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Pair-housing of male and female rats during chronic stress exposure results in gender-specific behavioral responses

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Abstract

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 footshock 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. In isolated males, chronic stress resulted in an increase in the time the animals spent in the tube. While stress did not affect this parameter in socially housed males, males with a stressed partner showed a similar response as isolated stressed males. 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. Socially housed stressed females spent less time in the 'safe' tube compared to control counterparts, indicating that stress has a gender-specific behavioral effect. In conclusion: pair-housing had a stress-reducing effect on behavior in males. Isolation of females was stressful by itself. Pair housing of females was not able to prevent stress-induced behavioral changes completely, but appeared to reduce the effects of chronic stress.

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Keywords: Open field; Locomotor activity; Adrenal; Social support; Affective disorders

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 (Ezquiaga et al., 1999; Hogan et al., 2002; Kruk et al., 1998; Oxman and Hull, 2001). More stressful life events and less

social support are associated with greater risk of disease progression in HIV patients (Leserman et al., 2000, 2002). 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 (Barefoot et al., 2000; Grace et al., 2002).

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 behavior and their stress response (Brotto et al., 1998; Ezquiaga et al., 1999). Since exposure

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to stress is a generally accepted animal model for affective disorders like major depression (Nestler et al., 2002) 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 behavioral and physiological effects of a social defeat (Ruis et al., 1999; Von Frijtag et al., 2000). Gender differences in the effects of housing conditions have also been found. While social instability affects females more than males (Haller et al., 1999), crowding is stressful for males but it actually calms females (Brown and Grunberg, 1995). 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 (Westenbroek et al., 2003b,c).

Affective disorders have a higher prevalence in women (Kessler et al., 1993), and even though this is widely recognized, preclinical research has mainly focused on male animals. In the present study, we investigated how the effects of mixed gender pair-housing during chronic stress exposure influenced behavior 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 (Westenbroek et al., 2003c), in that a tube was placed at the border of the open field arena to provide a shelter area. It was hypothesized that, since rats tend to avoid open spaces and show thigmotaxic behavior, stress would increase the time the rats spent in the tube. We have previously shown that with our stress and open field protocol, especially first minute, locomotor activity was increased in stressed animals. Also in the present experiment, we expected that the animals suffering most from the stress exposure would show the most pronounced increase in locomotor activity. 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 hypothesized that social housing therefore would be beneficial for both males and females, although for females, not necessarily to the same extent as social housing in a unisex group, since continuous sexual advances of the male could generate additional stress for the female.

Material and methods

Rats and 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. Group names used throughout the paper for

the socially housed males; control(C♀): control male–control female, control(S♀): control male–stressed female, stress(C♀): stressed male–control female. Group names for the socially housed females; control(C♂): control female–control male, control(S♂): control female–stressed male, stress(C♂): stressed female–control male.

A plastic tube ($\varnothing 8 \times 17$ cm.) was placed in each cage. This offers, in case of the socially housed rats, the 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 vasectomized under halothane anesthesia to prevent pregnancy of the females. The light–dark cycle was reversed (lights on 19.00–7.00 h) and water and food were provided ad libitum. 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). The estrus cycle of the females was monitored by stroking them gently on the back, which during estrus produced lordosis behavior, accompanied by weight loss on the day of estrus.

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 min session (0.8 mA in intensity and 8 s in duration). A light signal (10 s) 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. 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 anesthesia (1 ml, 6%). Upon termination, blood samples were taken by cardiac puncture and stored at -20°C to determine plasma epinephrine levels. The rats were transcardially perfused with 50 ml heparinized saline and 300 ml of a 4% paraformaldehyde solution in 0.1 M sodium phosphate buffer (pH 7.4), 2 h after the start of the last exposure to the stress box. Adrenal weights, corrected for body weight, were calculated and used as indication of the amount of stress perceived.

