Published in final edited form as:

Biol Psychiatry. 2014 October 1; 76(7): 542–549. doi:10.1016/j.biopsych.2014.01.013.

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

Andre Der-Avakian¹, Michelle S Mazei-Robison², James P Kesby¹, Eric J Nestler³, and Athina Markou^{1,*}

¹Department of Psychiatry; University of California San Diego, La Jolla, CA 92093-0603; USA

²Department of Physiology; Michigan State University, East Lansing, MI 48824; USA

³Fishberg Department of Neuroscience and Friedman Brain Institute; Icahn School of Medicine at Mount Sinai, New York, NY 10029; USA

Abstract

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 depression risk, although the effect of social stress on brain reward function remains incompletely understood.

Methods—We assessed: 1) brain reward function in rats (using the intracranial self-stimulation procedure) and protein levels of brain-derived neurotrophic factor (BDNF) and related signaling molecules in response to chronic social defeat; 2) brain reward function during social defeat and chronic treatment with the antidepressants fluoxetine (5 mg/kg/day) or 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, while resilient rats showed no long-term brain reward deficits. In the ventral tegmental area (VTA), social defeat, regardless of susceptibility or resilience, decreased and increased BDNF and phosphorylated AKT, respectively, whereas only susceptibility was associated with increased phosphorylated mammalian target of rapamycin (mTOR). 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.

© 2014 Society of Biological Psychiatry. Published by Elsevier Inc. All rights reserved.

*Address correspondence to: Athina Markou, Ph.D., Department of Psychiatry, M/C 0603, University of California San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0603, Tel: (858) 534-1572, Fax: (858) 534-9917, amarkou@ucsd.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

FINANCIAL DISCLOSURES: AD, MSM-R and JPK report no biomedical financial interests or potential conflicts of interest.

Conclusions—These results suggest that the differential persistent anhedonic response to psychosocial stress may be mediated by VTA signaling molecules independent of BDNF, and indicate that greater stress-induced anhedonia is associated with antidepressant treatment resistance. 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.

Keywords

Anhedonia; BDNF; Depression; ICSS; Stress; mTOR

INTRODUCTION

Mood disorders, like 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 even after antidepressant treatment (3).

Exposure to stress precipitates the development of neuropsychiatric disorders characterized by anhedonia (4, 5). Studies in healthy humans and non-human animals demonstrated that stress induced 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). Thus, this procedure is considered analogous to psychosocial stressors in humans.

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, others 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 to animals with low exploratory behaviors and high corticosterone levels, respectively, 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). Indeed, in humans, stress triggers depressive symptoms in vulnerable individuals, while resilient individuals adopt active coping strategies that promote healthy outcomes (23, 24). Thus, animal procedures modeling susceptibility and resilience to stress-induced anhedonia, like those described above, can provide insight into the factors that mediate stress-induced psychopathology.

Anhedonia reflects dysfunctional brain reward circuitries (25–27), yet there have been surprisingly few direct studies of the impact of social defeat on brain reward function. The goals of the present studies 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 (SSRI) fluoxetine or the tricyclic antidepressant (TCA) 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 reliably quantify the development and reversal of anhedonia after chronic stress and antidepressant administration, respectively. Furthermore, we 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 (NAc), central nucleus of the amygdala (CeA) 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, NC, USA) were used as intruders and residents, respectively, for the social defeat procedure (see below and Supplemental Methods 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

ICSS surgery and apparatus details are in the Supplemental Methods and previous publications (e.g., 28, 29). 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 Plexiglas operant testing chambers containing a metal wheel manipulandum (Med Associates; St. Albans, VT, USA) and connected to constant current stimulators (Model 1200; Stimtek; Acton, MA, USA) that delivered electrical stimulation upon 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 and colleagues (30) (for details, see Supplemental Methods and 28, 29). Each trial initiated with rats receiving a non-contingent stimulation (100 Hz electrical pulse ranging between 100–250 μ A) 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 non-contingent 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 non-contingent 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). 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 (61×43×20 cm) behind a perforated Plexiglas partition physically separating the rats, with food and water available *ad libitum*. At 8:00 h the following day, the females and partitions were removed. Social defeat was defined as the intruder displaying a defensive, supine posture for 3 consecutive seconds. 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 following day's social defeat session, which 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 prior to 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 then returned to their original vivarium room that was separate from the residents' vivarium room and single-housed; ICSS thresholds were assessed for an additional 21 days. Twenty-four hr after the final ICSS test, rats were euthanized and the VTA, NAc, CeA and whole hippocampus were dissected, prepared and analyzed by Western blot for BDNF, insulin receptor substrate 2 (IRS2), and phosphorylated AKT, mammalian target of rapamycin (mTOR) and ERK1,2 proteins as described previously (see Supplemental Methods and 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. Thirty min after ICSS testing beginning on social defeat day 14, rats were administered either 5 mg/kg fluoxetine or vehicle daily for 14 days. ICSS testing continued for 7 to 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 hr later (see Supplemental Methods 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 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 (generously donated by Eli Lilly and Co., Indianapolis, IN, USA) and desipramine hydrochloride (Sigma Aldrich, St. Louis, MO, USA) were mixed fresh daily, dissolved in 0.9% sterile saline (vehicle), and administered i.p. in a volume of 3 ml/kg. The 5 mg/kg fluoxetine dose was selected based on reports that higher doses elevated thresholds in naïve rats (32, 33). The 10 mg/kg desipramine dose was selected based on reports that this dose did not alter thresholds in naïve rats, but decreased cocaine withdrawal-induced threshold elevations, reflecting an antidepressant-like effect (34, 35).

