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Differential effects of social defeat in rats with high and low locomotor response to novelty

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Abstract

We compared the response to repeated social defeat in rats selected as high (HR) and low (LR) responders to novelty. In experiment 1, we investigated the behavioral and neuroendocrine effects of repeated social defeat in HR-LR rats. By the last defeat session, HR rats exhibited less passive-submissive behaviors than LR rats, and exhibited higher corticosterone secretion when recovering from defeat. Furthermore, in the forced swim test, while HR defeated rats spent more time immobile than their undefeated controls, LR rats' immobility was unaffected by defeat. In experiment 2, we compared the effects of repeated social defeat on body, adrenal, thymus and spleen weights in HR-LR rats; moreover, we compared the effects of repeated social defeat on stress related molecules' gene expression in these two groups of rats. Our results show that HR rats exhibited a decrease in thymus weight after repeated social defeat that was not present in LR rats. Analyses of *in situ* hybridization results found HR-LR differences in 5HT_{2a} mRNA levels in the parietal cortex and 5HT_{1a} mRNA levels in the dorsal raphe. Moreover, LR rats had higher glucocorticoid receptor (GR) mRNA expression than HR rats in the dentate gyrus, and repeated social defeat decreased this expression in LR rats to HR levels. Finally, hippocampal MR/GR ratio was reduced in HR rats only.

Taken together, our results show a differential response to social defeat in HR-LR rats, and support the HR-LR model as a useful tool to investigate inter-individual differences in response to social stressors.

Keywords

individual differences; behavioral phenotyping; *in situ* hybridization; repeated social defeat; corticosterone

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1. Introduction

Stressful life events, particularly those of a social nature, are important predisposing factors in the etiology of human psychiatric disorders such as anxiety and depression (Anisman and Zacharko, 1992). Thus, animal models of social stress have been developed in order to investigate the neurobiological changes associated with affective disorders and to identify possible therapeutic strategies. The “resident-intruder” paradigm is one such animal model that incorporates social stress with well-defined behavioral endpoints. In this behavioral paradigm, a male rodent (the intruder) is introduced into the cage of another male (the resident) and they are allowed to interact for a limited time. Within a very short time, the resident demonstrates dominant behaviors towards the intruder and prompts him to display submissive behaviors. This experimental model produces a classical stress response in both the resident and the intruder (Miczek, 1979, Meerlo et al., 1996, Ruis et al., 1999, Blanchard et al., 2001, Calfa et al., 2006). Marked and persistent behavioral and physiological changes, similar to those observed in anxiety and depression, are observed in the intruder (Albonetti and Farabollini, 1994, Koolhaas et al., 1997, Bhatnagar and Vining, 2003).

A strong correlation between personality traits and response to social stress has been reported (see for example (Pruessner et al., 1997, Takahashi, 2005)). To investigate the interplay between individual differences and stress response, we and others have developed an animal model in which male rats are separated into high responders (HR) and low responders (LR) based on their locomotor activity during exposure to the mild stress of a novel environment. Studies from our laboratory have shown that chronic social defeat inhibits drug-self administration in HR rats while promoting the same behavior in LR rats (Kabbaj et al., 2001). Moreover, we have reported that repeated social defeat leads to differential alterations in hippocampal gene expression, as evidenced by a microarray analysis, with many genes showing opposite regulation between HR and LR rats (Kabbaj et al., 2004).

Studies investigating the effects of chronic stress on gene expression in the brain have consistently shown an up-regulation of cortical 5-HT_{2A} receptors, an increase in 5-HT metabolism and a down-regulation of hippocampal 5-HT_{1A}, MR and GR receptors (Berton et al., 1998, Lopez et al., 1998, Berton et al., 1999, Blanchard et al., 2001). Accordingly, in this study, we examined the behavioral and physiological effects of social defeat in HR-LR rats. We measured agonistic behaviors in these two groups of rats during social defeat and, at the end of the last session, we tested HR-LR rats in the forced-swim test, a model of depressive-like behavior (Porsolt et al., 1977). We also examined a possible differential effect of social defeat on gene expression in HR-LR rats in specific brain areas implicated in depression. Accordingly, we examined mRNA expression of key components of the Hypothalamo-Pituitary-Adrenal (HPA) axis and the serotonergic system which are altered by clinical depression and chronic stress (Berton et al., 1998, Lopez et al., 1998, Blanchard et al., 2001). Finally, we examined HR-LR differences in the effects of chronic social defeat on other common indices of stress such as body weight, adrenals, thymus, and spleen weights.

