Effects of Subanesthetic Intravenous Ketamine Infusion on Corticosterone and Brain-Derived Neurotrophic Factor in the Plasma of Male Sprague-Dawley Rats

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Posttrauma anesthetic agents influence neuroendocrine responses that may affect fear memory. The effects of a subanesthetic intravenous (IV) ketamine infusion on mediators of stress and memory in rodents are unknown. Therefore, we used a clinically relevant method to administer a 2-hour subanesthetic IV ketamine infusion following a rodent fear-conditioning paradigm (paired tone plus foot shock) to evaluate the effects on corticosterone and brain-derived neurotrophic factor in the plasma of male Sprague-Dawley rats. We found that subanesthetic ketamine infusions (5 and 20 mg/kg/h) dose-dependently increased plasma corticosterone levels. Ketamine at 20 mg/kg/h significantly reduced plasma brain-derived neurotrophic factor measured 2 hours after the conclusion of the ketamine infusion. These results demonstrate that a subanesthetic IV ketamine infusion maintained a heightened neuroendocrine stress response after fear conditioning and reduced levels of a neurotrophin associated with memory, which may influence fear memory processing. The behavioral outcomes of these effects are unknown and warrant future investigation.

Keywords: BDNF, corticosterone, fear conditioning, ketamine, rats.

Ketamine, a multimodal potent dissociative anesthetic, is often administered in the immediate posttrauma period to provide sedation and analgesia. First responders and clinicians may favor ketamine as a trauma anesthetic because of its cardiovascular stability, maintenance of spontaneous respiration, and high safety ceiling.[1] Likewise, anesthesia professionals may administer perioperative subanesthetic intravenous (IV) ketamine infusions to manage acute pain and reduce opioid consumption.[2] However, the effect of immediate posttrauma ketamine administration on mediators of stress and memory are often not considered. Stress hormones expressed following trauma serve to either enhance or impair memory formation based on their concentration and duration of exposure in the brain.[3] It remains unclear how a prolonged exposure to ketamine, a sympathomimetic agent, may affect the stress response and brain proteins associated with memory formation. Therefore, understanding the effects of ketamine on mediators of stress and memory is an important first step before addressing larger questions related to the potential impact of peritrauma anesthetics on fear memory and stress-related disorders.

Trauma survivors with a diagnosis of a stress-related disorder such as posttraumatic stress disorder (PTSD) often experience dysfunctional and intrusive fear memories of an adverse event that fail to extinguish.[4] Fear memory, similar to other memory types, is formed and regulated through a series of complex and dynamic stages within the limbic regions of the brain, which include the hippocampus, amygdala, and the prefrontal cortices.[5] In the hours following trauma, a traumatic memory is consolidated from a short-term labile memory to a long-term stable memory through an enhancement of neural networks in limbic structures.[6] Memory consolidation is of particular interest because it occurs during the posttrauma period when healthcare providers are administering various pharmaceutical agents that may have an impact on memory development and storage. Memory consolidation comprises a series of nonlinear interactions between various mediators and signaling pathways. In particular, glucocorticoids and brain-derived neurotrophic factor (BDNF) are of great interest because of their relationship with ketamine.

Corticosterone (CORT; in rodents) and cortisol (in humans) are a type of glucocorticoid hormone secreted from the adrenal glands that mediate various vital functions in response to a stressor. A particular function of CORT is to regulate memories associated with strong emotions.[7] Corticosterone enters the brain and binds

to mineralocorticoid and glucocorticoid receptors in limbic structures,[7] which triggers signaling cascades that either impair or enhance fear memory. The opposing effects of CORT on memory are time, dose, and brain region dependent. Intermediate CORT levels strengthen memory, whereas low and high levels either impair or have no effect on memory consolidation, suggesting an inverted U dose relationship.[8,9] For example, intermediate CORT elevations enhance fear memory consolidation in the amygdala,[8] whereas either low CORT or prolonged CORT exposure may disrupt memory consolidation in the hippocampus through dendritic atrophy.[10,11]

