

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

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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

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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 hopefully these discoveries will lead to therapeutic interventions in humans, 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.

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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 training and this effect was prevented by prophylactic ketamine. These findings demonstrate that the protective effect of ketamine extends at least 4 weeks postinjection. To our knowledge, this is the first study to examine the potential of psychopharmaceuticals to provide long-term prophylactic protection against the induction of stress-related disorders.

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 (Supplemental Figure S2A), 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 (Supplemental Figure S3). 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 (Supplemental Figure S4). 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.

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

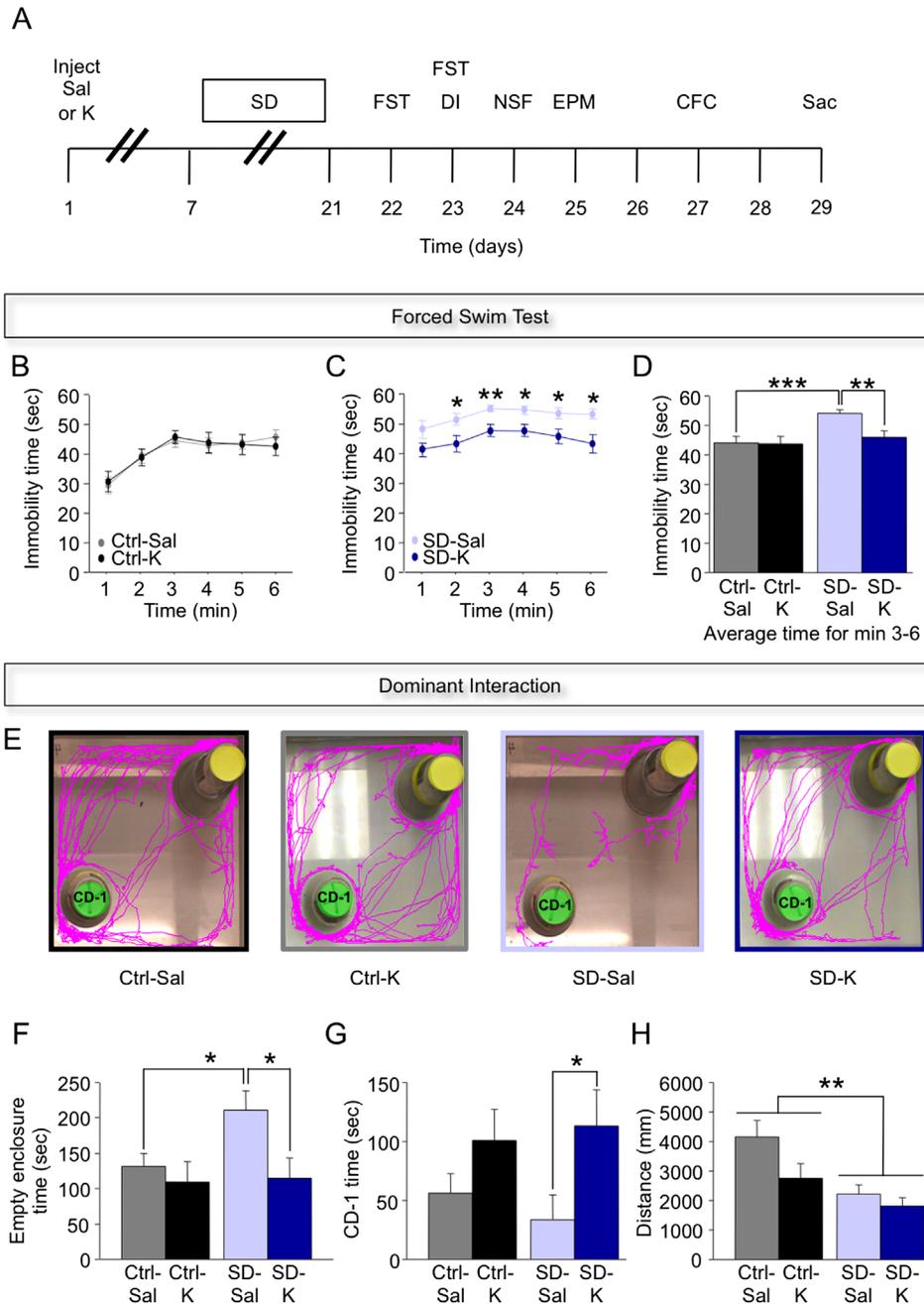