Statistical Analyses

ICSS thresholds were calculated as a percentage of 5-day average baseline thresholds before stress (see Supplemental Results) and analyzed with a mixed-design analysis of variance (ANOVA) with *Stress* (i.e., social defeat, control; between-subjects) and *Days* (within-subjects) as factors. *Stress* groups were further categorized using two independent statistical criteria. Rats with average thresholds during social defeat (Experiment 1: days 17–21; Experiments 2 and 3: days 10–14) within or greater than two standard deviations above the 5-day average baseline thresholds were categorized as resilient or susceptible, respectively (Criterion 1). Separately, two-step cluster analysis using Schwarz's Bayesian Criterion was performed with 5-day average stress-induced thresholds as dependent variables to categorize resilient and susceptible rats (Criterion 2). Both criteria were used and yielded identical categorizations of susceptible and resilient rats. *Stress Response* (i.e., susceptible, resilient, control; between-subjects) was analyzed with an ANOVA. Antidepressant-treated susceptible rats were categorized into two subgroups after treatment using the same Criterion 1 described above. Antidepressant treatment was considered effective or ineffective if average thresholds (antidepressant days 10–14) were within or greater than two standard deviations above baseline levels, respectively. Data were analyzed with an ANOVA with *Antidepressant Response* (between-subjects) and *Days* as factors. For Experiments 2 and 3, multiple linear regressions for fluoxetine, desipramine and vehicle (pooled) treatments were conducted in susceptible rats only between average stress-induced (social defeat days 10–14) and antidepressant/vehicle-induced (antidepressant days 10–14) thresholds. Western blot data were analyzed with a *t*-test between *Stress* groups (i.e., social defeat, control) and an ANOVA between *Stress Response* groups (i.e., susceptible, resilient, control). Forced swim test immobility counts were analyzed with an ANOVA and a Pearson's correlational analysis with ICSS thresholds measured prior to habituation and test sessions as variables. Significant main effects and interactions were further analyzed with pairwise *t*-tests. The significance level was $\alpha=0.05$.

RESULTS

Experiment 1: Effects of chronic social defeat on brain reward function

Social defeat elevated reward thresholds compared to controls [*Stress* $\times$ *Day* interaction: $F_{41,1025}=4.98$, $p<0.001$]. *Post hoc* analyses revealed significant stress-induced threshold elevations that returned to baseline after termination of social defeat (Figure 1A). Cluster analysis of social defeat and control groups revealed the existence of two discrete clusters; all resilient rats based on Criterion 1 (i.e., stress-induced threshold elevations within two standard deviations above baseline levels) were grouped into one cluster together with all control rats, while all susceptible rats based on Criterion 1 (i.e., stress-induced threshold elevations greater than two standard deviations above baseline levels) were grouped into a second cluster, confirming the distinctiveness of both stress subgroups regardless of the independent grouping criterion used. Furthermore, all susceptible rats classified using either of the two criteria above notably had threshold elevations greater than three standard deviations beyond baseline levels, indicating that a potential third criterion of three standard deviations for the susceptible group would have yielded identical findings. When categorized into resilient and susceptible subgroups based on either criterion, ANOVA confirmed a significant *Stress Response* $\times$ *Day* interaction [$F_{82,984}=4.04$, $p<0.001$]. *Post hoc* tests revealed significant stress-induced threshold elevations in susceptible rats during and after social defeat, while thresholds in resilient rats were only acutely elevated, compared to controls (Figure 1B).

In the VTA, social defeat decreased BDNF [$t_{25}=2.22$, $p<0.05$] and increased pAKT [$t_{25}=3.0$, $p<0.01$], compared to controls; measurable protein levels did not differ between susceptible and resilient rats (Figure 1C). pmTOR was increased in susceptible, but not resilient, rats [$F_{2,26}=5.25$, $p<0.05$]. No significant effects emerged in the NAc, CeA and hippocampus (see Supplemental Figure S1).

Experiment 2: Effects of repeated fluoxetine treatment on social defeat-induced deficits in brain reward function

Social defeat elevated reward thresholds compared to controls [*Stress* main effect: $F_{1,45}=14.37$, $p<0.001$] (Figure 2A). Cluster analysis revealed the existence of two distinct clusters; rats categorized as resilient or susceptible using the two standard deviation Criterion 1 were grouped into separate clusters, confirming the distinctiveness of both stress subgroups regardless of the independent grouping criterion used. All control rats were grouped into the resilient cluster. Seventeen of 25 (68%) susceptible rats characterized using either of the two criteria above had threshold elevations greater than three standard deviations beyond baseline levels. When categorized into resilient and susceptible subgroups based on either criterion, ANOVA revealed significant stress-induced threshold elevations in susceptible, but not resilient, rats compared to controls [*Stress Response* main effect: $F_{2,44}=27.58$, $p<0.001$] (Figure 2B).

