Ketamine as a Prophylactic Against Stress-Induced Depressive-Like Behavior

Rebecca A. Brachman, Josephine C. McGowan, Jennifer N. Perusini, Sean C. Lim, Thu Ha Pham, Charlene Faye, Alain M. Gardier, Indira Mendez-David, Denis J. David, René Hen, and Christine A. Denny

ABSTRACT

BACKGROUND: Stress exposure is one of the greatest risk factors for psychiatric illnesses like major depressive disorder and posttraumatic stress disorder. However, not all individuals exposed to stress develop affective disorders. Stress resilience, the ability to experience stress without developing persistent psychopathology, varies from individual to individual. Enhancing stress resilience in at-risk populations could potentially protect against stress-induced psychiatric disorders. Despite this fact, no resilience-enhancing pharmaceuticals have been identified.

METHODS: Using a chronic social defeat (SD) stress model, learned helplessness (LH), and a chronic corticosterone (CORT) model in mice, we tested if ketamine could protect against depressive-like behavior. Mice were administered a single dose of saline or ketamine and then 1 week later were subjected to 2 weeks of SD, LH training, or 3 weeks of CORT.

RESULTS: SD robustly and reliably induced depressive-like behavior in control mice. Mice treated with prophylactic ketamine were protected against the deleterious effects of SD in the forced swim test and in the dominant interaction test. We confirmed these effects in LH and the CORT model. In the LH model, latency to escape was increased following training, and this effect was prevented by ketamine. In the CORT model, a single dose of ketamine blocked stress-induced behavior in the forced swim test, novelty suppressed feeding paradigm, and the sucrose splash test.

CONCLUSIONS: These data show that ketamine can induce persistent stress resilience and, therefore, may be useful in protecting against stress-induced disorders.

Keywords: Depression, Ketamine, Mice, PTSD, Stress, Stress resilience

http://dx.doi.org/10.1016/j.biopsych.2015.04.022

Stress commonly precipitates psychiatric illness, particularly in vulnerable populations. For example, one in five soldiers returns from combat with posttraumatic stress disorder or combat-associated major depressive disorder (MDD) (1). Perhaps more surprising is that many soldiers do not develop psychopathology. While there has been extensive research on factors promoting susceptibility to psychiatric illnesses, few studies have examined what makes individuals resistant or stress resilient. Until recently, the sparse research on stress resilience has been predicated on the assumption that it is a passive property—more or less the absence of the risk factors that make individuals susceptible to stress-induced pathology (2). Recent work in animal models suggests that stress resilience is mediated through active processes and often distinct, parallel mechanisms to those of susceptibility (3–5).

The idea that increasing stress resilience could protect against the development of psychiatric disorders is appealing, but treatments to increase resilience are still in their infancy. Current interventions fall predominantly on the behavioral side, with psychotherapy and exercise being the best available tools to increase resilience (6–8). Rodent studies further support a role for exercise and enriched environment in stress resilience (9–11). Beyond behavioral manipulations in mice, researchers have successfully increased resilience biochemically through viral and transgenic overexpression methods (12), optogenetic activation (4), and chronic blockade of stress hormones (13,14). However, none of these interventions translates to the clinic. Most promisingly, we have identified the immune system as a novel target for enhancing resilience. Our recent work has shown that manipulating leukocytes is sufficient to increase stress resilience (15) and Hodes et al. (16) have shown a similar effect by modulating cytokines. Though these reports suggest that these discoveries are promising, they are not yet clinic ready.

Antidepressants are typically used to treat existing depressive symptoms, but chronic antidepressant treatment also protects against subsequent depressive episodes (17–21). Maintenance treatment in MDD patients is often referred to as prophylaxis against the development of additional depressive episodes (22). Whether this prophylactic effect against symptomatic episodes in disordered individuals extrapolates out to preventing de novo psychiatric disorders remains to be tested.
Ketamine has been shown to have antidepressant effects as rapidly as 2 hours following a single injection in patients with MDD (23). Whereas classic antidepressants require ongoing daily administration to maintain therapeutic efficacy, ketamine has the benefit of being administered as a single dose (23,24). Because ketamine has a window of therapeutic efficacy far beyond its half-life of a few hours (23–25), it is an excellent candidate for a plausible approach to pharmacologically increasing stress resilience.

Therefore, we first utilized social defeat (SD) to examine whether ketamine could increase stress resilience and, thereby protect against de novo induction of psychopathology. We hypothesized that ketamine would confer stress resiliency to mice if administered before stress. We chose to perform SD in 129S6/SvEvTac mice, which robustly and reliably develop a depressive-like phenotype following SD (26). Mice were administered either saline or a single subanesthetic injection of ketamine, and 1 week later, SD was administered to half of the mice. We found that a single injection of ketamine induced robust stress resilience that persisted for at least 3 weeks postinjection. Moreover, we confirmed our effects in two additional models in which depressive/anxious behavior is induced by chronic elevation of glucocorticoids in C57BL/6NTac mice (27) or by repeated, unescapable shocks [learned helplessness (LH)] (28–30).

Again, a single subanesthetic dose of ketamine, administered 4 weeks before behavioral assessment, decreased immobility in the forced swim test (FST) and protected against depressive-like behavior in the novelty suppressed feeding (NSF) paradigm and the sucrose splash test (ST). In the LH model, the latency to escape a shock increases with LH (NSF) paradigm and the sucrose splash test (ST). In the LH model, the latency to escape a shock increases with LH. One week later, mice either remained housed (Ctrl) or underwent SD. After 2 weeks of SD, mice were weighed (Figure S2A in Supplement 1), and behavior was assessed.

Classically, immobility in the FST has been interpreted as an index of hopelessness or a negative mood (31). Rodents given acute or chronic antidepressants exhibit decreased immobility (32). Here, on day 2 of the FST, there was an overall effect of SD on immobility time. Ctrl-saline (Sal) and Ctrl-ketamine (K) mice displayed equal levels of immobility time (Figure 1B). In SD mice, ketamine (SD-K) significantly decreased immobility time when compared with saline (SD-Sal) (Figure 1C,D). These data indicate that ketamine increases resilience to behavioral despair as measured by the FST.

Dominant interaction is a robust way of testing the induction of depressive-like behavior by SD (10) (Figure 1E). As expected, SD-Sal mice spent significantly more time investigating an empty enclosure quadrant than Ctrl-Sal mice (Figure 1F). Ctrl (Sal or K) mice spent an equivalent amount of time investigating the empty enclosure quadrant. SD-K mice exhibited significantly less time investigating the empty enclosure quadrant when compared with SD-Sal mice. Similarly, SD-K mice exhibited a significantly increased willingness to interact with the CD-1 when compared with SD-Sal mice (Figure 1G). There was an overall effect of SD and of ketamine on decreasing the distance traveled, but the interaction was not significant (Figure 1H).

To determine if this exploration deficit extended to neutral environments, open field exploration was investigated in an arena scented with female urine (Figure S3 in Supplement 1). We did not detect any differences in the empty quadrant or the urine quadrant between Ctrl and SD mice. Furthermore, to determine if social avoidance generalized to other mice, we also assessed social interaction with a novel mouse (Figure S4 in Supplement 1). We did not find an effect of SD or ketamine on social interaction. In summary, these data suggest that SD decreases exploration and willingness to interact with a CD-1 aggressor and that prior ketamine administration protects against this deleterious effect of SD on social behavior.