2. Experimental Procedures

2.1 Subjects

Seventy-two male Sprague-Dawley rats from Charles River (Wilmington, Mass.), weighing 250–300 g were used in this study. Rats were pair-housed in 43 × 21.5 × 24.5 cm Plexiglas cages, and kept on a 12 hr light/dark cycle (lights on at 6 am). Food and water were available *ad libitum*. In addition, 20 vasectomized male Long-Evans rats weighing 500–550 g were housed with female Long-Evans rats. These males were used as resident attackers in

the social defeat paradigm, and were chosen from an original group of 26 rats for their consistent aggressive behavior.

All experiments were conducted in accordance with the guidelines of the animal ethics committee at the University of Michigan following the Guide for the Care and Use of Laboratory Animals.

2.2 Experimental methods

2.2.1 Locomotor activity test—After 7 days of habituation to the housing conditions, rats were tested for locomotor activity during a 60 min exposure to a novel environment. The test was conducted during the light portion of the light/dark cycle. Each rat was placed in a 43 × 21.5 × 24.5 cm clear acrylic cages, and locomotor activity was monitored by means of two banks of 3 photocells each connected to a microprocessor. The locomotion testing rig and motion recording software were created in-house at the University of Michigan. Rats that exhibited locomotor counts in the highest third of the sample were classified as HR, whereas rats that exhibited locomotor counts in the lowest third of the sample were classified as LR (Jama et al., 2008).

2.2.2 Repeated social defeat—The repeated social defeat paradigm consisted of six encounters, carried out every other day, with an aggressive Long-Evans male rat. Each intruder was defeated by 6 different resident Long-Evans rats during the 6 sessions of defeat.

Prior to each agonistic interaction, the female housed with the resident was transferred to another cage. Either an HR or LR male rat was then weighted and placed as an intruder into the resident male's cage. Rats were allowed to interact for 5 min, after which the intruder rat was transferred to a protective metal cage and placed back into the resident's home cage for 10 more min. The metal cage allowed for intense visual, auditory, and olfactory interactions, and was of sufficient size to allow animals to move freely (10×10×15 cm). Following each defeat session, the intruders were returned to their home cage. HR and LR control groups were removed from their cages and gently handled. For each defeat session, the behaviors of the intruder rats were videotaped and later quantified by an experimenter blind to the experimental groups. A stopwatch was used to quantify the behaviors.

The behaviors of the intruders were divided up in three categories: proactive coping, neutral and passive-submissive behaviors (Gardner et al., 2005; table 1).

2.2.3 Forced swim test—For the forced swim test, we used a procedure similar to that described by Detke et al. (Detke et al., 1995). In this procedure, each rat was subjected to 2 swim sessions. Two days before the first social defeat encounter, HR and LR rats underwent a pretest session lasting 15 min during which they were individually placed inside a cylindrical Plexiglas tank (46 cm high × 20 cm diameter) filled with water (25 ± 1 °C) to a depth of 30 cm. Two days after the last social defeat encounter, rats were subjected to a second 5 min forced swim session (Test). Following both swim sessions, the rats were removed from the cylinders, dried with paper towels and placed in a heated enclosure before being returned to their home cage. Both pretest and test sessions were videotaped and later quantified by an experimenter blind to the experimental groups. A stopwatch was used to quantify the various behaviors.

The duration of forced swim-related behaviors during the first 5 min of the pretest and during the test was quantified *a posteriori* by an experimenter blind to the rat phenotype and stress conditions. We then determined the duration (in sec.) of immobility, swimming and climbing (table 2).

2.2.4 Blood collection and RIA for corticosterone determination—This procedure required two experimenters. One experimenter was gently holding that rat while the other one was collecting blood from the tail. The collection of each blood samples never surpassed 2 min. Blood samples (about 75µl) were collected in chilled heparinized Eppendorf tubes by tail vein nick and then kept on ice until centrifuged (1500 g for 10 min at 4 °C). Plasma was collected, frozen immediately in dry ice and stored at –80 °C until further processing. Plasma corticosterone levels were determined in triplicates, by radioimmunoassay, using a highly specific antibody developed in our laboratory and characterized by Dr. D. L. Helmreich. Specific details of the antibody and the radioimmunoassay procedure have been published previously (Campeau et al., 1997). Assay sensitivity was 10 pg and the intra-assay coefficient of variation was 2–5 %.

2.2.5 Organ collection—Animals were euthanized by decapitation after the final blood sample was taken. Adrenals, thymus and spleen were quickly removed and weighted. Brains were frozen in liquid isopentane (–42 °C) and stored at –80°C for subsequent *in situ* hybridization studies.