There is converging evidence that CORT is one of several signaling molecules that regulate BDNF production.[12] Brain-derived neurotrophic factor is a protein required for neuronal growth, neuroprotection, and synaptic plasticity throughout the central nervous system. Altered BDNF levels are associated with dysregulated fear memory disorders such as PTSD.[13] Corticosterone affects BDNF differently depending on the brain region and duration of exposure.[12] Stress-induced CORT activation increases BDNF in the amygdala,[14] which promotes neuronal growth and contributes to the enhanced emotional valence associated with fear memory. Conversely, reduced BDNF in the prefrontal cortices and hippocampus leads to neuronal atrophy, impaired memory, and disrupted fear extinction, which is a hallmark characteristic of PTSD.[15,16] Brain-derived neurotrophic factor can also cross the blood-brain barrier,[17] allowing researchers to use peripheral blood BDNF levels as a potential biomarker for stress-related disorders.[18] Additionally, blood BDNF is positively correlated with brain BDNF concentrations in rodents, suggesting blood sampling as a potential noninvasive method for in vivo monitoring of neuronal health.[19]

Subanesthetic ketamine administration directly affects both CORT and BDNF levels. Low-dose IV ketamine infusions increase cortisol levels in blood and saliva in humans.[20] Similarly, a single intraperitoneal (IP) injection or IV ketamine bolus increases CORT levels in rodents.[21,22] However, what remains unknown is the effect of a prolonged subanesthetic IV ketamine infusion administered immediately after trauma on the plasma CORT response, which could mediate fear memory consolidation associated with trauma. Effects of ketamine on BDNF, a protein associated with memory formation, are less clear. A single, low-dose (10 mg/kg) IP ketamine injection increases BDNF in rodent depression models,[23] whereas long-term ketamine administration reduces BDNF production in both humans and rodents.[24,25] Similar to CORT, the effect of a subanesthetic IV ketamine infusion on plasma BDNF protein concentration after trauma remains unclear.

A major limitation of rodent behavioral research is ketamine delivery via IP injections into the abdomen, which may hinder translation to human investigations and clinical practice that use IV infusions. A one-time IP ketamine injection in rodents results in a short duration of action secondary to a short drug half-life.[26] On the other hand, an IV infusion maintains a steady-state drug-plasma concentration over an extended time, allowing for maximum impact on neurobiological mediators such as CORT and BDNF.

Clinically relevant medication delivery routes are poorly used in rodent models that aim to characterize drug effects on mechanisms of fear memory. The effects of immediate posttrauma subanesthetic IV ketamine infusions on the stress response and brain proteins associated with fear memory are unknown. Therefore, the purpose of our investigation was to determine the effects of subanesthetic IV ketamine infusions following fear conditioning on CORT and BDNF, which are mediators of stress and memory. Based on previous studies, we hypothesized that a subanesthetic IV ketamine infusion may increase both CORT and BDNF levels in the plasma of rats.

Materials and Methods

• ***Animals.*** Adult male Sprague-Dawley rats (Envigo Laboratories) weighing 250 to 300 g were housed individually in clear plastic glass (Plexiglas) shoebox cages in a climate-controlled environment with food and water ad libitum. Animals were habituated to a 12-hour reversed dark-light cycle (lights off at 6 AM; testing during dark cycle) and handled daily for 7 days before testing. All procedures were performed in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee at the Uniformed Services University of the Health Sciences, Bethesda, Maryland.

• ***Intravenous Catheter.*** A jugular venous catheter (3F, polyurethane; Instech Laboratories Inc) was surgically placed using isoflurane anesthesia by personnel at Envigo Laboratories before animal arrival at our institution. The catheter was tunneled under the skin and connected to a vascular access button (Instech) that exited the dorsal position between the front rodent scapulae. All rodents remained ketamine naïve throughout surgery and postoperative care following catheter placement. Catheters were flushed every 3 days to verify venous patency and were locked with 0.1 mL of heparin/glycerol solution (Braintree Scientific Inc).

• ***Grouping.*** Animals were randomly assigned to groups of 10 to 12 each as follows: group 1 (no fear conditioning and saline infusion), group 2 (no fear conditioning and ketamine bolus + 20 mg/kg/h infusion), group 3 (fear conditioning and saline infusion), group 4 (fear conditioning and ketamine bolus + 5 mg/kg/h infusion), and group 5 (fear conditioning and ketamine bolus + 20 mg/kg/h infusion).