Open field test

Animals were subjected to an open field test (OF) for a period of 8 min. 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 h 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 behavior

was recorded with a videotracking system (EthoVision 2.1®, Noldus information Technology, Wageningen, the Netherlands), with a sample rate of 5 samples/s. Distance moved per minute (cm) and time spent in the hiding tube (s) were analyzed.

Epinephrine assay

Epinephrine was extracted from plasma using liquid/liquid extraction with 3,4-dihydroxybenzylamine as internal standard (Smedes et al., 1982). Briefly, plasma epinephrine was bound to diphenylborate-ethanolamine at pH 8.6. The extraction was performed with *n*-heptane (containing 1% octanol and 25% tetraoctylammoniumbromide). Epinephrine was extracted from the organic phase with diluted acetic acid. Epinephrine (20 μ l acetic acid extract) was analyzed by using an HPLC/auto-injector (CMA, Sweden) and a Shimadzu LC-10AD pump (Kyoto, Japan) The detection limit was 0.1 mM.

Statistical analysis

Main effects of housing (individual–social), treatment (control–stress), treatment-partner (control–stress), and gender (males–female) and the interaction effects were analyzed by Multilevel (mixed model) analysis (MlwiN software, version 1.2) (Rasbash et al., 2001), with random effects for rats and cages, with rats (level 1) nested in cages (level 2). Weight gain was analyzed 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 analyzed 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 min (minute as level 1, OF as level 2, rat as level 3, and cage as level 4) (Snijders and Bosker, 1999). A natural log transformation was performed when the data showed a skewed distribution (time spent in tube, epinephrine). 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 \pm 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 ($Z = -4.243$, $P \leq 0.001$) and also significant interaction effects were found for treatment by day ($Z = -2.935$, $P = 0.003$)

and day by treatment-partner ($Z = 2.162$, $P = 0.03$). 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 ($F_{1,10} = 40.614$, $P \leq 0.001$) and 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 paired with a stressed female partner also showed a reduced growth compared to isolated control males ($F_{1,10} = 5.008$, $P = 0.049$). In females, the growth rate was reduced only in the socially housed stressed females, in comparison to control(C♂) females ($F_{1,10} = 5.846$, $P = 0.036$) (Fig. 1).

Endocrine parameters

Adrenal weight

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

Chronic stress exposure increased adrenal weight in isolated ($F_{1,10} = 24.960$, $P = 0.001$) and socially housed males (compared to control(C♀): $F_{1,10} = 28.984$, $P \leq 0.001$ and control(S♀) males: $F_{1,10} = 41.739$, $P \leq 0.001$). Control(C♀) males also developed higher adrenal weights

