Influence of gender and social environment in an animal model of affective disorders
Westenbroek, Christel
Pair-housing of male and female rats during chronic stress exposure results in gender-specific behavioural responses

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Social support has a positive influence on the course of a depression and social housing of rats could provide an animal model for studying the neurobiological mechanisms of social support. Male and female rats were subjected to chronic foot-shock stress for 3 weeks and pair-housing of rats was used to mimic social support. Rats were isolated or housed with a partner of the opposite sex. A plastic tube was placed in each cage, and subsequently used as a ‘safe’ area in an open field test. Time spent in the tube was used as a measurement of anxiety levels. Chronic stress increased adrenal weights in all groups, except for isolated females who showed adrenal hypertrophy in control conditions. Chronic stress increased the anxiety level in isolated males, as shown by increased time the animals spent in the tube. While stress did not affect anxiety in socially housed males, males with a stressed partner appeared more anxious. Even though adrenal weights showed that isolated females were more affected by stress, after chronic stress exposure they spent less time in the tube than socially housed females. Also socially housed stressed females spent less time in the ‘safe’ tube compared to control counterparts, indicating stress has a gender specific behavioural effect. In conclusion: Pair housing had a stress-reducing effect on behaviour in males. Isolation of females was stressful by itself. Pair housing of females was not able to prevent stress-induced behavioural changes completely, but appeared to ameliorate stress-coping.
Introduction

Social support is known to have a positive influence on mental and physical health, but surprisingly the neurobiological mechanisms that underlie these effects have hardly been investigated. In major depression, social support has been reported to have beneficial effects on the outcome of a depressive episode and prevention of relapse. More stressful life events and less social support are associated with greater risk of disease progression in HIV patients. Also in cardiac patients it is suggested that the amount of social support and psychosocial interventions to increase social support improve the quality of life and length of survival.

A suitable animal model for studying social support would provide means to investigate what occurs in the brain and give a better understanding in the neurobiological mechanisms associated with social support. Social housing of rodents could provide such a model. During recent years increased attention is being paid to the effects of housing conditions on rodent behaviour and their stress response. Since exposure to stress is a generally accepted animal model for affective disorders like major depression, and social support has a positive influence on the outcome of a depressive episode, stress parameters may provide a useful indication of the effects of social housing and social support. In rats social housing can reduce the effect of a stressful experience, counteracting for example the behavioural and physiological effects of a social defeat. Gender differences in the effects of housing conditions have also been found. While social instability affects females more than males, crowding is stressful for males but it actually calms females. We have previously shown that female rats living in unisex groups have improved stress-coping, whereas males housed in unisex groups appear to be more stressed than isolated males.

Affective disorders have a higher prevalence in women, and even though this is widely recognised, preclinical research has mainly focussed on male animals. In the present study we investigated how the effects of mixed gender pair-housing during chronic stress influenced behaviour by measuring locomotor activity during repeated open field tests. Rats were subjected to an open field test with a slight modification in comparison to the previous experiment, in that a tube was placed at the border of the open field arena, to provide a shelter area. It was hypothesised that, since rats tend to avoid open spaces and show thigmotaxic behaviour, stress would increase the time the rats spent in the tube. With no other males present, the possibility of increased stress levels as a result of aggressive encounters is eliminated in the pair-housed males. We hypothesised that social housing therefore would be beneficial for both male and females, although for females not necessarily to the same extend as social housing in a unisex group, since continuous sexual advances of the male could
generate additional stress for the female.

Material & Methods

Rats & housing conditions

Female (n=30) and male (n=30) Wistar rats were either individually (n=24) or socially housed (n=36) with a rat of the opposite sex (n=6 per group), in the following combinations; control male with a control female, control male with a stressed female, and a stressed male with a control female (See for group names Table 1).

A plastic tube (Ø 8 x 17 cm.) was placed in each cage to offer the socially housed females some way of escape from the males. Ten days before the start of the experiment and 3 days before being housed with a female, the male rats were vasectomised under halothane anaesthesia, to prevent pregnancy of the females. 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±3 g. and females 233 ± 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. The oestrous cycle of the females was monitored by stroking them gently on the back, which during oestrus produced lordosis behaviour, accompanied by weight loss on the day of oestrus.

Rats were subjected to a chronic inescapable stress protocol for 3 weeks. Daily, at different times, rats in the stress group were placed in a box with a metal grid floor and received 5 inescapable footshocks with changing 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 subjected to the light stimulus only, so plasma adrenaline and corticosterone changes would reflect the ‘psychological’ aspect of stress exposure and not that of a foot shock related pain response. Control rats were handled daily but were not exposed to the adverse environment. All rats were weighed daily.