Figure 1. Ketamine protects against depressive-like behavior following social defeat (SD). **(A)** Experimental design. **(B)** On day 2 of the forced swim test (FST), group housed (Ctrl)-saline (Sal) and Ctrl-ketamine (K) mice did not differ from each other. **(C)** SD-K mice exhibited significantly less immobility time when compared with SD-Sal mice. **(D)** Average immobility time for minutes 3 to 6 was increased in the SD-Sal mice when compared with Ctrl-Sal mice. The SD-K mice displayed an average immobility time that was less than the SD-Sal mice. **(E)** Dominant interaction (DI) traces. **(F)** SD-Sal mice spent significantly more time investigating the empty enclosure when compared with the Ctrl-Sal mice. Conversely, SD-K mice spent significantly less time investigating the empty enclosure when compared with the SD-Sal mice. **(G)** SD-K mice spent considerably more time investigating a CD-1 mouse when compared with SD-Sal mice. **(H)** There was an overall effect of SD and of ketamine on decreasing the distance traveled during social interaction, but the interaction did not reach significance. ($n = 13-15$ male mice per group). Error bars represent \pm SEM. * $p < .05$, ** $p < .01$, *** $p < .001$. CFC, contextual fear conditioning; EPM, elevated plus maze; NSF, novelty suppressed feeding; Sac, sacrifice.

