Chapter 3

Housing familiar male Wildtype rats together reduces the long-term adverse behavioural and physiological effects of social defeat

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Abstract

Social stress in rats is known to induce long-lasting, adverse changes in behaviour and physiology, which seem to resemble certain human psychopathologies, such as depression and anxiety. The present experiment was designed to assess the influence of individual or group housing on the vulnerability of male Wildtype rats to long-term effects of inescapable social defeat. Group-housed rats were individually exposed to an aggressive, unfamiliar male conspecific, resulting in a social defeat. Defeated rats were then either individually housed or returned to their group. The changes in their behaviour and physiology were then studied for 3 weeks. Results showed that individually housed rats developed long-lasting, adverse behavioural and physiological changes after social defeat. Their body growth was significantly retarded \( (p<0.05) \) between 7 and 14 days after defeat. When individually and group-housed rats were exposed to a mild stressor (sudden silence), 2 days after defeat, both groups became highly immobile. However, when exposure was repeated at day 21, individually housed rats were still highly immobile compared to group-housed rats which regained their normal mobility after only 7 days. In an open field test, also regularly repeated, individually housed rats took significantly longer to leave their home base and were also significantly less mobile than group-housed rats over the entire 3-week test period as well as at specific timepoints. When the rats were placed in an elevated plus-maze 14 days after defeat, those that were individually housed were significantly more anxious than those that were group-housed. When tested at 21 days after defeat in a combined dexamethasone (DEX)/corticotrophin-releasing factor (CRF) test, results showed that the hypothalamic-pituitary-adrenocortical (HPA) activity in individually housed rats was higher. This was evidenced in the latter animals by the fact that DEX was significantly less able to suppress the secretion of ACTH and corticosterone, and by a significantly higher release of ACTH after administration of CRF. Although the weights of the spleen and testes of the two groups did not differ, the adrenals of individually housed rats were larger and the thymus and seminal vesicles were smaller. We conclude that when rats are isolated after defeat, they show long-lasting, adverse behavioural and physiological changes that resemble symptoms of stress-related disorders. In contrast, when familiar rats are housed together these effects of a social defeat are greatly reduced. These findings show that housing conditions importantly influence the probability of long-term adverse behavioural and physiological effects of social defeat in male wildtype rats.
Introduction

Major life events have been shown to be involved in the development of stress-related disorders in humans, including affective disorders such as depression and anxiety (Biondi and Picardi, 1996; Brown, 1993). The physiological mechanisms underlying psychopathologies have not only been directly investigated in human patients, but also indirectly in animal models. Although few animal studies have evaluated the effects of single life events, recent studies in rats demonstrate that long-lasting, adverse effects arise after a single social defeat (Koolhaas et al., 1997b; Miczek et al., 1990).

Social defeat has been shown to cause several behavioural and physiological changes in rats. Although the time courses of these changes differ greatly (Koolhaas et al., 1997b), there are several long-lasting effects of social defeat. These are, for example, retardation of body growth (Meerlo et al. 1996c, 1997), reduced mobility in response to a mild stressor (Koolhaas et al., 1990), decreased open field activity (Meerlo et al., 1996b), high anxiety in a plus-maze test (De Boer et al., unpublished data), and increased hypothalamic-pituitary-adrenocortical (HPA) activity (Buwalda et al., 1999). Koolhaas et al. (1990, 1997b) have suggested that the social defeat model in rats seems to be a promising model for human depression, but the interpretation of long-lasting changes following social defeat in rats in terms of pathologies similar to those in humans is still an important fundamental issue. However, the fact that certain behavioural and physiological changes can be counteracted by depriving rats of sleep (Koolhaas et al., 1990; Meerlo et al., 1996b) and by the use of antidepressants (Fuchs et al., 1996; Koolhaas et al., 1990; Sampson et al., 1991) shows that stress disorders exist in animals that may resemble those in humans.

However, the social defeat model in rats was developed using rats that were individually housed, and until now it has not been known whether rats that are housed with familiar congeners would also develop long-lasting, adverse behavioural and physiological changes. Wild rats live in groups and we speculate that the presence of familiar group members is beneficial for health and well-being by moderating or buffering rats against the adverse effects of stress (social support). A number of health studies in humans have also shown that social support reduces the frequency of depression (Biondi and Picardi, 1996; Coyne and Downey, 1991; Elmore, 1984, Miller and Surtees, 1995, Paykel, 1994) and the incidence of disease-related mortality and morbidity (Cohen, 1988). However, little has been published on this subject related to animals and results are contradictory. Although Van Dijken et al. (1992) found that housing rats in groups did not
moderate the pathological effects of foot-shocks, Boccia et al. (1997) suggested that social support in nonhuman primates did lessen adverse effects of stress.