Of the susceptible rats, fluoxetine was effective in 7 rats and ineffective in 9 rats based on the *a priori* criterion that threshold elevations fell to within or remained elevated beyond two standard deviations above baseline levels, respectively [*Fluoxetine Response* main

effect: $F_{2,22}=8.45, p<0.01$]. *Post hoc* analyses confirmed that Susceptible/Vehicle and Susceptible/Ineffective fluoxetine rats had significantly greater threshold elevations than Susceptible/Effective fluoxetine rats (Figure 2C). Linear regression analysis revealed a significant positive correlation between thresholds in response to stress (days 10–14) and fluoxetine treatment (days 24–28) [$F_{1,14}=16.46, p<0.01$], indicating that greater stress-induced threshold elevations in susceptible rats were associated with a lower likelihood of fluoxetine-induced reversal of threshold elevations (Figure 4). Fluoxetine did not affect thresholds in resilient or control rats (data not shown).

During the forced swim habituation session, a strong trend emerged for a *Stress Response* $\times$ *Antidepressant* interaction [$F_{2,41}=2.92, p=0.065$]. *Post hoc* analyses confirmed that fluoxetine treatment significantly decreased immobility in control, but not susceptible or resilient rats (Figure 2D). In addition, there was significantly less immobility in vehicle-treated susceptible, but not resilient, rats compared to vehicle-treated controls. There was no significant correlation between immobility counts and ICSS thresholds and no significant effects emerged during the next day's test session (data not shown).

Experiment 3: Effects of repeated desipramine treatment on social defeat-induced deficits in brain reward function

Social defeat elevated reward thresholds compared to controls [*Stress* main effect: $F_{1,53}=16.30, p<0.001$] (Figure 3A). Cluster analysis revealed the existence of two distinct clusters; rats categorized as resilient or susceptible using the two standard deviation Criterion 1 were grouped into separate clusters, confirming the distinctiveness of both stress subgroups regardless of the independent grouping criterion used. All control rats were grouped into the resilient cluster. Nineteen of 23 (83%) susceptible rats characterized using either of the two criteria above had threshold elevations greater than three standard deviations beyond baseline levels. When categorized into resilient and susceptible subgroups based on either criterion, ANOVA revealed significant stress-induced threshold elevations in susceptible, but not resilient, rats compared to controls [*Stress Response* main effect $F_{2,52}=42.64, p<0.001$] (Figure 3B).

Of the susceptible rats, desipramine was effective in 7 rats and ineffective in 8 rats based on the *a priori* criterion that threshold elevations fell to within or remained elevated beyond two standard deviations above baseline levels, respectively [*Desipramine Response* main effect: $F_{2,19}=6.30, p<0.01$]. *Post hoc* analyses confirmed that Susceptible/Vehicle and Susceptible/Ineffective desipramine rats had significantly greater threshold elevations than Susceptible/Effective desipramine rats (Figure 3C). Linear regression analysis revealed a significant positive correlation between thresholds in response to stress (days 10–14) and desipramine treatment (days 24–28) [$F_{1,13}=7.01, p<0.05$], indicating that greater stress-induced threshold elevations in susceptible rats were associated with a lower likelihood of desipramine-induced reversal of threshold elevations (Figure 4). No significant correlation emerged between thresholds in response to stress and subsequent vehicle treatment, indicating that stress-induced thresholds remained elevated in vehicle-treated susceptible rats (Figure 4). Desipramine did not affect thresholds in resilient or control rats (data not shown).

DISCUSSION

Chronic exposure to social defeat immediately elevated ICSS reward thresholds in all rats, reflecting a stress-induced decrease in brain reward function. Thresholds remained elevated in a subset of susceptible rats, whereas thresholds in resilient rats were only acutely elevated during the initial four days of social defeat and were subsequently unaffected despite ongoing stress exposure. While stress-induced thresholds remained elevated in all susceptible rats, larger threshold elevations were associated with a diminished response to antidepressant treatment. Thus, the stress-induced anhedonia procedure presented here may provide a translational model with relevance to key features of MDD, including clinical observations that stress precipitates MDD in a subset of vulnerable individuals (23, 24), and increased anhedonia severity confers resistance to antidepressant treatment (36–38).

Susceptible rats displayed reward threshold elevations immediately and persistently throughout the 21-day stress and 21-day post-stress periods. Resilient rats also displayed an immediate elevation of reward thresholds, reflecting an acute stress response, but in contrast to susceptible rats, thresholds quickly returned to baseline despite continued social defeat. These divergent responses highlight one advantage of the ICSS procedure that is difficult to reproduce with other animal procedures; that is, observation of temporal changes in anhedonia and the development of susceptibility and resilience throughout the stress experience. Importantly, two independent statistical criteria were used to determine susceptibility and resilience, both of which classified rats identically across all three experiments. Furthermore, cluster analyses from all three experiments were unable to distinguish between resilient and control rats, suggesting that brain reward function was indeed unimpaired in resilient rats exposed to social defeat. Thus, defining susceptibility and resilience was not dependent on the statistical method used, as classification methods determined by the experimenter (two standard deviation Criterion 1) or completely blind to the experimenter (cluster analysis Criterion 2) yielded identical results.