(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

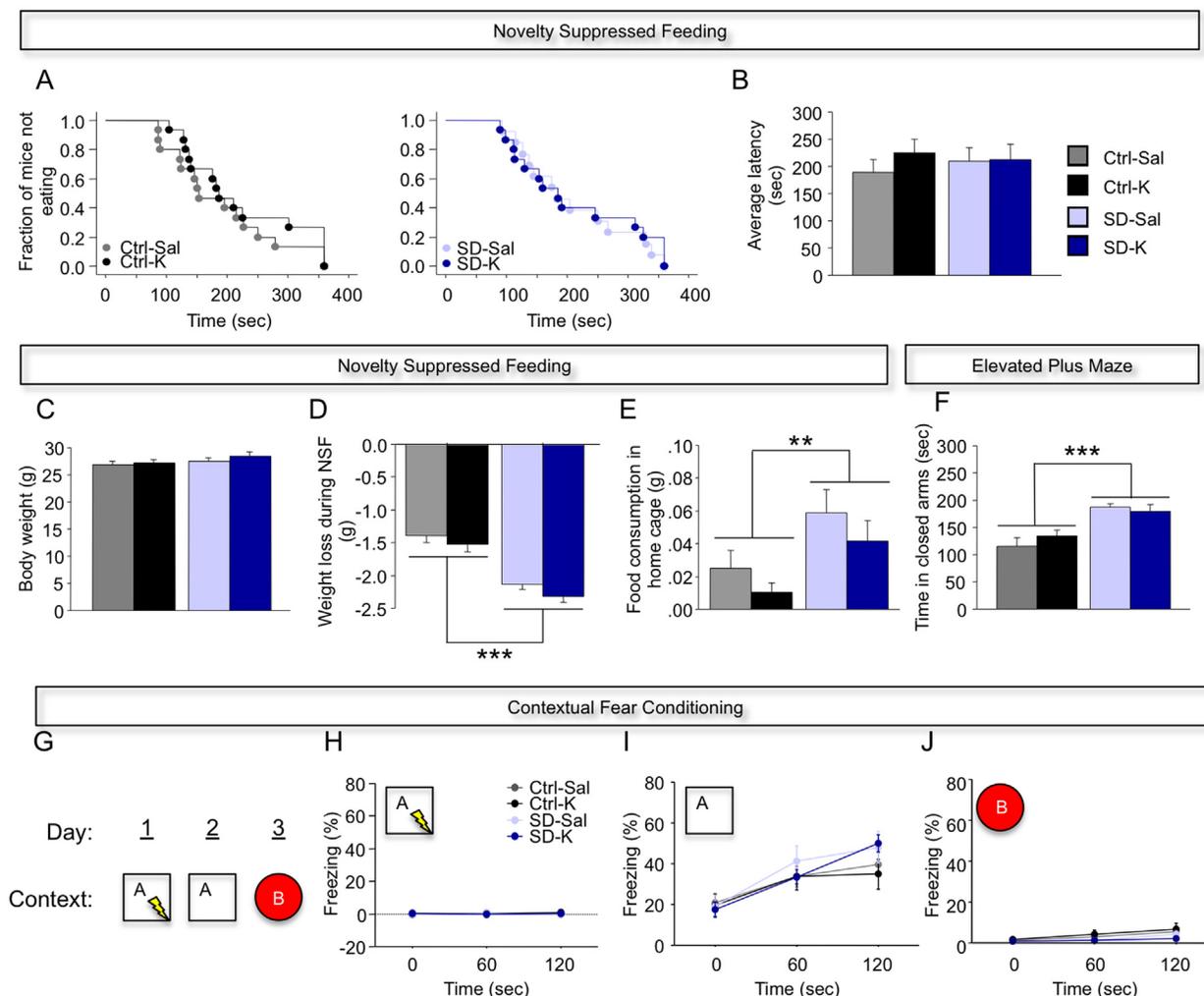


Figure 2. Ketamine does not protect against anxiety-like behavior or impair contextual fear conditioning learning following social defeat (SD). **(A,B)** In the novelty suppressed feeding (NSF) paradigm, all groups had equivalent average latencies to approach the food pellet. **(C)** Body weight did 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 \pm SEM. $**p < .01$, $***p < .001$. K, ketamine; Sal, saline.

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, E).