The purpose of our study was to assess the influence of individual or group-housing on the vulnerability of rats to the long-term adverse effects of an inescapable social defeat stress. Group-housed rats were exposed to social defeat by an aggressive noncongener, after which they were either individually housed (social isolation) or returned to their familiar group (group-housed). For 3 weeks after defeats, rats were weighed regularly and exposed to various behavioural and physiological tests. At the end of the 3-week period, part of the rats was killed and their adrenals, thymus, spleen, testes and seminal vesicles were removed for determinations of weights.

Materials and methods
Animals and housing

The experiment was performed with 24 male Wildtype rats, originating from 16 socially housed groups, each consisting of 3 littermates. Each group was housed in a cage of 40 x 30 x 20 cm. The rats were bred in the laboratory in Haren, were approximately 4 months old and weighed 399±6.6 g (mean±SEM) at the start of the experiment. Room temperature was maintained at about 21 °C. The light/dark cycle (12 hours/12 hours) was reversed (lights on from 20.00 until 08.00 h). Food and water were available ad libitum. After social defeat, the rats were either returned to their groups or were housed individually (cage size: 25 x 25 x 30 cm; clear Plexiglas). All experimental procedures took place in the dark (active) phase between 10.00 and 15.00 h. Cages were cleaned once a week and at least 2-3 days before experimental procedures.

Social defeats and housing conditions

Rats were removed from their social groups and transferred to a test room. In this room, previously selected aggressive male rats were housed in large cages (80 x 55 x 40 cm), along with one sterilized female. In a resident-intruder paradigm, an experimental rat (intruder) was introduced into the home cage of an unfamiliar aggressive rat (resident), from which the female was removed, for a standard period of 1 hour (Meerlo et al., 1996a,b,c). Experimental rats were attacked and defeated as indicated by fleeing, freezing and submissive behaviour. When a full submissive posture occurred, with the experimental rat lying motionless on its back, the animal was protected from further attacks by placing it in a small wire mesh cage (30 x 15 x 15 cm), within the home cage of the resident,
for the rest of the hour. This allowed auditory, olfactory and visual contact between intruder and resident. Defeat procedures took place under dim red light conditions. The defeated rats were returned either to their familiar groups or were housed individually. For each treatment, 12 rats were randomly chosen from 8 different social groups.

**Cannulations**

A total of 1 week after the social defeats, half of the animals (n = 6 for each treatment) received a silicon heart catheter (0.95 mm OD, 0.5 mm ID), enabling blood samples to be collected in freely moving rats. The catheter was inserted through the right jugular vein, under halothane anaesthesia, according to Steffens (1969). The external part of the cannula of the group-housed rats was protected by a small metal cap. Immediately after surgery, the rats were injected subcutaneously with 100 000 IE sodium-penicillin® G (Yamanouchi Pharma B.V., The Netherlands). The antibiotic treatment was repeated at 3 and 7 days after surgery.

**Behaviour and body weight**

Changes in the behaviour of non-cannulated rats (n = 6 for each treatment) were tested before social defeat and for 3 weeks thereafter. This was done 2 days before, and 2, 7, 14 and 21 days after defeat, with repeated open field and sudden silence tests. At day 14 after defeat an elevated plus-maze test was also performed. Body weights were measured on the days of behavioural testing.

*Suddenly silence test.* Rats were placed in a large perspex cage (60 x 30 x 40 cm), within a soundproof wooden box with dim white lights (5 lux) and a glass front enabling observation. Each test consisted of two trials performed on 2 consecutive days. In the first trial, the rats were exposed to a constant 70 dB background noise for 5 min. During the last 3 min, the relative duration (% of time) of immobility was derived from direct recording with a keyboard. In the second trial (at the above mentioned time-points), the same procedure was followed, except that the 70 dB background noise was switched off after 2 min. Again, immobility was recorded during 3 min. The response to the sudden silence was considered to be the difference in immobile behaviour between trial one (with the noise) and trial two (without the noise). The test was described and validated by Koolhaas et al. (1990).