Susceptibility and resilience is likely not due to differences in resident aggression, as susceptible and resilient rats had similar numbers of injuries and time spent in physical contact with the residents (see Supplemental Table S1). It should be noted that aggression was not quantified when intruders were housed behind the protective barrier, and likely varied across residents each day. However, intruder/resident pairings were alternated daily and each intruder (susceptible and resilient) was exposed to each resident only once throughout each experiment. Thus, it is unlikely that variation in aggression throughout each day contributed to overall susceptibility and resilience. Furthermore, both subgroups of stressed rats similarly lost weight during social defeat (see Supplemental Figures S2–S4), again suggesting similar stress experiences. Nonetheless, assessment of physiological stress markers is warranted to confirm stress severity. Similarities in the glucocorticoid response to social defeat between susceptible and resilient mice have been reported (15), although different hypothalamic-pituitary-adrenal axis responses were also observed in rats with different forced swim responses to social defeat (39).

Three weeks after termination of social defeat, susceptible rats had elevated pmTOR levels in the VTA relative to controls. mTOR is a signaling molecule that regulates cell

metabolism, growth, proliferation and survival (40). Because stimulation of VTA dopaminergic neurons was shown to underlie susceptible behavior in socially defeated mice (15, 22) and stress increases synaptic strength at VTA dopaminergic neurons (41), chronically increased pmTOR signaling in the VTA may possibly facilitate the firing of dopaminergic neurons that contributes to susceptible behavior. However, because pmTOR was intermediately expressed in resilient rats compared to susceptible and control rats, further investigation may elucidate the exact role of this signaling molecule in regulating stress-induced brain reward deficits.

Interestingly, phosphorylation of AKT, an upstream regulator of mTOR, was also increased 3 weeks post-stress, albeit similarly between susceptible and resilient rats. Previous work in mice showed that chronic social defeat decreased pAKT in the VTA and that this decrease mediated susceptibility (42). This observation raises the possibility that the pAKT increase observed here in both susceptible and resilient subgroups represents an adaptive response. Similarly, BDNF levels were equally decreased in susceptible and resilient rats 3 weeks post-stress, consistent with a previous report that a similar stress procedure in rats decreased VTA BDNF levels (14). However, chronic social defeat in mice induced BDNF signaling from VTA to NAc in susceptible mice only (15, 43), suggesting that the decrease observed here, like the increase in pAKT, may be adaptive. Alternatively, BDNF and pAKT expression patterns in the present study may represent post-stress recovery-induced changes that are independent of any putative changes during stress exposure. Nonetheless, our results show that chronic adaptations in VTA BDNF-AKT signaling *per se* appear to be independent of persistent anhedonic behavior in our rat model. Moreover, chronic stress exposure had no long-term effects on IRS2 and pERK1,2, two intracellular signaling molecules involved in neurotrophin-mediated neuronal survival.

Clinically, the persistence of anhedonia makes it one of the most treatment-resistant symptoms of MDD (44). In humans, 47–52% of patients with MDD positively responded to an initial antidepressant medication (45, 46). In the present studies, antidepressant treatment was considered effective if reward thresholds returned to within two standard deviations of average pre-stress baseline levels (i.e., similar to resilient and control rats). In susceptible rats, thresholds returned to baseline in 44% treated with fluoxetine and 47% treated with desipramine, reflecting an antidepressant-like response. By contrast, thresholds remained elevated in vehicle-treated rats. Interestingly, the significant correlation between reward thresholds after stress and antidepressant treatment indicates that susceptible rats for which antidepressant treatment was effective had relatively lower stress-induced threshold elevations before antidepressant treatment compared to susceptible rats that were unaffected by antidepressant treatment. This pattern was observed for both fluoxetine and desipramine and is consistent with clinical observations that greater anhedonia severity predicts poorer antidepressant response (36–38, 47). Although the reported clinical studies did not differentiate between the different aspects of reward processing deficits that are often indiscriminately measured in humans, ICSS of the lateral hypothalamus of rats, which mediates motivated behaviors (48, 49), suggests that antidepressant efficacy may be associated with degree of avolition. Thus, consideration of motivational deficits may provide insights about the neurobiological factors underlying antidepressant resistance.

Finally, we assessed whether stress and antidepressant treatment altered forced swim test behavior. Originally developed to screen novel antidepressant medications, the forced swim test is widely used as a putative assessment of depression-like behavior, with increased immobility often interpreted as behavioral despair. The forced swim response to social defeat was assessed previously with mixed results. Some studies demonstrated increased immobility after social defeat (12, 13, 18), though others showed no effect (13, 15, 50) or even decreased immobility after stress (51). Here, fluoxetine decreased immobility in non-stressed rats compared to vehicle treatment, indicating that the swim test was effective in detecting SSRI activity, for which it was originally designed. However, immobility was also decreased in susceptible rats compared to controls that received vehicle treatment and was similar between susceptible and resilient rats. Because social defeat decreased immobility (an antidepressant-like effect), any potential further change in immobility in either direction with antidepressant treatment would be uninterpretable. Accordingly, the forced swim test was not repeated in the desipramine study. Interestingly, no correlation emerged between immobility and reward thresholds that were previously assessed. One explanation for this discrepancy is that the ICSS and forced swim test procedures may reflect two unique aspects of depression-like behavior – respectively, anhedonia and behavioral despair – that may not always correlate. Interestingly, chronic variable stress also decreased immobility in the forced swim test, an effect that was attributed to increased impulsivity (52, 53). Moreover, impulsivity correlates strongly with anhedonia and avolition in depressive disorders (54). Thus, increased impulsivity in susceptible rats may have contributed to decreased immobility, although this speculation requires further investigation.

