

## Social environment determines the long-term effects of social defeat

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### Abstract

A single social defeat by a dominant conspecific induces long-term changes in several physiological and behavioral parameters in rats. These changes may represent an increased vulnerability to subsequent stress and stress-related pathology. Environmental factors, in particular possibilities for social interactions, could modulate these effects. Therefore, we assessed the influence of social environment on susceptibility for the long-term effects of social defeat. Socially housed males of an unselected strain of wild-type rats were equipped with radio-telemetry transmitters that recorded heart rate, temperature and activity. They were individually subjected to defeat and subsequently either housed alone or returned to their group. Behavioral and physiological responses to various novelty stressors were determined during a three-week period after the social defeat. Furthermore, changes in baseline behavior and physiology following defeat were studied in the rat's home cage. The results show a complex interaction between defeat and housing conditions. Depending on the parameters measured, effects were caused by both isolation alone, defeat alone or a combination of both defeat and isolation. Individual housing alone caused a characteristic hyperactive response to novelty stress. Though defeat did not affect behavioral responses, it amplified the physiological response to novelty and social housing did not attenuate this effect. However, social housing did reduce the effects of defeat on heart rate, temperature and activity in the home cage and completely prevented defeat-induced weight loss. Together these results indicate that social housing may indeed positively affect the animal's capacity to cope with stressors.

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### 1. Introduction

Major life events appear to play an important role in the etiology of stress-related disorders, ranging from cardiovascular disease to psychopathologies such as depression and drug abuse [1–3]. One of the mechanisms underlying this phenomenon may be that the experience of a major stressor sensitizes an individual to subsequent stress and thereby increases their risk of developing such disorders [4].

Few animal studies have focused on the long-term effects of a single severe stressor. Most use repeated stress exposures or study changes observed relatively short (hours or days) after the inducing stressor. Furthermore, the stressors used, such as repeated footshock or restraint, often

bear little or no resemblance to challenges an animal may encounter in its daily life [5]. Social defeat by an aggressive male rat is a natural stressor and induces a very strong acute stress response when measured by the amount of corticosterone and catecholamines released [6,7]. Following a single defeat, long-term changes in behavior and physiology develop, including changes in body growth, circadian rhythmicity, neuroendocrine functioning and behavioral responses to novel stressors [5,8–15]. These effects strongly differ in time-course and some of the changes suggest that the social defeat experience increases the susceptibility of animals to the effects of subsequent stress, i.e. the defeat induces stress-sensitization [5,6,16].

Because of their potential role in the development of stress pathology, it is of interest to know the conditions that influence the development of such enduring changes following a single stressful episode. Both human and animal

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studies suggest that the social environment may have a strong influence on the effects of stress on the organism [17–21]. Individuals with greater social support seem to be better protected against excessive neuroendocrine activation, thereby reducing the adverse effects of stress [22]. Community based studies also document an association between the extent and quality of an individual's social relationships and better health and longevity [17,19,23,24]. Animal studies likewise have reported that contact with others reduces physiological arousal in response to stressors and prevents many of the long-term effects of stress [9,21,25,26,28]. On the other hand, although supportive social relationships appear beneficial for health and are associated with reduced patterns of HPA and SNS activity, non-supportive social relationships and competition or aggression within a group are associated with enhanced reactivity to stress [22,24].

Wild rats are a social species with a complex and flexible social structure [27], however, the social defeat model has been developed using individually housed rats. It may be hypothesized that returning animals after defeat to a familiar social group may serve as a buffer to the adverse effects of social stress. Indeed, social housing counteracts defeat-induced changes in reward and social behavior [26] and prevented changes in the dopaminergic system [25]. Previous experiments in our laboratory showed that animals housed alone following defeat reacted more strongly than socially housed animals to various behavioral tests and showed an increased HPA-axis reactivity in a combined dexamethasone (DEX)/corticotrophin-releasing factor (CRF) test [9]. Therefore, social housing may reduce or even prevent the long-term behavioral and physiological effects of social defeat and thereby reduce its sensitizing effects.

Other studies have shown that individually housed animals in general show larger responses to common laboratory procedures, such as a clean cage [28], and react with increased locomotor activity in novel environments [29,30]. Because social isolation alone may also induce hyper-responsiveness to relatively mild stressors, some of the effects of individual housing following social defeat may have been caused by an effect of the isolation as such.

The present experiment was designed to test the assumption that social housing attenuates the long-term effects of social defeat. We used males of an unselected strain of wild type rats (Wildtype Groningen, WTG) because of their high levels of social activity [31]. To avoid possible confounding effects of competition and aggression, groups consisted of siblings that were housed together since birth. Animals equipped with radio-telemetry transmitters that recorded heart rate, temperature and activity, were individually subjected to social defeat and subsequently either housed alone or returned to their original group. Behavioral and physiological responses to various novelty stressors were determined during a 3-week period after the social defeat. Furthermore, changes in baseline behavior and

physiology following defeat were studied in the rat's homecage.