The rats were sacrificed on day 22 using sodium pentobarbital anaesthesia (1 ml, 6%). Upon termination blood samples were taken by cardiac puncture and stored at –20°C to determine plasma corticosterone and adrenaline levels. 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. Adrenal and thymus weights, corrected for body weight, were calculated and used as indication of the amount of stress perceived.

<table>
<thead>
<tr>
<th>Table 1. Group names pair-housed rats</th>
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<tbody>
<tr>
<td><strong>male</strong></td>
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<tr>
<td><strong>female</strong></td>
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**Open field test**

Animals were subjected to an open field test (OF) 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 3 times, on days 2, 14 and 21. The tube from the home cage of the rat was placed at the border of the open field, to provide a ‘safe’ and familiar area in the open field arena. Rats were gently placed in the tube in 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 (EthoVision 2.1®, Noldus information Technology, Wageningen, the Netherlands), with a sample rate of 5 samples/sec. Distance moved per minute (cm), and time spent in the hiding tube (sec) were analysed.

**Hormone assays**

Adrenaline was extracted from plasma using liquid/liquid extraction with 3,4-dihydroxybenzylamine as internal standard. Briefly, plasma adrenaline was bound to diphenylborate-ethanolamine at pH 8.6. The extraction was performed with n-heptane (containing 1% octanol and 25% tetraoctylammonium bromide). Adrenaline was extracted from the organic phase with diluted acetic acid. Adrenaline (20 μl acetic acid extract) was analysed by using an HPLC/auto-injector (CMA, Sweden) and a Shimadzu LC-10AD pump (Kyoto, Japan) The detection limit was 0.1mM.

For quantification of the corticosterone concentration, dexamethason was used as internal standard. Plasma was extracted with 3ml of diethylether. The extraction procedure was repeated twice. The organic phase was evaporated to dryness in a 50°C water bath. The residue was reconstituted with 200 μl of mobile phase and 50 μl was injected into the HPLC system. The concentration of both corticosterone and the internal standard was determined with UV detection at a wavelength of 254 nm. The detection limit of corticosterone was 10nM.

**Statistical analysis**

Main effects of housing (individual-social), treatment (control-stress), treatment-partner (control-stress) and gender (males-female) and the interaction effects were analysed by Multilevel (mixed model) analysis (MlwiN software, version 1.2), 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). Total distance moved and time spent in the tube were analysed similarly with open field test (OF) as level 1, rat as level 2 and cage as level 3. Because of the shape of the curve found for distance moved per minute, these curves were approximated by 2 quadratic spline functions for the first and second 4 minutes (minute as level 1, OF as level 2, rat as level 3 and cage as level 4). A natural log transformation was performed when the data showed a skewed distribution (time spent in tube, adrenaline). Effects were tested by Z tests. For the multilevel analysis the number of rats was 60, so effective degrees of freedom were large enough for a Z test. When the main effects were found to be significant further pairwise comparisons were performed by using ANOVA in SPSS 10.0. Data are presented as group means ± SEM.
Results

Weight

All rats continued to grow, as shown by a significant effect of day on weight gain ($Z = 8.792, p \leq 0.001$). Treatment had a significant effect on the growth rate ($Z = -4.243, p \leq 0.001$). The interaction effects treatment by day ($Z = -2.935, p = 0.003$) and day by treatment-partner ($Z = 2.162, p = 0.03$) were significant. Housing conditions affected the growth rate response to stress, as shown by an interaction effect of housing and treatment ($Z = 2.660, p = 0.008$). Chronic stress exposure decreased the growth rate of isolated ($F_{1,10} = 40.614, p \leq 0.001$) as well as that of socially housed males (compared to: control($C♀$): $F_{1,10} = 25.288, p \leq 0.001$; control($S♀$): $F_{1,10} = 12.676, p = 0.005$). Males housed together with a stressed female partner showed a reduced growth when compared to isolated controls ($F_{1,10} = 5.008, p = 0.049$). In females, stress reduced growth rate only in the socially housed females, in comparison to control($C♂$) females ($F_{1,10} = 5.846, p = 0.036$) (Figure 1).

Behaviour

Distance moved per minute

The most relevant differences were found between OF1 and OF3, so these data will be described in the results section. OF2 showed results intermediate of OF1 and OF3, and will for reasons of clarity not be described in detail, but significant differences are presented in tables 2A and B.