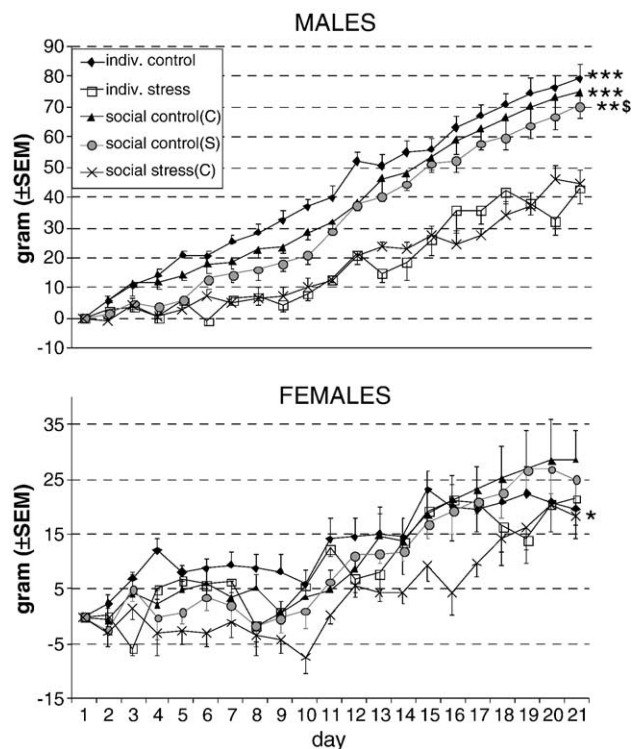


Fig. 1. Weight gain, expressed as delta weight in grams (mean \pm SEM, $n = 6$ per group) from day 1 of the experiment. Differences between controls and stressed counterparts (** $P \leq 0.01$; *** $P \leq 0.001$) and differences between individually and socially housed counterparts ($^{\$}P \leq 0.05$) are indicated.

than isolated controls ($F_{1,10} = 4.992$, $P = 0.049$), but the difference with control(S♀) males was not significant. In addition, the socially housed stressed males showed higher adrenal weights than isolated stressed males ($F_{1,10} = 12.488$, $P = 0.005$). Chronic stress exposure in females significantly increased 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 ($F_{1,20} = 3.609$, $P = 0.072$) (Fig. 2A).

Epinephrine

Treatment did not affect plasma epinephrine levels, but housing conditions did ($Z = -8.763$, $P \leq 0.001$). 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 the plasma epinephrine levels. Control males paired with a stressed female partner showed significantly increased plasma epinephrine levels compared to the stressed males paired with a control female ($F_{1,9} = 7.048$, $P = 0.026$). In addition,

the control(C♀) and stressed(C♀) males showed significantly lower plasma epinephrine levels than the isolated counterparts (resp. $F_{1,8} = 8.325$, $P = 0.02$ and $F_{1,8} = 53.308$, $P \leq 0.001$). Isolated control females had significantly higher plasma epinephrine levels than socially housed control females (control(C♂): $F_{1,9} = 10.966$, $P = 0.009$; control(S♂): $F_{1,10} = 15.142$, $P = 0.003$), and also stressed isolated females had higher epinephrine levels ($F_{1,9} = 15.396$, $P = 0.003$) than their socially housed counterparts (Fig. 2B).

Behavior

Distance moved per minute

The most relevant differences in locomotor activity 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.

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 ($Z = -3.859$, $P \leq 0.001$), 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.

Males. Chronic stress exposure and housing conditions did not have major effects on locomotor activity in the open field in male rats. The most pronounced difference after 3 weeks of stress was found between the socially housed stressed males and their control counterparts. The latter showing a pattern of declining activity in the first 4 min of the open field (stressed compared to control(C♀) males; $F_{1,10} = 5.496$, $P = 0.041$ and to control(S♀) males; $F_{1,10} = 9.255$, $P = 0.012$) (Fig. 3).

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. 3).

In isolated females, the most prominent changes occurring with repeated exposure to the open field was an increase in first minute activity (control; $P = 0.001$, stressed ($P = 0.02$). Only in the socially housed control females activity levels changed during repeated OF exposures. Control(S♂) females showed a decrease in activity between the first and third exposure to the open field (resp. ($P = 0.003$). In control(C♂) females, however this decrease did not reach significance ($P = 0.052$).