Social stress is a ubiquitous construct that may or may not have detrimental consequences depending on individual characteristics. Thus, investigating the factors that underlie susceptibility and resilience to stress may provide a more promising means of understanding stress-induced psychopathology compared to investigating stress *per se* (55). Similarly, low response and remission rates of currently available antidepressant medications suggest that investigating the factors that underlie treatment response and resistance using procedures and methods of analyses as described above may facilitate the development of more effective treatment strategies than those currently available.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by National Institutes of Health National Research Service Award Individual Postdoctoral Fellowship F32MH080585 (AD); Interdisciplinary Research Fellowship in NeuroAIDS MH81482 (JPK); and grants 2R01 MH062527 (AM), R01 MH51399 (EJN) and P50 MH096890 (EJN). The authors wish to thank Mr. Michael Arends for editorial assistance and Jessica Benedict, Angelica Campos, Kimberley Edwards, Tania Karapetian, Brian Kwan and Edwin Obana for technical assistance.

AM has received contract research support from Bristol-Myers Squibb Co., Forest Laboratories and Astra-Zeneca, and honoraria/consulting fees from AbbVie during the past 2 years. AM has a patent on the use of metabotropic glutamate compounds for the treatment of nicotine dependence that is unrelated to the present research. EJN is a consultant to Merck Research Laboratories and PsychoGenics, Inc. and has received contract research support from Johnson & Johnson during the past 2 years.

Abbreviations

ANOVA	analysis of variance
BDNF	brain-derived neurotrophic factor
CeA	central nucleus of the amygdala
ICSS	intracranial self-stimulation
IRS2	insulin receptor substrate 2
MDD	major depressive disorder
mTOR	mammalian target of rapamycin
NAc	nucleus accumbens
SSRI	selective serotonin reuptake inhibitor
TCA	tricyclic antidepressant
VTA	ventral tegmental area

REFERENCES

1. American Psychiatric Association. Diagnostic and statistical manual of mental disorders: DSM-5. 5th ed.. Arlington, VA: American Psychiatric Association; 2013.
2. Loas G. Vulnerability to depression: a model centered on anhedonia. *J Affect Disord.* 1996; 41:39–53. [PubMed: 8938204]
3. Schrader GD. Does anhedonia correlate with depression severity in chronic depression? *Compr Psychiatry.* 1997; 38:260–263. [PubMed: 9298317]
4. Charney DS, Manji HK. Life stress, genes, and depression: multiple pathways lead to increased risk and new opportunities for intervention. *Sci STKE.* 2004; 2004:re5. [PubMed: 15039492]
5. Kendler KS, Karkowski LM, Prescott CA. Causal relationship between stressful life events and the onset of major depression. *Am J Psychiatry.* 1999; 156:837–841. [PubMed: 10360120]
6. Zacharko RM, Bowers WJ, Kokkinidis L, Anisman H. Region-specific reductions of intracranial self-stimulation after uncontrollable stress: possible effects on reward processes. *Behav Brain Res.* 1983; 9:129–141. [PubMed: 6603854]
7. Bogdan R, Pizzagalli DA. Acute stress reduces reward responsiveness: implications for depression. *Biol Psychiatry.* 2006; 60:1147–1154. [PubMed: 16806107]
8. Brown GW, Harris TO, Hepworth C. Loss, humiliation and entrapment among women developing depression: a patient and non-patient comparison. *Psychol Med.* 1995; 25:7–21. [PubMed: 7792364]
9. Gilbert P, Allan S, Brough S, Melley S, Miles JN. Relationship of anhedonia and anxiety to social rank, defeat and entrapment. *J Affect Disord.* 2002; 71:141–151. [PubMed: 12167510]
10. Allan S, Gilbert P. Submissive behaviour and psychopathology. *Br J Clin Psychol.* 1997; 36(Pt 4): 467–488. [PubMed: 9403141]
11. Miczek KA, Yap JJ, Covington HE 3rd. Social stress, therapeutics and drug abuse: preclinical models of escalated and depressed intake. *Pharmacol Ther.* 2008; 120:102–128. [PubMed: 18789966]
12. Rygula R, Abumaria N, Havemann-Reinecke U, Ruther E, Hiemke C, Zernig G, et al. Pharmacological validation of a chronic social stress model of depression in rats: effects of reboxetine, haloperidol and diazepam. *Behav Pharmacol.* 2008; 19:183–196. [PubMed: 18469536]
13. Covington HE 3rd, Vialou VF, LaPlant Q, Ohnishi YN, Nestler EJ. Hippocampal-dependent antidepressant-like activity of histone deacetylase inhibition. *Neurosci Lett.* 2011; 493:122–126. [PubMed: 21335060]