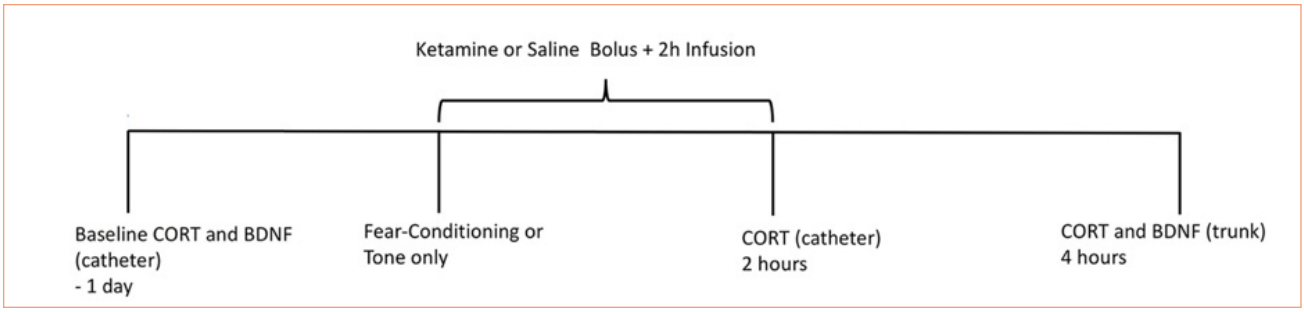

Figure 1. Experimental Timeline[a]
Abbreviations: CORT, corticosterone; BDNF, brain-derived neurotrophic factor.
[a]All blood samples were collected as plasma. Baseline and 2-hour samples were collected from an implanted jugular catheter in vivo. The sample collected at 4 hours was from trunk blood.

• ***Fear Conditioning.*** The fear conditioning box had a metal grid floor surrounded by clear plastic glass (Plexiglas) walls and was dimly lit by a single house light (2-3 lux). The fear conditioning box was housed inside a larger sound-attenuating chamber with a background noise level of 55 dB (Coulbourn Instruments). Groups 3, 4, and 5 underwent auditory cued fear conditioning as previously described[27] at 8 AM. After a 3-minute acclimation period, rats were presented with 3 pairings of an auditory tone (5 kHz, 75 dB, 20 seconds) that coterminated with a mild foot shock (0.8 mA, 0.5 seconds). There was an intertrial interval of 90 to 120 seconds to prevent tone prediction. Fear-conditioned rats were removed 60 seconds after the final tone-foot shock pairing. Groups 1 and 2 were placed into the chamber and underwent the same sequence of events at 8 AM, but were exposed to the tone without foot shock and thus did not undergo fear conditioning.

• ***Ketamine Infusion.*** Immediately after fear conditioning, each rat was placed into an operant conditioning chamber (Med Associates Inc). Each chamber was equipped with an infusion pump (Harvard Pump 11 Elite, Harvard Apparatus) using a 10-mL syringe connected to a fluid swivel (Instech) by polyurethane tubing encased in a metal spring wire tether to prevent chewing and tubing entanglement. The tether was attached to the vascular access button on the rat using a luer-lock connection. All tethered rats had free mobility in the chamber during saline or ketamine infusions. A dim red-light illuminated each box to facilitate observation by the study team.

• ***Ketamine Dosing.*** Racemic (+/- isomers) ketamine hydrochloride (100 mg/mL, Mylan Institutional LLC) was diluted in 0.9% sterile saline before dosing. Groups 2 and 5 received a ketamine bolus (5 mg/kg, IV) and then a 2-hour ketamine infusion (20 mg/kg/h, IV). Group 4 received a ketamine bolus (2 mg/kg, IV) and then a 2-hour ketamine infusion (5 mg/kg/h, IV). These subanesthetic IV ketamine bolus and infusion doses were previously determined in our laboratory.[28] Groups 1 and 3 received a saline bolus and 2-hour saline infusion (1 mL/h). All ketamine and saline bolus doses were delivered in a volume of 1 mL/kg.