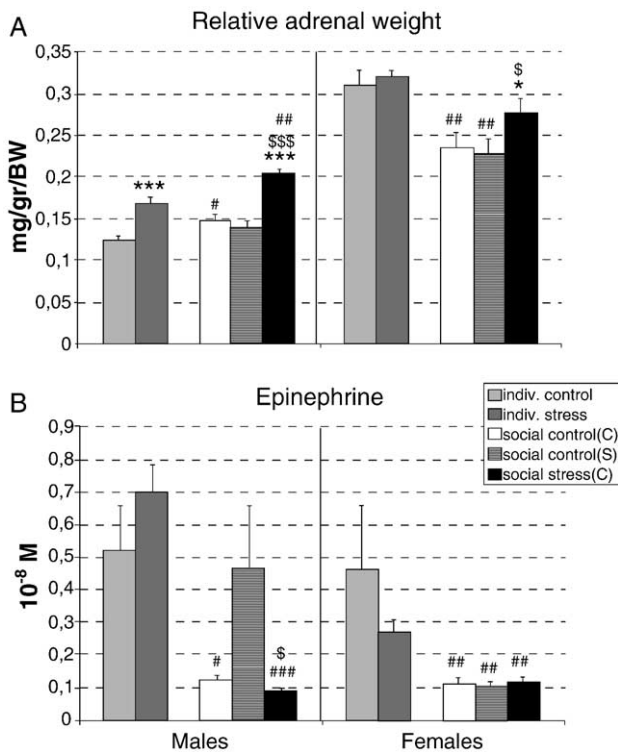


Fig. 2. (A) Relative adrenal weights expressed as mg/g body weight (mean \pm SEM, $n = 6$ per group). (B) Plasma epinephrine concentrations in 10^{-8} M (mean \pm SEM, $n = 5$ or 6 per group). Significant stress effects within housing conditions (* $P \leq 0.05$, *** $P \leq 0.001$), housing effect within treatment conditions (# $P \leq 0.05$, ## $P < 0.01$). Effect of stress compared to control (S) ($^{\$}P \leq 0.05$, $^{$$$}P \leq 0.001$).

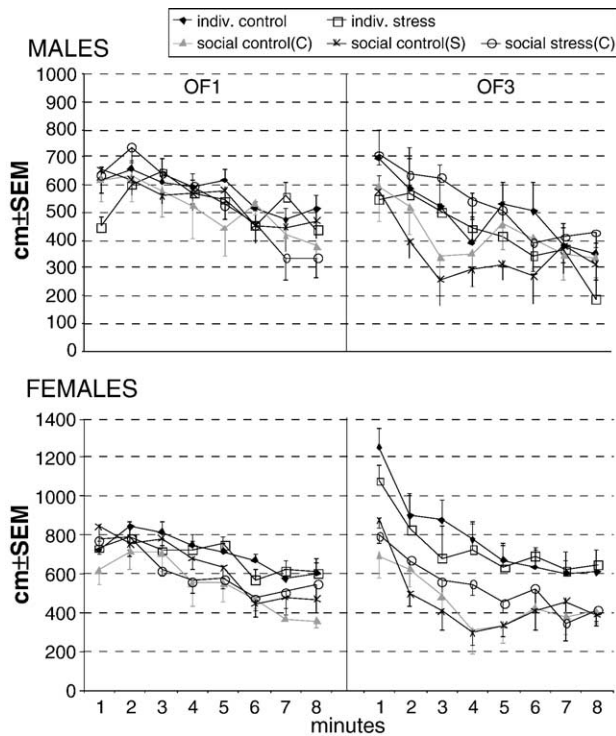


Fig. 3. Distance moved per minute in centimeters (mean \pm SEM, $n = 6$ per group) for the first (OF1) and third open field tests (OF3).

No locomotor differences were found between the isolated control and stressed females, however isolated controls were more active than control(C♂) females during all 3 OF tests (OF1: $F_{1,10} = 9.219$, $P = 0.013$; OF3: $F_{1,10} = 26.340$, $P \leq 0.001$). During OF3, isolated controls were also more active than the control(S♂) females ($F_{1,10} = 18.802$, $P = 0.001$).

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. Within group effects. After 3 weeks of stress exposure, isolated males significantly increased the time spent in the “safe” tube ($P = 0.023$). Socially housed control(C♀) males did not change the time spent in the tube but males with a stressed female partner significantly increased this behavior during OF3 ($P = 0.005$). On the other hand, socially housed stressed males decreased the time spent in the tube with repeated exposures, although this was only significant during OF2 ($P = 0.006$).