2.2.6 *In situ* hybridization—In this study, we compared the effects of repeated social defeat on gene expression in the brain of the HR and LR intruder rats. In this study, we examined 5-HT_{2A} receptor mRNA expression in the parietal cortex (PrCx, bregma –3.8 to –4.52mm; Paxinos and Watson Atlas), 5-HT_{1A} receptor mRNA and 5-HT transporter mRNA levels in the dorsal raphe (DR, bregma –7.3 to 8.0mm; Paxinos and Watson Atlas), and 5-HT_{1A}, MR mRNA and GR mRNA levels in the hippocampus proper (HIPP, bregma –3.14 to –3.8mm; Paxinos and Watson atlas) and dentate gyrus (DG, same coordinates than HIPP) in HR-LR rats. Moreover, we also examined MR/GR ratio in HIPP and DG as an index associated with control of the feedback of the HPA axis (Lopez et al., 1998).

The *in situ* hybridization method used in this study is detailed elsewhere (Isgor et al., 2003). Briefly, tissue was sectioned at –20°C at a thickness of 12 µm in a Leica microtome, mounted onto poly(L-lysine)-coated slides, and stored at –80°C until use. Before probe hybridization, tissue was fixed in 4% paraformaldehyde at room temperature, rinsed with aqueous buffers, and dehydrated with graded alcohols. Riboprobes were synthesized with incorporation of ³⁵S-UTP and ³⁵S-CTP and hybridized to tissue overnight at 55°C. Sections were then washed with in increasingly stringent solutions of SSC (1X SSC is 150 mM sodium chloride and 15 mM sodium citrate), dehydrated with graded alcohols, air-dried, and exposed to film. Exposure time was chosen to maximize signal.

All riboprobes were synthesized from cDNA fragments. 5-HT_{1A} and 5-HT_{2A} receptors were 908 and 903 nucleotide fragments (courtesy of O. Civelli, UC Irvine, and P. Seeburg, Max Plank Inst.) spanning nucleotides 333–1241 and 782–1685 of the coding regions of the rat's 5-HT_{1A} and 5-HT_{2A} genes, respectively. Serotonin transporter (5HTT) was 579 nucleotide fragment (courtesy of S. Burke, University of Michigan) spanning nucleotides 655–1234 of the coding region of the rat 5HTT gene. Glucocorticoid receptor (GR) was a 402 nucleotide fragment (courtesy of S. Burke, University of Michigan) spanning nucleotides 765–1167 of the coding region of the rat GR gene. Finally, mineralocorticoid receptor (MR) was a 327 nucleotide fragment (courtesy of P. Patel, University of Michigan) spanning nucleotides 4260–4587 of the coding region of the rat MR gene.

For all probes, eight sections per region per rat were used. Digital images of the brain sections were captured from X-ray films in the linear range of the gray levels using a CCD camera (TM-745; Pulnix, USA). The relative optical density for each probe was determined using the Micro Computer Imaging Device (Ontario, Canada) image analysis system. Optical density values were then corrected for background, averaged to produce one data

point for each brain region for each animal and averaged per group for statistical comparisons.

2.3 Experimental design

2.3.1 Experiment 1: behavioral effects of social defeat in HR-LR rats—Thirty-six Sprague-Dawley rats were acclimated to the animal colony for 1 week, and HR-LR animals were then separated according to their locomotor activity in a novel environment. The day after the locomotion test, equal numbers of HR and LR animals were assigned to one of two groups: socially defeated or handled control. This experimental protocol yielded 4 experimental groups: HR-Defeated (HR-Def), HR-control (HR-C), LR-Defeated (LR-Def) and LR-control (LR-C) (n=6 per group).

One week after the locomotor activity test, animals were subjected to the pretest phase of the forced swim test, and forty-eight hours later underwent the social defeat paradigm. Forty-eight hours after the last defeat session, rats were tested in the forced swim test, and were euthanized immediately afterwards.

2.3.2 Experiment 2: effects of social defeat on weight and gene expression in the brain of HR-LR animals—Thirty-six Sprague-Dawley rats were used for this experiment. HR-LR rats were separated according to their locomotor activity in a novel environment, and assigned to the socially defeated or handled control group as described above. This experimental protocol yielded 4 experimental groups: HR-Def, HR-C, LR-Def and LR-C (n=6 per group).

One week after the locomotor activity test, rats were subjected to the defeat paradigm and were euthanized 24 hours after the last defeat session. Weight of adrenals, thymus and spleen were obtained. Brains were quickly frozen and stored for *in situ* hybridization studies.

Rats' body weights were monitored throughout the experiment. Moreover, three blood samples were collected by tail vein nick in these animals 30 min before, immediately after and 4 hours after the last defeat session. Corticosterone levels in these samples were later quantified to investigate possible differential HPA axis activation during social defeat in HR-LR rats.