METHODS AND MATERIALS

Mice

Male 129S6/SvEvTac mice were purchased from Taconic (Hudson, New York). CD-1 mice were purchased from Charles River Laboratories (Wilmington, Massachusetts) at 8 to 10 weeks of age and housed individually until the start of SD. The procedures described herein were conducted in accordance with the National Institutes of Health regulations and approved by the Institutional Animal Care and Use Committees of Columbia University and the New York State Psychiatric Institute.

Male C57BL/6NTac mice were purchased from Taconic Farms (Lille Skensved, Denmark) at 8 weeks of age and were housed five per cage before the start of corticosterone (CORT) treatment. All testing was conducted in compliance with the laboratory animal care guidelines and with protocols approved by the Institutional Animal Care and Use Committee (European Directive, 2010/63/EU for the protection of laboratory animals, permissions # 92-256B, authorization ethical committee CEEA n° 26 2012_098).

All mice were housed in a 12-hour (600–1800) light-dark colony room at 22 C. Food and water were provided ad libitum. Behavioral testing was performed during the light phase.

RESULTS

Ketamine Administration Before SD Protects Against the Induction of Depressive-Like Behavior

Mice were administered a single injection of saline or ketamine (30 mg kg⁻¹) (Figure 1A). One week later, mice either remained group housed (Ctrl) or underwent SD. After 2 weeks of SD, mice were weighed (Figure S2A in Supplement 1), and behavior was assessed.

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An Injection of Ketamine Before SD Does Not Impact Anxiety-Like Behavior or Contextual Fear Memory

We next examined the effects of ketamine on anxiety-like behavior and cognitive tests. In the NSF paradigm, we found no significant effect of SD or ketamine on the latency to feed (Figure 2A). In fact, all groups showed similar latencies (Figure 2B). This effect is confounded: despite having comparable body weights before and after SD (Figure 2C), SD mice
lost significantly more weight during the 12-hour fast preceding NSF than Ctrl mice (Figure 2D). Possibly as a result, SD mice ate more in a home cage following NSF when compared with Ctrl mice (Figure 2E). These findings suggest that SD significantly alters metabolism in 129S6/SvEv mice.

We observed a significant effect of SD in an anxiety-related test, the elevated plus maze (EPM). SD mice spent more time in the closed arms than Ctrl mice (Figure 2F). However, there was no significant effect of ketamine in either group. The absence of an effect of ketamine in the EPM is consistent with previous studies (33,34), as it remains to be established if ketamine is as robust an anxiolytic as it is an antidepressant (35).

Finally, we assessed the impact of prior treatment with ketamine on one-shock contextual fear conditioning (CFC) (36,37) (Figure 2G). One group previously found that SD increased context-elicited fear following three-shock CFC (38). However, we chose to utilize a weak CFC training paradigm, as we have previously shown this one-shock CFC paradigm to be sensitive to the ablation of adult hippocampal neurogenesis (36,37) and to SD (26). Here, we found no effect of either SD or ketamine on baseline freezing levels on day 1 of
CFC training (Figure 2H). Ketamine or SD had no effect on freezing during exposure to the fearful context A (Figure 2I) or a novel context B (Figure 2J). Though this does not allow us to assess any stress resilience effect of ketamine, as there is no effect of stress to protect against, it does at least demonstrate that a single injection of ketamine does not appear to interfere with the ability to form contextual memories in mice.

The Ketamine-Induced Improvement Is Dose-Specific

We next examined a dose titration curve of ketamine. Mice were administered 0, 10, 30, or 90 mg kg\(^{-1}\) of ketamine before the start of SD. After 2 weeks of SD, mice underwent the FST and CORT levels were measured following a brief stressor. We replicated our previous SD effect, as SD-Sal mice displayed significantly more immobility time in the FST when compared with Ctrl-Sal (Figure 3A). However, SD-Sal and SD-K (10 mg kg\(^{-1}\)) mice did not differ in immobility time (Figure 3B). SD-K (30 mg kg\(^{-1}\)) mice again displayed significantly less immobility when compared with SD-Sal mice (Figure 3C). SD-Sal and SD-K (90 mg kg\(^{-1}\)) mice did not differ in immobility time (Figure 3D).

As the hypothalamic-pituitary-adrenal (HPA) axis is dysregulated in mice following SD (14), we also tested whether ketamine protected against the deleterious effect of SD on the stress response. Following a brief stressor, SD-Sal mice had significantly lower levels of CORT than Ctrl-Sal mice (Figure 3F), suggesting that SD blunts the response of the HPA axis. However, all ketamine-injected mice did not differ from Ctrl-Sal mice, suggesting that ketamine partially restores the HPA axis. To determine if adult hippocampal neurogenesis

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**Figure 2.** Ketamine does not protect against anxiety-like behavior or impair contextual fear conditioning learning following social defeat (SD). (A,B) In the novelty suppressed feeding (NSF) paradigm, all groups had equivalent average latencies to approach the food pellet. (C) Body weight did not differ between any of the groups before the start of NSF. (D) SD mice lost approximately 25% more body weight than group housed (Ctrl) mice. (E) SD mice consumed significantly more food than Ctrl mice. (F) In the elevated plus maze test, SD mice spent significantly more time in the closed arms when compared with Ctrl mice. (G–J) All groups of mice had comparable levels of freezing during contextual fear conditioning training in context A, following re-exposure to fearful context A, and during exposure to a novel context B. (\(n = 13–15\) male mice per group). Error bars represent ± SEM. **\(p < .01\), ***\(p < .001\). K, ketamine; Sal, saline.
was modulating, as least in part, these effects, we measured maturation of newborn neurons and proliferation of newborn neurons by quantifying the levels of doublecortin and Ki67, respectively. We did not observe an effect of ketamine on adult hippocampal neurogenesis (Figure S5 in Supplement 1). These data suggest that the ketamine improvement in depressive-like behavior may be mediated in part by changes in HPA functionality but not necessarily by adult hippocampal neurogenesis.

Prophylactic Ketamine Alters Fighting Behavior During SD Bouts

To determine if ketamine also affected behavior during SD, we analyzed individual fighting bouts. The total fighting bout length did not differ between groups (Figure S6A in Supplement 1). However, the average immobility during week 2 was significantly decreased in SD-K mice when compared with SD-Sal mice (Figure S6B in Supplement 1). The percent of time vocalizing (Figure S6C in Supplement 1) and number of approaches to the CD-1 (Figure S6D in Supplement 1) did not differ between the groups. These data suggest that mice administered ketamine may not be as fearful of the CD-1 mice and, therefore, spend less time immobile.

We next analyzed the latency of the CD-1 to attack the 129S6/SvEv mouse (Figure S6E–G in Supplement 1). CD-1s comparably attacked SD-Sal and SD-K (10 or 90 mg kg⁻¹) mice. However, at the start of SD, CD-1s attacked SD-K (30 mg kg⁻¹) mice significantly later than SD-Sal mice. These data suggest that perhaps the mice receiving K (30 mg kg⁻¹) have an advantageous ongoing response to SD when compared with Sal mice.