*Open field test.* Group-housed rats were temporarily separated from their groups and housed in individual cages from 1 day before the test until shortly after the test. After being transferred to a test room, the rat with its cage was put into the
centre of a round arena. This wooden arena was divided in two concentric zones, the inner zone with a diameter of 60 and the outer zone with a diameter of 120 cm, and was surrounded by a 30 cm high wall. At the start of the test, the cover of the cage was removed, leaving the rat on the bottom of the cage, representing the home base. The rat was allowed to explore the arena for 5 min. Behaviour was recorded with a camera and automatically analyzed with the software programme Ethovision® (Noldus Information Technology, Wageningen, The Netherlands). The following parameters were determined: (1) the latency period that elapsed before the rat left the home base to enter the outer zone; (2) locomotion, as expressed by the distance travelled in the outer zone; and (3) percentage of time spent in the outer zone (time in outer zone/(time in inner zone + time in outer zone) x 100). The test was performed under dim white light conditions (6 lux).

**Elevated plus-maze test.** The maze was a black wooden plus-shaped construction, elevated to a height of 50 cm. It consisted of two open arms (50 x 10 cm) opposite to each other, and two closed arms (50 x 10 x 40 cm; with an open roof), also opposite to each other (Korte et al., 1995; Pellow et al., 1985). A desk lamp was placed on one side of the maze so that the open arms were lighted, but closed arms remained dark. The light intensity on the open arms varied, ranging from 10 lux close to the center of the maze to 80 lux on the end of the open arms. In the closed arms it was less than one lux. Rats were placed individually in the center of the maze facing a closed arm. The number of entries into open and closed arms was scored with a keyboard for 5 min. In addition, the percentage time spent on open arms (time on open arms/(time on open arms + time on closed arms) x 100), the percentage entries into open arms (entries open/total entries x 100), and total entries were determined.

**Organ weights**

A total of 3 weeks after the social defeats, non-cannulated rats were killed and the adrenals, thymus, spleen, testes and seminal vesicles were removed and weighed. Organ weights were expressed relative to body weights (mg/100 g body weight).

**Combined DEX/CRF challenge test and blood sampling**

HPA activity was tested in a combined dexamethasone (DEX)/corticotrophin-releasing factor (CRF) challenge test. First the capacity of DEX to suppress the secretion of corticosterone and ACTH was determined, then the responsiveness of these two hormones to CRF was determined (Hatzinger et al.,
Social defeat and housing conditions of rats

1996; Heuser et al., 1994). The test was performed 21 days after the social defeats. Just before the test, group-housed cannulated rats were temporarily transferred to individual cages for the collection of blood samples. Individually housed cannulated rats were also put in new and clean individual cages. Immediately after transfer, rats were connected with a polyethylene blood sampling tube (40 cm length, 1.45 mm OD and 0.75 mm ID). DEX® (Sigma, St. Louis, MO) was injected SC to suppress HPA activity, in a concentration of 25 µg/kg, dissolved in a volume of 1 ml/kg of 50% polyethylene glycol in saline. At 89 min after DEX injection (1 min prior to CRF administration), a blood sample was collected to determine basal ACTH and corticosterone concentrations. Blood samples were drawn in amounts of 0.5 ml. Immediately thereafter, CRF® (oCRF, American Peptide, Sunnyvale, CA; 0.5 µg/kg per ml saline) was administered IV and blood samples were collected at 5, 15, 30, 60 min thereafter to measure hormone responses to CRF. Blood samples were immediately stored in chilled centrifuge tubes containing 0.3% EDTA as anticoagulant and 30 µl aprotinin (10 000 KUI/ml) as protease inhibitor. Blood was centrifuged at 4°C for 10 min at 2600 g. Amounts of 100 µl and 75 µl of the supernatants were stored at -20°C, awaiting ACTH and corticosterone measurements, respectively. A two-site radioimmunoassay (Nichols Inst. Diagnostics, CA) with an intra-assay variance of 3.2% and an inter-assay variance of 7.8% was used to measure plasma ACTH. Reversed phase high performance liquid chromatography (HPLC) was used to measure plasma corticosterone, as described by Dawson et al. (1984). The detection limit of the assay was 0.8 µg corticosterone/100 ml, and the intra-assay and inter-assay variances were, respectively, 3 and 8%.