2.4 Statistical analysis

Data were analyzed using Statview 5 software. Data for social defeat in Experiment 1 were analyzed using a two-way analysis of variance (ANOVA) with repeated measures. The independent variables were phenotype (HR-LR; between subjects), and time (within subjects). Data for forced swim test in experiment 1, and organ weight and *in situ* hybridization in experiment 2 were analyzed using a two-way ANOVA. The independent variables were phenotype (HR-LR), and treatment (defeat or no defeat). Finally, data for changes in body weight and corticosterone in experiment 2 were analyzed using a three-way ANOVA with repeated measures. The independent variables were phenotype (HR-LR; between subjects), treatment (defeat or no defeat; between subjects) and time (within subjects). Whenever there were significant main effects and/or significant interactions a Bonferroni/Dunn test was applied. Also, all means are reported as mean $\pm$ SEM.

3. Results

3.1 Experiment 1: differential response to social defeat and subsequent forced swim test in HR-LR rats

Social Defeat: LR rats show higher levels of passive-submissive behaviors than HR rats—Locomotor activity counts followed a unimodal distribution. Locomotor counts for HR and LR rats were 777 ± 36 and 412 ± 23 respectively.

Proactive coping during social defeat showed a tendency for higher levels of the behavior in HR animals ($F_{1,10}=4.222$, $p=0.06$), with no time effect or phenotype X time interaction (Figure 1A).

Neutral behaviors significantly decreased over time ($F_{5,50}=10.362$, $p<0.01$) regardless of rats' phenotype (Figure 1B). Passive-submissive behaviors showed significantly higher levels in LR animals overall ($F_{1,10}=5.408$, $p<0.05$), and a significant increase over time ($F_{5,50}=3.254$, $p<0.05$). Subsequent post-hoc analyses indicated that LR animals displayed significantly higher levels of passive-submissive behavior than HR during the 6th session of social defeat ($p<0.05$; figure 1C).

Forced swim test: repeated social defeat increased immobility in the forced swim test in HR rats—During pretest, LR rats showed higher immobility than HR rats ($F_{1,20}=6.344$, $p<0.05$), with no significant HR-LR differences in swimming and climbing (data not shown).

During the test, immobility time was significantly affected by defeat ($F_{1,20}=32.079$, $p<0.01$), and there was a significant defeat X phenotype interaction ($F_{1,20}=13.047$, $p<0.01$). Subsequent post-hoc analyses indicated that LR-C rats displayed higher immobility than HR-C, similar to the pretest ($p<0.01$). Moreover, social defeat significantly increased immobility in HR but not LR animals ($p<0.01$; Figure 2A).

For swimming behaviors, there was a significant defeat X phenotype interaction ($F_{1,20}=4.377$, $p<0.05$), with no significant main effects. Subsequent post-hoc analyses revealed a significant decrease in swimming for HR-Def rats compared to HR-C ($p<0.05$; Figure 2B).

Finally, no significant interaction or main effects were found for climbing (Figure 2C).

3.2 Experiment 2: repeated social defeat differentially affects corticosterone secretion, thymus weight and hippocampal gene expression in HR-LR rats

Social defeat significantly decreased weight gain in rats regardless of phenotype—Locomotor activity in experiment 1 and experiment 2 were similar [$F_{1,44}=0.004$; $p=0.98$]. In experiment 2, locomotor activity for HR and LR rats were respectively 773 ± 24 and 383 ± 21 .

Animals that were subjected to social defeat showed less weight gain compared to controls regardless of phenotype as demonstrated by significant main effect of defeat ($F_{1,20}=19.559$, $p<0.01$), a significant effect of time ($F_{5,100}=3.702$, $p<0.01$) and a significant defeat X time interaction ($F_{5,100}=3.402$, $p<0.01$), but no significant phenotype effects. Subsequent post-hoc analyses indicated that defeated animals had less weight gain than handled controls on sessions 2 ($p<0.05$), 3 ($p<0.01$) and 4 ($p<0.05$) of the social defeat protocol (Figure 3). Average animals' weight in grams at the end of the repeated defeat protocol was 454 ± 11.1 for HR controls, 439 ± 11.9 for LR controls, 412 ± 6.8 for HR defeated and 418 ± 11.1 for LR defeated animals.

HR-Def rats showed higher corticosterone levels than LR-Def during recovery from social defeat—Corticosterone secretion during social defeat showed significant effects of time ($F_{2,38}=261.805$, $p<0.01$) and defeat ($F_{1,19}=54.163$, $p<0.01$), and significant time X defeat ($F_{2,38}=91.24$, $p<0.01$), time X phenotype ($F_{2,38}=11.786$, $p<0.01$) and time X defeat X phenotype ($F_{2,38}=3.514$, $p<0.05$) interactions. Post-hoc analyses revealed that social defeat significantly increased corticosterone secretion regardless of rats' phenotypes ($p<0.01$). Moreover, during recovery, HR-Def rats had significantly higher corticosterone levels than their LR counterparts ($p<0.05$; Figure 4), and had a tendency to have higher corticosterone levels than HR-C ($p=0.07$).