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

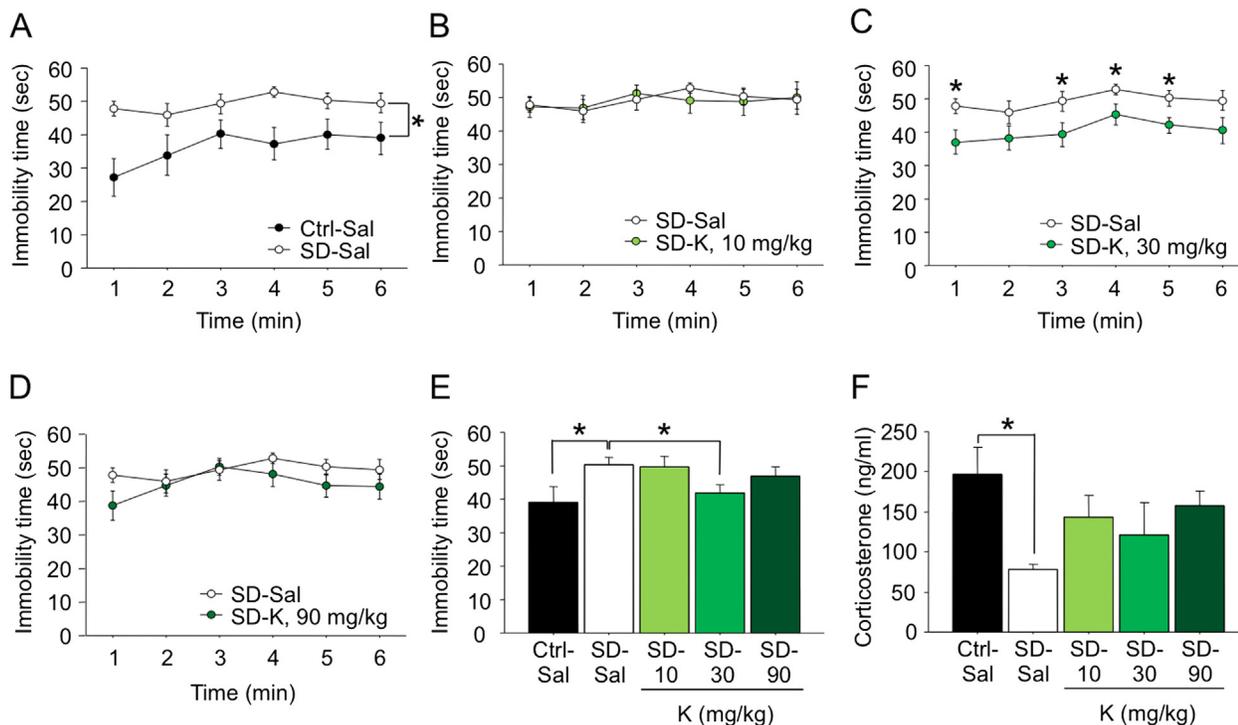


Figure 3. The ketamine-induced protection against depressive-like behavior is dose-specific. **(A)** On day 2 of the forced swim test (FST), social defeat (SD)-saline (Sal) mice displayed significantly more immobility time in the FST when compared with group housed (Ctrl)-Sal mice. **(B)** SD-Sal and SD-ketamine (K) (10 mg kg^{-1}) mice did not differ in immobility. **(C)** SD-K (30 mg kg^{-1}) mice displayed significantly less immobility time when compared with SD-Sal mice. **(D)** SD-Sal and SD-K (90 mg kg^{-1}) mice did not differ in immobility time. **(E)** For minutes 3 to 6 (averaged), SD-Sal mice displayed significantly more immobility in the FST when compared with Ctrl-Sal mice. SD-K (30 mg kg^{-1}) mice again displayed decreased immobility when compared with SD-Sal mice. **(F)** Following a brief stressor, SD-Sal mice had significantly lower levels of corticosterone when compared with Ctrl-Sal mice. ($n = 7\text{--}8$ male mice per group). Error bars represent \pm SEM. * $p < .05$.

from Ctrl-Sal mice, suggesting that ketamine partially restores the HPA axis. To determine if adult hippocampal neurogenesis 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 (Supplemental Figure S5). 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 (Supplemental Figure S6A). However, the average immobility during week 2 was significantly decreased in SD-K mice when compared with SD-Sal mice (Supplemental Figure S6B). The percent of time vocalizing (Supplemental Figure S6C) and number of approaches to the CD-1 (Supplemental Figure S6D) 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 (Supplemental Figure S6E–G). 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; Supplemental Figure S7). On day 2 of the FST, Ctrl-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 (Supplemental Figure S8). Fluoxetine treatment did not significantly alter anxiety or cognition but did affect metabolism (Supplemental Figure S8G–H).