• ***Blood Sampling.*** Baseline blood samples were taken 1 day before fear conditioning at consistent times (10 AM), 2 hours after fear conditioning (the conclusion of the ketamine or saline infusion at 10 AM), and 4 hours after fear conditioning (2 hours after the end of the infusion at noon) (Figure 1). Blood was collected in vivo from the implanted catheter at the baseline and at the 2-hour time point. Blood (0.2 mL) was withdrawn to clear the catheter of medication and prime with fresh blood. Then 0.3 mL of blood was withdrawn and placed into 1.5-mL Eppendorf tubes with 10 µL of ethylene-diamine-tetra-acetic-acid (EDTA) additive to prevent blood clotting. Trunk blood was collected following decapitation at the 4-hour time point and placed in EDTA tubes. Blood samples were centrifuged at 4,000 rpm for 20 minutes at 4°C (Eppendorf Model 5415 R). Plasma supernatant was collected and stored at –80°C for future analysis.

• ***Corticosterone Assay.*** CORT was quantified using a corticosterone enzyme-linked immunosorbent assay (ELISA) kit (DetectX, Arbor Assays) per manufacturer protocol. Plasma samples (10 µL) were combined with a dissociation reagent (10 µL), then diluted to 1:100 with an assay buffer. Standards were prepared in serial dilutions (10,000 to 78.125 pg/mL). Next, standards or samples (50 µL) were added to each well in duplicate using a 96-well plate. Then, CORT conjugate (25 µL) and antibody (25 µL) were added to the samples. After a 1-hour incubation at room temperature on a plate shaker, the wells were aspirated and washed 3 times with wash buffer (300 µL). Then, 3,3´, 5,5´-tetramethylbenzidine (TMB) substrate was added per well (100 µL) and allowed to incubate for 30 minutes. Finally, stop solution (50 µL) was added to each well, and the plate optical density was measured at 450 nm (200 Pro Microplate Reader, Tecan) and calculated as nanograms per milliliter.

• ***Brain-Derived Neurotrophic Factor Assay.*** Plasma BDNF levels were quantified using Emax Immunoassay System (Promega) according to manufacturer protocol.

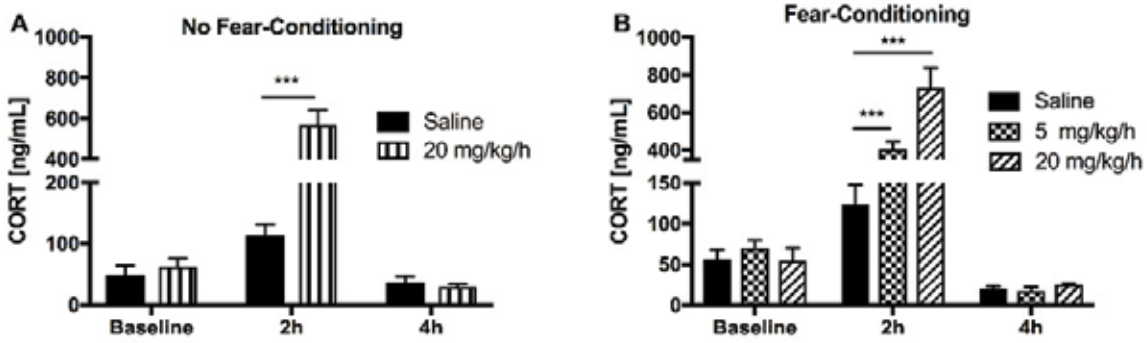

Figure 2. Effect of Ketamine Infusion on Corticosterone (CORT)[a]

A. Ketamine infusion (20 mg/kg/h for 2 hours) significantly increased CORT concentration in rats that did not undergo fear conditioning. B. Ketamine infusions (5 and 20 mg/kg/h for 2 hours) dose-dependently increased CORT concentrations compared with a saline infusion among rats that experienced fear-conditioning.

[a] CORT was measured at baseline, immediately after the 2-hour infusion (2h), and again at 2 hours after the infusion (4h). Data are shown as mean ± standard error of the mean.

