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RATS WITH HIGH OR LOW SOCIABILITY ARE DIFFERENTLY AFFECTED BY CHRONIC VARIABLE STRESS

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Abstract—Depression is strongly related to social behavior. We have previously shown that social behavior of rats is individually stable. The purpose of the present study was to compare the sensitivity of animals with different sociability to chronic variable stress (CVS). Four social interaction tests were performed with 60 single-housed male Sprague–Dawley rats. Twenty rats with the lowest and 20 with the highest average social activity time were selected as low sociability (LS) and high sociability (HS) rats, respectively. Both groups were further divided into control and stress groups with equal average body weight. The CVS procedure lasted for 3 weeks. The stressors applied were cold water and wet bedding, imitation of injection, stroboscopic light, movement restriction in a small cage, tail pinch with a clothespin, and strong illumination during the predicted dark phase. In HS-rats, but not in LS-rats, CVS reduced sucrose intake compared with baseline after 3 weeks, suggesting that HS-rats are more vulnerable to anhedonia elicited by CVS. LS-animals were more anxious in the social interaction and open field tests, but stress eliminated differences with HS-animals in the social interaction test and increased their activity in the forced swimming test. In LS-rats stress increased *ex vivo* dopamine levels and reduced 5-HT levels in the frontal cortex, suggesting that the increased behavioral activity after stress may be related to increased impulsivity. This study thus revealed that animals with high sociability trait are more vulnerable to anhedonia elicited by chronic stress in conditions of single housing.

Key words: sucrose intake, anxiety, depression, anhedonia, single housing.

Stress is a major contributor in the etiology of depression, anxiety, and other disorders (Bale, 2005). Stressful life events precipitate the onset of depressive episodes in humans (Gilmer and McKinney, 2003), and a large number of studies have used chronic mild/variable stress in animals to induce behavioral changes that resemble the symptoms of depression (Willner, 2005). Stress response itself is essential for adaptation, maintenance of homeostasis, and survival (Bale, 2005). Therefore the sensitivity of the individual to stressful encounters is important in the development of mood disorders (Harro and Oreland, 2001).

Studies with chronic variable/mild stress have shown that this procedure elicits helplessness or anhedonia in some but not all animals, which suggests an important role of individual vulnerability (Henn and Vollmayr, 2005). Using the elevated plus-maze test, rats bred for either high or low anxiety–related behavior differ in their stress coping strategies, the former being more susceptible and vulnerable to stressor exposure and preferring more passive strategies (Landgraf and Wigger, 2002). Acute tail pinch stressor, but not chronic social defeat stress increases alcohol preference in Maudsley reactive rats, originally selected for high and low open-field defecation (Blizard and Adams, 2002).

The majority of stressful stimuli that lead to psychopathology in humans are of social nature (Buwalda et al., 2005) and a decrease in social functioning is a major symptom of depressed patients (Nemeroff, 1998). Coping with stress depends on effective social relations, and improved social circumstances are effective in therapy of depression (Brown et al., 1988; Sloman et al., 2003). Forebrain 5-HT-ergic neurotransmission is important in reducing the impact of environmental aversive conditions and in protecting against the psychosocial depressogenic influence (Deakin, 1996). Drugs that are effective in the treatment of depression and influence central serotonergic function also modulate dimensions of normal personality, reducing negative affective experience and increasing affiliative behavior in healthy persons (Knutson et al., 1998). Social support in humans and affiliative behaviors in animals can provide a buffer against stress and have a positive impact on measures of health and well-being, thus social behavior can protect from stress-related diseases (DeVries et al., 2003). Deficits in social skills may be a cause of depression (Davison and Neale, 1998), and it has been described that low social competence predicts the onset of depression among elementary-school-age children (Cole et al., 1990), and poor interpersonal problem solving skills predict increases in depression among adolescents (Davila et al., 1995).

In humans, social behavior is a very consistent disposition (Depue and Collins, 1999), which is linked to the regulation of affects and behaviors, which in turn are linked to stress systems and implicated in depression (Sloman et

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Abbreviations: ANOVA, analysis of variance; CRF, corticotropin-releasing factor; CVS, chronic variable stress; DA, dopamine; DOPAC, dihydroxyphenylacetic acid; D_2, dopamine receptor subtype 2; EDTA, ethylenediamine tetraacetic acid; GTPγS, guanosine-triphosphate-gamma-sulfate; HS, high sociability; HVA, homovanillic acid; K-Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid potassium salt; LS, low sociability; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT_{1A}, 5-HT receptor 1A.

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al., 2003). We have previously shown that social behavior of rats is individually stable and that sociability is related to 5-HT metabolism in the prefrontal cortex (Tõnissaar et al., 2004). Sociability and anxiety are closely related domains. Many anxiety disorders include difficulties in creating or maintaining social contacts, such as social anxiety disorder, several personality disorders, autism, etc. The social interaction test, developed to measure effects of anxiogenic and anxiolytic drugs (File and Hyde, 1978), entirely relies on social behavior. Importantly, basal social activity in this test does not correlate well with performance in other animal tests of anxiety (Ramos et al., 1997). This suggests that in addition to anxiety, behavior in the social interaction test reflects other, distinct psychological domains. The purpose of the present study was to compare how animals with traitwise different social activity vary in sensitivity to chronic variable stress (CVS). Low and high sociability rats were also compared with regard to 5-HT transporter, 5-HT receptor 1A (5-HT_{1A}) and dopamine receptor subtype 2 (D_2) binding in the present study, because of their relevance to anxiety and motivational state which are important in social behavior (Beneytez et al., 1998; Dekeyne et al., 2000; McGregor et al., 2003; Panksepp, 1998; Thompson et al., 2004).