Main effects

Repetition of the open field test (OF) had a significant effect on distance moved per minute ($Z = -4.483, p \leq 0.001$). Interaction effects were found for OF by housing, OF by treatment by housing and OF by housing by gender (resp. $Z = -2.553, p = 0.011$; $Z = 1.991, p = 0.047$ and $Z = 2.127, p = 0.033$). Main effects of minute ($Z = -

Figure 1. Weight gain, expressed as delta weight (gram ± SEM) from day 1 of the experiment. Differences between controls and stressed counterparts (**$p \leq 0.01$; ***$p \leq 0.001$) and differences between individually and socially housed counterparts ($p \leq 0.05$) are indicated.
3.859, \( p \leq 0.001 \), minute by OF \( (Z=3.749, \ p \leq 0.001) \), and minute by treatment \( (Z=-2.124, \ p=0.034) \) were also found, indicating that treatment and OF affected the time course pattern of distance walked per minute. Locomotor activity during the first (min \( \leq 4 \)) and second part (min \( \geq 5 \)) of the open field test were differently affected by treatment, housing conditions and repetition of the test. Both intervals had a significant main effect on the distance moved per minute (resp. \( Z=-3.393, \ p < 0.001 \) and \( Z=-2.0832, \ p=0.038 \)). Significant interaction effects for min \( \leq 4 \) by housing by OF \( (Z=3.226, \ p=0.001) \), and min \( \geq 5 \) by OF \( (Z=3.058, \ p=0.002) \) were observed.

**Males**

Overall, socially housed control males showed a decrease in locomotor activity with repeated exposures to the OF test, indicating they were habituating to the open field, but this effect was not found in the socially housed stressed males. The socially housed males also showed a small stress-induced increase in locomotor activity, whereas isolated males showed little effect of stress exposure (Figure 2).

**Within group effects:** (Table 2A) Total locomotor activity (over 8 minutes) was changed only in control(S♀) males, and was decreased during OF3 compared to OF1 \( (p=0.017) \). **First 4 minutes:** Only the socially housed control males showed significant changes in activity between the open field tests. Locomotor activity of activity of control(S♀) males was decreased during the third OF exposure in the first
four minutes (p=0.018). Second 4 minutes: Locomotor activity of control(S♀) males in the second half of the open field test was decreased in OF3 compared to OF1 (p=0.03). Isolated stressed males showed a decreased activity in OF3 (p=0.007).

**Between groups effects:** (Table 2B) Socially housed stressed males demonstrated more total locomotor activity than control(S♀) males (F_{1,10} = 6.712, p=0.027) after 3 weeks of stress exposure, which could be attributed mostly to increased activity during the first 4 minutes (F_{1,10} = 9.255, p=0.012). The stress(C♀) males also showed a higher locomotor activity than control(C♀) males (F_{1,10} = 5.496, p=0.041) during the first 4 minutes of OF3. A single stress exposure (OF1) decreased the activity in the open field of isolated males in the first minute (F_{1,10} = 9.312, p=0.012). Moreover, these males were also less active than socially housed stressed males in the first minute after introduction into the arena (F_{1,10} = 5.624, p=0.039).

**Females**

Isolated and socially housed females showed opposite responses in locomotor activity after repeated open field exposures. Socially housed females decreased the distance moved, whereas the isolated females increased their locomotor activity. Isolated females were also more active than socially housed females (Fig. 2 and 4).

**Within group effects** (Table 2A): Individually housed females: Of control females, especially the activity in the first minute was increased after repeated OF exposures (p=0.001) and total locomotor activity was increased during the third exposure (p=0.02). This increase in total distance moved was caused by increased activity in

<table>
<thead>
<tr>
<th>Table 2A. Differences in locomotor activity between open field tests</th>
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<tr>
<td><strong>MALES</strong></td>
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<tr>
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<tr>
<td><strong>8 minutes</strong></td>
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<td>≤ 4 min.</td>
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<td>≥ 5 min.</td>
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<td><strong>8 minutes</strong></td>
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<td>1st minute</td>
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<td>≥ 5 min.</td>
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<td><strong>FEMALES</strong></td>
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<tr>
<td><strong>1st minute</strong></td>
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<td>≤ 4 min.</td>
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<td>≥ 5 min.</td>
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</table>

Down arrows show a decrease in distance moved. ≤ min 4; first 4 minutes of the open field test, ≥ 5 min; second 4 minutes of the open field test. *p=0.052, *p<0.05, **p<0.01, ***p<0.001
the first 4 minutes (p=0.022). Chronically stressed isolated females only showed an increased first minute activity after repeated OF exposures (p=0.016). **Socially housed females:** The only effect found in locomotor activity of control(C♂) females after repeated OF exposures was an almost significant decrease in total locomotor activity during OF3 (p=0.052). Locomotor activity of control(S♂) females was significantly decreased during OF3 (p=0.003). This reduction in activity was observed during both the first (p=0.008) and second half of the test (p=0.035). Stressed(C♂) females showed a reduction in distance moved only during the second half of OF3 (p=0.05).