Fluoxetine Treatment Before SD Does Not Protect Against the Induction of Depressive-Like Behavior

We next determined if this protective effect of ketamine extended to other antidepressants. Mice were administered 3 weeks of fluoxetine (Flx) (18 mg kg⁻¹) treatment before the start of SD (Figure 4A; Figure S7 in Supplement 1). On day 2 of the FST, Ctrl-Vehicle (Veh) and Ctrl-Flx displayed equal levels of immobility time (Figure 4B). In SD mice, fluoxetine did not improve immobility time induced by SD (Figure 4C, D). These data indicate that fluoxetine, unlike ketamine, is not capable of preventing stress-induced behavioral despair as measured by the FST.

We also assessed a number of other behaviors following fluoxetine treatment (Figure S8 in Supplement 1). Fluoxetine treatment did not significantly alter anxiety or cognition but did affect metabolism (Figure S8G–S8H in Supplement 1). Interestingly, unlike SD-K (30 mg kg⁻¹) mice, SD-Flx mice do not display differences during SD bouts when compared with
SD-Veh mice (Figure S9 in Supplement 1). These data suggest that fluoxetine treatment cannot protect against depressive-like behavior as ketamine does.

**Ketamine Administered After SD Does Not Improve Depressive-Like Behavior**

To compare the robustness of prophylactic ketamine relative to its typical use as an antidepressant, we next asked if ketamine could improve behavioral despair if administered after SD (Figure 5A). Mice were administered 2 weeks of SD and then received one injection of saline or ketamine the day after the final SD session. On day 2 of the FST, Ctrl-Sal and Ctrl-K mice did not display different immobility time (Figure 5B). SD-Sal and SD-K mice had similar levels of immobility time (Figure 5C). We averaged minutes 3 to 6 and found that SD increased immobility time, but ketamine given after SD did not decrease immobility time (Figure 5D). These data indicate that ketamine more potently decreases behavioral despair in the FST when given as a prophylactic before SD than after SD.

We also assessed a number of other behaviors following ketamine treatment (Figure S10 in Supplement 1). In Ctrl mice,
ketamine decreased the latency to eat in the NSF when compared with saline (Figure S10A in Supplement 1). This effect was abolished in the SD mice, most likely due to weight loss differences between Ctrl and SD mice (Figure S10B–S10C in Supplement 1). Interestingly, ketamine lessened the percentage of weight loss in the SD mice when compared with saline, possibly by protecting against stress-induced hypophagia (Figure S10D in Supplement 1). Ketamine also did not impact CFC learning (Figure S10G in Supplement 1). Most importantly, although we did not detect differences from prophylactic ketamine treatment, we did determine that ketamine administered after SD significantly increases the number of Ki67+ cells in the dentate gyrus (Figure S10K in Supplement 1).

Prophylactic Ketamine Protects Against Learned Helplessness

We hypothesized that ketamine would protect against LH, a paradigm in which a mouse is exposed to inescapable shocks (28–30). Mice were injected with saline or ketamine and administered an inescapable shock stress protocol (LH training) 1 week later (Figure 6A). Two weeks later, mice were administered a shock escape protocol (LH testing) and the latency to escape the shock was measured. The activity in the habituation phase during testing did not differ between mice administered saline or ketamine (Figure 6B). However, mice injected with ketamine had a decreased latency to escape the shock when compared with mice injected with saline (Figure 6C,D). Moreover, the session length was significantly shorter in the ketamine mice than in the saline mice (Figure 6E). These data indicate that ketamine protection is not just limited to SD stress.

Prophylactic Ketamine Protects Against the Depressive-Like Effects of Chronic Corticosterone Treatment

To address whether ketamine was protective in a third stress model, we utilized a mouse model of anxiety/depression based on elevation of glucocorticoids (3 weeks of chronic CORT administration in C57BL/6NTac mice) (27). We tested the protective effects of a chronic fluoxetine treatment (18 mg kg⁻¹ for 3 weeks) or a single injection of ketamine (10, 30, or 90 mg kg⁻¹) given before CORT administration (Figure 7A). We found that ketamine (90 mg kg⁻¹) and fluoxetine prevented the CORT-induced increase in body weight (Figure S11A in Supplement 1).

Both ketamine (90 mg kg⁻¹) and fluoxetine decreased immobility time on day 2 in the FST (Figure 7B,C). Chronic CORT induced depressive-like symptoms (e.g., increased grooming latency) in the ST (Figure 7D). Here, ketamine (90 mg kg⁻¹), but not fluoxetine, prevented the chronic CORT-induced depressive-like phenotype (Figure 7D). These data indicate that the protective effect of ketamine extends to a third depression model.

In the NSF, ketamine (10 and 90 mg kg⁻¹) prevented the chronic CORT-induced increase in latency to feed (Figure 7E; Figure S11 in Supplement 1). However, only ketamine (90 mg kg⁻¹) increased home cage food consumption (Figure 7F). Finally, we assayed anxiety behavior using the EPM (Figure S12 in Supplement 1). CORT-Veh mice spent more time in the closed arms than Veh-Veh mice. Neither ketamine nor fluoxetine robustly protected against this anxiety-like phenotype. In summary, these data suggest that 90 mg kg⁻¹ of ketamine is the most effective dose in protecting against depressive-like behavior following chronic CORT treatment in C57BL/6NTac mice.

Ketamine Administered After Chronic Corticosterone Does Not Improve Depressive-Like Behavior

Finally, as in the SD model, we measured the behavioral impact of ketamine when given after CORT (Figure S13A in Supplement 1). In this experimental design, mice were administered 4 weeks of CORT and then received either one injection of saline or ketamine, or vehicle or fluoxetine for 2 weeks. Here, we utilized the tail suspension test (TST) and the NSF to test the same behavior on multiple occasions. Fluoxetine, but not ketamine, decreased immobility time in the TST at both time points tested following CORT (Figure S13B–S13C in Supplement 1). In the NSF, CORT treatment increased the latency to feed when compared with Veh treatment. Fluoxetine, but not ketamine, decreased the latency.
to feed 14 days, but not 7 days, after the start of treatment (Figure S13D–S13I in Supplement 1). In summary, as previously demonstrated in the SD model, these data further indicate that ketamine more potently improves depressive-like behavior when given as a prophylactic before CORT treatment rather than after CORT treatment.

**DISCUSSION**

Here, we have shown that a single injection of ketamine administered before SD protected mice against stress-induced increased immobility time in the FST. Additionally, ketamine protected mice against stress-induced social avoidance of an aggressor mouse. We found that mice administered ketamine before SD were protected against stress-induced depressive-like behavior, but consistent with the literature definition of stress resilience, their behavior in anxiety tests and levels of adult hippocampal neurogenesis were not significantly altered. Interestingly, in the SD paradigm, only a subanesthetic dose (30 mg kg\(^{-1}\)) of ketamine was found to be effective.

The prophylactic effect of ketamine was recapitulated in two additional models. In LH, ketamine decreased depressive-like, helpless behavior. In the CORT model, ketamine was protective against depressive-like behaviors (FST, ST), anxiety (NSF), and metabolic changes (body weight), albeit at a slightly higher dose (90 mg kg\(^{-1}\)) than in SD or LH. The efficacy of the higher dose in the CORT model is perhaps attributable to mouse strain differences (C57BL/6NTac versus 129S6/SvEv). Nevertheless, the dose administered in the CORT model is in the anesthetic range, whereas the dose in the SD/LH model is subanesthetic. If an equivalent anesthetic dose were required to obtain prophylactic efficacy in humans, acute side effects would need to be considered when developing treatment regimens.