Repeated social defeat decreased thymus weight in HR but not LR rats—These data were analyzed as relative organ weight (in mg) per 100g of body weight. Two-way ANOVA for thymus weight revealed a significant defeat X phenotype interaction ($F_{1,20}=4.28$, $p<0.05$). Subsequent post-hoc analyses indicated that defeat significantly decreased thymus weight in HR ($p<0.01$) but not LR animals (Figure 5A). By contrast, statistical analyses indicated that defeat or phenotype had no significant effects on weight of adrenals and spleen (Figures 5B and 5C respectively).

Repeated social defeat differentially affected hippocampal GR mRNA expression in HR-LR rats—LR rats had higher 5HT_{2a} mRNA levels in the PrCx than HR rats ($F_{1,16}=16.894$, $p<0.01$), and that defeat did not affect 5HT_{2a} expression in this brain region. Moreover, HR rats had higher 5HT_{1a} mRNA expression in the DR than LR rats ($F_{1,16}=8.379$, $p<0.01$), and defeat did not affect the expression of this gene. By contrast, levels of expression for 5HTT in the same brain region were similar in HR and LR rats, and defeat decreased its expression in both phenotypes ($F_{1,16}=17.349$, $p<0.01$). Finally, neither phenotype nor defeat affected 5HT1a mRNA levels in HIPP or DG (Table 3).

MR expression levels in HIPP and DG showed a significant decrease in mRNA levels in both brain regions after social defeat ($F_{1,18}=5.636$, $p<0.05$, and $F_{1,18}=37.856$, $p<0.01$ respectively) regardless of rats' phenotype.

GR expression in HIPP showed no phenotype or defeat effects. By contrast, GR mRNA levels in DG revealed significant effects of defeat ($F_{1,18}=5.309$, $p<0.05$) and phenotype ($F_{1,18}=4.469$, $p<0.05$). Subsequent post-hoc analyses indicated that LR-C rats had significantly higher GR levels than HR-C ($p<0.01$), and that defeat significantly decreased GR mRNA expression in the DG of LR animals only ($p<0.05$; Table 3).

Finally, MR/GR ratio in HIPP and DG revealed significant effects of defeat ($F_{1,16}=7.384$, $p<0.05$ and $F_{1,16}=13.738$, $p<0.01$) and a significant defeat X phenotype interaction ($F_{1,16}=4.103$, $p<0.05$ and $F_{1,16}=18.14$, $p<0.01$ respectively) in both hippocampal regions. Subsequent post-hoc analyses revealed a significant decrease in the ratio after defeat in HR rats ($p<0.01$; Figure 6).

4. Discussion

The results from this study show that: a) greater novelty-seeking behavior is associated with less passive-submissive behavior during social defeat; b) compared to LR rats, HR rats showed higher corticosterone levels during recovery from social defeat; c) repeated social defeat reduces GR expression in the dentate gyrus of LR but not HR rats; d) repeated social defeat decreases thymus weight and hippocampal GR/MR ratio in HR rats only.

4.1 Effects of social defeat on behavior

Overall, animals that underwent repeated social defeat increased the amount of passive-submissive behavior over time. These data are in agreement with previous reports of an increase in passive defense postures subsequent to a repeated social defeat paradigm (see for example (Kudryavtseva et al., 1991)). HR animals however decreased the amount of passive-submissive behaviors at the end of our social defeat paradigm to a level that became significantly lower than LR rats. Frank and colleagues showed that rats bred for low trait anxiety exhibit less passive coping behavior when exposed to social defeat (Frank et al., 2006), suggesting a link between anxiety-like behaviors and coping strategies during defeat. HR animals display less anxiety-like behaviors than LR rats in the light/dark box and the elevated plus-maze tests (Kabbaj et al., 2001). Our data therefore suggests a direct association between low novelty-seeking behavior, anxiety and passive coping behavior during repeated social defeat. More broadly, our results add to the evidence of different coping strategies in HR-LR rats when rats are faced with a stressful situation. When challenged with a stressor, LR animals cope by being more passive and submissive, while HR rats show less anxiety-like behavior and take a more active stance. In agreement with this hypothesis, when selectively bred HR (bHR) and LR (bLR) animals generated in our lab were tested for aggressivity against an intruder rat introduced in their own environment, bHR rats showed a much more aggressive behavior than bLR rats (Abraham et al., 2006).