**Between groups effects** (Table 2B): Always when significant differences in locomotor activity between groups were found, the stress-exposed females and females with a stressed male partner showed a higher locomotor activity. Moreover, also isolation was a cause of higher locomotor activity. No locomotor differences were found between the isolated control and stressed females, however isolated controls were more active than control(C♂) females (OF1: $F_{1,10} = 9.219$, p=0.013; OF3: $F_{1,10} = 26.340$, p≤0.001). During OF3 isolated controls were also more active than the control(S♂) females ($F_{1,10} = 18.802$, p=0.001). The difference between isolated stressed and socially housed stressed females did not reach significance after 3 weeks of stress exposure ($F_{1,10} = 4.024$, p=0.073).

**First 4 minutes:** Living with a stressed male partner increased locomotor activity of socially housed control females in OF1 ($F_{1,10} = 13.227$, p=0.005), and resulted in higher first minute activity ($F_{1,10} = 5.602$, p=0.039). No differences were observed between control(S♂) and stress(C♂) females. Isolated controls were more active

| Table 2B. Locomotor differences between groups within open field tests |
|-----------------|-----------------|-----------------|
|                  | OF1             | OF2             | OF3             |
| **MALES**        |                 |                 |                 |
| 8 minutes        |                 |                 |                 |
| 1st minute       |                  |                 |                 |
| ≤ 4 min.         | isol. control< isol. stress* | control(C♂)<stress(C♂)* | control(S♂)<stress(C♂)* |
|                  | isol. stress>control(C♂)* | control(C♂)<stress(C♂)* | control(S♂)<stress(C♂)* |
| 8 minutes        |                 |                 |                 |
| 1st minute       |                  |                 |                 |
| ≤ 4 min.         | control(C♂)<control(S♂)* | control(C♂)<control(S♂)* | isol. control>control(C♂)** |
|                  | isol. control>control(C♂)* | control(C♂)<stress(C♂)** | isol. control>control(S♂)** |
| ≥ 5 min.         | isol. control>control(C♂)* | control(C♂)<control(S♂)* | isol. control>control(C♂)** |
|                  | isol. control>control(S♂)** | isol. control>control(S♂)** | isol. control>control(S♂)** |
|                  | isol. stress>stress(C♂)* | isol. control>control(S♂)** | isol. control>control(S♂)** |

Group differences in distance moved per open field test. ≤min 4; first 4 minutes of the open field test, ≥5 min; second 4 minutes of the open field test. *p≤0.05, **p<0.01, ***p≤0.001
than control(C♂) females during the first 4 minutes of OF1 and OF3 (resp. $F_{1,10} = 7.033, p=0.024$ and $F_{1,10} = 15.340, p=0.003$). Also during the first minute of OF3 isolated controls were more active than social(C♂ ) females ($F_{1,10} = 22.507, p=0.001$). The difference between isolated controls and control(S♂ ) females was only significant during OF3. The isolated females were more active during the first 4 minutes, as well as in the first minute (resp. $F_{1,10} = 16.275, p=0.002$ and $F_{1,10} = 7.988, p=0.018$). No significant differences in locomotor activity were found between isolated and socially housed stressed females.

**Second 4 minutes:** There were no activity differences between control(C♂) and stress(C♂ ) females during the second 4 minutes, nor between control(S♂) and stress(C♂ ) females. Also isolated control and stressed females did not differ in locomotor activity. However, isolated control females were more active than socially housed control(C♂) females during OF1 and OF3 (resp. $F_{1,10} = 9.024, p=0.013$ and $F_{1,10} = 14.662, p=0.003$). A similar effect was found between isolated control and control(S♂ ) females (resp. $F_{1,10} = 4.694, p=0.055$ and $F_{1,10} = 13.007, p=0.005$). Only during OF3 isolated stressed females were more active than socially housed stressed females ($F_{1,10} = 6.298, p=0.031$).

**Time spent in the tube:**

**Main effects**

The time the rats spent in the tube showed a significant treatment effect ($Z=3.341, p=0.006$), and also the treatment of the partner affected this parameter ($Z=-2.911, p=0.004$). Interaction effects were observed for gender by treatment by OF ($Z=2.593, p=0.01$) and gender by treatment by housing by OF ($Z=-2.767, p=0.006$).