Administration of the classic antidepressant fluoxetine before stress did not consistently or robustly protect against stress-induced depressive-like behavior. In the SD model, fluoxetine did not improve immobility time in the FST, but in the CORT model, fluoxetine protected against immobility time in the FST and body weight alterations. Thus, it remains to be fully determined if antidepressant drugs other than ketamine can protect against depressive-like behavior. Perhaps other drugs may be more useful in protecting against coincident stress-induced pathologies (e.g., anxiety, cognitive deficits, metabolic disturbances).

**Figure 7.** Ketamine (K) protects against depressive-like and anxiety behavior induced with a neuroendocrine model. (A) Experimental paradigm schematic. (B, C) Corticosterone (CORT) mice administered K (90 mg kg\(^{-1}\)) or fluoxetine (Flx) (18 mg kg\(^{-1}\) /day) exhibited significantly reduced immobility in the forced swim test (FST). (D) Chronic CORT increased the latency to groom during the sucrose splash test (ST). In contrast to Flx, K for the highest doses tested (90 mg kg\(^{-1}\)) decreased the latency to groom in the novelty suppressed feeding (NSF). (E) K (90 mg kg\(^{-1}\)) increased home food consumption. (n = 10–15 male mice per group). Error bars represent SEM. *p < .05, **p < .01. EPM, elevated plus maze; Sal, saline; Veh, vehicle.
Though preventing psychopathology has obvious advantages over noncurative medication regimens, we also wanted to assess the relative potencies of ketamine’s protective and antidepressant effects. Interestingly, when ketamine was administered following stress, we did not observe a significant decrease in immobility time in the FST or TST. In our SD model, we utilized a 30 mg kg$^{-1}$ dose, but in the CORT model, we utilized a 10 mg kg$^{-1}$ dose to compare with more recent studies using ketamine as an antidepressant in C57BL mice (12,33). This suggests that the beneficial effects of ketamine on stress-induced pathology may be more robust when given before stress. In contrast, Donahue et al. (12) recently found the converse when they administered ketamine either 1 hour after the final SD session or 24 hours before the first SD session. A high (20 mg kg$^{-1}$)—but not low (2.5 mg kg$^{-1}$)—dose of ketamine following the final SD session attenuated social avoidance but not anhedonia. Conversely, when ketamine (20 mg kg$^{-1}$) was administered before SD, it did not attenuate social avoidance. The lack of effect in their experiments, however, does not mean that ketamine’s protective effect is not as robust as we suggest. The effect of ketamine is less likely effective, as a C57BL/6J strain is utilized in the Donahue et al. (12) study, but as we have shown in the CORT model, a higher dose is necessary for prophylactic efficacy in C57BL mice. For future studies, we believe that a dose titration curve is necessary in each model. Based on our data, we predict that ketamine dosing for prophylactic administration may likely differ from antidepressant administration.

Ketamine-induced resilience is robust and long lasting—persisting at least 3 weeks postinjection in the SD model and 4 weeks postinjection in the CORT model. It is worth noting that as the half-life of ketamine is only a few hours in rodents (39), ketamine is not bioactive at any point during the SD fighting bouts, LH, or CORT administration. Thus, the process by which ketamine protects against depressive-like behavior is necessarily self-maintaining. Further investigation will be required to identify the mechanisms underlying this process. We have shown, however, that ketamine can alter ongoing response to a chronic stressor. In the SD model, our data suggest that ketamine alters the way in which mice react to the fighting bouts, which may contribute to the differences in developing depressive-like behavior at a later time point. Not only do the SD-K (30 mg kg$^{-1}$) mice have a decreased immobility time during the fighting bouts, but also the CD-1 mice attack the SD-K (30 mg kg$^{-1}$) mice at greater latencies. Work done characterizing stress resilience in other models has implicated a series of mechanisms, including adult hippocampal neurogenesis, HPA axis output (10,13,14), ΔFosB expression in the prefrontal cortex (11,40) and striatum (12), activation of the infralimbic cortex and the mesolimbic dopaminergic system (4,5,11,12,41–44), glutamatergic tone (38), and altered leukocyte and cytokine profiles (15,16). Additionally, ketamine has been shown to induce rapid and persistent remodeling of synapses (45). In our model, ketamine administration acutely, but transiently, increased proliferative adult hippocampal neurogenesis. Whether this contributes to mechanisms of prophylactic or antidepressant ketamine remains to be determined. As ketamine prevents SD-induced HPA axis dysregulation, we hypothesize that the HPA axis may partially mediate the differences in how the SD-K mice respond to the SD fighting bouts. Further analysis will be needed to elucidate the mechanisms underlying ketamine’s resilience-enhancing properties. It is worth noting that these mechanisms are likely divergent from those of ketamine’s antidepressant effects.

In summary, these experiments demonstrate that ketamine has a long-lasting resilience-enhancing effect and protects against the deleterious effects of chronic stress on depressive-like behaviors. Because the protective effect of ketamine persists beyond its half-life of 2 to 2.5 hours, assuming the prophylactic effect translates to humans, it is potentially useful as a vaccine-like strategy in at-risk populations where high-stress conditions can be predicted. Active combat soldiers offer a good example of a predictably at-risk patient population. Administration of ketamine before deployment may mitigate the emergence of posttraumatic stress disorder or other stress-related disorders in this vulnerable population. How far out this prophylaxis persists is as of yet unknown. Whether subsequent injections would have a similar, increasing, or deleterious effect on stress resilience also has yet to be tested. If these effects do translate from mice to humans, ketamine may offer a novel, clinic-ready approach to protect and prevent at-risk patients from developing stress-induced disorders.

ACKNOWLEDGMENTS AND DISCLOSURES

RAB was supported by National Institute of Child Health and Human Development T32HD07430. DJD currently was supported by Lundbeck and the Brain & Behavior Research Foundation (formerly National Alliance for Research on Schizophrenia and Depression). The lab UMR S 1178 is funded by Agence Nationale pour la Recherche SAMENTA (ANR-12-SAMA-0007). RH was supported by R37 MH088542; R01 AG043688, and a Hope for Depression Research Foundation grant. CAD was supported by National Institutes of Health T32 MH015174-36, National Institutes of Health DPS OD017908-01, a National Alliance for Research on Schizophrenia and Depression Young Investigator Grant from the Brain & Behavior Research Foundation, and New York Stem Cell Science N13S-006. Cognitive and behavioral phenotyping experiments utilized the facilities of the Rodent Models Neurobehavioral Analysis Core of the Lieber Center for Schizophrenia Research at Columbia University and the New York State Psychiatric Institute.

We thank the animal care facility ANIMEX of SFR-UMRS Institut Paris-Saclay d’Innovation Thérapeutique de Universite Paris-Sud for their technical assistance. We thank members of the Denny and Hen laboratories for insightful comments on this project and manuscript and M. Drew and Z. Donaldson for assistance with statistical analyses. We thank A. Jonathan for his constant assistance in the Rodent Models Neurobehavioral Analysis Core.

DJD serves as a consultant for Lundbeck, Roche, and Servier. RH receives compensation as a consultant for Lundbeck, Servier, and Roche. All other authors report no biomedical financial interests or potential conflicts of interest.