## 2. Materials and methods

### 2.1. Animals and housing conditions

All procedures in this study were approved by the Committee on Animal Bioethics of the University of Groningen, The Netherlands. For the experiment, we used 72 male wildtype rats (Wildtype Groningen, WTG), originating from 12 groups of six siblings. The wildtype strain was originally caught in the wild, but has been bred in our laboratory for 26 generations. The strain is known for its high levels of social activity [31].

Rats were divided into 24 groups of three siblings and subsequently assigned to one of four treatments: control/isolation, defeat/isolation, control/social housing, and defeat/social housing. To reduce the number of animals, we used all but two rats in the control/social housing groups for the experiments and combined the control/isolation and defeat/isolation groups before defeat. This resulted in a total of 40 experimental rats and 32 animals that were only used as companions. Experimental animals were equipped with a radiotelemetry (ECG/temperature/activity) transmitter for continuous registration of heart rate, temperature and activity (see below). Due to the limited availability of transmitters, the experiment was conducted in three cohorts, each consisting of eight groups of three rats.

The animals were 3 months of age at the start of the experiment and weighed  $295 \pm 3.5$  g (mean  $\pm$  SEM). They were housed in clear Plexiglas cages on a layer of wood shavings and remained socially housed until the social defeat procedure. Following defeat, they were either isolated or returned to their group (group cage:  $55 \times 35 \times 20$  cm and individual cage:  $40 \times 23 \times 15$  cm). The light/dark cycle was reversed and fixed at 12/12 h (lights on at 20:00 h) and room temperature was maintained at 21 °C. Food and water were available ad libitum. All experimental procedures were conducted between 10:00 and 16:00 h.

### 2.2. Data collection

The telemetry system consisted of a small ECG transmitter (model TA11CTA-F40, Data Sciences, St. Paul, MN, USA), which was implanted intraperitoneally under iso-flurane/O<sub>2</sub>/N<sub>2</sub>O anesthesia. The two electrodes of the transmitter were attached to the dorsal surface of the xiphoid process and in the anterior mediastinum close to the right atrium respectively, as previously described by Ref. [32]. Following surgery, rats were briefly isolated to recover and then reintroduced into the same group. Experiments started no sooner than 2 weeks after regrouping. Data were collected via a receiver underneath the homecage (model RA1010, Data Sciences) and processed by a PC with a

specialized recording and analysis system (Dataquest IV, Data Sciences). Heart rate and temperature were sampled for 12 s every 5 min. Locomotor activity was measured continuously and stored at 5 min intervals.

### 2.3. Social defeat

Before social defeat, rats were removed from their groups, weighed, and transferred in a separate cage to the test room. Social defeat consisted of placing the experimental rat (intruder) in the cage of an aggressive male conspecific (resident). Resident rats were of the same strain as the experimental animals (WTG) and housed in large cages (80×55×40 cm) with a female to stimulate territorial aggression. They were trained on a regular basis by confronting them with naive male intruders and only animals with attack latencies shorter than 2 min were used. One hour before the start of the defeat, females were removed from the resident's cage. The total social stress procedure lasted 1 h, during which rats were attacked for a standard period of 15 min. Subsequently, animals were removed from the cage, placed in a protective wire mesh cage (30×15×15 cm) and returned to the resident's cage for the remainder of the hour. During this period, rats were protected from further attacks and injury, but remained in full auditory, olfactory and visual contact with the resident. This period of close proximity of the resident is known to be highly stressful for the intruder rat [33]. Control animals were also removed from their social groups and placed in a separate cage for a period similar to the defeat procedure. Following defeat or control treatment, animals were either regrouped with their original group members in a clean cage or housed individually. The social defeat procedure started at 10:00 h and ended at 12:00 h. Telemetry measurements started again at 14:00 h, when the acute response to defeat had ended.

### 2.4. Stress-reactivity

Reactivity to mild stressors was determined at several intervals after social defeat. On days 3, 16, and 23 after social defeat, behavioral and heart rate responses to novelty were determined in a small open field, on days 6 and 21 locomotor activity was measured in a large open field, and the temperature response to a clean home cage was determined 8 days following defeat.

#### 2.4.1. Small open field

Animals were individually transported to a separate test room and placed into a Perspex cage (60×30×40 cm) within a soundproof wooden box with a glass front. The box was illuminated by dim white light and fitted with a telemetry receiver connected to a PC with a specialized recording and analysis system (Cardia). The system allowed for continuous and simultaneous measurements of heart rate and behavior. During the 7-min test, we measured heart rate and scored the following behaviors: explore, rear, groom, digging, and

immobility. The layer of wood shavings in the cage was replaced between trials.

