Behavioural Brain Research 378 (2020) 112238

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Prophylactic (*R,S*)-ketamine selectively protects against inflammatory stressors

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ARTICLE INFO

Keywords:
Depression
Stress
Fear
Viral
Inflammation
Mice

ABSTRACT

Individuals with peripheral inflammation are a particularly vulnerable population for developing depression and are also more resistant towards traditional antidepressants. This signals the need for novel drugs that can effectively treat this patient population. Recently, we have demonstrated that (*R,S*)-ketamine is a prophylactic against a variety of stressors, but have yet to test if it is protective against inflammatory-induced vulnerability to a stressor. Here, male 129S6/SvEv mice were administered saline or (*R,S*)-ketamine (30 mg/kg) 6 days before an injection of vehicle (VEH) or lipopolysaccharide (LPS) (0.83 or 1.0 mg/kg, serotypes O111:B4 or O127:B8). Twenty-four hours after LPS administration, mice were administered a contextual fear conditioning (CFC) paradigm, followed by a context re-exposure and the forced swim test (FST). In a separate cohort, we tested if (*R,S*)-ketamine was effective as a prophylactic against polyinosinic-polycytidylic acid (PIC), a viral mimetic. (*R,S*)-ketamine was effective as a prophylactic for attenuating learned fear in the O111:B4 and O127:B8 strains of LPS. (*R,S*)-ketamine was also effective as a prophylactic for decreasing stress-induced depressive-like behavior in the O111:B4 and O127:B8 strains of LPS. Both of these effects were limited to administration of 1.0, but not 0.83 mg/kg of the O111:B4 and O127:B8 strains of LPS. (*R,S*)-ketamine was not effective against either stress phenotype following PIC administration. These data suggest that prophylactic (*R,S*)-ketamine may protect against selective inflammation-induced stress phenotypes following an inflammatory challenge. Future studies will be necessary to determine if (*R,S*)-ketamine can be useful in patient populations with peripheral inflammation.

1. Introduction

Major depressive disorder (MDD) is a debilitating condition characterized by symptoms such as depressed mood, fatigue, weight loss, anhedonia, and suicidal thoughts [1]. It is estimated that MDD affects 6.7% of the US adult population in a given year, and in 2010, the annual cost of depression for the healthcare system and for patients combined was $210.5 billion [2]. A well-known risk factor for depression is peripheral inflammation, and patients with inflammation-induced depression are more resistant to classical antidepressants, such as selective serotonin reuptake inhibitors (SSRIs) [3]. For example, Wright and colleagues found that humans injected with a *Salmonella enterica* serotype typhi vaccine experienced negative mood symptoms in the absence of illness symptoms [4]. Additionally, patients with significantly elevated inflammatory markers such as interleukin 6 (IL-6), at baseline, were less likely to respond to SSRIs and benzodiazepines [5].

The relationship between inflammation and depression is also particularly relevant for patients on pro-inflammatory treatments such as interferon, commonly used to treat cancer and hepatitis-C [6]. Musselman and colleagues found that chronic interferon therapy typical for cancer treatment induced MDD in 30–50% of patients [7]. Additionally, studies have demonstrated that SSRIs given to patients before and during interferon treatment is ineffective or, at best, inconsistent

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https://doi.org/10.1016/j.bbr.2019.112238
Received 3 July 2019; Received in revised form 4 September 2019; Accepted 12 September 2019
Available online 26 September 2019

[8–10]. These studies demonstrate that classical antidepressants lack efficacy for treating inflammation-induced depression, which signals the need for novel therapeutics for treating depression in this patient population.