ARTICLE INFORMATION

From the Departments of Neuroscience (RAB) and Psychiatry (JCM, JNP, SCL, RH, CAD), Columbia University, New York; and Division of Integrative Neuroscience (JCM, JNP, SCL, RH, CAD), New York State Psychiatric Institute/Research Foundation for Mental Hygiene, Inc., New York; New York State Psychiatric Institute, New York; Institut National de la Santé et de la Recherche Médicale UMR-S 1178 Santé Mentale et Santé Publique, Université Paris-Sud, Fac Pharmacie, Université Paris Saclay, Châttenay-Malabry, France; and Department of Pharmacology (RH), Columbia University, New York, New York.

Address correspondence to Christine Ann Denny, Ph.D., Columbia University/Research Foundation for Mental Hygiene, Inc., Psychiatry, NYSPH Kolb Research Annex, Room 777, 1051 Riverside Drive, Unit 87, New York, NY 10032-2695; E-mail: cad2125@cumc.columbia.edu.

Received Dec 30, 2014; revised Apr 14, 2015; accepted Apr 14, 2015.
Supplementary material cited in this article is available online at http://dx.doi.org/10.1016/j.biopsych.2015.04.022.

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Supplemental Information

Supplemental Methods and Materials

Drugs

Experiment 1 (Pre-ketamine in social defeat (SD)): At 8 weeks of age, a single injection of saline (0.9% NaCl) or ketamine (K) (10, 30, or 90 mg kg\(^{-1}\)) (Ketaset III, Ketamine HCl, Fort Dodge Animal Health, Forth Dodge, IA) was administered. Ketamine was prepared in physiological saline and all injections were administered intraperitoneally (i.p.) in volumes of 0.1 cc per 10 mg body weight. A dose of 30 mg kg\(^{-1}\) of ketamine was chosen for the 129S6/SvEv experiments, as pilot studies indicated that this dose did not affect immobility 2 h after administration (Figure S1). At 9 weeks of age, 1 week following this injection, SD was started.

Experiment 2 (Post-ketamine in SD): At 9 weeks of age, SD was administered for 2 weeks. Twenty-four h after the last bout of SD, a single injection of saline or ketamine (30 mg kg\(^{-1}\)) was administered (mice were 11 weeks old). The forced swim test (FST) was administered 2 h following the injection.

Experiment 3 (Pre-fluoxetine in SD): At 6 weeks of age, fluoxetine hydrochloride (Flx) (BioTrend Chemicals AG, BG197) was administered in the drinking water (18 mg kg\(^{-1}\)/day) for 3 weeks before the start of SD. Starting at 9 weeks of age, SD was then administered for 2 weeks without vehicle or Flx in the drinking water.
Experiment 4 (Pre-ketamine in learned helplessness (LH)): At 8 weeks of age, a single injection of saline or ketamine (30 mg kg$^{-1}$) was administered. One week following this injection, LH training (inescapable shock) was administered. Two weeks later, mice were administered LH testing (shock escape).

Experiment 5 (Pre-ketamine and pre-fluoxetine in corticosterone (CORT)): C57BL/6NTac mice were injected with ketamine (10, 30, 90 mg kg$^{-1}$, i.p.) 1 week before the start of CORT, or administered fluoxetine (18 mg kg$^{-1}$ per day) in the drinking water for 3 weeks before the start of CORT. Ketamine was purchased from Merial (100 mg/ml stock solution). Fluoxetine hydrochloride (160 μg/mL, equivalent to 18 mg kg$^{-1}$ per day in the drinking water) was purchased from Anawa Trading (Zurich, Switzerland) and dissolved in 0.45% hydroxypropyl-β-cyclodextrin (β-CD) (Sigma-Aldrich, Saint-Quentin Fallavier, France) solution. Following ketamine or fluoxetine treatment, CORT was administered for 3 weeks in the drinking water. CORT (4-pregnen-11b-DIOL-3 20-DIONE 21-hemisuccinate) (35 μg/ml) from Sigma Aldrich (Saint-Quentin Fallavier, France) was dissolved in vehicle (0.45% β-CD). Control animals received vehicle (0.45% β-CD) throughout the duration of the experiment.

Experiment 6 (Post-ketamine and post-fluoxetine in CORT): In C57BL/6NTac mice, vehicle or CORT was administered for 4 weeks in the drinking water. While administration with 0.45% β-CD or CORT continued, mice were injected with saline or ketamine (10 mg kg$^{-1}$), or were administered vehicle or chronic fluoxetine (18 mg kg$^{-1}$ per day in the drinking water).
Social Defeat

Adult aggressor CD-1 male mice were single-housed in Macrolon® polycarbonate resin cages (15.25 in x 7.8 in x 9.5 in) (Animal Care Systems, Inc., Centennial, CO). Male 129S6/SvEvTac mice (9 weeks of age) were subsequently placed into the resident CD-1 mouse’s cage. Three antagonistic encounters were allowed between the CD-1 mouse and the intruder. Each of these encounters typically lasted less than 5 min. Following the encounters, mice were separated by a polished stainless steel cage divider (Animal Care Systems, Inc., Centennial, CO, P/N C79171), which allowed for olfactory and auditory communication, but limited visual or tactile contact. The partition was removed daily for 2 weeks and 3 antagonistic encounters were allowed between the mice each day. This procedure consistently yielded a submissive phenotype in the experimental intruder mice. Control (Ctrl) mice were group housed 4 to 5 per cage in Macrolon® polycarbonate resin cages without dividers.

Corticosterone Model

The hypothalamic-pituitary-adrenal (HPA) axis is often dysregulated in clinical depression. In this model, glucocorticoid levels are exogenously increased. This chronic CORT elevation results in dysregulation of the HPA axis. For example, there is a blunting of the HPA axis response to stress in CORT-treated mice, as shown by marked attenuation of stress-induced CORT levels (1, 2). This model reliably induces anxious and depressive-like behavior in mice.

The dose and duration of CORT treatment was selected based on previous studies (1, 2, 3). CORT (35 μg/ml, equivalent to approximately 5 mg/kg/d) or vehicle (0.45 % β–CD) was available ad libitum in the drinking water in opaque bottles to protect it from light. CORT-treated water was changed every 3 d to prevent any possible degradation.
Learned Helplessness

The LH paradigm was used to induce depressive-like behavior (4). In this paradigm, mice are exposed to unpredictable and uncontrollable stress (shocks) and then develop coping deficits to deal with the inescapable shocks. We modified a previously published paradigm (5). Briefly, the procedure was conducted in a two-chamber shuttle box (model ENV 010MD; Med Associates, St. Albans, VT) located within a sound-attenuated cubicle. The grid floor was made of stainless steel and connected to a shock generator. The scrambled shock generator (model ENV 414S, Med Associates) created varying electrical potential differences between bars preventing an animal from avoiding shock.

Inescapable shock (training): At approximately 9 weeks of age, mice were trained in the LH paradigm. For each shuttle box, 2 animals were administered the protocol at the same time; the central door was closed, with one animal in the chamber on each side. After a 3 min habituation period, the shock deliveries began. The training protocol consisted of 70 shocks, each with a 3 s average duration, at 0.5 mA, and with an intertrial interval (ITI) of approximately 15 s. This protocol was the same as the “Med Protocol” published by Muller et al. (2011) (5), with the exception of the intensity (0.5 mA versus 0.6 mA).

