Effects of Striatal ΔFosB Overexpression and Ketamine on Social Defeat Stress–Induced Anhedonia in Mice

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Background: Chronic social defeat stress (CSDS) produces persistent behavioral adaptations in mice. In many behavioral assays, it can be difficult to determine if these adaptations reflect core signs of depression. We designed studies to characterize the effects of CSDS on sensitivity to reward because anhedonia (reduced sensitivity to reward) is a defining characteristic of depressive disorders in humans. We also examined the effects of striatal ΔFosB overexpression and the *N*-methyl-D-aspartate receptor antagonist ketamine, both of which promote resilience, on CSDS-induced alterations in reward function and social interaction.

Methods: Intracranial self-stimulation (ICSS) was used to quantify CSDS-induced changes in reward function. Mice were implanted with lateral hypothalamic electrodes, and ICSS thresholds were measured after each of 10 daily CSDS sessions and during a 5-day recovery period. We also examined if acute intraperitoneal administration of ketamine (2.5–20 mg/kg) reverses CSDS-induced effects on reward or, in separate mice, social interaction.

Results: ICSS thresholds were increased by CSDS, indicating decreases in the rewarding impact of lateral hypothalamic stimulation (anhedonia). This effect was attenuated in mice overexpressing ΔFosB in striatum, consistent with pro-resilient actions of this transcription factor. High, but not low, doses of ketamine administered after completion of the CSDS regimen attenuated social avoidance in defeated mice, although this effect was transient. Ketamine did not block CSDS-induced anhedonia in the ICSS test.

Conclusions: This study found that CSDS triggers persistent anhedonia and confirms that ΔFosB overexpression produces stress resilience. The findings of this study also indicate that acute administration of ketamine fails to attenuate CSDS-induced anhedonia despite reducing other depression-related behavioral abnormalities.

Key Words: Anhedonia, antidepressant, defeat, intracranial self-stimulation (ICSS), ketamine, social interaction, stress

Chronic stress is implicated in the etiology and pathophysiology of anxiety and depressive disorders (1–3). These disorders are increasingly prevalent (4) and tend to be persistent and resistant to current treatments (5,6), and the mechanisms by which stress triggers them remain poorly understood (7). Validating depression models is crucial to better understand the consequences of stress, elucidate the neurobiology of affective disorders, and develop novel antistress and antidepressant treatments.

Animal models of depression rely on an ability to mimic or produce core symptoms of the disorder in humans, including social avoidance and anhedonia (reduced sensitivity to reward) (8,9). Chronic social defeat stress (CSDS) is an increasingly popular model that exploits the ethological relevance of territorial aggression (10,11) and produces these core symptoms as assessed in tests quantifying social interaction (SI) and preference for sucrose and other natural rewards (12–14). The effects of CSDS are reversed by long-term administration, but not acute administration, of fluoxetine or imipramine (12,15,16), standard antidepressant medications widely used to treat depressive disorders in humans. In contrast, standard anxiolytic medications are ineffective (12). The CSDS model is considered to have construct, face, and predictive validity (10). However, it has been proposed that CSDS-triggered behaviors have their basis in anxiety (17,18), and what is often interpreted as anhedonia in sucrose preference tests may actually reflect anxiety-enhanced neophobia.

The primary goal of the present study was to examine the ability of CSDS to produce anhedonia, a core feature of depressive, but not anxiety, disorders (8). We used intracranial self-stimulation (ICSS), an operant paradigm in which mice self-administer rewarding electrical brain stimulation, to assess the effects of CSDS directly on reward sensitivity (19,20). In rodents, ICSS behavior is attenuated under conditions that cause depressive-like states in humans, including drug withdrawal (21–25), unpredictable and chronic mild stress (26,27), and administration of kappa-opioid receptor agonists (28,29). Specifically, these treatments increase the threshold frequency at which the stimulation supports responding, an indicator of anhedonia (19). The ICSS paradigm also allows for the study of manipulation-induced alterations in reward sensitivity over time and is impervious to anxiety-related and satiety-related factors that confound other paradigms used to assess reward system function (e.g., sucrose preference, sex, drugs of abuse) (19).

In parallel, we examined the ability of ketamine, an *N*-methyl-D-aspartate receptor antagonist (30), to mitigate the effects of CSDS on social avoidance and ICSS thresholds. Although standard antidepressant treatments have delayed therapeutic efficacy (often several weeks), more recent studies demonstrate that a single dose of ketamine can produce rapid (although transient)

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antidepressant responses in depressed patients (31–37), including patients who are resistant to treatment (32,34,38,39), and in numerous models of depression (40–51). To determine if the therapeutic-like actions of ketamine are accompanied by amnestic (learning-disrupting and memory-disrupting) effects often associated with *N*-methyl-D-aspartate receptor antagonists (52,53) or anxiolytic effects (43), we examined performance in the passive-avoidance and elevated plus maze (EPM) tests. To evaluate whether the effects of CSDS on ICSS can be mitigated, we included studies using ΔFosB-overexpressing mice, which are less sensitive (resilient) to CSDS (54).