#### 2.4.2. Open field

Animals were individually transported to a separate test room and subjected to an open field test. The open field consisted of a round wooden arena with a diameter of 120 cm and a surrounding wall of 30 cm high. The arena was divided into two concentric zones: an inner and an outer zone (diameter 60 and 120 cm, respectively). The test was performed under dim white light conditions and lasted 5 min. At the start of the test, rats were placed into the center of the arena. Behavior was recorded with a video camera and automatically analyzed with a special software package (Ethovision, Noldus Information Technology, Wageningen, The Netherlands). We recorded the following parameters: time moving, distance moved, time spent in inner zone, average distance to arena border and rear. The open field was cleaned between trials.

### 2.5. Statistical analysis

Results are presented as means±SEM. Statistical analysis was performed using the SPSS software package (version 11.0) and a probability level of  $p<0.05$  was considered significant.

The effects of social defeat and housing conditions on body weight gain were assessed via analysis of variance (ANOVA) for repeated measurements with days as within subjects factor and defeat (control, defeat) and housing (isolation, social) as between subjects factors. Homecage heart rate, temperature and activity were averaged over 12 h. Telemetry measurements from 08:00 and 14:00 h on the day of defeat were excluded from analysis. Changes in basal heart rate, temperature or activity were expressed as percentage increase or decrease compared to the 2 days preceding defeat and/or isolation. Data from dark and light periods were analyzed separately via ANOVA for repeated measurement as described above.

Changes in temperature response to a clean cage following defeat and/or isolation were assessed by ANOVA for repeated measurements with time as within subjects factor and defeat (control, defeat) and housing (isolation, social) as between subjects factors. Temperature responses expressed as area under the curve were determined for the first 20 min and first 2 h after the cage change and analyzed with univariate ANOVA with defeat (control, defeat) and housing (isolation, social) as between subjects factors. Heart rate responses to the small open field were also expressed as area under the curve and analyzed with ANOVA for repeated measurements with test day as within subjects factor and defeat (control, defeat) and housing (isolation, social) as between subjects factors. Further analysis of the different test days was performed via univariate ANOVA. Effects of defeat and/or isolation on behavior in the small and large open field were assessed similarly.

### 3. Results

#### 3.1. Body weight

Over the whole three week period following defeat and/or isolation, there was a significant effect of housing on body growth, with the individually housed animals gaining less weight compared to the socially housed animals ( $F_{1,36}=4.128$ ,  $p=0.050$ ). Body weight gain over the first 10 days after defeat is depicted in Fig. 1. During this time period, ANOVA for repeated measurements revealed, apart from a significant effect of housing ( $F_{1,36}=7.289$ ,  $p=0.011$ ), also interaction effects of time $\times$ defeat ( $F_{6,216}=2.475$ ,  $p=0.025$ ) and time $\times$ housing ( $F_{6,216}=2.529$ ,  $p=0.022$ ). Posthoc multiple comparisons (LSD) showed that these effects were due to a reduced body growth of the isolated defeat group compared to both socially housed groups ( $p=0.003$  and  $p=0.011$  for control and defeat groups respectively). Apart from a small non-significant dip in body weight on the first day after isolation, isolated control animals showed a comparable growth curve to the socially housed animals. There was a trend towards a reduced body weight gain in the defeated isolated animals compared to the isolated control groups as well ( $p=0.052$ ).

#### 3.2. Homecage heart rate, temperature, and activity

##### 3.2.1. Dark period

Social defeat caused a reduction in heart rate, core body temperature, and activity during the dark, normally active phase (Fig. 2, panel A). However, these effects were generally stronger in animals housed alone. For heart rate, ANOVA for repeated measurements revealed significant effects of time ( $F_{4,144}=53.548$ ,  $p<0.001$ ) and defeat ( $F_{1,36}=$

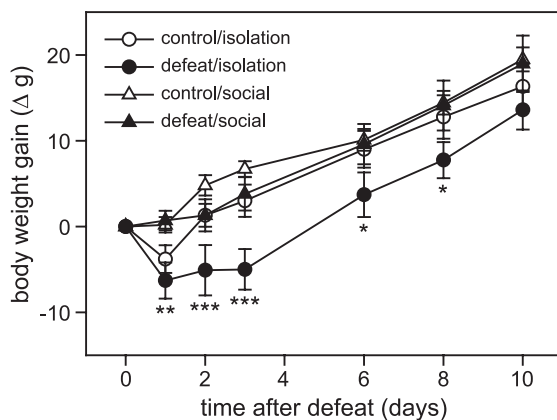


Fig. 1. Effects of social defeat and subsequent housing conditions on body growth. On day 0, socially housed rats were individually subjected to social defeat and subsequently either housed alone or returned to their group. Data are presented as delta increases in body weight compared to body weight shortly preceding social defeat and/or isolation (mean $\pm$ SEM). Significant effect of housing ( $p=0.011$ ) and significant interaction effects of time $\times$ defeat ( $p=0.025$ ) and time $\times$ housing ( $p=0.022$ ). \* $p<0.05$ , \*\* $p<0.01$ , \*\*\* $p<0.001$ .