Shock escape (testing): Mice were tested in the same shuttle box used in the inescapable shock training. The box consisted of two identical chambers (17 l x 20 w x 17 h), separated by an automated door that opened vertically. The shuttle box was equipped with 8 infrared beams (4 on each side) for detecting position and activity of the animal (Med Associates, St. Albans, VT). Each mouse was placed into the right chamber with the door raised and was allowed to freely explore both chambers for 3 min. Then the door then closed automatically.
At the beginning of each trial, the door was raised and 5 s later a foot shock (0.5 mA) was delivered. The subject's exit from the shocked side ended the trial. If the mouse did not exit after 15 s, the shock was turned off and the trial ended. The door was lowered at the end of the trial. A session consisted of 30 trials, each separated by a 30 s ITI. Escape latencies were computed as the time from shock onset to the end of trial. If the subject failed to make a transition the maximum 15 s was used for the escape latency score.

**Forced Swim Test**

The FST is typically used in rodents to screen for potential human antidepressants (6-7). In fact, many papers examining ketamine in mouse models only observe effects in the FST (8-10). In the FST, time spent immobile, as opposed to swimming, is used as a measure of depressive behavior.

The FST was administered as previously described (11). Briefly, mice were placed into clear plastic buckets 20 cm in diameter and 23 cm deep filled 2/3 of the way with 22°C water. Mice were videotaped from the side for 6 min and were exposed to the swim test on 2 consecutive days. For the SD experiments, scoring was performed using an automated Viewpoint Videotrack software package ([http://www.viewpoint.fr/en/a/anxiety-and-depression](http://www.viewpoint.fr/en/a/anxiety-and-depression)). For the CORT experiments, automated scoring was done using the automated X’PERT FST software suite (Bioseb, Vitrolles, France). The dependent variable was immobility.

**Tail Suspension Test (TST)**

The TST was administered as previously described (2). Briefly, animals were suspended for 6 min and immobility during this period was assessed using an automated TST apparatus (Bioseb,
Vitrolles, France). A specific strain gauge linked to a computer quantifies the time spent by each mouse trying to escape.

**Dominant Interaction**

Dominant interaction was performed as previously described (12). Briefly, mice were placed into a large open field containing 2 upside-down wire mesh pencil cups. One pencil cup served as a novel object or empty container. The other pencil cup contained the CD-1 aggressor mouse. Mice were placed in the middle of the open field arena and allowed to explore for 10 min. Sessions were videotaped and the first 5 min were later analyzed using behavioral tracking software (TopScan, CleverSys, Reston, VA).

**Social Interaction**

Mice were placed into a large open field containing 2 upside-down wire mesh pencil cups. One pencil cup served as a novel object or empty container. The other pencil cup contained a novel 129S6/SvEv male mouse. Mice were placed in the middle of the open field arena and allowed to explore for 10 min. Sessions were videotaped and the first 5 min were later analyzed using behavioral tracking software (TopScan, CleverSys, Reston, VA).

**Novelty Suppressed Feeding (NSF)**

Testing was performed as previously described (13). Briefly, the testing apparatus consisted of a plastic box (50 x 50 x 20 cm). The floor was covered with approximately 2 cm of wooden bedding and the arena was brightly lit (1100-1200 lux). For 129S6/SvEv experiments, mice were food restricted for 12 h. For the C57BL/6N experiments, mice were food restricted for 24 h. All
food was removed from the home cage. At the time of testing, a single pellet of food (regular chow) was placed on a white paper platform positioned in the center of the box. Each animal was placed in a corner of the box, and a stopwatch was immediately started. The latency of the mice to begin eating was recorded. Immediately after the latency was recorded, the food pellet was removed from the arena. The mice were then placed into a cage and the amount of food consumed in 5 min was measured (home cage consumption), followed by an assessment of post-restriction weight. We used the Kaplan-Meier survival analysis due to the lack of normal distribution of data. The Mantel-Cox log-rank test was used to evaluate differences between the experimental groups.

**Elevated Plus Maze (EPM)**

Testing was performed as previously described (14). Briefly, the maze is a plus-cross-shaped apparatus consisting of four arms, two open and two enclosed by walls, linked by a central platform at a height of 50 cm from the floor. Mice were individually placed in the center of the maze facing an open arm and were allowed to explore the maze for 5 min. The time spent in and the number of entries into the open arms was used as an anxiety index. For the 129S6/SvEv experiments, videos were scored using behavioral tracking software (TopScan, CleverSys, Reston, VA). For the C57BL/6Ntc experiments, all parameters were measured using a videotracker (EPM3C, Bioseb, Vitrolles, France).

**Contextual Fear Conditioning (CFC)**

The 1-shock CFC procedure was performed as previously published (15). Briefly, mice were placed into context A and administered a 2-s shock (0.75 mA) 180 s later. Mice were removed
from the context 15 s following the termination of shock (at 197 s). For context retrieval, mice were placed back into context A for 180 s. For alternate contexts, mice were placed into the contexts for 180 s. A table of context information is listed below.

<table>
<thead>
<tr>
<th></th>
<th>Context A</th>
<th>Context A'</th>
<th>Context B</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Scent</strong></td>
<td>Lemon</td>
<td>No scent</td>
<td>Anise</td>
</tr>
<tr>
<td><strong>Grids</strong></td>
<td>Exposed</td>
<td>Exposed</td>
<td>Covered with a white plastic and bedding on top</td>
</tr>
<tr>
<td><strong>Room Lights</strong></td>
<td>ON</td>
<td>ON</td>
<td>OFF</td>
</tr>
<tr>
<td><strong>Red Lamps</strong></td>
<td>OFF</td>
<td>OFF</td>
<td>ON</td>
</tr>
<tr>
<td><strong>House Fan</strong></td>
<td>ON</td>
<td>ON</td>
<td>ON</td>
</tr>
<tr>
<td><strong>House Lights</strong></td>
<td>ON</td>
<td>ON</td>
<td>ON</td>
</tr>
<tr>
<td><strong>Transfer Cage</strong></td>
<td>Square cage</td>
<td>Square cage</td>
<td>White bucket</td>
</tr>
<tr>
<td><strong>Cleaning</strong></td>
<td>95% EtOH</td>
<td>95% EtOH</td>
<td>Saniwipes</td>
</tr>
<tr>
<td><strong>Box Doors</strong></td>
<td>Closed</td>
<td>Closed</td>
<td>Open</td>
</tr>
<tr>
<td><strong>Modified Walls</strong></td>
<td>-</td>
<td>Rounded green walls</td>
<td>Rounded green walls</td>
</tr>
</tbody>
</table>

**Splash Test**

This test consisted of squirting 200 µl of a 10% sucrose solution on the mouse’s snout. The grooming duration was quantified using Stopwatch+ (Center for Behavioral Neuroscience, Georgia State University).
Open Field (OF)

The open field protocol was administered as previously described (11), with the exception that 1 quadrant was scented with female urine. Total distance traveled in each quadrant was quantified in 1-min bins.

Corticosterone Enzyme Immunoassay (EIA)

Mice were exposed to a minor stressor for 10 min and then returned to their home cages. Blood was collected from awake mice 70 min after the start of the stressor from the retro-orbital sinus using hematocrit tubes. Blood was then transferred to Eppendorf tubes pre-coated with 5 μl 0.5 M EDTA, placed on ice, and immediately spun down to obtain plasma for subsequent EIA. Plasma was stored at -80°C until analysis. EIA was performed using a DetectX Corticosterone EIA Kit (#K014-H1, Arbor Assays).