Lipopolysaccharide (LPS) administration is a common model for examining inflammation-induced neuropsychiatric disorders such as depression [11]. LPS is the principal component of Gram-negative bacteria (e.g., *Escherichia coli* (*E. coli*)) and elicits an immune response when administered in animals. LPS administration causes sickness behavior in mice such as weight loss and decreased locomotor activity [11]. LPS acts on the central nervous system (CNS) through indoleamine 2,3-dioxygenase (IDO), a heme-containing enzyme in peripheral macrophages, which metabolizes tryptophan into kynurenine. Kynurenine enters the brain through the blood-brain barrier (BBB) and is further metabolized into the neurotoxic compounds, kynurenic acid and quinolinic acid. The LPS mouse model is clinically relevant because there is evidence that interferon- and LPS-induced depression work through similar mechanisms of action to induce depression [12]. For example, kynurenic acid and quinolinic acid are both increased in the cerebral spinal fluid (CSF) of patients on interferon treatment, which suggests interferon and LPS activate similar pathways in the CNS [13]. Furthermore, interferon-stimulated monocytes and lymphocytes also activate IDO [14]. Therefore, the findings of the present study may be relevant for patients suffering from interferon-induced depression.

In addition to LPS administration, the viral mimetic polyinosinic-polycytidylic acid (PIC) has been widely used to induce inflammation in rodents [15–17]. PIC is a synthetic analog of double-stranded RNA (dsRNA), that induces the acute phase response (APR) via multiple inflammatory pathways, including nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) and interferon regulatory factor (IRF) [18,19]. Systemic administration of PIC has been shown to induce behavioral phenotypes consistent with neuropsychiatric disorders, such as schizophrenia and autism in rodents [20,21].

Previously, we have found that a single, prophylactic injection of (*R,S*)-ketamine, an *N*-methyl-D-aspartate (NMDA) receptor antagonist, protects against a variety of stressors in mice [22]. Specifically, when (*R,S*)-ketamine is administered 1 week before a 3-shock contextual fear conditioning (CFC) paradigm, it attenuates learned fear and stress-induced depressive-like behavior when compared with saline [23,24]. These results have also been replicated in 4 different mouse models of stress in a dose-specific manner, in both mice and rats [22,25], as well as in male [25] and female rodents [26,27]. In addition to protecting against depressive-like behavior, prophylactic (*R,S*)-ketamine has been found to be efficacious in post-traumatic stress disorder (PTSD) [28] and postpartum depression (PDD) in humans [29].

Here, we tested whether prophylactic (*R,S*)-ketamine can protect against LPS- and PIC-induced inflammatory challengers when paired with a CFC stressor. Saline or (*R,S*)-ketamine (30 mg/kg) was administered 6 days before a single dose of VEH or LPS (0.83 or 1.0 mg/kg, serotypes O111:B4 or O127:B8). The 2 doses and serotypes of LPS were chosen based on previous studies showing their efficacy for inducing inflammatory responses and sickness behavior [12,30,31]. The dose of (*R,S*)-ketamine was based on our previous studies demonstrating a dose-specific efficacy to prevent stress-induced depressive-like behavior and attenuate learned fear in 129S6/SvEv mice [22–24]. To compare an LPS-induced inflammatory challenge to a viral mimetic, in 2 separate experiments, saline or (*R,S*)-ketamine was administered prior to an injection of VEH or PIC. (*R,S*)-ketamine was effective as a prophylactic for attenuating learned fear in the O111:B4 and O127:B8 strains of LPS. (*R,S*)-ketamine was also effective as a prophylactic for decreasing stress-induced depressive-like behavior in the O111:B4 and O127:B8 strains of LPS. Both of these effects were limited to administration of 1.0 mg/kg, but not 0.83 mg/kg of serotypes O111:B4 and O127:B8 of LPS. (*R,S*)-ketamine was not effective against either stress phenotype following PIC administration. These data suggest that prophylactic (*R,S*)-ketamine may protect against selective inflammation-induced stress phenotypes following an inflammatory challenge, but is completely ineffective against a viral challenge.

2. Methods and materials

2.1. Mice

Male 129S6/SvEvTac male mice were purchased from Taconic (Hudson, New York) at 8 weeks of age and housed 4–5 per cage in a 12-h (06:00– 18:00) light–dark colony room at 22 °C. Food and water were provided *ad libitum*. All behavioral testing was performed during the light phase. All the procedures described herein were conducted in compliance with the NIH regulations and approved by the Institutional Animal Care and Use Committee (IACUC) of the New York State Psychiatric Institute (NYSPI).