8.779,  $p=0.005$ ) and significant interaction effects of time $\times$ defeat ( $F_{4,144}=8.757$ ,  $p<0.001$ ) and time $\times$ housing $\times$ defeat ( $F_{4,144}=3.263$ ,  $p=0.014$ ). Posthoc multiple comparisons (LSD) showed that this effect was mainly caused by a reduced heart rate in the defeat/isolation animals compared to both control groups ( $p=0.029$  and  $p=0.023$  for isolation and social groups, respectively). The heart rate in the socially housed defeated animals was not significantly different from controls. During the dark period directly following defeat (day 0), heart rate in the socially housed defeated animals appeared increased and was in fact significantly higher than that of the isolated defeated group ( $p=0.025$ ). Still, over the whole period there was a clear trend to a reduced heart rate in this group as well ( $p=0.079$  and  $p=0.064$  compared to the isolation and social control groups, respectively).

Core body temperature of the defeated animals was also reduced during the dark phase, resulting in significant effects of time ( $F_{4,144}=17.663$ ,  $p<0.001$ ) and defeat ( $F_{1,36}=13.666$ ,  $p=0.001$ ), and an interaction effect of time $\times$ defeat ( $F_{4,144}=3.264$ ,  $p=0.014$ ). The effects were due to a significantly decreased dark phase temperature in defeat/isolation animals compared to both control groups ( $p=0.006$  and  $p=0.001$  for isolation and social groups, respectively) and a significant reduction in defeat/social animals compared to the control/social group ( $p=0.027$ ).

Locomotor counts during the dark period were strongly reduced in the isolated defeated animals, but only slightly so in the socially housed defeated rats. ANOVA for repeated measurements showed significant effects of time ( $F_{4,144}=8.263$ ,  $p<0.001$ ) and defeat ( $F_{1,36}=6.836$ ,  $p=0.013$ ) and a significant interaction effect of time $\times$ defeat ( $F_{4,144}=3.257$ ,  $p=0.014$ ). The effects were due to a strong reduction in homecage activity in animals isolated after defeat compared to both control groups ( $p=0.011$  and  $p=0.012$  for isolation and social groups, respectively). There was no significant difference in activity during the dark phase between the control groups and the socially housed defeated animals.

##### 3.2.2. Light period

The heart rate, temperature, and activity data during the light phase are more complex. For heart rate, ANOVA for repeated measurement revealed, apart from an effect of time ( $F_{4,144}=9.903$ ,  $p<0.001$ ), significant interaction effects of time $\times$ defeat ( $F_{4,144}=7.030$ ,  $p<0.001$ ) and housing $\times$ defeat ( $F_{1,36}=4.657$ ,  $p=0.038$ ). Part of the results can be explained by an effect in the control/social group, which shows a reduced heart rate compared to both the control/isolation and the defeat/social group ( $p=0.047$  and  $p=0.015$ , respectively). Secondly, animals in the socially housed defeat group show an increased light phase heart rate during the first day after defeat compared to both control groups ( $p=0.014$  and  $p<0.001$  for isolation and social groups, respectively) and a trend toward an increased heart rate compared to the defeat/isolation group ( $p=0.076$ ).