**Males**

![Figure 3. Time (sec) spent in the tube. *p*≤0.05: stressed compared to control(C), **p**≤0.05, $$$p$$≤0.001: compared to indiv. counterparts, $^\dag p$$≤0.05: compared to control counterparts, $^\ddag p$$≤0.05, $^\S p$$≤0.01: compared to stressed counterparts. $^\# p$$≤0.05, $^\#\# p$$≤0.01: comparison between open field tests](image-url)
Summarising, chronic stress exposure increased the time isolated males spent in the tube. This stress response was prevented by social housing, whereas a stressed female partner increased the time the control males spent in this sheltered area (Figure 3).

Within group effects: After 3 weeks of stress exposure isolated males significantly increased the time spent in the “safe” tube (p=0.023). While socially housed control(C♀) males did not change the time spent in the tube, males with a stressed female partner showed a significant increase during OF3 (p=0.005). Socially housed stressed males on the other hand, showed a decrease in time spent in the tube with repeated exposures, although this was only significant during OF 2 (p=0.006).

Between group effects: Housing conditions had significant effects on the time rats spent in the tube. During OF1 socially housed control(C♀) males spent significant more time in the familiar tube than isolated counterparts (F_{1,8} =8.295, p=0.021). Control(S♀) males also spent more time in the tube than the isolated controls in OF1 and OF3 (resp. F_{1,10} =7.701, p=0.02 and F_{1,10} =4.939, p=0.05). Socially housed stressed males spent less time in the tube than isolated stressed males in OF2 and OF3 (resp. F_{1,10} = 5.110, p=0.047 and F_{1,10} =10.049, p=0.01). A single stress exposure session led to increased time in the tube in isolated males (F_{1,10} =8.693, p=0.015). However, this effect was not found to be significant after 3 weeks of stress exposure due to high variation in this group. Socially housed stressed males showed the opposite response and spent significantly less time in the tube during OF2 and OF3 (compared to control(C♀)): resp. F_{1,8} =4.614, p=0.064 and F_{1,8} =6.608, p=0.033; compared to control(S♀): F_{1,10} =9.749, p=0.011 and F_{1,10} =16.299, p=0.002).
Females

In general, repeated exposures to the open field increased the time socially housed control females spent in the tube, but this response was absent in socially housed stressed females and isolated controls. Isolated females even decreased the time spent in the tube after stress exposure (Figure 3).

Within groups: Repeated exposures to the open field increased the time the socially housed control(C♂ ) females spent in the tube (OF1 vs.OF2; p=0.04, OF1 vs.OF3; p=0.02). Socially housed females with a stressed male partner only showed a significant increase during the third exposure (OF1 vs. OF3: p=0.023, OF2 vs. OF3: p=0.029). The time the socially housed stress females spent in the tube was not affected by repeated exposures. In contrast to socially housed control females, the isolated control rats, did not show a change with repeated open field exposures, whereas isolated stressed females even slightly decreased the time spent in the tube after chronic stress exposure (OF1 vs. OF3: p=0.007).

Between groups: No significant differences in the time spent in the tube were found between control females housed with a control or a stressed partner. During OF2 and OF3 socially housed stressed females spent less time in the tube than control(C♂ ) females (resp. F_{1,10} =5.399, p=0.043, F_{1,10} =6.100, p=0.033). The behaviour of control(S♂ ) females did not differ from stressed counterparts. During OF3 isolated stressed females spent less time in the tube than isolated control females (F_{1,10} =4.794, p=0.053). This latter group spent less time in the tube than control(C♂ ) females during OF1 and OF3 (resp: F_{1,10} =9.523, p=0.012 and F_{1,10} =22.389, p=0.001), and control (S♂ ) females during OF3 (F_{1,10} =6.988, p=0.025). Isolated stressed females also spent less time in the tube than their socially housed counterparts (F_{1,10} =8.111, p=0.017).

Endocrine parameters

Adrenal weight: Treatment and housing conditions had significant effects on
adrenal weight (resp. $Z=5.366$, $p<0.001$ and $Z=-2.232$, $p=0.026$). Also main effects of gender and gender by housing were observed (resp. $Z=16.883$, $p<0.001$ and $Z=5.962$, $p<0.001$), showing that housing conditions differently affected adrenal weight in males and females (Fig. 5).

