Enduring Deficits in Brain Reward Function after Chronic Social Defeat in Rats: Susceptibility, Resilience, and Antidepressant Response

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Background: Anhedonia, or diminished interest or pleasure in rewarding activities, characterizes depression and reflects deficits in brain reward circuitries. Social stress induces anhedonia and increases risk of depression, although the effect of social stress on brain reward function is incompletely understood.

Methods: This study assessed the following: 1) brain reward function in rats (using the intracranial self-stimulation procedure) and protein levels of brain-derived neurotrophic factor and related signaling molecules in response to chronic social defeat, 2) brain reward function during social defeat and long-term treatment with the antidepressants fluoxetine (5 mg/kg/day) and desipramine (10 mg/kg/day), and 3) forced swim test behavior after social defeat and fluoxetine treatment.

Results: Social defeat profoundly and persistently decreased brain reward function, reflecting an enduring anhedonic response, in susceptible rats, whereas resilient rats showed no long-term brain reward deficits. In the ventral tegmental area, social defeat, regardless of susceptibility or resilience, decreased brain-derived neurotrophic factor and increased phosphorylated AKT, whereas only susceptibility was associated with increased phosphorylated mammalian target of rapamycin. Fluoxetine and desipramine reversed lower, but not higher, stress-induced brain reward deficits in susceptible rats. Fluoxetine decreased immobility in the forced swim test, as did social defeat.

Conclusions: These results suggest that the differential persistent anhedonic response to psychosocial stress may be mediated by ventral tegmental area signaling molecules independent of brain-derived neurotrophic factor and indicate that greater stress-induced anhedonia is associated with resistance to antidepressant treatment. Consideration of these behavioral and neurobiological factors associated with resistance to stress and antidepressant action may promote the discovery of novel targets to treat stress-related mood disorders.

Key Words: Anhedonia, BDNF, depression, ICSS, mTOR, stress

Mood disorders, such as major depressive disorder (MDD), are characterized by deficits in reward processing (1). Clinically, these deficits are manifested in the symptom of anhedonia, or diminished interest or pleasure in rewarding stimuli and activities. Anhedonia is a trait feature of MDD (2) and may persist after antidepressant treatment (3).

Exposure to stress precipitates the development of neuropsychiatric disorders characterized by anhedonia (4,5). Studies in healthy humans and nonhuman animals demonstrated that stress induces anhedonia (6,7). In humans, social loss, humiliation, entrapment, and submissive behavior are associated with MDD (8–10). The social defeat procedure in rodents involves an ethological stressor whereby the experimental animal displays submissive behaviors when confronted by an aggressive, socially dominant conspecific (11). This procedure is considered analogous to psychosocial stressors in humans.

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Repeated social defeat has been used to assess anhedonia-like behaviors in rodents, although almost exclusively with sucrose-based measures. Some studies demonstrated stress-induced decreases in preference for (12–15) and anticipation of (16) sucrose. Conversely, other studies reported unaffected sucrose consumption (16,17) or preference (13,18) after social defeat. Variability within experimental groups may partly explain these discrepancies. For example, rodents with high baseline exploratory activity (19) or low basal plasma corticosterone levels (20) displayed less sucrose preference after social defeat compared with animals with low exploratory behaviors or high corticosterone levels, suggesting intrinsic susceptibility and resilience to social defeat–induced anhedonia. Similar differential effects were observed in mice showing high or low social avoidance after chronic social defeat (15,21), an effect mediated by the firing and neurotrophic signaling of ventral tegmental area (VTA) dopaminergic neurons (15,22). In humans, stress triggers depressive symptoms in vulnerable individuals, whereas resilient individuals adopt active coping strategies that promote healthy outcomes (23,24). Animal procedures modeling susceptibility and resilience to stress-induced anhedonia, similar to the above-described procedures, can provide insight into the factors that mediate stress-induced psychopathology.

Anhedonia reflects dysfunctional brain reward circuitries (25–27); however, there have been few direct studies of the impact of social defeat on brain reward function. The goals of the present study were to determine 1) if chronic exposure to social defeat in rats produces deficits in brain reward function in all or only a subgroup of susceptible rats and 2) whether repeated treatment with the selective serotonin reuptake inhibitor fluoxetine or

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tricyclic antidepressant desipramine would reverse such deficits. Brain reward function was assessed using the intracranial self-stimulation (ICSS) procedure that allows for daily assessment of reward thresholds to quantify reliably the development and reversal of anhedonia after chronic stress and antidepressant administration, respectively. We also assessed whether stress-induced deficits in reward thresholds correlated with changes in brain-derived neurotrophic factor (BDNF) and related signaling molecules in depression-related brain areas, including the VTA, nucleus accumbens, central nucleus of the amygdala, and hippocampus. The forced swim test, a widely used assessment of antidepressant efficacy and a putative measure of behavioral despair, was also conducted after social defeat and fluoxetine treatment to determine whether brain reward function correlated with behavioral despair.