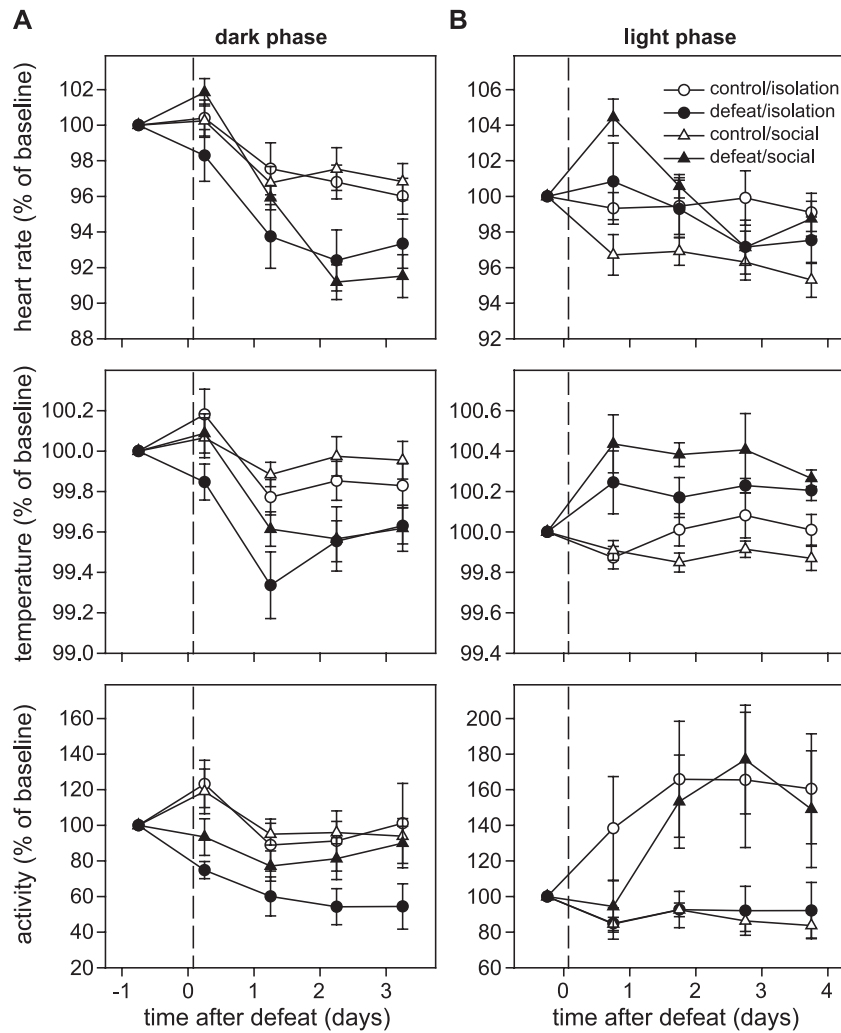


Fig. 2. Effects of social defeat and subsequent housing conditions on homecage heart rate, core body temperature and activity. Data from dark phase (A) and light phase (B) are presented separately.  $T=0$  indicates the start of the dark phase (08:00 h) on the day of defeat. The social defeat procedure started at 10:00 and ended at 12:00 h, indicated by the dotted line. Telemetry measurements from 08:00 and 14:00 h on the day of defeat were excluded from analysis. Changes are expressed as percentage increase compared to the average of the corresponding phase over the two days preceding defeat and/or isolation (mean  $\pm$  SEM). (A) Analysis of the dark (active) phase revealed the following significant effects: for heart rate: effects of defeat ( $p=0.005$ ) and time ( $p<0.001$ ) and interaction effects of time  $\times$  defeat ( $p<0.001$ ) and time  $\times$  housing  $\times$  defeat ( $p=0.014$ ); for temperature: effects of defeat ( $p=0.001$ ) and time ( $p<0.001$ ) and an interaction effect of time  $\times$  defeat ( $p=0.014$ ); for activity: effects of defeat ( $p=0.013$ ) and time ( $p<0.001$ ) and an interaction effect of time  $\times$  defeat ( $p=0.014$ ). (B) Analysis of the light (inactive) phase revealed the following significant effects: for heart rate: effect of time ( $p<0.001$ ) and significant interaction effects of time  $\times$  defeat ( $p<0.001$ ) and housing  $\times$  defeat ( $p=0.038$ ); for temperature: effects of defeat ( $p<0.001$ ) and time ( $p<0.001$ ) and interaction effects of time  $\times$  defeat ( $p<0.001$ ) and housing  $\times$  defeat ( $p=0.047$ ); for activity: effect of time ( $p=0.001$ ) and interaction effects of housing  $\times$  defeat ( $p=0.004$ ) and time  $\times$  housing  $\times$  defeat ( $p<0.001$ ).

Core body temperature during the light phase was increased in both the defeat/isolation and defeat/social groups, however, the effect was stronger in the defeated animals which remained socially housed. ANOVA for repeated measurements revealed significant effects of time ( $F_{4,144}=2.762$ ,  $p=0.030$ ) and defeat ( $F_{1,36}=29.296$ ,  $p<0.001$ ) and significant interaction effects of time  $\times$  defeat ( $F_{4,144}=5.736$ ,  $p<0.001$ ) and housing  $\times$  defeat ( $F_{1,36}=4.249$ ,  $p=0.047$ ). Core body temperature in the defeat/isolation group was increased compared to both control groups ( $p=0.023$  and  $p=0.001$  for isolation and social groups, respectively). Light phase temperature in the defeat/social group was also increased compared to both control groups

( $p<0.001$  for both isolation and social groups), but in addition, posthoc multiple comparisons also revealed a trend towards an increased temperature compared to the defeat/isolation group ( $p=0.089$ ).

Activity results during the light, normally inactive, period are more difficult to interpret. Following defeat and/or isolation, activity increased in the control/isolation animals as well as in the defeat/social animals. This resulted in a significant effect of time ( $F_{4,144}=4.855$ ,  $p=0.001$ ) and significant interaction effects of housing  $\times$  defeat ( $F_{1,36}=9.412$ ,  $p=0.004$ ) and time  $\times$  housing  $\times$  defeat ( $F_{4,144}=6.523$ ,  $p<0.001$ ). Posthoc multiple comparisons showed a significant increase in activity in the control/isolation animal

compared to the control/social and the defeat/isolation groups ( $p=0.018$  and  $p=0.024$ , respectively) and a significant increase in activity in the defeat/social group compared to control/social animals ( $p=0.050$ ) and a trend towards an increase compared to the defeat/isolation animals as well ( $p=0.071$ ).