Chronic stress exposure increased adrenal weight in isolated ($F_{1,10} = 24.960$, $p=0.001$) and socially housed males (compared to control(C♀): $F_{1,10} =28.984$, $p<0.001$ and control(S♀) males: $F_{1,10} =41.739$, $p<0.001$). Socially housed control(C♀) males also developed higher adrenal weights than isolated controls ($F_{1,10} =4.992$, $p=0.049$), but the difference with control(S♀) males was not significant. In addition, socially housed stressed males showed higher adrenal weights than isolated stressed males ($F_{1,10} =12.488$, $p=0.005$). In females chronic stress exposure significantly increased relative adrenal weight in the socially housed rats (compared to control(C♂): $F_{1,20} =4.541$, $p=0.046$; control(S♂): $F_{1,20} =6.427$, $p=0.02$). Isolated control females had higher adrenal weights than social control(C♂) and control(S♂) females (resp. $F_{1,20} =11.087$, $p=0.003$ and $F_{1,20} =13.455$, $p=0.002$), but the difference between isolated and socially housed stressed animals did not reach significance ($F_{1,20} =3.609$, $p=0.072$) (Figure 5A).

**Thymus weight:** Treatment had a significant main effect on thymus weight ($Z=-3.261$, $p<0.001$) also the interaction gender by treatment-partner was significant ($Z=-2.373$, $p=0.018$). Thymus weight was significantly reduced in socially housed stressed males compared to both males housed with a control and a stressed partner (resp. $F_{1,10} =7.613$, $p=0.02$ and $F_{1,10} =14.299$, $p=0.004$). However no groups differences were found in the females, indicating that the significant main effects were due to significant group differences in male rats (Table 3).

### Table 3. Endocrine parameters

<table>
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<tr>
<th></th>
<th>thymus (mg/gr BW)</th>
<th>CORT ($10^{-8}$ M)</th>
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<tbody>
<tr>
<td><strong>Male</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>indiv. controls</td>
<td>1.727(0.09)</td>
<td>655.8(44)</td>
</tr>
<tr>
<td>indiv. stressed</td>
<td>1.570(0.12)</td>
<td>502.8(38)</td>
</tr>
<tr>
<td>social controls(C♀)</td>
<td>2.06(0.18)</td>
<td>724.2(71)</td>
</tr>
<tr>
<td>social controls(S♀)</td>
<td>1.812(0.05)</td>
<td>647.3(67)</td>
</tr>
<tr>
<td>social stressed(C♀)</td>
<td>1.523(0.06)***$$</td>
<td>584.5(161)</td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>indiv. controls</td>
<td>1.840(0.111)</td>
<td>1173.3(191)</td>
</tr>
<tr>
<td>indiv. stressed</td>
<td>1.643(0.114)</td>
<td>1139.0(209)</td>
</tr>
<tr>
<td>social controls(C♂)</td>
<td>1.627(0.141)</td>
<td>1112.5(102)</td>
</tr>
<tr>
<td>social controls(S♂)</td>
<td>1.862(0.102)</td>
<td>1161.7(179)</td>
</tr>
<tr>
<td>social stressed(C♂)</td>
<td>1.611(0.138)</td>
<td>1209.2(251)</td>
</tr>
</tbody>
</table>

Significant stress effects within housing conditions (**p<0.001), effects of stress compared to control(S) ($$p<0.001).
Adrenaline: Treatment did not affect plasma adrenaline levels, but housing conditions did (Z = -8.763, p ≤ 0.001). Also the treatment of the partner (Z = 2.038, p = 0.041), gender (Z = -2.820, p = 0.005), gender by treatment-partner (Z = 2.173, p = 0.03), and gender by housing by treatment (Z = 2.943, p = 0.003) had a significant effect on plasma adrenaline levels. Socially housed control males with a stressed female partner showed significant increased plasma adrenaline levels compared to the socially housed stressed males (F1,9 = 7.048, p = 0.026). In addition, the socially housed control (C♀) and stressed (S♀) males showed significantly lower plasma adrenaline levels than the isolated counterparts (resp. F1,8 = 8.325, p = 0.02 and F1,8 = 53.308, p ≤ 0.001). Isolated control females had significantly higher plasma adrenaline levels than socially housed control females (control (C♂): F1,8 = 10.966, p = 0.009; control (S♂): F1,10 = 15.142, p = 0.003), and also stressed isolated females had higher adrenaline levels (F1,9 = 15.396, p = 0.003) than their socially housed counterparts (Figure 5B).

Corticosterone: No significant effects of stress or housing conditions on plasma corticosterone levels were found two hours after stress exposure. But a significant gender effect was found (Z = 6.393, p ≤ 0.001), with females demonstrating higher plasma corticosterone levels than males (Table 3).