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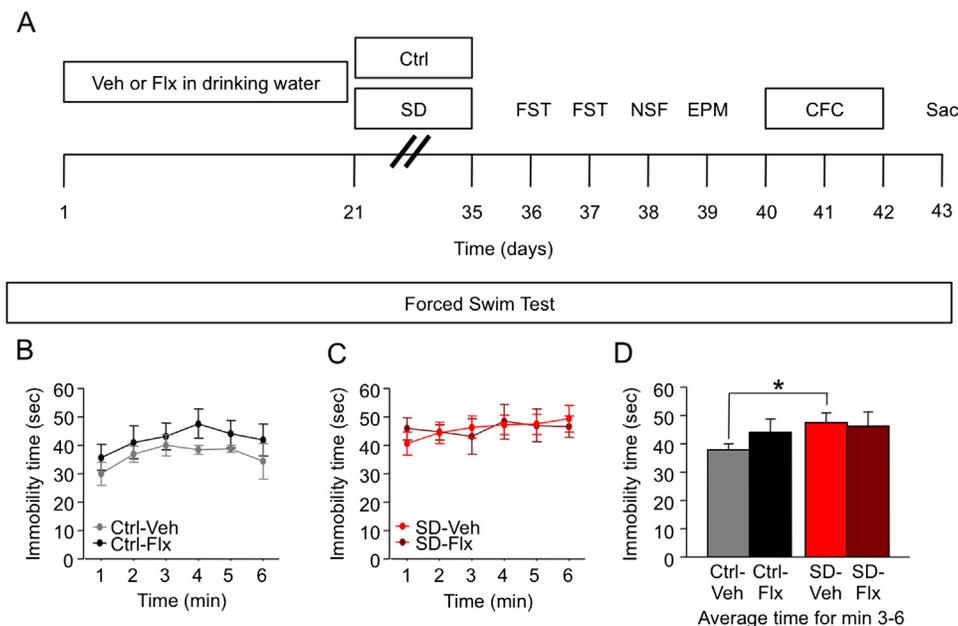


Figure 4. Fluoxetine (Flx) does not protect against depressive-like behavior following social defeat (SD). **(A)** Experimental design. **(B)** On day 2 of the forced swim test (FST), group housed (Ctrl)-vehicle (Veh) and Ctrl-Flx mice did not differ from each other. **(C)** SD-Veh and SD-Flx mice exhibited similar amounts of immobility time. **(D)** Average immobility time for minutes 3 to 6 was increased in the SD-Veh mice when compared with Ctrl-Veh mice. (*n* = 8 male mice per group). Error bars represent ± SEM. **p* < .05. CFC, contextual fear conditioning; EPM, elevated plus maze; NSF, novelty suppressed feeding; Sac, sacrifice.

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 (Supplemental Figure S9). 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.

