenic effects. *Am J Psychiatry* 2001;158:587-593
32. **Kirschbaum C, Klauer T, Filipp SH, Hellhammer DH**. Sex-specific effects of social support on cortisol and subjective responses to acute psychological stress. *Psychosom Med* 1995;57:23-31
33. **Koob GF**. Corticotropin-releasing factor, norepinephrine, and stress. *Biol Psychiatry* 1999;46: 1167-1180
34. **Kruk MR, Westphal KG, Van Erp AM, et al.** The hypothalamus: cross-roads of endocrine and behavioural regulation in grooming and aggression. *Neurosci Biobehav Rev* 1998;23:163-177
35. **Lopez JF, Akil H, Watson SJ**. Neural circuits mediating stress. *Biol Psychiatry* 1999;46:1461-1471
36. **Ma XM, Lightman SL**. The arginine vasopressin and corticotrophin-releasing hormone gene transcription responses to varied frequencies of repeated stress in rats. *J Physiol* 1998;510 (Pt 2): 605-614
37. **Maswood S, Barter JE, Watkins LR, Maier SF**. Exposure to inescapable but not escapable shock increases extracellular levels of 5-HT in the dorsal raphe nucleus of the rat. *Brain Res* 1998;783: 115-120
38. **Matthews K, Forbes N, Reid IC**. Sucrose consumption as an hedonic measure following chronic unpredictable mild stress. *Physiol Behav* 1995;57:241-248
39. **Nemeroff CB, Krishnan KR, Reed D, Leder R, Beam C, Dunnick NR**. Adrenal gland enlargement in major depression. A computed tomographic study. *Arch Gen Psychiatry* 1992;49:384-387
40. **Neumann ID, Kromer SA, Toschi N, Ebner K**. Brain oxytocin inhibits the (re)activity of the hypothalamo-pituitary-adrenal axis in male rats: involvement of hypothalamic and limbic brain regions. *Regul Pept* 2000;96:31-38
41. **Oxman TE, Hull JG**. Social support and treatment response in older depressed primary care patients. *J Gerontol B Psychol Sci Soc Sci* 2001;56:35-45
42. **Petersson M, Hulting AL, Uvnas-Moberg K**. Oxytocin causes a sustained decrease in plasma levels of corticosterone in rats. *Neurosci Lett* 1999;264:41-44
43. **Piccinelli M, Wilkinson G**. Gender differences in depression. Critical review. *Br J Psychiatry* 2000;177:486-492
44. **Price ML, Curtis AL, Kirby LG, Valentino RJ, Lucki I**. Effects of corticotropin-releasing factor on brain serotonergic activity. *Neuropsychopharmacology* 1998;18:492-502
45. **Quintana SM, Maxwell SE**. A Monte Carlo comparison of seven e-adjustments procedures in repeated measures designs with small sample sizes. *Journal of Educational Statistics* 1994;19:57-71
46. **Ramos A, Mormede P**. Stress and emotionality: a multidimensional and genetic approach. *Neurosci Biobehav Rev* 1998;22:33-57
47. **Rhodes ME, Rubin RT**. Functional sex differences ('sexual diergism') of central nervous system

cholinergic systems, vasopressin, and hypothalamic-pituitary-adrenal axis activity in mammals: a selective review. *Brain Res Brain Res Rev* 1999;30:135-152
48. **Rivier C**. Gender, sex steroids, corticotropin-releasing factor, nitric oxide, and the HPA response to stress. *Pharmacol Biochem Behav* 1999;64:739-751
49. **Rubin RT, Phillips JJ, McCracken JT, Sadow TF**. Adrenal gland volume in major depression: relationship to basal and stimulated pituitary-adrenal cortical axis function. *Biol Psychiatry* 1996;40:89-97
50. **Ruis MA, te Brake JH, Buwalda B, et al.** Housing familiar male wildtype rats together reduces the long-term adverse behavioural and physiological effects of social defeat. *Psychoneuroendocrinology* 1999;24:285-300
51. **Schank JC**. Do Norway rats (Rattus norvegicus) synchronize their estrous cycles? *Physiol Behav* 2001;72:129-139
52. **Senba E, Ueyama T**. Stress-induced expression of immediate early genes in the brain and peripheral organs of the rat. *Neurosci Res* 1997;29:183-207
53. **Spina MG, Basso AM, Zorrilla EP, et al.** Behavioral effects of central administration of the novel CRF antagonist astressin in rats. *Neuropsychopharmacology* 2000;22:230-239
54. **Staley JK, Malison RT, Innis RB**. Imaging of the serotonergic system: interactions of neuroanatomical and functional abnormalities of depression. *Biol Psychiatry* 1998;44:534-549
55. **Stamp J, Herbert J**. Corticosterone modulates autonomic responses and adaptation of central immediate-early gene expression to repeated restraint stress. *Neuroscience* 2001;107:465-479
56. **Stamp JA, Herbert J**. Multiple immediate-early gene expression during physiological and endocrine adaptation to repeated stress. *Neuroscience* 1999;94:1313-1322
57. **Taylor SE, Klein LC, Lewis BP, Gruenewald TL, Gurung RA, Updegraff JA**. Biobehavioral responses to stress in females: tend-and-befriend, not fight-or-flight. *Psychol Rev* 2000;107:411-429
58. **Trentani A, Kuipers SD, Ter Horst GJ, Den Boer JA**. Selective chronic stress-induced in vivo ERK1/2 hyperphosphorylation in medial prefrontocortical dendrites: implications for stress-related cortical pathology? *Eur J Neurosci* 2002;15:1681-1691
59. **Troisi A**. Gender differences in vulnerability to social stress. A Darwinian perspective. *Physiol Behav* 2001;73:443-449
60. **Uvnas-Moberg K**. Oxytocin linked antistress effects--the relaxation and growth response. *Acta Physiol Scand Suppl* 1997;640:38-42
61. **Uvnas-Moberg K**. Physiological and endocrine effects of social contact. *Ann N Y Acad Sci* 997;807:146-163
62. **Viau V, Meaney MJ**. Variations in the hypothalamic-pituitary-adrenal response to stress during the estrous cycle in the rat. *Endocrinology* 1991;129:2503-2511
63. **Von Frijtag JC, Reijmers LG, Van der Harst JE, Leus IE, Van den BR, Spruijt BM**. Defeat followed by individual housing results in long-term impaired re. *Behav Brain Res* 2000;117:137-146
64. **Weinstock M, Razin M, Schorer-Apelbaum D, Men D, McCarty R**. Gender differences in sympathoadrenal activity in rats at rest and in response to footshock stress. *Int J Dev Neurosci* 1998;16:289-295
65. **Willner P**. Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. *Psychopharmacology (Berl)* 1997;134:319-329
66. **Willner P, Moreau JL, Nielsen CK, Papp M, Sluzewska A**. Decreased hedonic responsiveness following chronic mild stress is not secondary to loss of body weight. *Physiol Behav* 1996;60:129-134
67. **Windle RJ, Shanks N, Lightman SL, Ingram CD**. Central oxytocin administration reduces stress-induced corticosterone release and anxiety behavior in rats. *Endocrinology* 1997;138:2829-2834
68. **Zelena D, Haller J, Halasz J, Makara GB**. Social stress of variable intensity: physiological and behavioral consequences. *Brain Res Bull* 1999;48:297-302
69. **Zimmermann A, Stauffacher M, Langhans W, Wurbel H**. Enrichment-dependent differences in novelty exploration in rats can be explained by habituation. *Behav Brain Res* 2001;121:11-20