Discussion

Chronic stress exposure and pair-housing with a rat of the opposite sex differentially affected behavioural and endocrine parameters in male and female rats. Repetition of the open field tests, housing conditions and stress did not have major effects on locomotor activity of male rats. In females however, especially locomotor activity of the isolated rats was affected by repeated open field exposures, irrespective of the fact whether or not they were exposed to stress. Chronic stress exposure increased the time males spent in the tube during the open field test, and this response could be prevented by pair-housing with a female. Interestingly the presence of a stressed female partner resulted in a response similar to that of isolated stress males. Females showed an opposite response than males on the parameter time spent in the tube, and showed under stress-free conditions an increase in the time they spent in the tube with repeated exposures, which was inhibited by chronic stress and isolation.

Exposure to stress reduced the growth rate of both individually and socially housed males, which corroborates other studies, and was also accompanied by adrenal hypertrophy showing the chronicity of the stress. Social housing with a female slightly reduced the growth rate in control males, which is likely due to increased activity in the home cage. Especially males with a stressed female partner showed a reduced growth rate. The absence of increased adrenal weight in this
group also suggests that these control(S ♀) males were probably not more stressed than control(C ♀) males. The lack of a stress effect on weight gain in isolated females corroborates previous results from our group,46 and results reported by Duncko and co-workers.13 However, a stress effect on weight gain was observed in the socially housed females, who showed a significant reduced weight gain compared to control(C♂ ) females. The stress-exposed female most likely induced an increased interest of the male partner leading to more social activity in the home cage resulting in a reduction in weight gain of the females. The slightly reduced weight gain of the male partners of these stressed females supports this.

The most pronounced differences in locomotor activity in males were found in socially housed males after 3 weeks of either control or stress treatment. Chronically stressed socially housed males showed increased locomotor activity in the first four minutes compared to control counterparts. The higher activity level in the first four minutes could be due to a stress-induced increase in responsivity to a change of environment, although it is unclear why isolated males did not show this response to stress. Chronic stress exposure induced a clear reduction in weight gain and an increase in adrenal weight in both isolated and socially housed males, eliminating the possibility that these isolated male rats did not suffer from stress. A reduction in open field activity, was most evident in control(S♀) males. This could either be related to habituation to the open field, or to the fact that these males also spent significantly more time in the tube, resulting in less locomotor activity, similar as seen in isolated stressed males. Isolated females, controls as well as stressed, showed an increase in locomotor activity with repeated exposures to the open field, corroborating previous results.46 Together with the observed adrenal hypertrophy, this shows that isolated females, irrespective of treatment, demonstrated signs of stress exposure. Socially housed females who did suffer from stress-induced adrenal hypertrophy, although to a lesser extent than isolated females, demonstrated a decrease in locomotor activity whenever a change in activity was observed. This could indicate that socially housed females were habituating to the open field and isolated females were not. A stress-induced reduction of locomotor activity as usually reported was not found in the previous46 and current study. Most likely this is due to differences in design and circumstances of test performance, like testing in the light period or shortly after stress exposure, as was done in other studies.11,16,47 Increased initial open field activity was also found by Duncko and co-workers who reported an stress-induced increase in first minute open field activity.13 The significant stress effects on first minute locomotor activity, as reported previously46 were not observed in the present study. Likely this is caused by the presence of the tube in the open field test in which the animals were placed at the beginning of the test.

Whereas locomotor activity did not show clear stress- and housing-induced
changes, the parameter time spent in the tube was affected by gender, stress and pair-housing. In isolated males, as expected, stress exposure increased the time the animals spent in the shelter of the tube. Socially housed stressed males did not show this response but showed a decrease in the time spent in the tube, suggesting that the presence of a female can improve stress-coping in males. Interestingly, males housed with a stressed female partner appeared to be more anxious as suggested by them spending more time in the tube during the open field test. One could state that control(S ♀) males are exposed daily to a mild variant of communication stress. In this stress paradigm rats are placed in a so called communication box, which exposes them to visual, olfactory and auditory stimuli produced by foot-shocked rats. In our experiment, control(S ♀) males are exposed to an “unexplainable” of “fear-smelling” female partner in their home cage. This appeared to result in an increased sensitivity to a mild stressor like a change in environment, however without having a chronic impact on these males, since no adrenal hypertrophy occurred. Increased behavioural reactivity to stress could also relate to the observed elevated plasma adrenaline levels in these control(S♀) males that illustrates increased autonomic nervous system activity.