### 3.3. Stress-reactivity

#### 3.3.1. Cage cleaning

The temperature response to a clean cage 8 days after social defeat is depicted in Fig. 3. The initial temperature response to the novel cage was increased in the defeated animals, but the response was prolonged in the individually housed animals regardless of defeat status. Over the first 2 h ANOVA for repeated measurements revealed a significant effect of time ( $F_{24,864}=38.924$ ,  $p<0.001$ ) and significant

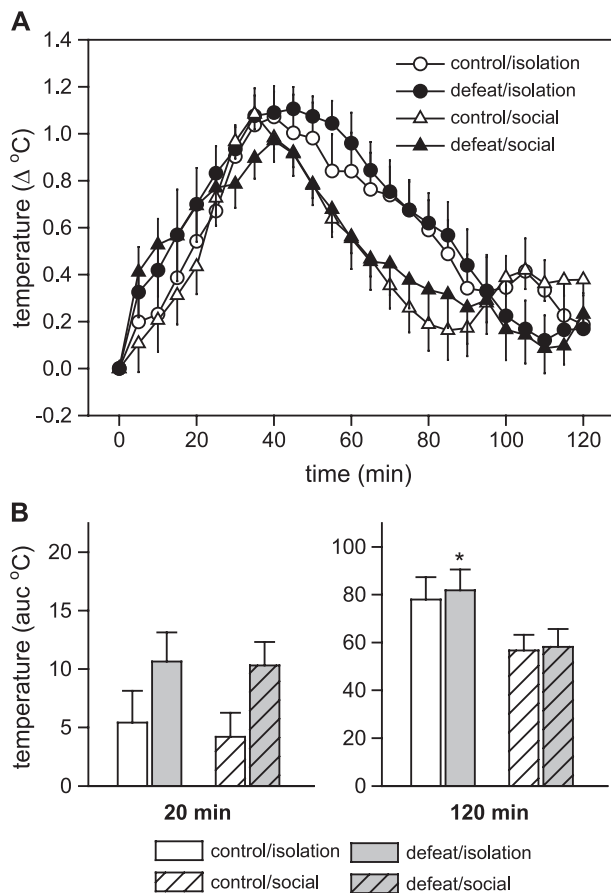


Fig. 3. Effects of social defeat and subsequent housing conditions on the hyperthermic response to a clean cage 8 days later. (A) Temperature response to a clean cage at  $t=0$ . Data are expressed as delta increase in core body temperature compared to the 30 min preceding the cage change (mean $\pm$ SEM). Significant effect of time ( $p<0.001$ ) and significant interaction effects for time $\times$ defeat ( $p=0.001$ ) and time $\times$ housing ( $p<0.001$ ). (B) Temperature response expressed as area under the curve for the first 20 min and the whole 120 min period after the cage change (mean $\pm$ SEM). Significant effect of defeat ( $p=0.020$ ) for the first 20 min and a significant effect of housing ( $p=0.009$ ) for the whole 120 min. \* $p<0.05$  vs. defeat/social.

interaction effects of time $\times$ defeat ( $F_{24,864}=2.163$ ,  $p=0.001$ ) and time $\times$ housing ( $F_{24,864}=2.572$ ,  $p<0.001$ ). When the response over the first 20 min was expressed as area under the curve, univariate ANOVA showed an effect of defeat only ( $F_{1,36}=5.881$ ,  $p=0.020$ ). Yet, the area under the curve over the whole 120 min produced an effect of housing ( $F_{1,36}=7.720$ ,  $p=0.009$ ), but no effect of defeat.

#### 3.3.2. Small open field

The heart rate response to the novel environment of the small open field was increased by defeat (Fig. 4). These effects were almost exclusively due to an effect of defeat in the social housing group. ANOVA for repeated measurements showed an effect of defeat on heart rate as expressed as area under the curve (defeat effect,  $F_{1,36}=7.072$ ,  $p=0.012$ ). Analysis by univariate ANOVA of the different test days revealed a trend towards a defeat effect on day 3 ( $F_{1,36}=3.185$ ,  $p=0.083$ ), and significant defeat effects on day 16 ( $F_{1,36}=5.128$ ,  $p=0.030$ ) and day 23 ( $F_{1,36}=6.422$ ,  $p=0.016$ ). The socially housed defeated animals had a higher heart rate response than controls on all test days ( $p=0.021$ ,  $p=0.031$  and  $p=0.006$  for days 3, 16 and 21, respectively). Individually housed defeated animals did not differ from their individually housed controls, but did respond more strongly than the socially housed control animals on days 16 and 23 ( $p=0.006$  and  $p=0.047$ , respectively).