Methods and Materials

Animals and Drugs

Male C57BL/6J mice (6–8 weeks old) were purchased from Jackson Laboratories (Bar Harbor, Maine), and male CD-1 mice (retired breeders) were purchased from Charles River Laboratories (Wilmington, Massachusetts). Inducible bitransgenic male mice that overexpress ΔFosB were generated from crosses of NSE-tTA (line A) and TetOP-ΔFosB (line A11) mice and fully backcrossed to a C57BL/6J background, using a tetracycline-regulated gene expression system (55). The ΔFosB mice were raised on water containing doxycycline (DOX) (100 μg/mL) (Sigma-Aldrich, St. Louis, Missouri), which represses transgene expression. Experiments were conducted ~8 weeks after DOX discontinuation, when transgene expression of ΔFosB is maximal (ΔFosB-ON group) (55). Half of the mice remained on DOX for the duration of the experiment to serve as controls (ΔFosB-Control group). Mice had free access to food and water and were maintained on a 12-hour light/dark cycle. All procedures were conducted in accordance with National Institutes of Health and McLean Hospital policies. Ketamine was obtained from Sigma-Aldrich, dissolved in .9% saline (vehicle), and administered intraperitoneally at 10 mL/kg. Control mice received identical treatments as defeated mice.

Behavioral Manipulations and Tests

CSDS was performed as described previously (10,12). The CD-1 mice (residents) were screened for consistent aggressive behavior (attack latencies <30 sec for three consecutive screening tests). On each of 10 consecutive days, the intruder (defeated) mouse was placed in the home cage of a resident mouse and subjected to 10 min of social defeat stress. After the defeat session, the mice were separated in the cage with a perforated Plexiglas divider, which allowed for continuous protected sensory exposure. Defeated mice were exposed to a new resident and cage each day. Control mice were handled daily and housed in identical cage setups as the defeated mice but opposite a conspecific mouse. Separate cohorts were used for ICSS and SI experiments.

The ICSS test was performed as previously described (19,28). Briefly, mice (25–30 g) were implanted with monopolar electrodes aimed at the lateral hypothalamus. Mice were trained daily with four descending series (or "passes") of 15 stimulation frequency trials (.05 $\log_{10}$ unit steps), at the minimum effective current. The defeat and control groups had equivalent minimum currents (~75 μA). The ICSS thresholds (theta-0) were calculated using a least-squares line of best-fit analysis (19,56). After stable baseline thresholds were established (±15% for 5 consecutive days [BL1–5]), mice were subjected to CSDS for 10 days (D1–10). Mice were initially separated into two groups to test whether the effects of CSDS on ICSS thresholds depend on the interval of time between the defeat session and ICSS testing: mice in the long interval (LInt) group were tested in ICSS ~16 hours after defeat, whereas mice in the short interval (ShInt) group were tested in ICSS ~6 hours after defeat (Figure 1A). After CSDS, mice were returned to their home cages and tested postdefeat in ICSS for 5 days (P1–5). For ketamine experiments, mice received either vehicle or ketamine (20 mg/kg) 1 hour after the final defeat session.

To assess ketamine effects on SI, mice received vehicle, a low (2.5 mg/kg) dose of ketamine, or a high (20 mg/kg) dose of ketamine 24 hours before the first day of CSDS (day 0) or 1 hour after the final defeat session (day 10). Mice were habituated to the interaction arena in red light for 15 min on days 8–10 of CSDS. Social approach behavior in the presence of an unfamiliar CD-1 mouse enclosed in a wire cage was assessed 24 hours after the final defeat session (day 11), as previously described (12,57), with minor modifications. The SI scores were defined as the amount of time the mouse spent near an enclosure containing a CD-1 mouse (social target) over a 2.5-min period compared with when the target enclosure was empty. Because control mice spend more time interacting with a social target, an SI score of 1 (equal time near social target vs. empty enclosure) was used as a cutoff: SI scores >1 were considered "stress-resilient" and scores <1 were considered "stress-susceptible" (13). The segregation of defeated mice into susceptible and resilient subpopulations is supported by extensive behavioral, neurobiological, and electrophysiological analyses (13,54).

Passive-avoidance conditioning was conducted in a GEMINI Avoidance System apparatus (San Diego Instruments, San Diego, California) as previously described (50) with minor modifications. During training, mice were given 1 min of acclimation to the light compartment before access to the dark compartment. When a cross was made to the dark compartment, mice were conditioned with two consecutive 2-sec (inescapable) foot shocks (.2 mA), followed by a 1-min time-out. Mice were given either vehicle or ketamine (20 mg/kg) 1 hour after conditioning. Step-through latencies were measured 24 hours later. To assess the effects of ketamine on anxiety-like behavior, a separate cohort of mice was given either vehicle or ketamine (20 mg/kg) 24 hours before EPM testing. Mice were placed in the center of an EPM (each arm 33 cm long × 5 cm wide, with two opposite arms closed by 16.5-cm-high walls, maze elevated 81 cm from floor) in red light and allowed to explore for 5 min.

Statistical Analysis

Two-way and three-way repeated measures analyses of variance were performed for CSDS, ICSS, and passive-avoidance data. Significant analysis of variance results were analyzed further with Bonferroni post hoc tests. Effects of ketamine on SI were analyzed with preplanned contrasts (Bonferroni tests) between control and defeated mice within each treatment group, based on a specific a priori hypothesis that ketamine treatment would mitigate depressive-like behavior in defeated mice. Effects on EPM behavior were analyzed using Student *t* test. The SI and EPM tests were videotaped and scored by raters blinded to treatment conditions.

Results

The effects of social defeat on ICSS thresholds were evaluated after each episode of defeat, enabling tracking of changes in responsiveness across the entire CSDS regimen (Figure 1A,B). To facilitate a side-by-side comparison of interval duration effects