2.2. (R,S)-ketamine

A single injection of saline (0.9% NaCl) or (*R,S*)-ketamine (30 mg/kg) (Ketaset III, Ketamine HCl injection, Fort Dodge Animal Health, Fort Dodge, Iowa) was administered 1 week before the start of 3-shock CFC according to our previous studies [22–24]. In our previous studies, we report that 30 mg/kg was the most effective prophylactic dose in male 129S6/SvEv mice.

2.3. Lipopolysaccharide (LPS)

An injection of VEH (saline; 0.9% NaCl) or LPS (0.83 or 1.0 mg/kg, serotypes O111:B4 and O127:B8, Sigma, St Louis, Missouri) was administered once during the course of the experiment. On the day of injection, LPS was dissolved in 0.9% sterile NaCl and administered in a volume of 0.1 ml/mouse by intraperitoneal (i.p.) route. The doses of LPS were based on a previous study [12].

2.4. Polyinosinic-polycytidylic acid (PIC)

An injection of VEH (saline; 0.9% NaCl) or ultrapure PIC (12 mg/kg) (Invivogen, San Diego, California) was administered once during the course of the experiment. On the day of injection, PIC was dissolved in 0.9% sterile NaCl and administered in a volume of 0.1 ml/mouse by intraperitoneal (i.p.) route. The dose of PIC was chosen from a previously published study [32].

2.5. Contextual fear conditioning (CFC)

A 3-shock CFC paradigm was administered as previously described [33,34]. Context re-exposure consisted of a 5-minute exposure to the aversive training context. All sessions were scored for freezing using FreezeFrame4.

2.6. Forced swim test (FST)

The FST was administered as previously described [22]. Average immobility time for day 2 of the FST was calculated for min 3–6 (4 min in total).

2.7. Statistical analysis

All data were analyzed using StatView 5.0 software (SAS Institute, Cary, North Carolina) or Prism 7 (Graphpad Software, Inc., La Jolla, California). Alpha was set to 0.05 for all analyses. All statistical tests and p values are listed in **Supplemental Table S01**.