*** $P < .001$ (10-12 rats per group).

The well bottoms of a 96-well plate were coated with anti-BDNF monoclonal antibody and incubated at 4°C overnight. Standards were prepared in serial dilutions (500-7.8 pg/mL), and plasma samples were diluted 1:4 in buffer. Wells were blocked with Block & Sample buffer (Emax Immunoassay System) for 1 hour and washed with Tris-buffered saline containing 0.1% Tween 20 (TBST). Next, samples or standards (100 µL) were added to each well in duplicate and incubated for 2 hours on a plate shaker. After TBST washing, plates were incubated for 2 hours with anti-human BDNF polyclonal antibody (100 µL). Then, after TBST washing, plates were incubated for 1 hour with anti-IgY horse radish peroxidase (100 µL). After a final TBST washing, plates were incubated for 10 minutes with TMB One solution (100 µL, Promega), and the reaction was stopped with 100 µL of 1N solution of hydrochloric acid. The optical densities of standards and samples were measured at 450 nm (200 Pro Microplate Reader, Tecan) and calculated as picograms per milliliter. The BDNF Emax Immunoassay System sensitivity is 15.6 pg/mL.

• ***Statistical Analysis.*** All data are presented as mean ± standard error of the mean (SEM) and were analyzed using GraphPad Prism (GraphPad Software, version 7.0). Repeated-measures 2-way analysis of variance (ANOVA) with Time and Ketamine as factors, 1-way ANOVA, and unpaired *t* tests were used for CORT and BDNF assays as appropriate. Bonferroni post hoc tests were used to compare significant ANOVA differences. The BDNF data were normalized to the nonfear-conditioned saline control group. The accepted level of significance was $P < .05$.

Results

• ***Corticosterone.*** We compared the effect of a 2-hour ketamine infusion on plasma CORT concentrations at 3 time points (baseline, 2 hours, and 4 hours) in both nonfear-conditioned and fear-conditioned rats using treatment and time as factors (Figure 2). There were no differences between groups at baseline. First, we compared nonfear-conditioned rats and found significant main effects of the following: treatment, F (1,22) = 35.48 ($P < .001$); time, F (2,44) = 60.57 ($p < 0.001$); and a Treatment × Time interaction, F (2,44) = 35.01 ($P < .001$). Post hoc testing showed that ketamine infusion (20 mg/kg/h) induced a significant increase in plasma CORT concentration (568.1 ± 70.81 ng/mL) compared with a saline vehicle at 2 hours (114 ± 17.85 ng/mL; $P < .001$; Figure 2a). Next, we compared fear-conditioned rats and found significant main effects of the same factors: treatment, F (2,33) = 19.87 ($P < .001$); time, F (2,66) = 92.38 ($P < .001$); and a Treatment × Time interaction, F (4,66) = 19.63 ($P < .001$). Post hoc testing revealed that ketamine infusions at 5 mg/kg/h (405 ± 41.03 ng/mL) and 20 mg/kg/h (731 ± 106.5 ng/mL) dose-dependently increased CORT concentrations compared with the saline vehicle at 2 hours (122 ± 25.04 ng/mL; $P < .001$; Figure 2b). Saline infusions induced mild elevations in CORT concentration at 2 hours in both fear-and nonfear-conditioned rats compared with baseline values, but these differences were not significant. Additionally, we observed depressed CORT concentrations at 4 hours compared with baseline measures, but these differences were also not significant. Lastly, we compared fear-conditioned with nonfear-conditioned groups at each time point (baseline, 2 hours, and 4 hours). There were no differences in CORT concentrations between fear-conditioned and nonfear-conditioned groups at the baseline or 4-hour time points. Additionally, there were no significant differences between the ketamine infusion (20 mg/kg/h) in the nonfear-conditioned group and ketamine infusions (5 and 20 mg/kg/h) in the fear-conditioned group.