14. Miczek KA, Nikulina EM, Shimamoto A, Covington HE 3rd. Escalated or suppressed cocaine reward, tegmental BDNF, and accumbal dopamine caused by episodic versus continuous social stress in rats. *J Neurosci.* 2011; 31:9848–9857. [PubMed: 21734276]
15. Krishnan V, Han MH, Graham DL, Berton O, Renthal W, Russo SJ, et al. Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. *Cell.* 2007; 131:391–404. [PubMed: 17956738]
16. Von Frijtag JC, Reijmers LG, Van der Harst JE, Leus IE, Van den Bos R, Spruijt BM. Defeat followed by individual housing results in long-term impaired reward- and cognition-related behaviours in rats. *Behav Brain Res.* 2000; 117:137–146. [PubMed: 11099767]
17. Kanarik M, Althoff A, Matrov D, Koiv K, Sharp T, Panksepp J, et al. Brain responses to chronic social defeat stress: effects on regional oxidative metabolism as a function of a hedonic trait, and gene expression in susceptible and resilient rats. *Eur Neuropsychopharmacol.* 2011; 21:92–107. [PubMed: 20656462]
18. Hollis F, Wang H, Dietz D, Gunjan A, Kabbaj M. The effects of repeated social defeat on long-term depressive-like behavior and short-term histone modifications in the hippocampus in male Sprague-Dawley rats. *Psychopharmacology (Berl).* 2010; 211:69–77. [PubMed: 20454892]
19. Hollis F, Duclot F, Gunjan A, Kabbaj M. Individual differences in the effect of social defeat on anhedonia and histone acetylation in the rat hippocampus. *Horm Behav.* 2011; 59:331–337. [PubMed: 20851702]
20. Bowens N, Heydendael W, Bhatnagar S, Jacobson L. Lack of elevations in glucocorticoids correlates with dysphoria-like behavior after repeated social defeat. *Physiol Behav.* 2012; 105:958–965. [PubMed: 22108507]
21. Vialou V, Robison AJ, Laplant QC, Covington HE 3rd, Dietz DM, Ohnishi YN, et al. DeltaFosB in brain reward circuits mediates resilience to stress and antidepressant responses. *Nat Neurosci.* 2010; 13:745–752. [PubMed: 20473292]
22. Chaudhury D, Walsh JJ, Friedman AK, Juarez B, Ku SM, Koo JW, et al. Rapid regulation of depression-related behaviours by control of midbrain dopamine neurons. *Nature.* 2013; 493:532–536. [PubMed: 23235832]
23. Zisook S, Paulus M, Shuchter SR, Judd LL. The many faces of depression following spousal bereavement. *J Affect Disord.* 1997; 45:85–94. discussion 94–85. [PubMed: 9268778]
24. Bonanno GA, Wortman CB, Lehman DR, Tweed RG, Haring M, Sonnega J, et al. Resilience to loss and chronic grief: a prospective study from preloss to 18-months postloss. *J Pers Soc Psychol.* 2002; 83:1150–1164. [PubMed: 12416919]
25. Der-Avakian A, Markou A. The neurobiology of anhedonia and other reward-related deficits. *Trends Neurosci.* 2012; 35:68–77. [PubMed: 22177980]
26. Gorwood P. Neurobiological mechanisms of anhedonia. *Dialogues Clin Neurosci.* 2008; 10:291–299. [PubMed: 18979942]
27. Keedwell PA, Andrew C, Williams SC, Brammer MJ, Phillips ML. The neural correlates of anhedonia in major depressive disorder. *Biol Psychiatry.* 2005; 58:843–853. [PubMed: 16043128]
28. Der-Avakian A, Markou A. Neonatal maternal separation exacerbates the reward-enhancing effect of acute amphetamine administration and the anhedonic effect of repeated social defeat in adult rats. *Neuroscience.* 2010; 170:1189–1198. [PubMed: 20691770]
29. Markou A, Koob GF. Construct validity of a self-stimulation threshold paradigm: effects of reward and performance manipulations. *Physiol Behav.* 1992; 51:111–119. [PubMed: 1741436]
30. Kornetsky C, Esposito RU, McLean S, Jacobson JO. Intracranial self-stimulation thresholds: a model for the hedonic effects of drugs of abuse. *Arch Gen Psychiatry.* 1979; 36:289–292. [PubMed: 420547]
31. Mazei-Robison MS, Koo JW, Friedman AK, Lansink CS, Robison AJ, Vinish M, et al. Role for mTOR signaling and neuronal activity in morphine-induced adaptations in ventral tegmental area dopamine neurons. *Neuron.* 2011; 72:977–990. [PubMed: 22196333]
32. Harrison AA, Markou A. Serotonergic manipulations both potentiate and reduce brain stimulation reward in rats: involvement of serotonin-1A receptors. *J Pharmacol Exp Ther.* 2001; 297:316–325. [PubMed: 11259559]