The forced swim test revealed higher immobility in LR rats when compared to HR rats during the pretest. The behavioral performance of rats on the first day of the forced swim test is believed to primarily reflect coping strategies in a stressful environment (Armario et al., 1988, Marti and Armario, 1993, Liebsch et al., 1998). In agreement with our social defeat results, our pretest data again suggests a more passive coping strategy for LR animals when under stress. During the test phase of the forced swim test, HR rats exposed to repeated defeat exhibited an increase in immobility compared to HR non-defeated, while LR rats' immobility was unaffected by defeat. Hollis and colleagues have recently reported a similar pattern of behavior while looking at a measure of anhedonia in HR and LR rats that had been previously defeated: repeated social defeat significantly decreases sucrose preference in HR but not LR animals (Hollis et al., 2010). A more recent finding also suggests that HR rats are more sensitive to the long term "depressive" effects of social defeat (Duclot et al., 2011). These findings suggest a greater behavioral vulnerability of HR rats to repeated stress, an effect that might be due to higher corticosterone levels during recovery from defeat, lower thymus weight and decreased hippocampal GR/MR ratio.

4.2 Effects of social defeat on organ and body weights

Social defeat significantly decreased thymus weight in HR but not LR animals. It has been long known that stress modulates immune function (for review see (McEwen et al., 1997)). In 1936, Hans Selye introduced experimental data showing that a variety of stressors were associated with an involution of the thymus (Selye, 1936); this effect was accompanied by enlarged adrenal glands, also a consequence of stress. In our study, we did not observe any effect of social defeat on adrenals or spleen weights. It is possible that our social defeat protocol was not severe enough to induce changes in these organ weights. Indeed, published data show that Sprague-Dawley rats subjected to chronic mild stress present a decrease in thymus weight and no changes in adrenals, while rats that undergo a more severe stress protocol present increased adrenals weight in addition to the effects on thymus (Kioukia-Fougia et al., 2002). It is also possible that the effects of stress on adrenals and spleen are dependent on the type of stressors applied, social defeat being not as effective as other stressor. Indeed, social defeat has been previously shown to have only a limited effect on relative adrenal weights in Lewis rats, and no effect in spontaneously hypertensive or Sprague-Dawley animals (Berton et al., 1998, Bhatnagar and Vining, 2003).

Rats subjected to social defeat showed a significant reduction in total body weight gain compared to handled controls on sessions 2, 3 and 4 of the social defeat paradigm regardless of phenotypes. Consistent with our results, a previous report has shown a similar decrease in body weight during a 7-day protocol of repeated social defeat stress, with the effect mostly concentrated on days 3 to 5 of the social defeat paradigm (Bhatnagar et al., 2006). These changes in weight gain cannot be fully explained by changes in food intake, and most likely reflect a decreased caloric efficiency that is consequential to changes in metabolic or other physiological functions in socially stressed rats (Bhatnagar et al., 2006).

4.3 Effects of social defeat on gene expression and corticosterone secretion

LR-C animals showed significantly higher GR mRNA expression in the dentate gyrus when compared to HR-C. This is consistent with previously reported data that LR animals have higher GR mRNA levels and higher corticosteroid receptor affinity than HR in the hippocampus (Maccari et al., 1991, Kabbaj et al., 2000, Kabbaj et al., 2007). Direct microinjection of the glucocorticoid receptor antagonist RU38486 in the hippocampus significantly decreases anxiety-like behavior in LR in the light-dark box test, an indication that the GR levels in the hippocampus are directly correlated to anxiety-like behavior (Kabbaj et al., 2000), and might be implicated in the higher anxiety levels displayed by LR rats. Consistent with this hypothesis, pregnant LR females selectively bred for low novelty-seeking behavior that undergo 18 days of chronic unpredictable stress show less anxiety-like behavior than unstressed controls in the elevated plus-maze test (Clinton et al., 2008), and our data show that repeated stress significantly decreases GR mRNA expression in LR animals.

Repeated social defeat significantly decreased MR/GR ratio in the hippocampus of HR animals only. The balance in actions mediated by GR and MR receptors in hippocampal neurons appears critical for neuronal excitability, stress responsiveness, and behavioral adaptation (De Kloet et al., 1998). Neonatal handling, which has been shown to improve spatial learning and memory in male rats, increases MR/GR ratio in the hippocampus of the same animals (Garoflos et al., 2005). By contrast, negative experiences such as stress decrease hippocampal MR/GR ratio. For example single-prolonged stress, a proposed model for post-traumatic stress disorder significantly decreases MR/GR ratio in the hippocampus (Zhe et al., 2008). Prolonged maternal deprivation in infant rats also results in a decrease in hippocampal MR/GR ratio. This decrease is associated with an enhanced and sustained corticosterone response when animals are challenged with a saline injection after deprivation (Vazquez et al., 1996). Interestingly, in our study, we observed a sustained corticosterone response after repeated social defeat in HR animals only. Our results indicate that HR and LR rats are differentially affected by repeated social stress at the neural level, and add to the evidence that a stress-induced decrease in hippocampal MR/GR ratio correlates with, and possibly underlies a delayed shut-off of the stress response.