• ***Brain-Derived Neurotrophic Factor.*** We compared plasma BDNF concentrations at baseline and 2 hours

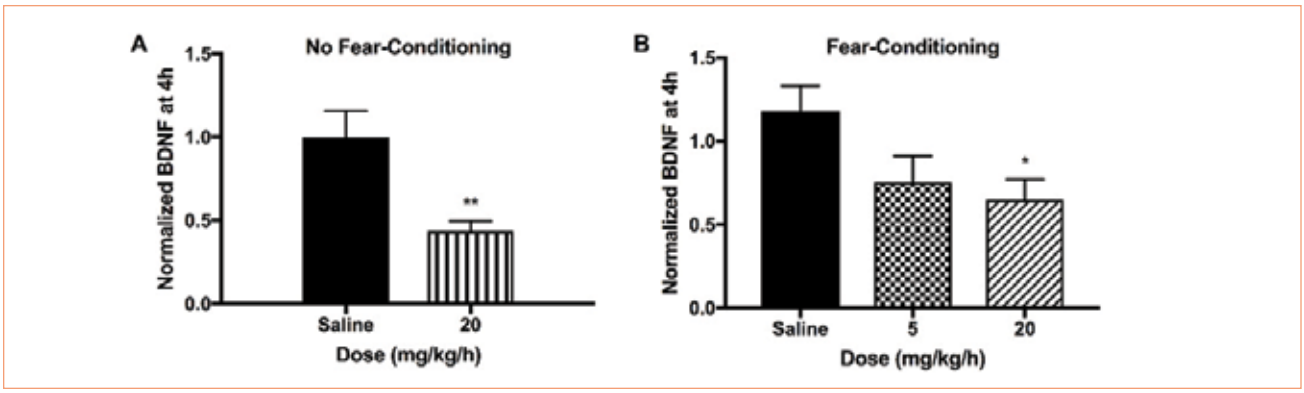

Figure 3. Effect of Ketamine Infusion on Brain-Derived Neurotrophic Factor (BDNF)[a]

A. Ketamine infusion (20 mg/kg/h for 2 hours) significantly decreased plasma BDNF concentrations (pg/mL) in rats that did not undergo fear conditioning. B. Ketamine infusion (20 mg/kg/h for 2 hours) significantly reduced plasma BDNF concentration among rats that experienced fear conditioning.

[a]BDNF was measured 2 hours after the conclusion of a ketamine infusion (4h). Data are shown as mean ± standard error of the mean.

* $P < .05$ (10-12 rats per group).

** $P < .01$ (10-12 rats per group).

after a ketamine infusion (4 hours) in both nonfear-conditioned and fear-conditioned rats (Figure 3). Data are reported as normalized BDNF values to the nonfear-conditioned saline control. There were no significant group differences in BDNF concentrations at baseline. First, we compared nonfear-conditioned rats and found that ketamine infusion at 20 mg/kg/h induced a significant reduction in plasma BDNF concentrations (0.44 ± 0.06 pg/mL) compared with the saline vehicle (1.0 ± 0.16 pg/mL; t (3.248), $P < .01$, two-tailed) measured at 4 hours (Figure 3A). Next, we compared fear-conditioned rats and found significant differences between the ketamine infusions and the saline vehicle F (2,32) = 3.89 ($P < .05$). Post hoc testing revealed that a ketamine infusion at 20 mg/kg/h significantly reduced BDNF concentration (0.65 ± 0.13 pg/mL) compared with saline at 4 hours (1.2 ± 0.15 pg/mL; $P < .05$; Figure 3B). Although the ketamine infusion at 5 mg/kg/h also showed a reduction in BDNF (0.75 ± 0.16 pg/mL), the result was not statistically significant.

Discussion

Although clinicians typically administer ketamine to humans using the IV route, most preclinical researchers administer ketamine via IP injections to rodents, which limits translational value to clinical practice. There are no published reports, to our knowledge, that have measured the effects of a subanesthetic IV ketamine infusion administered immediately after trauma on biomarkers of stress and fear memory. Therefore, we administered a subanesthetic IV ketamine infusion immediately after fear conditioning and measured effects on plasma CORT and BDNF levels in rats.