Between group effects. Housing conditions had significant effects on the time rats spent in the tube. Control(S♀) males spent more time in the tube than the isolated controls during OF3 ($F_{1,10} = 4.939$, $P = 0.05$). Also

isolated stressed males spent more time in the tube than socially housed stressed males during OF3 ($F_{1,10} = 10.049$, $P = 0.01$). A single stress exposure session led to increased time in the tube in isolated males ($F_{1,10} = 8.693$, $P = 0.015$), however, due to high variation in this group, this effect was not significant after 3 weeks of stress exposure. Socially housed stressed males spent significantly less time in the tube than control males 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 $F_{1,10} = 16.299$, $P = 0.002$).

Summarizing, chronic stress exposure increased the time the 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 (Fig. 4).

Females. Within groups effects. 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

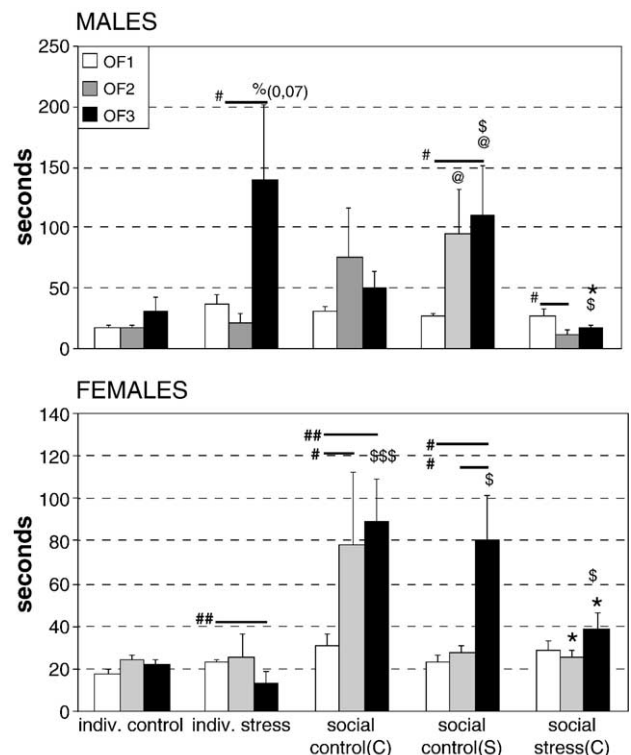


Fig. 4. Time spent in the tube (mean \pm SEM, $n = 5$ or 6). * $P \leq 0.05$: stressed compared to control (C), \$ $P \leq 0.05$, \$\$\$ $P \leq 0.001$: compared to individual counterparts, % $P \leq 0.05$: compared to control counterparts, @ $P \leq 0.05$, @@ $P \leq 0.01$: compared to stressed counterparts. # $P \leq 0.05$, ## $P \leq 0.01$: comparison between open field tests.

females spent in the tube was not affected by repeated exposures. Repeated open field exposures did not affect the time in tube of isolated females, whereas isolated stressed females even showed a slight decrease in time spent in tube after chronic stress exposure (OF1 vs. OF3: $P = 0.007$).

Between groups. No significant differences were found between control females paired 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 time in tube behavior 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 ($F_{1,10} = 4.794$, $P = 0.053$), and the latter less than control(C♂) ($F_{1,10} = 22.389$, $P = 0.001$) and control (S♂) females ($F_{1,10} = 6.988$, $P = 0.025$). Isolated stressed females also spent less time in the tube than their socially housed counterparts ($F_{1,10} = 8.111$, $P = 0.017$).

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 (Figs. 4 and 5).