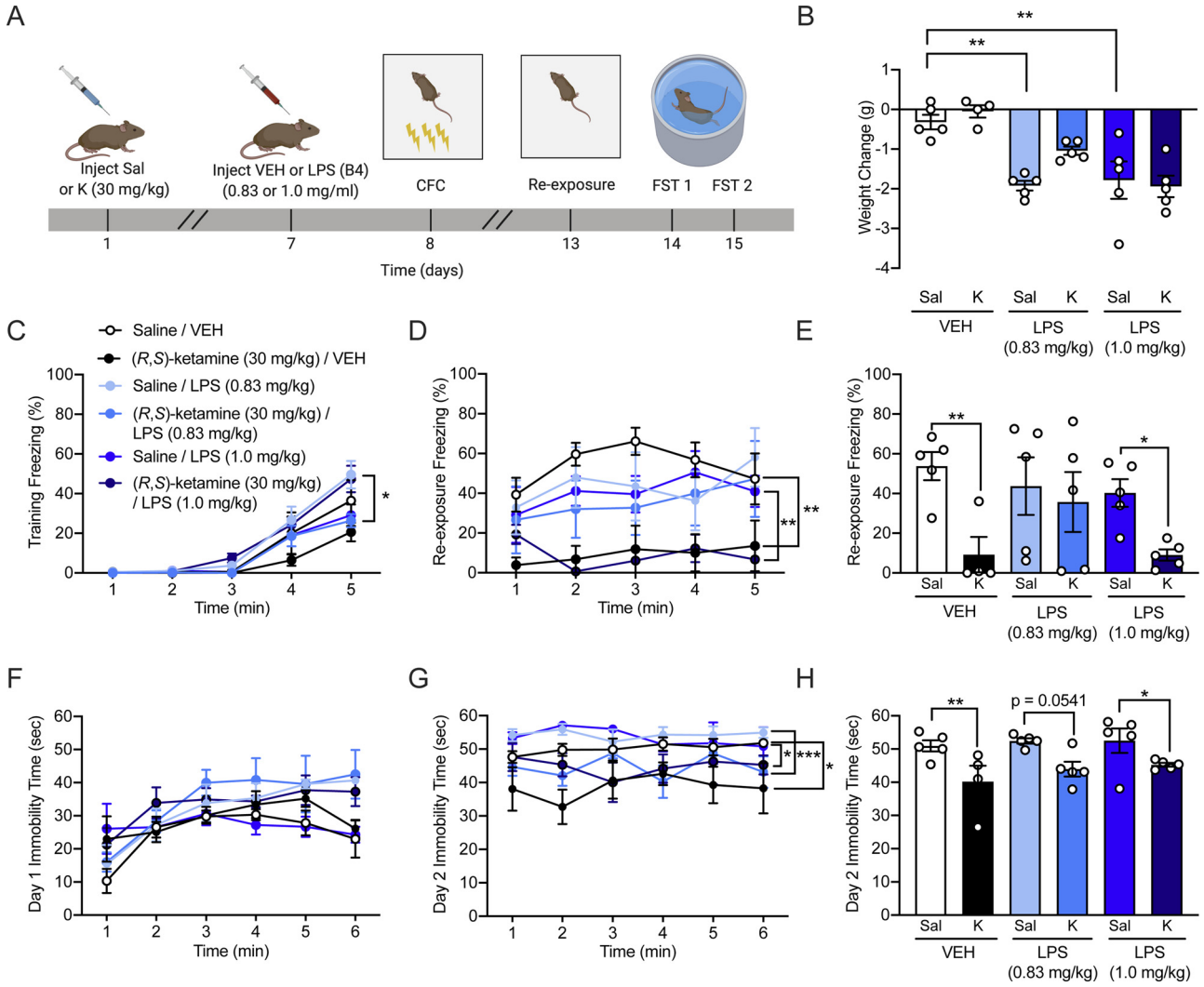

Fig. 1. (*R,S*)-ketamine is efficacious as a prophylactic when administered prior to a single injection of LPS serotype O111:B4 in mice. (**A**) Experimental design. (**B**) Mice injected with LPS lost significantly more weight when compared with mice injected with VEH. (**C**) During CFC training, there was no effect of LPS or of Drug, but there was a significant interaction. (*R,S*)-ketamine significantly attenuated fear encoding in LPS-injected mice administered 0.83 mg/kg of serotype O111:B4 when compared with saline administration. (**D-E**) During context re-exposure, there was a significant effect of Drug, but not of LPS or an interaction on fear expression. In VEH-injected mice, (*R,S*)-ketamine significantly attenuated learned fear when compared with saline. (*R,S*)-ketamine significantly attenuated learned fear in LPS-injected mice administered 1.0 mg/kg of serotype O111:B4, but not in LPS-injected mice administered 0.83 mg/kg of serotype O111:B4. (**F**) All groups of mice exhibited a comparable amount of immobility time during day 1 of the FST. (**G-H**) During day 2 of the FST, there was a significant effect of Drug, but not of LPS or an interaction. In VEH-injected mice, (*R,S*)-ketamine significantly decreased immobility time when compared with saline. In LPS-injected (0.83 mg/kg) mice, (*R,S*)-ketamine did not significantly decrease immobility time when compared with saline, although this effect was trending. In LPS-injected (1.0 mg/kg) mice, (*R,S*)-ketamine significantly decreased immobility time when compared with saline. (n = 4–5 male 129S6/SvEv mice per group). Error bars represent ± SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Sal, saline; K, (*R,S*)-ketamine; VEH, vehicle; LPS, lipopolysaccharide; CFC, contextual fear conditioning; FST, forced swim test; sec, seconds; min, minutes.

3. Results

3.1. (R,S)-ketamine is efficacious as a prophylactic when administered prior to a single injection of LPS serotype O111:B4 in mice

To determine if (*R,S*)-ketamine can protect against an inflammatory challenge paired with a fear-based stressor, male 129S6/SvEv mice were administered a single injection of saline or (*R,S*)-ketamine (30 mg/kg) 6 days before a single injection of VEH or LPS (0.83 or 1.0 mg/kg, serotype O111:B4) (Fig. 1A). Twenty-four hours later, mice were weighed and then administered a 3-shock CFC stressor, followed by context re-exposure and the FST, in order to measure depressive-like behavior.