EXPERIMENTAL PROCEDURES

Animals

Male Sprague–Dawley rats (Scanbur BK AB, Sollentuna, Sweden) were single-housed at age 2 months in transparent macrolone cages under controlled light cycle (lights on from 08:30 h to 20:30 h) and temperature (19–21 °C), with free access to tap water and food pellets (diet R70, Lactamin, Sweden). All handling of animals, including the behavioral experiments, was carried out during the light phase. All procedures were carried out in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals. All experiments were carried out as approved by the national committee on the ethical use of animals. The number of animals used was minimal and their suffering was minimized.

General procedure

Sixty rats were single-housed 11 days before the first social interaction test (see Fig. 1). Altogether four tests, always with unfamiliar partners, were performed with the interval of 7–8 days. Thereafter, animals were allocated to high or low sociability group. Twenty rats with the lowest (LS-rats) and 20 with the highest (HS-rats) average social activity time were selected. Three days after the last social interaction test, the first sucrose preference test was carried out and the CVS procedure was started. Rats were divided into Stress and Control groups on the basis of controlled body weight, so that their average body weight was similar before the onset of stress procedure. Animals belonging to the Stress groups were transported to another room for all manipulations included in the CVS procedure. Three subsequent sucrose preference tests were performed after 8, 16 and 24 days from the first test. Another social interaction test was carried out on the next day after the fourth sucrose preference test. On the next day thereafter, an elevated plus-maze test was performed. The method first described by Handley and Mithani (1984) was used as modified in our laboratory for animals with low behavioral activity (Harro et al., 1990) but because only a few rats entered the open arms the results were considered unreliable and were not included in data analysis. The forced swimming test was carried out 2 days after the elevated plus-maze test, on the two subsequent days. Then, 2 days later, the rats were tested in the open field test, and immediately killed thereafter. The brains were quickly dissected on ice and the brain tissue was stored at −80 °C in a deep freezer. Body weight changes were measured between consecutive sucrose preference tests. First 3 “weeks” of the study (CVS regimen) lasted 8 days, the last week (the session of behavioral experiments) 7 days. The respective body weight gain data were, to enable comparison, adjusted on the basis of average daily weight gain.

CVS procedure

The CVS procedure was based on our previous experiments (Harro et al., 1999, 2001; Tõnissaar et al., 2000). Various stressors of different duration were applied, one stressor per day. Each stressor was used three times. The stressors were presented in the following order: cold (4 °C) water and wet bedding (initially, 400 ml of water was poured on a rat, and the sawdust bedding was kept wet for the following 17 h), imitation of injection (the rat was captured and a syringe without a needle was pressed against its abdomen), stroboscopic light (for 13 h, 10 Hz, 2 lx), movement restriction in a small cage (11×16×7 cm) for 2 h, cage tilt at 45 °C (for 20 h), tail pinch with a clothespin placed 5 cm distal from the base of tail (5 min), strong illumination (900 lx) during the predicted dark phase (for 12 h). In order to avoid the direct effect of a single stressor on body weight gain and on sucrose consumption in the sucrose preference test, a day without stress preceded

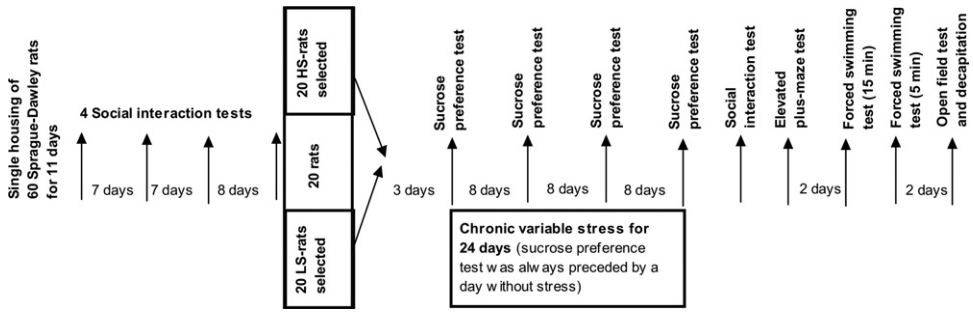

Fig. 1. Study design. Four social interaction tests were performed with 60 male Sprague–Dawley rats. Twenty rats with the lowest and 20 with the highest average social activity time were selected. Both groups were further divided into control and stress group with similar average body weights. CVS consisted of seven stressors: cold water and wet bedding, imitation of injection, stroboscopic light, movement restriction in a small cage, tail pinch with a clothespin, strong illumination during the predicted dark phase. Each stressor was used three times and one stressor per day was applied. One day without stress was always left before sucrose preference test. The effect of CVS was measured in several behavioral tests: sucrose preference test, social interaction test, elevated plus-maze test, forced swimming test and open field test.