Exploration of the small open field was increased by isolation, however, with ANOVA for repeated measurement showing a significant effect of housing ( $F_{1,36}=9.606$ ,  $p=0.004$ ). When the separate tests were analyzed, the effect was significant only during the second ( $F_{1,36}=16.165$ ,  $p\leq 0.001$ ) and third exposure to the small open field ( $F_{1,36}=4.789$ ,  $p=0.035$ ), with only a trend toward an effect of housing on day 3 ( $F_{1,36}=3.298$ ,  $p=0.078$ ). On day 16, both control/isolation and defeat/isolation animals were significantly more explorative than their socially housed counterparts ( $p=0.002$  and  $p=0.031$ , respectively). Isolated animals showed less grooming behavior ( $F_{1,36}=5.879$ ,  $p=0.020$ ) and immobility ( $F_{1,36}=7.582$ ,  $p=0.009$ ). The effect on grooming behavior was only significant on day 16 ( $F_{1,36}=6.740$ ,  $p=0.014$ ), whereas the effect of housing on immobility was significant on day 16 ( $F_{1,36}=6.856$ ,  $p=0.013$ ) and day 23 ( $F_{1,36}=4.987$ ,  $p=0.032$ ), with a trend towards an effect on day 3 as well ( $F_{1,36}=3.298$ ,  $p=0.078$ ). There were no effects of housing or defeat on digging and rearing behavior in the small open field.

#### 3.3.3. Open field

Locomotor activity in the open field 6 and 21 days after defeat and/or isolation was also increased in the individually housed animals (Fig. 5). This effect was already present 6 days after isolation and although locomotor activity was reduced in the second test, isolated animals were still more active than socially housed groups 21 days after isolation. There was no effect of defeat on locomotor activity in the

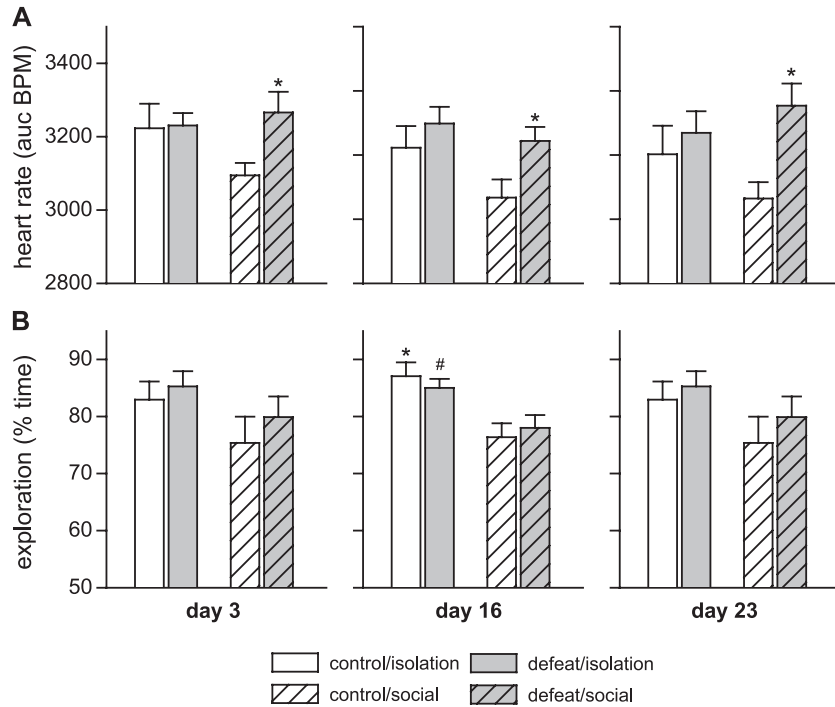


Fig. 4. Effects of social defeat and subsequent housing conditions on behavioral and heart rate responses to a small open field. Animals were exposed to a small open field on days 3, 16 and 23 following defeat and/or isolation. (B) Heart rate (beats per minute) in the small open field expressed as area under the curve (mean±SEM). Significant effect of defeat ( $p=0.012$ ). (A) Percentage time spent on exploring the small open field (mean±SEM). Significant effect of housing ( $p=0.004$ ). \* $p<0.05$  vs. control/social, # $p<0.05$  vs. defeat/social.

open field. Over both tests, ANOVA for repeated measurements revealed significant effects of test ( $F_{1,36}=22.353$ ,  $p<0.001$ ) and housing ( $F_{1,36}=16.730$ ,  $p<0.001$ ) on total time moving. Univariate ANOVA for the different test days, showed both a significant effect of housing in the first ( $F_{1,36}=16.337$ ,  $p<0.001$ ) and the second open field exposure ( $F_{1,36}=7.379$ ,  $p=0.010$ ). Similar results were obtained for total distance moved ( $F_{1,36}=9.306$ ,  $p=0.004$ ), time spend in inner zone ( $F_{1,36}=7.692$ ,  $p=0.009$ ) and distance to arena border ( $F_{1,36}=7.675$ ,  $p=0.009$ ), which were all increased by isolation during both tests. There were no

effects of housing or defeat on rearing behavior in the large open field.

#### 4. Discussion

The results confirm the hypothesis that the long-term consequences of social defeat are modulated by the social housing conditions after the defeat. However, there appears to be a complex interaction between social defeat and social isolation even within the same test situation. Depending on the parameters measured, effects were caused by both isolation alone, defeat alone or a combination of both defeat and isolation.