Statistical analysis

Data on body weights, levels of passivity in the repeated sudden silence test, and latencies and locomotions in the repeated open field test, were analyzed with a split-plot analysis of variance model for the repeated measurements (Snedecor and Cochran, 1967). After fitting the model to the data, residuals (predicted error terms within animals) were inspected for homogeneity of variance, which is an important model assumption. Data on body weights and on latencies and locomotions in the open field tests showed heterogeneity, mostly due to an increase of variance with an increasing mean. They were logarithmically transformed and re-analyzed, i.e. treatment effects were expressed by multiplicative factors between treatment means, rather than by their differences. Fixed effects in the split-plot model were main effects and interactions for the factors housing (individually
or group) and time relative to social defeat (-2, 2, 7, 14 and 21 days). Random effects were animal specific effects and residual environmental effects, which account for variation between and within animals. Initially, a first order autoregressive process was assumed for the residual terms within animals (Chatfield, 1989). Because differences between split-plot analyses with or without residual auto-correlation were negligible, results are only shown for split-plot analyses without residual auto-correlation. As a follow up, estimated means were compared with Fisher’s Least Significant Difference method (LSD method; Snedecor and Cochran, 1967). One-way analysis of variance was performed to test the effect of housing on plus-maze behaviour, organ weights, and some characteristics of the hormone responses in the DEX/CRF tests. All calculations were performed with the statistical programming language Genstat 5	extsuperscript{®} (1993), employing some of the restricted extended maximum likelihood (REML) facilities from the beta-test version of release 4.1 to fit an additional auto-regressive process. Effects were considered significant if $p<0.05$. Unless stated otherwise, data are presented as mean (±SEM).

**Results**

**Body weight**

At the start of the experiment, the mean body weight of the animals being individually housed was higher than that of animals being group-housed (418±15.9 vs. 380±12.7 g; NS). Therefore, body weights were expressed as percentage change relative to the weight shortly before the start of experimental procedures (Figure 1). Averaged over the entire 3-week period, the way that rats were housed significantly affected body weight gain, shown by a significant main effect for the factor housing ($p<0.05$). Body weight gain was lower in individually housed rats. When analyzed within separate timepoints, individually housed rats had gained significantly less weight at 7 and 14 days after defeats, but caught up with their group-housed counterparts between 14 and 21 days after defeats.

**Behavioural observations**

*Immobility in the repeated sudden silence test.* The type of housing significantly influenced durations of immobility when averaged over the 3-week period, shown by a significant main effect for the factor housing ($p<0.001$). Relative durations of immobility were 31.2±2.1 and 16.3±2.4% for individually and group-housed rats, respectively. Moreover, a significant interaction was found between the factors housing and time ($p<0.001$).
Immobility increased significantly 2 days after the defeats for both individually housed rats and group-housed rats. However, at day 21 individually housed rats were still highly immobile (even a gradual increase) compared to group-housed rats which regained their normal mobility after only 7 days (Figure 2).

Latency period and locomotion in the repeated open field test. The average latency period (during the 3-week period after defeat) to leave the home base, lasted a factor 1.72±0.48 longer for individually housed rats when compared to their group-housed counterparts (significant main effect for the factor housing: $p<0.05$). By day 21, the difference between the two groups was significant (Figure 3A). A general decrease in latency times in the course of 3 weeks, was responsible for a main effect for the factor time ($p<0.05$).
The type of housing did not affect the amounts of time that rats spent in the outer zone of the open field. However, during the 3-week period after defeat, group-housed rats moved a factor 1.23±0.07 more in the outer zone than individually housed rats (significant main effect for housing: \( p<0.01 \)). Within the different timepoints, differences between both groups were significant at 2 and 21 days after defeat (Figure 3B). In the course of weeks after defeat, locomotions decreased in both groups, shown by a significant main effect for the factor time (\( p<0.001 \)).

Figure 2. Time courses of changes in responses to a sudden silence. Immobility responses to a sudden silence were determined as the change (mean±SEM) from baseline values observed during exposure to the noise. DI: defeat, individual housing (n = 6); and DG: defeat, group-housing (n = 6). Significant interaction between the factors housing and time (\( p<0.05 \)), and a significant main effect for the factor housing (\( p<0.05 \)). *Asterisks indicate significant differences (\( p<0.05 \)) between the two groups.
Figure 3. Time courses of changes in open field behaviour. DI: defeat, individual housing (n = 6); and DG: defeat, group-housing (n = 6). (A) Latency period that elapsed before the home base was left to enter the outer zone; (B) Total travelled distance in the outer zone. Data are expressed as mean±SEM. Significant main effects for the factors housing and time (*p*<0.05). *Asterisks indicate significant differences (p<0.05) between the two groups.

