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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.

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 (∅ 8 × 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