Immunohistochemistry

Mice were deeply anesthetized with ketamine (100 mg kg⁻¹) and transcardially perfused with 1X phosphate buffer saline (PBS), followed by chilled 4% paraformaldehyde (PFA)/1X PBS (15). Brains were postfixed overnight in 4% PFA at 4°C, then cryoprotected in 30% sucrose/1X PBS, and stored at 4°C. Serial coronal sections (35 μm) were cut through the entire hippocampus on a cryostat and stored in 1X PBS with 0.1% NaN₃. Doublecortin (DCX) immunohistochemistry was performed as previously described (15). For DCX immunohistochemistry, sections were washed in 1X PBS containing 0.5% Triton and then quenched in 0.3% H₂O₂ in 1X PBS/CH₃OH (1:1) for 15 min at room temperature. Sections were blocked in 10% normal donkey serum in 1X PBS with 0.5% Triton X-100 for 2 h at room temperature. Incubation with primary antibody was
performed at 4°C overnight (goat anti-DCX, 1:500, Santa Cruz Biotechnology, Santa Cruz, CA, #SC 8,066) in 1X PBS with 0.5% Triton X-100. Sections were then incubated with a biotinylated secondary antibody (donkey anti-goat; 1:250, Jackson ImmunoResearch, West Grove, PA) for 2 h at room temperature. Sections were treated with avidin-biotin-peroxidase complex (ABC Elite Kit, Vector Labs, Burlingame, CA) followed by 3,3’ diaminobenzidine as a substrate for staining (Vector, Burlingame, CA).

Ki67 immunohistochemistry was performed as previously described (16). For Ki67 immunohistochemistry, sections were washed in 1X PBS containing 0.5% Triton, and then blocked in 10% normal donkey serum in 1X PBS with 0.5% Triton X-100 for 2 h at room temperature. Incubation with primary antibody was performed at 4°C overnight (rabbit anti-Ki67, 1:100, Vector Laboratories, Burlingame, CA, #VP-RM04) in 1X PBS with 0.5% Triton X-100. Sections were then incubated with a biotinylated secondary antibody (donkey anti-rabbit; 1:250, Jackson ImmunoResearch, West Grove, PA) for 2 h at room temperature. Sections were treated with avidin-biotin-peroxidase complex (ABC Elite Kit, Vector Labs, Burlingame, CA) followed by 3,3’ diaminobenzidine as a substrate for staining (Vector, Burlingame, CA).

**Cell Quantification**

An investigator blind to treatment used a Zeiss Axioplan-2 upright microscope (Oberkochen, Germany) to count cells bilaterally in the granule cell layer of the dentate gyrus throughout the entire rostro-caudal axis of the hippocampus (HPC). Every sixth section throughout the entire extent of the HPC was included in the analysis. Cells were counted bilaterally using a 20X objective. The average cells per section are presented throughout the text.
**Statistical Analysis**

Results from data analyses are expressed as means ± SEM. Alpha was set to 0.05 for all analyses. Data were analyzed using StatView 5.0 software (SAS Institute, Cary, NC) for the SD experiments and were analyzed using GraphPad Prism v6.0f for the CORT experiments. For all experiments, one-way or one-way ANOVAs with repeated-measures were applied to the data as appropriate. Significant main effects and/or interactions were followed by Fisher’s protected least significant difference post-hoc analysis or unpaired *t*-tests. All main effects and interactions are noted throughout the text.
**Figure S1.** *Ketamine does not alter immobility long term in the FST.* (A) Mice were injected with saline or ketamine (10, 30, or 50 mg kg\(^{-1}\)) and administered the FST 1 h later. (B) Ketamine altered immobility on day 1. Mice injected with 30 mg kg\(^{-1}\) of ketamine were significantly different than mice injected with saline (0 mg kg\(^{-1}\)) at minutes 1, 3, 4, and 5 (p’s < 0.05). Asterisks indicated significance for the 30 mg kg\(^{-1}\) group. Mice injected with 50 mg kg\(^{-1}\) of ketamine were significantly different than mice injected with saline (0 mg/kg) at minutes 1, 4, 5, and 6 (p’s < 0.05). All groups had similar levels of immobility on day 2. (C) Mice were injected with saline or ketamine (30 mg kg\(^{-1}\)) and administered the FST 24 h and 48 h later. (D) All groups have similar levels of immobility on day 1 and day 2 of the FST. n = 8 male 129S6/SvEv mice per group. Error bars represent ± SEM. * p < 0.05, ** p < 0.01, *** p < 0.001.
Figure S2. Neither ketamine nor social defeat impact body weight. (A) Ctrl and SD mice had comparable body weights before the start of SD and following SD. Ketamine did not affect body weight in either of these groups. (B) Varying doses of ketamine did not alter body weight. (C-D) Ketamine or SD did not affect immobility time on day 1 of the FST. (E) The average immobility time for min 3-6 did not differ between groups. (F) Varying doses of ketamine did not alter immobility time on day 1 of the FST. (G) The average immobility time for minutes 3-6 did not differ between groups. n = 7-15 male 129S6/SvEv mice per group. Error bars represent ± SEM.
Figure S3. *Neither ketamine nor social defeat impact open field exploration.* (A) Experimental design. One quadrant of the open field was scented with female 129S6/SvEv urine. (B-C) All groups of mice displayed comparable levels of exploration in the empty quadrant and in the urine scented quadrant. n = 8 male 129S6/SvEv mice per group. Error bars represent ± SEM.
Figure S4. *Ketamine does not alter social interaction investigation time.* All groups of mice explored an empty enclosure and a novel 129S6/SvEv male for comparable amounts of time during a social interaction paradigm. n = 8 male 129S6/SvEv mice per group. Error bars represent ± SEM.
Figure S5. Ketamine-induced improvement in depressive-like behaviors is not mediated by proliferative adult hippocampal neurogenesis. (A) There was no effect of SD or of ketamine on the average number of Ki67+ cells. (B) Representative image of Ki67 immunohistochemistry in the dentate gyrus. The arrow indicates a Ki67+ cell. (C) There was no effect of SD or of ketamine on the average number of doublecortin+ (DCX+) cells. (D) Representative image of DCX immunohistochemistry in the dentate gyrus. n = 8-9 male 129S6/SvEv mice per group. Error bars represent ± SEM.
Figure S6. Ketamine alters fighting behavior during social defeat bouts. (A) The bout length did not differ for all groups of mice during week 1 or week 2. (B) Average immobility time during week 1 was not significantly different in all groups of mice. However, average immobility during week 2 of SD was significantly altered in mice administered ketamine. (C) The percent of time vocalizing did not differ between groups. (D) The number of approaches by the 129S6/SvEv mouse to the CD-1 did not differ between the groups. (E-G) Interestingly, the latency of the CD-1 to attack the 129S6/SvEv mouse only differed in SD-K (30 mg kg⁻¹) mice when compared with SD-Sal mice. n = 7-8 male 129S6/SvEv mice per group. Error bars represent ± SEM. * p < 0.05, ** p < 0.01; *** p < 0.001.
Figure S7. Fluoxetine treatment does not prevent behavioral despair following SD in the FST. (A) Experimental design. Mice were administered 3 weeks of Flx in the drinking water (18 mg/kg). Mice were then either group housed or administered SD for 2 weeks. The FST was administered for 2 days following SD. (B) SD-Flx mice display increased immobility time when compared with Ctrl-Veh mice. (C) SD-Flx mice have an increased average immobility time when compared with Ctrl-Sal mice. n = 6-8 male 129S6/SvEv mice per group. Error bars represent ± SEM. * p < 0.05; ** p < 0.01; *** p < 0.001.
Figure S8. Fluoxetine treatment before SD does not alter anxiety or cognition. (A) All groups had similar body weights before the start of SD. (B-D) All groups of mice had similar levels of immobility on day 1 of the FST. (E-F) Three weeks of fluoxetine before SD did not alter the latency to eat in the NSF. (G) SD and Ctrl mice differed in body weight following SD. Whereas Ctrl mice gained weight with Flx treatment, SD mice lost weight with Flx treatment. (H) SD mice lost less weight than Ctrl mice during the fast for NSF. Interestingly, SD-Flx mice lost less weight than SD-Veh mice. (I) SD mice consumed more food in the home cage when compared with Ctrl mice. (J-K) Ctrl and SD mice did not differ in freezing levels before administration of 1-shock CFC. (L) There was an overall effect of Drug on 1-shock CFC retrieval. However, the individual groups failed to reach significance. n = 8 male 129S6/SvEv mice per group. Error bars represent ± SEM. * p < 0.05; ** p < 0.01; *** p < 0.001.
Figure S9. Fluoxetine does not alter fighting behavior during SD bouts. In this experiment, fluoxetine was given for 3 weeks before the start of SD. (A) The bout length did not differ between the groups of mice during week 1 or week 2. (B) Average immobility did not differ between the groups of mice. (C) The percent of time vocalizing did not differ between groups. (D) The number of approaches by the 129S6/SvEv mouse to the CD-1 did not differ between the groups. (E) The latency of the CD-1 to attack the 129S6/SvEv mouse did not differ between the groups. n = 8 male 129S6/SvEv mice per group. Error bars represent ± SEM.
Figure 10. *Ketamine, as an antidepressant, increases adult hippocampal neurogenesis.* (A-B) Ketamine significantly decreased the latency to approach the food pellet in Ctrl mice. SD-Sal and SD-K mice did not differ from one another. (C) Body weight did not differ in any of the groups of mice before the start of NSF. (D) Before NSF, SD-Sal mice lost more body weight than Ctrl-Sal mice. Ketamine attenuated this weight loss. (E) SD mice lost more body weight than Ctrl mice during the fast for NSF. (F) SD mice consumed significantly more food than Ctrl mice. (G-J) All groups of mice had comparable levels of freezing during CFC training and following re-exposure to the fearful context A and to a similar context A’. n = 13-15 129S6/SvEv male mice per group. (K) Ketamine increases the number of Ki67+ cells when compared to saline in Ctrl mice but not in SD mice. (L) Ketamine does not alter DCX levels. n = 6-9 129S6/SvEv male mice per group. Error bars represent ± SEM. * p < 0.05, ** p < 0.01, *** p < 0.001.
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**Body Weight**