Subanesthetic IV ketamine infusions (5 and 20 mg/kg/h) dose-dependently increased CORT levels in rats (see Figure 2). These results are in line with previous investigations that observed elevated cortisol levels in the blood and saliva of humans following subanesthetic IV ketamine infusions (0.29-0.57 mg/kg over 40-60 minutes).[20,29] Similar CORT elevations were observed following anesthetic and subanesthetic IP ketamine injections (15-120 mg/kg) and single IV bolus doses (2 and 35 mg/kg) in rodents.[21,22,30] Some investigators attribute the sympathomimetic properties of ketamine to trigger the increased CORT response,[29] which suggests a direct action on the adrenal gland. In contrast, other studies reported no CORT effects when ketamine was administered to rats without a pituitary gland, which infers ketamine must act either at the hypothalamus or pituitary, not directly at the adrenal gland.[22] Similarly, ketamine added to isolated adrenal cells in vitro had no direct effect on CORT, whereas ketamine increased the expression of corticotropin (formerly called adrenocorticotropic hormone) when added to isolated anterior-pituitary cells.[31] Additionally, the hypothalamic neuroendocrine circuitry is rich in *N*-methyl-D-aspartate (NMDA) receptors, the principal receptor type for ketamine effects. Taken together, these data provide evidence that an IV ketamine infusion increases CORT levels through direct hypothalamic and/or pituitary stimulation, not through direct action at the adrenal glands.

Elevated CORT levels following trauma in humans, as well as fear conditioning in rodents, may have an impact on fear memory consolidation. An inadequate glucocorticoid-stress response following trauma may disrupt the information processing and predict PTSD symptoms.[32] Zohar et al[33] reported that high-dose hydrocortisone administered during emergency care following a traumatic event reduced core symptoms of PTSD up to 3 months after trauma. Similarly, CORT elevations induced by acute immobilization stress to rodents after fear conditioning reduced subsequent fear memory.[34] In contrast, other study authors have reported that increased CORT

expression during memory consolidation enhanced fear memory, which is an adverse effect.[9,35] The adverse effects of acute CORT are supported by other studies, which described fear memory consolidation as dependent on acute CORT elevations following a stressor.[7,8] Corticosterone binds to glucocorticoid receptors in fear memory structures such as the amygdala and hippocampus following trauma exposure to signal the production of proteins that enhance memory consolidation.[7] The memory-enhancing effects of CORT also require simultaneous noradrenergic activity.[8] Interestingly, ketamine is a sympathomimetic agent that also increases the CORT response. Therefore, it is plausible that our subanesthetic IV ketamine infusion administered immediately after fear conditioning may serve to enhance fear memory through CORT and sympathomimetic synergistic mechanisms.

Subanesthetic IV ketamine infusions (5 and 20 mg/kg/h) decreased plasma BDNF levels in rats (Figure 3). These results were not anticipated because they contradicted previous investigators who reported increased BDNF concentrations following single subanesthetic IP ketamine injection (10-15 mg/kg IP) in rodent depression models.[36] Our results, however, are in line with other studies that observed reduced blood BDNF levels among chronic ketamine abusers[25] and reduced brain BDNF concentrations among rats that received repeated ketamine injections (25 mg/kg IP) over 7 days.[24] Similarly, long-term subanesthetic ketamine injections (25 mg/kg IP) also reduced brain BDNF concentrations in a rodent model of schizophrenia.[37]

The mechanisms by which ketamine increases BDNF concentrations in rodent depression models are well studied, and a variety of receptors and signaling pathways are implicated. It is generally proposed that ketamine antagonizes the NMDA receptor on presynaptic γ-aminobutyric acid-ergic (GABAergic) neurons. The NMDA blockade disinhibits presynaptic GABAergic neurons, leading to a paradoxical increase in presynaptic glutamate release.[38] Glutamatergic signaling culminates in the postsynaptic production of BDNF via activation of the mammalian target of rapamycin (mTOR) pathway.[23]

On the other hand, prolonged NMDA blockade of presynaptic GABAergic neurons can induce excessive glutamate release and negative consequences. Hyperactive glutamate leads to the dysfunctional influx of calcium and sodium ions that trigger excitotoxicity and neuronal apoptosis.[39] Furthermore, glutamatergic dysregulation is implicated in a variety of psychiatric diagnoses, including PTSD.[40] The seemingly opposite effects of ketamine on BDNF may be related to the duration of the NMDA receptor blockade. A one-time, low-dose IP ketamine injection administered in rodent depression models appears to increase BDNF concentrations,[36] whereas repeated/long-term administration of ketamine in humans and other rodent models reduces BDNF.[24,25] The subanesthetic IV ketamine infusions administered in our investigation may exert a similar effect to that of long-term ketamine paradigms by antagonizing NMDA receptors over a sustained time, possibly leading to a dysregulated glutamate/BDNF mechanism and our reduced plasma BDNF results.