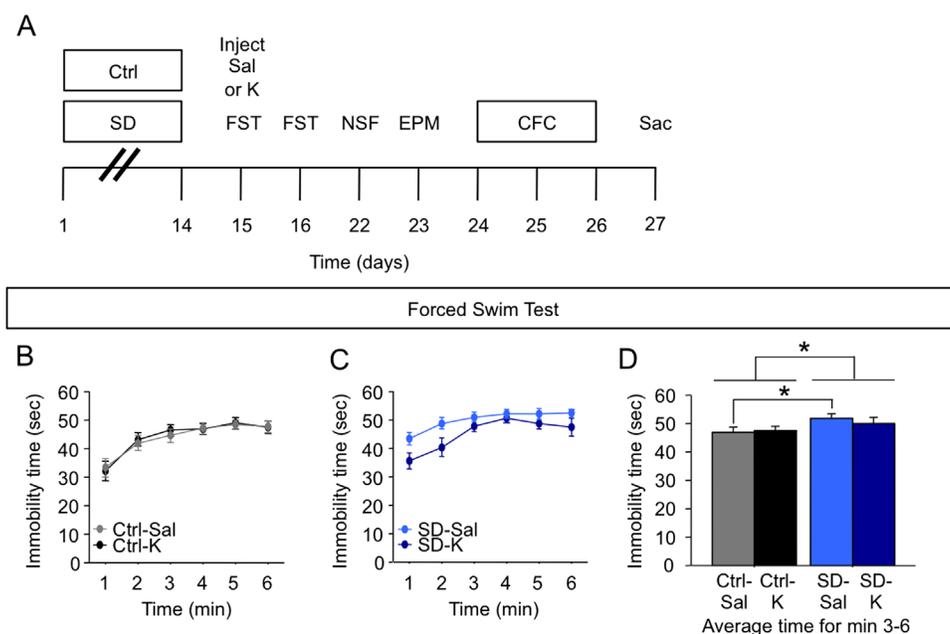


Figure 5. Ketamine (K) given after social defeat (SD) does not improve depressive-like behavior. **(A)** Experimental design. **(B)** On day 2 of the forced swim test (FST), group housed (Ctrl)-saline (Sal) and Ctrl-K mice did not differ from each other. **(C)** SD-Sal and SD-K mice had similar levels of immobility time. **(D)** Average immobility time for minutes 3 to 6 was increased in the SD-Sal mice when compared with Ctrl-Sal mice. (*n* = 12–15 male mice per group). Error bars represent ± SEM. **p* < .05. CFC, contextual fear conditioning; EPM, elevated plus maze; NSF, novelty suppressed feeding; Sac, sacrifice.

We also assessed a number of other behaviors following ketamine treatment (Supplemental Figure S10). In Ctrl mice, ketamine decreased the latency to eat in the NSF when compared with saline (Supplemental Figure S10A). This effect was abolished in the SD mice, most likely due to weight loss differences between Ctrl and SD mice (Supplemental Figure S10B–C). Interestingly, ketamine lessened the percentage of weight loss in the SD mice when compared with saline, possibly by protecting against stress-induced hypophagia (Supplemental Figure S10D). Ketamine also did not impact CFC learning (Supplemental Figure S10G–J). 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 (Supplemental Figure S10K).

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

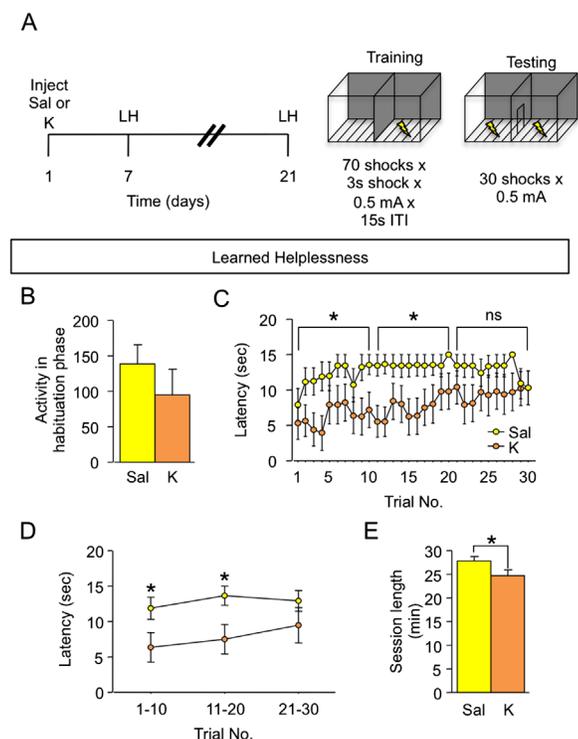


Figure 6. Ketamine (K) protects against depressive-like behavior in learned helplessness (LH). **(A)** Experimental design. **(B)** Activity in the habituation phase during testing did not differ between groups. **(C, D)** The latency to escape the shock was significantly less in the K-injected mice when compared with the saline (Sal)-injected mice. **(E)** The total session length for all 30 trials was significantly less in the K-injected mice when compared with the Sal-injected mice. ($n = 8-9$ male mice per group). Error bars represent \pm SEM. * $p < .05$. ITI, intertrial interval; ns, nonsignificant.

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 (Supplemental Figure S11A).

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; Supplemental Figure S11). However, only ketamine (90 mg kg⁻¹) increased home cage food consumption (Figure 7F). Finally, we assayed anxiety behavior using the EPM (Supplemental Figure S12). 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 (Supplemental Figure S13A). 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 (Supplemental Figure S13B, C). In the NSF, CORT treatment

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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 (Supplemental Figure S13D-I). 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 vs. 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