Methods and Materials

Subjects

Adult male Wistar and adult male and female Long-Evans rats (Charles River Laboratories, Raleigh, North Carolina) were used as intruders and residents, respectively, for the social defeat procedure (see below and Supplemental Methods in Supplement 1 for details). All procedures were conducted in accordance with the guidelines from the National Institutes of Health and the Association for the Assessment and Accreditation of Laboratory Animal Care and were approved by the institutional animal care and use committee.

ICSS Surgery and Apparatus

Supplement 1 and previous publications (28,29) provide details of ICSS surgery and apparatus. Briefly, rats were surgically prepared with bipolar stimulating electrodes aimed at the posterior lateral hypothalamus, part of the brain's reward circuitry. During the ICSS procedure, rats were placed inside operant testing chambers containing a metal wheel manipulandum (Med Associates Inc, St. Albans, Vermont) and connected to constant-current stimulators (Model 1200C; Stimtek, Acton, Massachusetts) that delivered electrical stimulation on rotation of the wheel manipulandum. Stimulation parameters, data collection, and test session functions were controlled by a computer.

ICSS Training and Testing Procedures

Brain reward function was assessed using a modified discrete-trial, current-intensity threshold procedure originally designed by Kornetsky *et al.* (30). For details, see Supplemental Methods in Supplement 1 and previous publications (28,29). Each trial was initiated with rats receiving a noncontingent stimulation (100 Hz electrical pulse ranging from 100–250 μA) and responding on the wheel manipulandum to receive a second, contingent stimulation identical in all parameters to the initial contingent stimulus. By systematically varying the current intensity of the noncontingent and contingent stimuli, a reward threshold was determined for each subject, defined as the point at which higher and lower current intensities delivered as the noncontingent stimulus would elicit a response or no response, respectively. After training, baseline thresholds were stabilized (i.e., $<$10% variation over 5 days). Elevations in reward thresholds indicated that greater stimulus intensities were necessary for positive reinforcement, reflecting decreased brain reward function and suggesting an anhedonic or depression-like state. Conversely, lowering of thresholds reflected a reward-enhancing effect.

Social Defeat Procedure

Rats were assigned to receive either social defeat or no stress (counterbalanced for baseline thresholds; see Supplemental Results in Supplement 1). Wistar rats receiving social defeat (i.e., intruders) were transported to a separate room housing Long-Evans rats (i.e., residents) selected for aggressive behavior. Each intruder was placed inside the residents' cage (61 cm × 43 cm × 20 cm) behind a perforated acrylic glass partition physically separating the rats, with food and water available ad libitum. At 8:00 AM the following day, the females and partitions were removed. Social defeat was defined as the intruder displaying a defensive, supine posture for 3 consecutive sec. For Experiments 2 and 3 (see below), time until social defeat and number of injuries (e.g., bite marks) were recorded. After social defeat or 3 min (whichever occurred first), intruders were transported to the laboratory for ICSS testing. Afterward, intruders were housed with different residents (separated by a partition) until the social defeat session the following day; this was repeated for 21 days. Intruders were never paired with the same residents twice. Control (i.e., no stress) rats were briefly handled in the vivarium before ICSS testing.

Experimental Designs and Procedures

Experiment 1: Effects of Chronic Social Defeat on Brain Reward Function and Neurotrophic Factor Signaling. Rats were exposed to social defeat or no stress followed by ICSS testing for 21 days. Rats were returned to their original vivarium room that was separate from the residents' vivarium room and single-housed, and ICSS thresholds were assessed for an additional 21 days. Rats were euthanized 24 hours after the final ICSS test, and the VTA, nucleus accumbens, central nucleus of the amygdala, and whole hippocampus were dissected, prepared, and analyzed by Western blot for BDNF, insulin receptor substrate 2, phosphorylated AKT, phosphorylated mammalian target of rapamycin (mTOR), and phosphorylated extracellular signal-regulated kinase 1/2 proteins as described previously (see Supplemental Methods in Supplement 1) (31).

Experiment 2: Effects of Repeated Fluoxetine Treatment on Social Defeat–Induced Deficits in Brain Reward Function. Rats were exposed to social defeat or no stress followed by ICSS testing for 21 days. Beginning on social defeat day 14, rats were administered either 5 mg/kg fluoxetine or vehicle 30 min after ICSS testing daily for 14 days. The ICSS testing was continued for 7–10 days after termination of fluoxetine treatment. The forced swim test was conducted on the final 2 days of ICSS testing, consisting of a 15-min habituation followed by a 5-min test 24 hours later (see Supplemental Methods in Supplement 1 for details).

Experiment 3: Effects of Repeated Desipramine Treatment on Social Defeat–Induced Deficits in Brain Reward Function. Experiment 3 was identical to experiment 2 except that rats were administered either 10 mg/kg desipramine or vehicle for 14 days, and ICSS testing was terminated after desipramine treatment. The forced swim test was not conducted in experiment 3 because the results of experiment 2 revealed that stress exposure decreased immobility to levels observed with antidepressant treatment alone (see below). Thus, social defeat produced an antidepressant-like effect that is not conducive to reversal with antidepressant treatment.

Drugs

Fluoxetine hydrochloride (donated by Eli Lilly and Co., Indianapolis, Indiana) and desipramine hydrochloride (Sigma-Aldrich,