The blunted response of BDNF observed in our investigation stimulates intriguing possibilities. Although BDNF is necessary to form long-term memories, reductions of BDNF can hinder various types of memory formation, including fear memory.[41] Disrupting fear memory consolidation could weaken fear memories and potentially prevent stress-related disorders. However, there is evidence that inadequate BDNF formation and reduced synaptic plasticity can dysregulate fear memory formation.[33] Impaired BDNF production, secondary to a *BDNF* gene mutation (*BDNF* val66met), may predispose certain individuals to the development of PTSD and anxiety disorders, while also impairing fear extinction.[42] Nevertheless, manipulating BDNF levels in either direction after trauma may affect fear memory formation and processing, and this possibility needs further investigation.

Overall, the clinical significance of our findings is difficult to interpret in the absence of behavioral data. Ketamine is a popular trauma anesthetic, and subanesthetic IV ketamine infusions are used in the perioperative period to manage acute pain and minimize opioid consumption. However, the long-term psychological impacts of the immediate posttrauma administration of ketamine are unclear. Human investigations of posttrauma ketamine administration are scant and have mixed results. McGhee et al[43] found no relationship between intraoperative ketamine administration and PTSD risk among burn patients; however, these ketamine doses were given significantly after the initial trauma and memory consolidation period. In contrast, Winter and Irle[44] reported increased rates of PTSD among burn patients who received 12 days of benzodiazepine/ketamine analgesia after injury. Schönenberg et al[45] administered ketamine in the immediate period after trauma and reported an increase in acute stress disorder and core symptoms of PTSD compared with patients who received opiate and nonopiate analgesics. Anesthetic and subanesthetic IP ketamine injections initiated during the fear memory consolidation phase in rodents have also produced mixed results, showing either improvement or no change in fear behaviors.[46,47] However, more recent investigations have shown ketamine administered after a rodent stressor to worsen fear memory and impair social interaction, which are characteristics of PTSD-like behavior.[48-50]

We caution clinicians not to compare our findings with those of recent investigations that have used ketamine to temporarily reverse depressive and PTSD symptoms. Timing of ketamine administration could produce different results. We contend that a subanesthetic ketamine infusion administered immediately after trauma

may affect fear memory acquisition and consolidation through dose-dependent alterations of stress and memory mediators. In contrast, ketamine administered months to years later as a treatment may affect different memory processes such as retrieval and reconsolidation and may prove beneficial.

Our investigation is not without limitations. We used only male rats, and therefore we cannot generalize our results to female rats. There are gender-related differences in response to stress and anxiety that require further investigation. Because we measured BDNF in plasma, we do not know the brain region-specific expression of BDNF. Regional differences in BDNF expression is an area for future investigation. Clinically, a benzodiazepine is often coadministered with ketamine to offset the experience of dissociative symptoms. We did not administer a benzodiazepine in our rodents because we wanted to examine ketamine-specific effects without a confounding psychoactive medication. Interestingly, benzodiazepines are GABA agonists that may blunt the potential hyperglutamatergic and excitotoxic effects of a prolonged ketamine infusion. Lastly, we did not measure behavioral effects because the biomarker results from this study were used to support a grant proposal for a future investigation that will explore subanesthetic ketamine infusion effects on fear memory and behavior.

In conclusion, subanesthetic IV ketamine infusions administered after rodent fear conditioning dose-dependently increased CORT and reduced plasma BDNF concentrations. These findings illustrate the impact of IV anesthetic agents administered after trauma on mediators of stress and memory. Alterations of these mediators may have long-term unintended psychological consequences. For instance, we do not know what effect, if any, elevated CORT levels for an extended period after trauma may have on memory and emotional processing of a traumatic event. To that end, we strongly encourage future studies that will extend our CORT and BDNF results and examine the effects of posttrauma subanesthetic IV ketamine infusions on fear memory and stress-related disorders.

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