33. Lin D, Koob GF, Markou A. Differential effects of withdrawal from chronic amphetamine or fluoxetine administration on brain stimulation reward in the rat--interactions between the two drugs. *Psychopharmacology (Berl)*. 1999; 145:283–294. [PubMed: 10494577]
34. Markou A, Hauger RL, Koob GF. Desmethylimipramine attenuates cocaine withdrawal in rats. *Psychopharmacology (Berl)*. 1992; 109:305–314. [PubMed: 1365631]
35. Phillips AG, Blaha CD, Fibiger HC. Neurochemical correlates of brain-stimulation reward measured by ex vivo and in vivo analyses. *Neurosci Biobehav Rev*. 1989; 13:99–104. [PubMed: 2530478]
36. Friedman ES, Davis LL, Zisook S, Wisniewski SR, Trivedi MH, Fava M, et al. Baseline depression severity as a predictor of single and combination antidepressant treatment outcome: results from the CO-MED trial. *Eur Neuropsychopharmacol*. 2012; 22:183–199. [PubMed: 21920711]
37. McMakin DL, Olino TM, Porta G, Dietz LJ, Emslie G, Clarke G, et al. Anhedonia predicts poorer recovery among youth with selective serotonin reuptake inhibitor treatment-resistant depression. *J Am Acad Child Adolesc Psychiatry*. 2012; 51:404–411. [PubMed: 22449646]
38. Warden D, Rush AJ, Wisniewski SR, Lesser IM, Kornstein SG, Balasubramani GK, et al. What predicts attrition in second step medication treatments for depression?: a STAR*D Report. *Int J Neuropsychopharmacol*. 2009; 12:459–473. [PubMed: 18611293]
39. Wood SK, Walker HE, Valentino RJ, Bhatnagar S. Individual differences in reactivity to social stress predict susceptibility and resilience to a depressive phenotype: role of corticotropin-releasing factor. *Endocrinology*. 2010; 151:1795–1805. [PubMed: 20160137]
40. Laplante M, Sabatini DM. mTOR signaling at a glance. *J Cell Sci*. 2009; 122:3589–3594. [PubMed: 19812304]
41. Saal D, Dong Y, Bonci A, Malenka RC. Drugs of abuse and stress trigger a common synaptic adaptation in dopamine neurons. *Neuron*. 2003; 37:577–582. [PubMed: 12597856]
42. Krishnan V, Han MH, Mazei-Robison M, Iniguez SD, Ables JL, Vialou V, et al. AKT signaling within the ventral tegmental area regulates cellular and behavioral responses to stressful stimuli. *Biol Psychiatry*. 2008; 64:691–700. [PubMed: 18639865]
43. Berton O, McClung CA, Dileone RJ, Krishnan V, Renthall W, Russo SJ, et al. Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. *Science*. 2006; 311:864–868. [PubMed: 16469931]
44. Boyer P, Tassin JP, Falissart B, Troy S. Sequential improvement of anxiety, depression and anhedonia with sertraline treatment in patients with major depression. *J Clin Pharm Ther*. 2000; 25:363–371. [PubMed: 11123488]
45. Trivedi MH, Rush AJ, Wisniewski SR, Nierenberg AA, Warden D, Ritz L, et al. Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: implications for clinical practice. *Am J Psychiatry*. 2006; 163:28–40. [PubMed: 16390886]
46. Rush AJ, Trivedi MH, Stewart JW, Nierenberg AA, Fava M, Kurian BT, et al. Combining medications to enhance depression outcomes (CO-MED): acute and long-term outcomes of a single-blind randomized study. *Am J Psychiatry*. 2011; 168:689–701. [PubMed: 21536692]
47. Klein DF. Endogenomorphic depression. A conceptual and terminological revision. *Arch Gen Psychiatry*. 1974; 31:447–454. [PubMed: 4420562]
48. DiLeone RJ, Georgescu D, Nestler EJ. Lateral hypothalamic neuropeptides in reward and drug addiction. *Life Sci*. 2003; 73:759–768. [PubMed: 12801597]
49. Berridge KC, Valenstein ES. What psychological process mediates feeding evoked by electrical stimulation of the lateral hypothalamus? *Behav Neurosci*. 1991; 105:3–14. [PubMed: 2025391]
50. Slattery DA, Uschold N, Magoni M, Bar J, Popoli M, Neumann ID, et al. Behavioural consequences of two chronic psychosocial stress paradigms: anxiety without depression. *Psychoneuroendocrinology*. 2012; 37:702–714. [PubMed: 21962377]
51. Ver Hoeve ES, Kelly G, Luz S, Ghanshani S, Bhatnagar S. Short-term and long-term effects of repeated social defeat during adolescence or adulthood in female rats. *Neuroscience*. 2013
52. Harro J, Tonissaar M, Eller M, Kask A, Oreland L. Chronic variable stress and partial 5-HT denervation by parachloroamphetamine treatment in the rat: effects on behavior and monoamine neurochemistry. *Brain Res*. 2001; 899:227–239. [PubMed: 11311884]

53. Harro J. Long-term partial 5-HT depletion: interference of anxiety and impulsivity? *Psychopharmacology (Berl)*. 2002; 164:433–434. [PubMed: 12457276]
54. Swann AC, Steinberg JL, Lijffijt M, Moeller FG. Impulsivity: differential relationship to depression and mania in bipolar disorder. *J Affect Disord*. 2008; 106:241–248. [PubMed: 17822778]
55. Strekalova T, Couch Y, Kholod N, Boyks M, Malin D, Leprince P, et al. Update in the methodology of the chronic stress paradigm: internal control matters. *Behav Brain Funct*. 2011; 7:9. [PubMed: 21524310]

Figure 1.