![Graph showing mouse body weight over weeks with different treatment groups.]

**Forced Swim Test (Day 1)**

![Graph showing immobility time in seconds for different treatment groups.]

**Novelty Suppressed Feeding**

![Graph showing fraction of animals not eating over time with different treatment groups.]

**Novelty Suppressed Feeding**

![Graph showing body weight after 24hr food deprivation with different treatment groups.]

**Weight Loss (%)**

![Graph showing weight loss (%) with different treatment groups.]

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### Additional Notes

- **Body Weight**
  - Treatment groups: Vehicle, CORT/Veh, CORT/Flx, CORT/K 10, CORT/K 30, CORT/K 90
  - Significant differences indicated by *** and **

- **Forced Swim Test (Day 1)**
  - Treatment groups: Vehicle, CORT/Veh, CORT/Flx, CORT/K 10, CORT/K 30, CORT/K 90

- **Novelty Suppressed Feeding**
  - Treatment groups: Vehicle, CORT/Veh
  - Significant differences indicated by **

- **Weight Loss (%)**
  - Treatment groups: Vehicle, CORT/Veh, CORT/Flx, CORT/K 10, CORT/K 30, CORT/K 90
  - Significant differences indicated by ***
Figure S11. Ketamine and fluoxetine both prevent chronic CORT-induced increases in body weight. (A) Of note, mice treated with CORT significantly gain weight. Administration of ketamine (90 mg kg\(^{-1}\)) or fluoxetine (18 mg kg\(^{-1}\)/day), but not ketamine (10 or 30 mg kg\(^{-1}\)), prevented the chronic CORT-induced increase in mouse body. Error bars represent ± SEM. (B-C) Ketamine injection for all the doses tested or fluoxetine did not induce antidepressant-like activity. Ketamine or fluoxetine treatment did not change immobility duration measured during the last 4-min of the test. (D-E) Ketamine prevents the chronic CORT-induced anxiety/depressive-like phenotype in the NSF. Chronic CORT increased the latency to feed in the NSF. Ketamine (90 mg kg\(^{-1}\)) prevented this chronic CORT-induced increase in latency (Survival plot shown here as opposed to the main text). (F) Food deprivation did not affect body weight differently among CORT-treated mice. (G) Mice treated with CORT lost a significantly greater percentage of body weight when compared with Veh mice during the 24-hour period of food deprivation preceding the NSF. n = 10-15 male C57BL/6NTac mice per group. Error bars represent ± SEM. * p < 0.05, ** p < 0.01, *** p < 0.001.
Figure S12. Ketamine does not prevent the chronic CORT-induced anxiety-like phenotype in the EPM. (A-C) Chronic CORT induced anxiety-like symptoms. Both ketamine injection for all of the doses tested and fluoxetine did not protect against the chronic CORT-induced anxiety-like phenotype. (D) No change in locomotor activity was observed between groups. n = 10-15 male C57BL/6NTac mice per group. Error bars represent ± SEM. * p < 0.05, ** p < 0.01.
Figure S13. Ketamine and fluoxetine given after CORT treatment are less effective than when given before CORT treatment. (A) Experimental design. Mice were administered Veh or CORT in the drinking water for 4 weeks. Mice were then administered saline, ketamine (10 mg/kg), or fluoxetine (18 mg/kg). (B-C) Fluoxetine, but not ketamine, decreases immobility time in the FST on day 5 and day 11 following CORT treatment. (D) At 7 days following CORT treatment, chronic CORT increased the latency to feed in the NSF. Ketamine or fluoxetine did not decrease the latency to feed in the NSF. (E) All groups of mice lost a comparable amount of body weight during the NSF fast. (F) All groups of mice consumed a comparable amount of food in the home cage. (G) At 14 days following CORT treatment, chronic CORT increased the latency to feed in the NSF. Fluoxetine, but not ketamine, decreased the latency to feed in the NSF. (H) All groups of mice lost a comparable amount of body weight during the NSF fast. (I) All groups of mice consumed a comparable amount of food in the home cage. n = 10-15 male C57BL/6NTac mice per group. Error bars represent ± SEM. * p < 0.05, ** p < 0.01, *** p < 0.001.
Supplemental References


13. David DJ, Klemenhagen KC, Holick KA, Saxe MD, Mendez I, Santarelli L, et al. (2007): Efficacy of the MCHR1 antagonist N-(3-(1-{(4-(3,4-difluorophenoxy)phenyl)methyl}(4-piperidyl))-4-methylphenyl)-2-m ethylpropanamide (SNAP 94847) in mouse models of