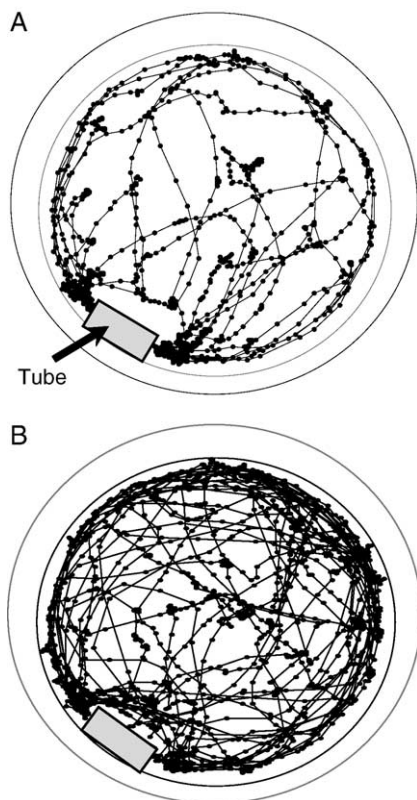


Fig. 5. Characteristic 8 min walking pattern of a pair-housed control female (A) and an isolated stressed female rat (B) in the open field arena during the third test. ● represents each sample taken by EthoVision.

Discussion

Chronic stress exposure and pair-housing with a rat of the opposite sex differentially affected behavioral and endocrine parameters in male and female rats. Both isolated and socially housed males showed chronic stress-induced adrenal hypertrophy and reduced growth rate. Whereas chronic stress and housing conditions did not have major effects on locomotor activity in the open field, stress increased the time the isolated males spent in the tube, while the presence of a female partner in the home cage appeared to prevent this response. This indicates that the presence of a female cage-mate had a moderate stress-reducing effect in males. Adrenal hypertrophy and plasma epinephrine levels showed that isolation was stressful for females which masked the effects of chronic stress. In socially housed females, chronic stress exposure increased adrenal weight, however not to the same level as in isolated female rats. Furthermore, isolated control and stressed females showed a higher activity level in the open field than socially housed females, corroborating previous results (Westenbroek et al., 2003c). Females show a different response than the male rats with respect to the behavioral parameter ‘time in tube.’ The most stressed female rats, as indicated by adrenal weights, spent the least time in the shelter of the tube.

Exposure to stress reduced the growth rate of both individually and socially housed males, which is supported by other studies (Harro et al., 1999; Kuipers et al., 2003; Westenbroek et al., 2003c), 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 paired with a stressed female partner showed this reduced growth rate. However, the absence of adrenal hypertrophy in this control 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 (Westenbroek et al., 2003c) and results reported by Duncko et al. (2001).

The most pronounced differences in locomotor activity in males were found after 3 weeks. Socially housed control males displayed a fast habituation to the open field, as shown by a rapid decline in locomotor activity in the first 4 min, an effect that was absent in the socially housed stressed and the isolated males. Isolated females, controls as well as stressed, showed an increase in locomotor activity with repeated exposures to the open field, corroborating previous results (Westenbroek et al., 2003c). Together with the observed adrenal hypertrophy, this shows that isolated females, irrespective of treatment, demonstrated signs of stress exposure. Socially housed control females showed decreased locomotor activity with repeated exposures to the open field, this could indicate that socially housed control females were habituating to the open field whereas isolated

and socially housed stressed females were not. A stress-induced reduction of locomotor activity as usually reported was not found in the previous (Westenbroek et al., 2003c) 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 most other studies (Ferretti et al., 1995; D'Aquila et al., 2000; Willner, 1997). The significant stress effects on first minute locomotor activity, as reported previously by our group and by Duncko and co-workers, were not observed in the present study (Duncko et al., 2001; Westenbroek et al., 2003c). Likely, this is caused by the presence of the familiar tube in the open field in which the animals were placed at the beginning of the test.