Defeated animals that were housed individually showed the most pronounced reductions in homecage heart rate, temperature, and activity during the dark phase and were the only ones to show a decrease in body weight. This long-term reduction in body growth is one of the most consistently found effects following social defeat [8,9,14], and it is therefore striking that this effect is completely prevented by social housing. The reduction in body weight gain may in part be due to a reduction in food intake [14]. Although food intake was not measured in this study, the observation that individually housed defeated animals show a strong reduction in dark phase activity, during which most food is ingested, may point to a reduced intake.

The defeat-induced reductions in heart rate, temperature, and locomotor activity during the dark period appear to

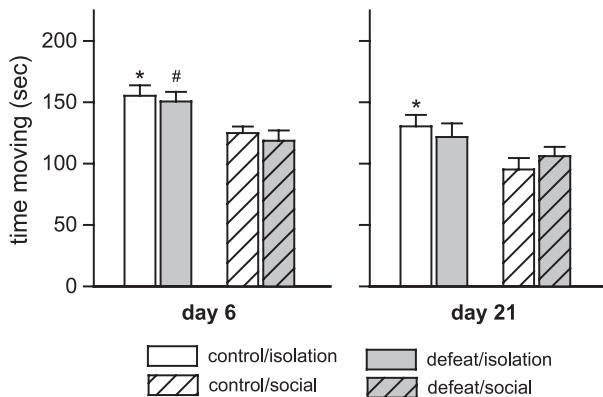


Fig. 5. Effects of social defeat and subsequent housing conditions on behavior in a large open field. Locomotor activity expressed as total time moving (mean±SEM) was measured on days 6 and 21 after social defeat and/or isolation. Significant effect of housing ( $p<0.001$ ). \* $p<0.05$  vs. control/social, # $p<0.05$  vs. defeat/social.

move in the same direction. Together with an increase in temperature during the light phase, they result in a decreased circadian rhythm amplitude as previously reported after defeat [13,15]. These effects are influenced by the housing conditions following the initial defeat. While the reductions during the dark phase are all stronger in the individually housed defeated animals, the increase in temperature during the light phase is augmented by social housing and is accompanied by an increase in locomotor activity in this group. It appears that socially housed defeated animals shift some of their activity to the resting phase, whereas individually housed defeated animals just show a general decrease of locomotor activity.

There were no effects of social defeat on the behavioral response to the small and large open field or a clean home cage. During the large open field test, social defeat did not result in a reduced locomotor activity on day 6. It has been reported previously that this activity is reduced 2 days after defeat [8,14]. Combined, the results indicate that the enhanced immobility response to a novel environment in defeated rats disappears within a few days. Unpublished data obtained in our lab measuring behavioral changes on the elevated plus-maze support the idea that this is due to a transient increase in anxiety following defeat.

Though the behavioral responses to the mild stressors used in this study were not affected by social defeat, defeat did result in an increased heart rate response to the small open field and a stronger initial increase in temperature in response to a clean cage. This suggests that although defeat did not result in a change in behavior, the physiological responses to subsequent mild stressors were augmented by defeat. Social housing did not ameliorate this effect. In fact social housing following defeat increased the heart rate response to the small open field, even though exploratory behavior was not changed.

In fact, all changes in behavioral response to the challenges used were produced by an effect of housing conditions alone. Individually housed animals were more active in the large open field and showed more exploratory behavior in response to the small open field, irrespective of social defeat. The prolonged temperature response to a clean cage in the isolated animals also points to an increased exploration of the novel bedding in the individually housed animals. These increases in locomotor activity during the behavioral tests are in correspondence with a general hyper-responsiveness to novelty in isolates observed by others [29,30]. There has been some debate of the isolation period necessary to induce this hyper-reactivity. Some authors suggest that a relative short period is sufficient, while others claim that the effect is specific for isolation rearing [29,34–36]. Our results show locomotor hyperactivity in the open field already after 6 days of isolation, persisting to at least day 21, and a significant increase in exploratory behavior in the small open field 16 days following individual housing. Furthermore, the effect is not just limited to responses to novelty, since the isolated control animals show an

increased home cage activity during the resting phase as well. Another study comparing individually housed rats to animals housed in groups reported increases in resting mean arterial blood pressure and heart rate in animals housed alone. In addition, these rats also showed increased responses to common laboratory procedures, including a prolonged response to routine cage cleaning, suggesting that the isolated rats were in general more stress responsive than group housed animals [28].

In conclusion, social housing prevented some of the commonly observed long-term effects of social defeat. Secondly, social isolation in itself induces long-term changes in behavior and physiology suggesting a hyper-reactivity to stressors. Together these results indicate that social housing may indeed positively affect the animal's capacity to cope with stressors; animals housed together with familiar conspecifics responded less strongly to mild stressors and were less susceptible to the long-term effects of a single severe stressor. However, group housing did not prevent all of the physiological changes induced by social defeat and it appears that some of the physiological responses to subsequent stressors were in fact even increased. Still, living in a familiar social environment appears to modulate the response to stressors. It remains to be answered what the role of this modulation is in the long-term adaptation to stress and the development of stress pathology.

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