(A) ICSS reward thresholds were significantly elevated in rats during exposure to chronic social defeat (n=13) compared to non-stressed controls (n=14). (B) Reward thresholds remained elevated in a subgroup of rats – termed susceptible (n=6) – after termination of the stressor, while threshold elevations were transient and indistinguishable from non-stressed controls in another subgroup of rats, termed resilient (n=7). Susceptibility and resilience were determined by average threshold elevations during days 17–21 of social defeat being greater than or within two standard deviations of average baseline threshold levels,

respectively (Criterion 1) and by cluster analysis (Criterion 2) (i.e., both criteria characterized stress responsiveness identically for each rat). (C) Three weeks after termination of social defeat, BDNF protein levels were decreased and phosphorylation of AKT and mTOR, expressed as a ratio with total levels of these proteins, were increased in the VTA. Only the pmTOR increase was specific for susceptibility. Levels of total AKT and mTOR were unaffected (not shown). * Reward thresholds in social defeat (A)/ susceptible (B) groups were significantly different from those of controls; @ Reward thresholds in resilient rats were significantly different from those of controls; # Reward thresholds in susceptible rats were significantly different from those of resilient rats; $p < 0.05$.

Figure 2.

(A) ICSS reward thresholds were significantly elevated in rats during exposure to chronic social defeat (n=37) compared to non-stressed controls (n=10). (B) Reward thresholds remained elevated in susceptible rats (n=25) during stress exposure, while threshold elevations returned to baseline in resilient rats (n=12). Susceptibility and resilience were determined by average threshold elevations during days 10–14 of social defeat being greater than or within two standard deviations of average baseline threshold levels, respectively (Criterion 1) and by cluster analysis (Criterion 2) (i.e., both criteria characterized stress responsiveness identically for each rat). * Reward thresholds in social defeat (A)/susceptible (B) groups were significantly different from those of controls; # Reward thresholds in susceptible rats were significantly different from those of resilient rats. (C) Stress-induced threshold elevations were significantly reversed in a subgroup of fluoxetine-treated susceptible rats (n=7) compared to vehicle-treated susceptible rats (n=9) and fluoxetine-treated susceptible rats that did not respond to fluoxetine (n=9). Ineffectiveness and effectiveness of fluoxetine treatment were determined by average threshold elevations during days 24–28 of testing (i.e., days 10–14 of fluoxetine treatment) being greater than or within two standard deviations of average baseline threshold levels, respectively (Criterion 1). * Reward thresholds in “Ineffective fluoxetine” rats were significantly different from those of “Effective fluoxetine” rats; @ Reward thresholds in “Ineffective fluoxetine” rats

were significantly different from those of vehicle-treated rats; # Reward thresholds of “Effective fluoxetine” rats were significantly different from those of vehicle-treated rats. **(D)** Repeated fluoxetine treatment significantly decreased immobility in the forced swim test in non-stressed controls, but not in resilient or susceptible rats. Vehicle-treated susceptible rats were also significantly less immobile than vehicle-treated non-stressed controls ($p<0.05$).

Figure 3.

(A) ICSS reward thresholds were significantly elevated in rats during exposure to chronic social defeat (n=38) compared to non-stressed controls (n=17). (B) Reward thresholds remained elevated in susceptible rats (n=22) during stress exposure, while threshold elevations returned to baseline in resilient rats (n=16). Susceptibility and resilience were determined by average threshold elevations during days 10–14 of social defeat being greater than or within two standard deviations of average baseline threshold levels, respectively (Criterion 1) and by cluster analysis (Criterion 2) (i.e., both criteria characterized stress

responsiveness identically for each rat). * Reward thresholds in social defeat (A)/susceptible (B) groups were significantly different from those of controls; # Reward thresholds in susceptible rats were significantly different from those of resilient rats. (C) Stress-induced threshold elevations were significantly reversed in a subgroup of desipramine-treated susceptible rats (n=7) compared to vehicle-treated susceptible rats (n=7) and desipramine-treated susceptible rats that did not respond to desipramine (n=8). Ineffectiveness and effectiveness of desipramine treatment were determined by average threshold elevations during days 24–28 of testing (i.e., days 10–14 of desipramine treatment) being greater than or within two standard deviations of average baseline threshold levels, respectively (Criterion 1). * Reward thresholds in “Ineffective desipramine” rats were significantly different from those of “Effective desipramine” rats; # Reward thresholds of “Effective desipramine” rats were significantly different from those of vehicle-treated rats ($p<0.05$).

Figure 4.

The degree of stress-induced reward threshold elevations in susceptible rats predicted the subsequent response to antidepressant treatment, with greater stress induced-anhedonia associated with less likelihood of an antidepressant-like effect with fluoxetine or desipramine. In contrast, reward thresholds remained elevated in vehicle-treated susceptible rats throughout testing, regardless of the degree of stress-induced threshold elevations. Data include susceptible rats treated with fluoxetine (Experiment 2), desipramine (Experiment 3) and vehicle (pooled between Experiments 2 and 3). * $p < 0.05$; ** $p < 0.01$ (significant correlation).