Times on and entries into the arms of the elevated plus-maze test. The type of housing significantly affected plus-maze behaviour when tested at 14 days after defeat (Figure 4). Individually housed rats spent less time on the open arms (*p*<0.01) and made fewer entries into the open arms (*p*<0.01). The total number of entries were about the same for individually and group-housed animals: 12±1 and 14±2, respectively.
Figure 4. Mean (±SEM) percentage of time on open arms and percentage open arm entries on an elevated plus-maze at 14 days after social defeats and the start of different housing procedures. DI: defeat, individual housing (n = 6); and DG: defeat, group-housing (n = 6). *Significant difference (p<0.05) between the two groups.

HPA-axis activity

The way in which rats were housed, significantly affected HPA responses to DEX in the combined DEX/CRF test 21 days after social defeat (Figure 5). At 89 min after administration of DEX (1 min prior to the CRF challenge), ACTH (p<0.01) and corticosterone concentrations (p<0.01) were lower in the group-housed than in the individually housed rats. The way in which rats were housed also significantly affected the release (response: t = 5 minus t = -1 min) of ACTH when CRF was administered (p<0.05). A total of 5 min after administration, group-housed rats released less ACTH than individually housed rats. However, corticosterone responses did not significantly differ between the two groups. A total of 15 min after administration of CRF, no significant differences in the decline (t = 5 minus t = 15 min) of ACTH and corticosterone concentrations between the two groups were observed. Type of housing had a tendency (p=0.06) to affect the areas under the curves (AUC) for ACTH concentrations. Group-
housed rats had a lower AUC (660±144) than individually housed rats (1070±228). AUC for corticosterone concentrations did not differ.

**Organ weights**

As shown in Table 1, at 3 weeks after social defeat, the way in which rats were housed significantly affected the weights of the adrenals (p<0.05), thymus (p<0.05), and seminal vesicles (p<0.01). The adrenals of individually housed rats were larger, but their thymuses and seminal vesicles were smaller. Weights of testes and spleens did not differ between the two groups.

![Figure 5](image-url) **Figure 5.** Mean (±SEM) plasma levels of ACTH and corticosterone (CORT) in a combined DEX/CRF test at 21 days after social defeats and the start of different housing procedures. DI: defeat, individual housing (n = 6); and DG: defeat, group-housing (n = 6). Animals were injected with DEX, 90 min prior to the CRF challenge. *Significant difference (p<0.05) in ACTH and corticosterone concentrations at -1 min, which was 89 min after DEX administration. **Significant difference (p<0.05) between the two groups in ACTH responses, 5 min after administration of CRF.
Table 1. Relative weights of various organs 21 days after social defeat and the start of different housing procedures (mean±SEM).

<table>
<thead>
<tr>
<th>Type of housing</th>
<th>Individually (n = 6)</th>
<th>Group (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenals (mg/100g)</td>
<td>14.3±0.5</td>
<td>12.8±0.3*</td>
</tr>
<tr>
<td>Thymus (mg/100g)</td>
<td>74.5±2.2</td>
<td>85.5±2.8*</td>
</tr>
<tr>
<td>Spleen (mg/100g)</td>
<td>252±9.8</td>
<td>222±18.5</td>
</tr>
<tr>
<td>Testes (mg/100g)</td>
<td>632±50.9</td>
<td>703±21.8</td>
</tr>
<tr>
<td>Seminal vesicles (mg/100g)</td>
<td>236±8.0</td>
<td>271±7.6*</td>
</tr>
</tbody>
</table>

*Asterisks indicate significant differences (p<0.05) between means.

Discussion

The results of this study show that the duration and severity of behavioural and physiological effects of a single social defeat depend on the way in which animals are subsequently housed. Long-term adverse effects of social defeat were greatly reduced in group-housed rats, when compared to those in defeated and individually housed rats.

Body growth of individually housed rats was significantly retarded in the first 2 weeks after defeat compared to rats that were reintroduced in their social groups (Figure 1). However, growth rate recovered and they caught up with the group-housed rats at 3 weeks after defeats. Meerlo et al. (1996c, 1997) also reported a low body weight gain in defeated and individually housed rats, recovering after about 5 days, but not catching up with isolated controls within 3 weeks. The retardation of growth may be at least partly due to the fact that food intake may be reduced (Meerlo et al., 1996c, 1997; Willner, 1993). Also, an increase in metabolic processes may be involved, reflected by an increase of body temperature during the circadian resting phase for several days after defeat (Meerlo et al., 1996a).