There was a significant effect of LPS, but not of Drug or an interaction on weight loss (2-way ANOVA, $p < 0.0001$) (Fig. 1B). Mice injected with either dose of LPS lost significantly more weight when compared with mice injected with VEH.

During CFC training, there was no effect of LPS or of Drug, but there was a significant interaction on freezing behavior (RMANOVA, $p = 0.0138$) (Fig. 1C). (*R,S*)-ketamine did not affect fear encoding in VEH-injected mice when compared with saline administration (**Fig. S1A**); however, (*R,S*)-ketamine significantly attenuated fear encoding in LPS-injected mice administered 0.83 mg/kg of serotype O111:B4 when compared with saline administration (RMANOVA, $p = 0.0164$) (**Fig. S1B**). (*R,S*)-ketamine did not affect fear encoding in LPS-injected mice administered 1.0 mg/kg of serotype O111:B4 when compared with saline administration (**Fig. S1C**).

During context re-exposure, there was a significant effect of Drug, but not LPS or an interaction on freezing behavior (Fig. 1D–E). (*R,S*)-ketamine significantly attenuated learned fear when compared with

saline in VEH-injected mice (RMANOVA, p = 0.0054). (*R,S*)-ketamine significantly attenuated learned fear in LPS-injected mice administered 1.0 mg/kg of serotype O111:B4, but not in LPS-injected mice administered 0.83 mg/kg of serotype O111:B4 (RMANOVAs, p's = 0.0031 and 0.7120, respectively).

All groups of mice exhibited a comparable amount of immobility time during day 1 of the FST (Fig. 1F). During day 2 of the FST, there was a significant effect of Drug, but not of LPS or an interaction (RMANOVA, p < 0.0001) (Fig. 1G–H). For average freezing, in VEH-injected mice, (*R,S*)-ketamine significantly decreased immobility time when compared with saline (p = 0.0089). In LPS-injected mice (0.83 mg/kg), (*R,S*)-ketamine did not significantly decrease immobility time when compared with saline, although this effect was trending (p = 0.0541). In LPS-injected mice (1.0 mg/kg), (*R,S*)-ketamine significantly decreased immobility time when compared with saline (p = 0.0243). These data suggest that (*R,S*)-ketamine is effective as a prophylactic against the LPS serotype O111:B4 strain, although at a specific dose, for stress-induced behavior.

3.2. (R,S)-ketamine is efficacious as a prophylactic when administered prior to a single injection of LPS serotype O127:B8 in mice

Next, we sought to determine if (*R,S*)-ketamine was effective against a different serotype of LPS. Here, we utilized LPS serotype O127:B8 based on previous studies investigating inflammation-induced behaviors in mice [12,30,31]. Male 129S6/SvEv mice were administered a single injection of saline or (*R,S*)-ketamine (30 mg/kg) 6 days before a single injection of VEH or LPS serotype O127:B8 (0.83 or 1.0 mg/kg) (Fig. 2A). Twenty-four hours later, mice were weighed and then administered a 3-shock CFC paradigm, followed by context re-exposure and the FST.

There was a significant effect of LPS and of Drug, but not a significant interaction on weight loss (2-way ANOVA, p < 0.0001, p = 0.0002, and p = 0.2340, respectively) (Fig. 2B). Mice injected with LPS at 0.83 mg/kg and 1.0 mg/kg lost significantly more weight when compared with mice injected with VEH (p's = 0.0002 and 0.0274, respectively). Prophylactic (*R,S*)-ketamine significantly attenuated weight loss in mice injected with LPS at 1.0 mg/kg when compared with saline (p = 0.0014).