In our study, we observed HR-LR differences in 5HT_{2a} mRNA levels in the PrCx and 5HT_{1a} mRNA levels in the DR that were unaffected by social defeat. LR animals showed higher 5HT_{2a} expression in the PrCx, and lower 5HT_{1a} expression in the DR than HR rats. Interestingly, in a study aimed at correlating serotonin 5HT_{2a} receptor binding and personality traits in healthy subjects, Moresco and colleagues have found an inverse correlation between harm avoidance, a personal trait linked to anxiety, and 5HT_{2a} receptor binding in the left PrCx (Moresco et al., 2002). Moreover, a decrease in 5HT_{1a} receptor function in the DR has been linked to anxiogenic behavior (Pobbe and Zangrossi, 2005). It is therefore possible that these differences in gene expression, together with the higher GR mRNA levels observed in the DG, might contribute to the higher anxiety levels reported in the literature in LR rats (see for example (Kabbaj et al., 2000, Clinton et al., 2008)). Further functional studies are needed to confirm or reject These hypotheses.

4.4 Conclusions

In this work we showed that differences in novelty-seeking behavior can predict inter-individual variability in the effects of social defeat on agonistic and depressive-like behaviors, thymus weight and hippocampal gene expression, and suggest that further studies based on this model of inter-individual variability in rats could lead to a better understanding of the biological mechanisms underlying individual differences in behavioral and physiological responses to social stress in humans. This work also provides an exciting animal model of two different coping strategies (active vs. passive) that have been shown in some human studies, but not all, to lead to different behavioral outcomes. Indeed, in one such study, aggressive coping style (like in HR rats) has been shown to predict later depression in human (Murberg and Bru, 2005).

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Abbreviations

5HTT	Serotonin Transporter
bHR	Bred High responder rats
bLR	Bred Low responder rats
DG	Dentate gyrus
DR	Dorsal raphe
GR	Glucocorticoid receptor
HIPP	Hippocampus proper
HPA axis	Hypothalamic-pituitary-adrenal axis
HR	High responder rats
LR	Low responder rats
MR	Mineralcorticoid receptor
PrCx	Parietal cortex

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Figure 1.

Proactive coping (A), neutral behavior (B) and passive-submissive behavior (C) in HR vs. LR rats during repeated social defeat. Panels on the left show the duration of these behaviors (in min) throughout the whole social defeat paradigm; panels on the right show the duration of proactive coping, neutral and passive-submissive behaviors during the 5 min of each defeat session (in sec). All values are mean $\pm$ SEM (n=6 per group). *= $p < 0.05$ compared to HR. Two-way ANOVA with repeater measures followed by Bonferroni/Dunn test for post-hoc comparisons.

Figure 2.

Behavioral effects of social defeat on immobility (A), swimming (B) and climbing (C) in HR vs. LR animals during the test phase of the forced swim test. All values are mean $\pm$ SEM (n=6 per group). * = p < 0.05, ** = p < 0.01 compared to HR-C animals. Two-way ANOVA followed by Bonferroni/Dunn test for post-hoc comparisons. C refers to handled controls rats and Def refers to defeated rats.

Figure 3. Effects of repeated social defeat on HR and LR rats' body weight. Data are expressed as changes, in grams, over the previous 2 days throughout the whole social defeat paradigm. All values are mean $\pm$ SEM (n=6 per group). *= $p < 0.05$, **= $p < 0.01$ compared to non-defeated animals. Three-way ANOVA with repeated measures followed by Bonferroni/Dunn test for post-hoc comparisons. C refers to handled controls rats and Def refers to defeated rats.

Figure 4.

Corticosterone secretion in HR-C, LR-C, HR-Def and LR-Def rats at the time of the 6th defeat session. All values are mean $\pm$ SEM (n=6 per group). ##=p<0.01 compared to non-defeated animals; *=p<0.05 compared to HR-Def rats. Three-way ANOVA with repeated measures followed by Bonferroni/Dunn test for post-hoc comparisons. C refers to handled controls rats and Def refers to defeated rats.