When individually and group-housed rats were exposed to sudden silence, both groups became highly immobile 2 days after defeat (Figure 2). However, upon repeated exposure, group-housed rats returned to their normal mobility after 7 days, whereas individually housed rats did not. Even after 21 days, individually housed rats were highly immobile, with immobility even increasing. Koolhaas et
al. (1990) also described extreme immobility in individually housed rats, lasting up to 10 weeks after defeat.

When rats were tested in an open field, individually housed rats were on average less active than group-housed rats (Figure 3). Although both groups became less active over time (probably because they became habituated to the repeated test), individually housed rats took on average significantly longer to leave their home base and moved significantly less in the outer zone. Latency periods for individually housed rats were significantly longer 21 days after defeat. They were also far less active in the outer zone of the open field 2 days after defeat compared to group-housed rats. However, differences in locomotion between the two groups were only significant at days 2 and 21 days after defeat. These findings agree with those of Meerlo et al. (1996b,c), reporting low levels of open field activity after social defeat in individually housed rats, being reduced when compared to isolated controls for a period of at least 7 days.

When rats were placed 14 days after defeat in an elevated plus-maze, validated for measuring anxiety (Pellow et al., 1985), those that were individually housed spent significantly less time on the open, unprotected arms of the maze than group-housed rats (Figure 4). This indicates that individually housed rats were more anxious than their group-housed counterparts. Similar levels of anxiety at 14 days after defeat were found in a previous study with individually housed rats (De Boer et al., unpublished data), in which defeated rats displayed a higher anxiety than their controls during 3 days following defeat.

Individually housing of rats after a social defeat also changed organ weights, measured 21 days after defeats (Table 1). It is well-established that stressful conditions enlarge the adrenals, diminish the thymus and spleen (Baldwin et al., 1995; Selye, 1950), and decrease the weight of reproductive organs (Selye, 1950). Although the spleen and testes of rats in our study did not differ in weight, the weight of the thymus and seminal vesicles of individually housed rats were significantly lower, and their adrenals were significantly larger.

When the DEX/CRF test was used, HPA activity was higher in individually housed rats at 21 days after defeat than in group-housed rats (Figure 5). In individually housed rats DEX was far less able to suppress the secretion of ACTH and corticosterone, and much more ACTH was released after CRF was administered. Moreover, in these rats, the AUC for the ACTH response tended to be higher. These results agree with those of Buwalda et al. (1999), who also showed a hyperactivity of the HPA-axis in defeated and isolated rats at 21 days after defeat. The high HPA activity in defeated and individually housed rats may be
explained by a reduced binding capacity of mineralocorticoid (MR) and glucocorticoid receptors (GR), as reported before by Sutanto et al. (1992), measured in the hippocampus 3 weeks after defeat. Human depression also coincides with increased HPA activity (Barden et al., 1995; Seckl et al., 1990). This may indicate that stress-related disorders in humans resemble those in rats following social defeat.

**Implications for further research**

Our results support the usefulness of the rat model using a social defeat as a stressor to study human psychopathologies, such as depression and anxiety (Koolhaas et al., 1997b). However, housing conditions seem to play an important role in the development of stress disorders in rats. Therefore the validity of the defeat model may be importantly increased by using housing conditions as one of the experimental variables. This will mimic different social settings, i.e. whether social support is available or not, which individuals may experience in everyday life.

Some factors that may affect our results were not within the scope of this study and should be mentioned. For instance, housing conditions before the stress treatments might influence comparisons between the two housing conditions (Brain and Benton, 1979). Furthermore, the degree of physical, olfactory, auditory, and visual contact with conspecifics, might influence the effect of isolation (Brain and Benton, 1979; Hurst et al., 1997). Also, characteristics of the social environment such as stability and quality of social relations are important determinants of social support (Seeman and McEwen, 1996). In addition, animals might be more or less vulnerable to pathological effects of stress, depending on the hierarchical position (social status) in a social group and on individual differences in coping style (Bohus et al., 1991; Fokkema et al., 1995; Koolhaas et al., 1997b; Mormède, 1990). Finally, the type of stress may determine whether social support will moderate the adverse effects of that stressor. For instance, after rats were exposed to a brief session of foot-shocks, group-housing did not moderate pathological effects (Van Dijken et al., 1992). This may be related to the non-social nature of foot-shock stress. Our contrasting results of a modulating role of type of housing in the detrimental effects of a social defeat, emphasizes that a single loss of social control differs from other, less naturalistic, stressors. Therefore, the social defeat model in rats may be more relevant for the biology of species, i.e. deals more with situations that organisms may meet in everyday life.