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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 LRs. Analyses of *in situ* hybridization results found HR-LR differences in 5-HT_{2a} mRNA levels in the parietal cortex and 5-HT_{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 mineralcorticoid receptor (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.

Key words: individual differences, behavioral phenotyping, *in situ* hybridization, repeated social defeat, corticosterone.

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}, mineralcorticoid receptor (MR) and glucocorticoid receptor (GR) receptors (Berton et al., 1998, 1999; Lopez et al., 1998; 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

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Abbreviations: 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; HR-Def, HR-defeated; LR, low responder rats; LR-C, LR-control; MR, mineralcorticoid receptor; PrCx, parietal cortex; 5HTT, serotonin transporter.

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Table 1. Intruder rat behaviors during the repeated social defeat paradigm

Intruder rat behavior	Behaviors comprised	Behavior description
Proactive Coping	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).
Neutral	Exploration/locomotion	Motor activity (walking around the cage).
	Self-grooming	Grooming or scratching of the animal directed towards face and flanks.
Passive-submissive	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).

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.

EXPERIMENTAL PROCEDURES

Subjects

Seventy-two male Sprague–Dawley rats from Charles River (Wilmington, MA, USA), weighing 250–300 g were used in this study. Rats were pair-housed in 43×21.5×24.5 cm^3 Plexiglas cages, and kept on a 12 h 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.

Experimental methods

Locomotor activity test. After 7 days of habituation to the housing conditions, rats were tested for locomotor activity during a 60 minute (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 cage, and locomotor activity was monitored by means of two banks of three 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).

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 six different resident Long-Evans rats during the six 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).

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 two 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 seconds (s) of immoblilty, swimming, and climbing (Table 2).

Blood collection and Radioimmunoassay for corticosterone determination. This procedure required two experimenters. One experimenter was gently holding the rat while the other one was collecting blood from the tail. The collection time for each blood sample never surpassed 2 min. Blood samples (about 75 μl) 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

Table 2. Rats' behaviors during the forced-swim test

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