Figure 5. Effects of repeated social defeat on thymus (A), adrenal (B) and spleen (C) weights in HR vs. LR animals. Results are expressed as weight (in mg) per 100g of body weights. All values are mean $\pm$ SEM (n=6 per group). **=p<0.01 compared to HR-C animals; #=p<0.05 compared to HR-Def rats. Two-way ANOVA followed by Bonferroni/Dunn test for post-hoc comparisons. C refers to handled controls rats and Def refers to defeated rats.

Figure 6.

Effects of repeated social defeat on MR/GR ratio in the HIPP (A) and DG (B) of HR vs. LR animals. All values are mean $\pm$ SEM (n=5–6 per group). *= $p < 0.05$, **= $p < 0.01$ compared to HR-C rats. Two-way ANOVA followed by Bonferroni/Dunn test for post-hoc comparisons. C refers to handled controls rats and Def refers to defeated rats.

Figure 7. Representative sections from the *in situ* hybridization of GR mRNA expression in the DG of HR-C, LR-C, HR-Def and LR-Def. Bar = 1 mm. C refers to handled controls rats and Def refers to defeated rats

Table 1

Intruder rat behaviors during the repeated social defeat paradigm

Intruder rat behavior	Behaviors comprised	Behavior description
<i>Proactive Coping</i>	Defensive upright	The intruder rat rears on his hind paws and extends the forepaws while facing the resident (Blanchard and Blanchard 1977, Miczek 1979).
	Rearing/escape	The intruder rat assumes bipedal posture with attempt to escape as described by De Boer and Koolhaas (De Boer and Koolhaas, 2003).
<i>Neutral</i>	Exploration/locomotion	Motor activity (Walking around the cage)
	Self-grooming	Grooming or scratching of the animal directed towards the face and flank.
<i>Passive-submissive</i>	Supine posture	The intruder rat lies flat on his back exposing his ventral surface (Tornatzky and Miczek, 1994).
	Freezing posture	The intruder rat assumes totally immobile crouched posture, with all four limbs on the ground and usually no activity except for the movement associated with respiration (Miczek, 1979).

Table 2

Rat behaviors during the forced-swim test

Rat behavior	Behavior description
<i>Immobility</i>	Rat remains immobile in the water, without struggling and making only occasional movements to keep the body balanced and the nose above water
<i>Swimming</i>	Rat moves all four limbs, swimming around the tank or diving
<i>Climbing</i>	Rat strongly moves all four limbs with the forepaws breaking the water surface and stretching the tank wall

Changes in serotonergic system and corticoid receptors after social defeat as measured by *in situ* hybridization. Results are expressed as optical density (O.D.). All values are mean $\pm$ SEM (n=5–6 per group).

Table 3

	HR-C	LR-C	HR-Def	LR-Def	
5HT _{2a}	24.5 $\pm$ 2.8	34.2 $\pm$ 2.1**	19.2 $\pm$ 1.5	36.1 $\pm$ 5.3**	PrCx
5HT _{1a}	50.8 $\pm$ 4.1	30.5 $\pm$ 3.4**	52.8 $\pm$ 5	34.5 $\pm$ 11.1**	DR
5HTT	134.4 $\pm$ 3.2	138.1 $\pm$ 4.1	112.7 $\pm$ 9##	110.5 $\pm$ 5.7##	DR
5HT _{1a}	29.9 $\pm$ 1	29.2 $\pm$ 1.1	27.7 $\pm$ 1.7	30.4 $\pm$ 1.9	HIPP
5HT _{1a}	70.4 $\pm$ 2.9	65.2 $\pm$ 1.7	58.7 $\pm$ 5.2	65.5 $\pm$ 3.8	DG
MR	21.2 $\pm$ 1.1	20.6 $\pm$ 2.9	14.4 $\pm$ 2.1#	18.8 $\pm$ 0.2#	HIPP
MR	15.6 $\pm$ 0.6	14.8 $\pm$ 1.5	9.5 $\pm$ 0.9##	10.1 $\pm$ 0.4##	DG
GR	21.5 $\pm$ 2.1	25.6 $\pm$ 1.2	24.7 $\pm$ 1.7	26.6 $\pm$ 1.5	HIPP
GR	17.8 $\pm$ 1.1	25.4 $\pm$ 1.2 $\psi\psi$	18.3 $\pm$ 0.9	17.5 $\pm$ 2.4 θ	DG

** p<0.01 compared to HR animals;

p<0.05,

p<0.01 compared to non defeated rats;

$\psi\psi$ p<0.01 compared to HR-C animals;

θ p<0.05 compared to LR-C rats. Two-way ANOVA followed by Bonferroni/Dunn test for post-hoc comparisons. C refers to handled controls rats and Def refers to defeated rats.