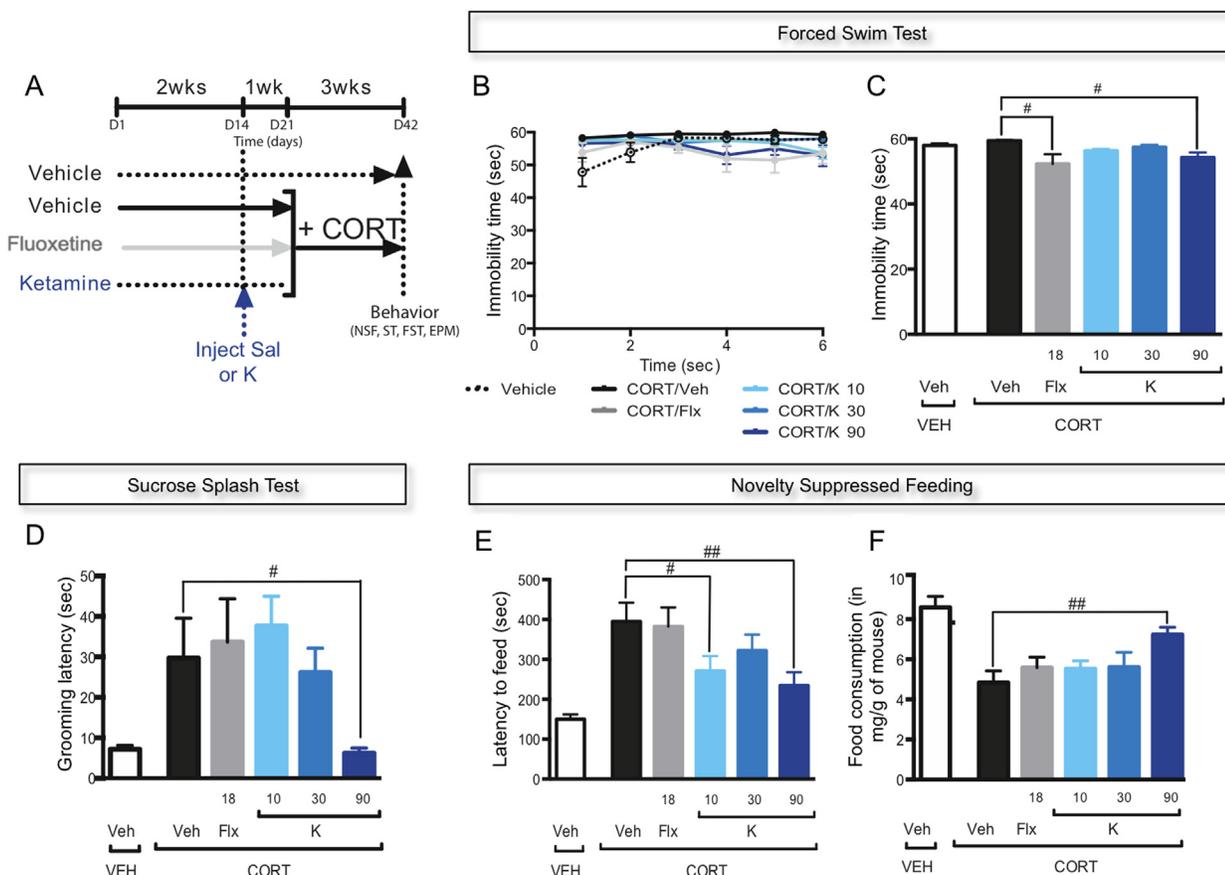


Figure 7. Ketamine (K) protects against depressive-like and anxiety behavior induced with a neuroendocrine model. (A) Experimental paradigm schematic. (B, C) Corticosterone (CORT) mice administered K (90 mg kg⁻¹) or fluoxetine (Flx) (18 mg kg⁻¹/day) exhibited significantly reduced immobility in the forced 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 during the ST. (E) K (10 and 90 mg kg⁻¹) decreased the latency to feed in the novelty suppressed feeding (NSF). (F) 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.

drugs may be more useful in protecting against coincident stress-induced pathologies (e.g., anxiety, cognitive deficits, metabolic disturbances).

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.

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