Whereas locomotor activity did not show distinct stress- and housing-induced changes, the parameter 'time in 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, however, did not show this response but decreased the time spent in the tube, suggesting that the presence of a female can improve stress-coping in males. Interestingly, males that were housed with a stressed female partner appeared to show signs of stress, as demonstrated 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 footshocked rats (Endo and Shiraki, 2000; Funada and Hara, 2001; Noguchi et al., 2001). 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 behavioral reactivity to stress could also relate to the observed elevated plasma epinephrine levels in these control(S♀) males that illustrates increased autonomic nervous system reactivity. In contrast to the males with a stressed female cage mate, the presence of a stressed male partner did not increase plasma epinephrine levels in females. However, like in the males, plasma epinephrine levels were higher in isolated females, indicating a higher autonomic stress responsivity in these isolated animals. The measured epinephrine levels were most likely the result of stress induced by the transport before the sacrifice and not of exposure to the footshock box 2 h before, since epinephrine levels rise within minutes after exposure to a stressor (Weinstock et al., 1998). One could argue that, since all rats were subjected to the same transport, differences in epinephrine levels would represent differences in stress-reactivity originating from the different treatments. Socially housed rats are familiar with a constantly changing environment (namely their home cage) and might therefore be less

affected by transport. This could explain the lower epinephrine levels of these socially housed rats at the time of sacrifice, whereas the presence of a stressed female cage mate eliminates this effect.

Female rats showed an opposite behavioral response to stress than males. In the current experiment, the 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, epinephrine levels, and reduced pCREB expression in the dentate gyrus of the hippocampus (Westenbroek et al., 2003a) demonstrated that isolated females were more affected by stress than socially housed females, implying that isolation by itself was stressful. It is tempting to speculate that in control, stress-free conditions, when an environment, like an open field, becomes familiar, the urge to explore it decreases and female rats spent more time in a relative shielded area such as the tube. Pairing with 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 behavioral stress response. In females, pairing with a male partner could not counteract these behavioral stress effects as was observed in males. Socially housed stressed females did not show an increased 'time in tube' but did spend more time in the shielded area than the isolated stressed females suggesting that they were less stressed than their isolated counterparts.

Gender differences in the behavioral effects of stress have been found previously, especially regarding performance in learning and memory tasks. In male rats, chronic stress reduced spatial memory, while it is improved in females (Bowman et al., 2001; Conrad et al., 2003; Krugers et al., 1997). Furthermore, classical eye blink conditioning is impaired in females after stress, whereas males show the opposite response (Wood and Shors, 1998). Studies by the group of File showed that validated anxiety tests have different outcomes in male and female rats (Fernandes et al., 1999; Johnston and File, 1991) and that behavioral responses of female rats are characterized by activity and those of males by anxiety and sexual preference. Maybe it is therefore not realistic to expect a similar response to stress and housing conditions in male and female rats on open field behavior, specifically on parameters like locomotor activity and the time the animals spent in a sheltered area.

Behavioral data indicate that pair-housed males were less affected by chronic stress exposure than isolated males. Surprisingly, the socially housed males did show higher adrenal weights than their isolated counterparts. Lemaire et al. (1997) reported 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 (Taylor et al., 1987). Since testosterone levels are negatively correlated with HPA-axis activity (Viau, 2002), 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. Reduced weight gain and adrenal hypertrophy generally are used as an indication of stress exposure. Moncek et al. (2004) however recently showed that environmental enrichment also leads to a reduced weight gain, elevated corticosterone levels, and increased adrenal weights. At the same time, it also results in increased neuronal plasticity and neurogenesis (Nilsson et al., 1999; Pham et al., 2002). This implies that stress parameters like increased adrenal weights and plasma corticosterone levels not necessarily are analogous to a negative influence on the brain and that, in this model, at least parts 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.

Summarizing, social housing modulates the response to chronic stress exposure in a sex-specific manner. Chronic stress resulted in adrenal hypertrophy in all groups except isolated females who showed high adrenal weights already in control conditions. 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. Concluding, in male rats, pair-housing with a (preferably unstressed) female is able to prevent several of the stress-induced behavioral and endocrine effects, whereas in females, isolation is stressful by itself and social housing cannot prevent the effects of chronic stress but is better for females than isolation.

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