Female rats showed an opposite behavioural response to stress than males. In the current experiment socially housed control females increased the time they spent in the tube, which was attenuated by stress. Isolated females did not show this increase and spent significantly less time in the tube after stress exposure. Adrenal hypertrophy, adrenaline levels and reduced pCREB expression in the dentate gyrus of the hippocampus demonstrated that isolated females were more affected by stress than socially housed females and that isolation by itself was stressful. It is tempting to speculate that under control, stress-free conditions, when an environment, like an open field, becomes familiar, the urge to explore it decreases and females rats spent more time in a relative shielded area such as the tube. Having a stressed male partner prolonged the time for this response to occur but apparently had no long-lasting effects. Male rats with a stressed female partner in contrast, did show a behavioural stress response. Social housing of females with a male partner was not able to counteract these behavioural stress-effects as was observed in males. Socially housed stressed females, although they did not show an increased time in the tube, spent more time in the shielded area than isolated stressed females. Gender differences in the behavioural effects of stress have been found previously, especially regarding learning and memory tasks. In male rats chronic stress reduced spatial memory, while it is improved in females. Also classical eyeblink conditioning is impaired in females after stress, whereas males show the opposite response. Although an open field test is not a learning task, apparently stress and housing conditions also have a gender-specific effect on open field behaviour of rats, specifically on the time
the animals spent in a sheltered area.

In contrast to the males, the presence of a stressed male partner, did not increase plasma adrenaline levels in females. However like in the males, plasma adrenaline levels were higher in isolated rats, indicating higher stress responsivity in isolated animals. Adrenaline levels rise within minutes after exposure to a stressor. One could argue that the adrenaline levels are the result of stress induced by the brief transport before the sacrifice and not of exposure to the footshock box. However, all rats were subjected to the same transport, so differences in adrenaline levels would still represent differences in stress-reactivity. Socially housed rats are used to an active, changing environment (namely their home cage), and might be less affected by transport. This could explain the lower adrenaline levels at the time of sacrifice of these socially housed rats, with the exception of control(S♀) males.

The lack of stress effects on plasma corticosterone levels in the current experiment does not necessarily represent an insufficient stress exposure or habituation. Blood samples were taken 2 hours after the last stress exposure, and therefore are not representative of the stress response. Also, exposure to stress during the active/dark period could be responsible for this lack of a plasma corticosterone effect. A study by Retana-Marquez also failed to show a footshock-induced increase in corticosterone when the footshock was given in the dark period, when baseline corticosterone levels were higher. With the current experimental set-up, adrenal weight likely provides a more reliable indicator of severity and susceptibility to chronic stress, and may serve as indicator of the chronicity of the stress and lack of habituation.

Behavioural data indicate that paired-housed males were less affected by chronic stress exposure than isolated males. Surprisingly, socially housed males did show higher adrenal weights than isolated counterparts. Lemaire and co-workers showed an increased adrenal weight in males exposed to female rats, so the somewhat higher adrenal weight in socially housed males might be caused by the continuous presence of a female. Taylor and co-workers also showed that the company of females increased adrenal weight under low-stress circumstances, and that the presence of females increased the plasma testosterone levels in males. Since testosterone levels are negatively correlated with HPA-axis activity, it is tempting to suggest that possibly elevated testosterone levels induced by the presence of a female may have decreased the impact of chronic stress exposure in the male, despite them showing higher adrenal weights than isolated counterparts.

Neurochemical and endocrine changes do not necessarily reflect the impact of chronic stress. In a visible burrow system, which provides a naturalistic colony system to study psychosocial stress in rats, male rats will establish a dominance hierarchy. Behavioural differences between the dominant and subordinate animals
are very clear, but in this paradigm endocrine and neurochemical changes are similar in dominant and subordinate rats. This indicates that, in this model, at least part of the changes are adaptations to environmental demands and not a sign of severe chronic stress. It is therefore possible that the higher adrenal weights in socially housed males found in the current study are not a sign of chronic stress, but also reflect an adaptation to the presence of a female.

Summarising, gender specific responses were found especially for the behavioural parameter time spent in the tube. Chronic stress increased the time male rats spent in the tube, which was prevented by pair-housing with a female. Under control conditions, socially housed females, increased the time they spent in the ‘safety’ of the tube, implying that this is the normal response to repeated open field exposures for females, which was inhibited by stress and even more by being housed individually. Male rats showed an increase in stress-sensitivity when housed with a stressed partner, whereas females were hardly affected by a stressed male partner. Concluding, in male rats, pair-housing with a (preferably unstressed) female is able to prevent several of the stress-induced behavioural and endocrine effects, whereas in females, social housing cannot prevent the effects of chronic stress, but is better than isolation.

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References

8. Brotto LA, Gorzaika BB, Hanson LA. Effects of housing conditions and 5-HT2A activation on male rat sexual behavior. Physiol Behav 1998;63:475-479

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. 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

14. Endo Y, Shiraki K. Behavior and body temperature in rats following chronic foot shock or psychological stress exposure [In Process Citation]. *Physiol Behav* 2000;71:263-268