All groups of mice exhibited comparable levels of freezing during the 3-shock CFC training, suggesting that LPS does not affect freezing levels during CFC training (Fig. 2C). During context re-exposure, there was a significant effect of Drug, but not of LPS or an interaction on freezing behavior (RMANOVA, p = 0.0002) (Fig. 2D–E). For average freezing, (*R,S*)-ketamine attenuated learned fear when compared with saline in VEH-injected mice (p = 0.0054). (*R,S*)-ketamine trended to decrease learned fear in LPS-injected mice administered 0.083 mg/kg of serotype O127:B8 (p = 0.0564) and significantly attenuate learned fear in LPS-injected mice administered 1.0 mg/kg of serotype O127:B8 (p = 0.0222) when compared with saline in LPS-injected mice (Fig. 2E).

All groups of mice exhibited a comparable amount of immobility time during day 1 of the FST (Fig. 2F). During day 2 of the FST, there was a significant effect of Drug (RMANOVA, p = 0.0172), but there was no effect of LPS and no significant interaction of Time x Drug and Time x Drug x VEH/LPS treatment (Fig. 2G and H). For average immobility time, (*R,S*)-ketamine significantly decreased immobility time when compared with saline in VEH-injected mice (p = 0.0009). (*R,S*)-ketamine did not decrease immobility time when compared with saline in LPS-injected mice administered 0.83 mg/kg of serotype O127:B8 (p = 0.8325). However, (*R,S*)-ketamine significantly decreased immobility time when compared with saline in LPS-injected mice administered 1.0 mg/kg of serotype O127:B8 (p = 0.0261). These data suggest that (*R,S*)-ketamine is effective as a prophylactic against the LPS serotypes O111:B4 and O127:B8 following 1.0, but not 0.83 mg/kg of administration.

3.3. (R,S)-ketamine is not efficacious as a prophylactic when administered prior to PIC, a viral mimetic, in mice

Next, we sought to determine if (*R,S*)-ketamine was effective against a different inflammatory challenge. Here, we modeled the induction of the APR via PIC [15]. Male 129S6/SvEv mice were administered a single injection of saline or (*R,S*)-ketamine (30 mg/kg) 6 days before a single injection of VEH or PIC (12 mg/kg) (Fig. 3A). Twenty-four hours later, mice were administered a 3-shock CFC paradigm, followed by context re-exposure and the FST.

During the 3-shock CFC training, there was a significant effect of PIC, but not of Drug or the interaction on fear encoding (RMANOVA, p = 0.0008) (Fig. 3B). PIC significantly increased freezing when compared with VEH in saline-injected mice (RMANOVA, p = 0.0016). PIC did not alter freezing behavior when compared with VEH in (*R,S*)-ketamine-injected mice.

During context re-exposure, there was a significant effect of PIC, Drug, and an interaction on freezing behavior (RMANOVAs, p's = 0.0267, 0.0001, 0.0241, respectively) (Fig. 3C and D). For average freezing, (*R,S*)-ketamine significantly reduced fear when compared with saline in VEH-injected mice (p = 0.0006). PIC did not alter freezing behavior when compared with VEH in saline-injected mice (p = 0.9023). Moreover, (*R,S*)-ketamine was ineffective in PIC-injected mice, as both saline and (*R,S*)-ketamine mice froze at comparable levels (p = 0.1687).

On day 1 of the FST, there was a significant effect of PIC, but not a significant effect of Drug or an interaction (RMANOVA, p = 0.0288) (Fig. 3E). During day 2 of the FST, all groups of mice exhibited a comparable amount of immobility time (Fig. 3F and G). These data suggest that (*R,S*)-ketamine is not effective as a prophylactic against PIC; specifically, PIC blocks (*R,S*)-ketamine's efficacy as a prophylactic against fear.

3.4. (R,S)-ketamine is not efficacious as a prophylactic when PIC is administered following CFC in mice

Next, we sought to determine if (*R,S*)-ketamine was effective as a prophylactic if PIC was administered following 3-shock CFC rather than administered prior to 3-shock CFC. Male 129S6/SvEv mice were administered a single injection of saline or (*R,S*)-ketamine (30 mg/kg) 1 week before 3-shock CFC. Twenty-four hours following CFC, a single injection of PIC (12 mg/kg) was administered (Fig. 4A). Context re-exposure and the FST occurred as aforementioned.

Both groups of mice exhibited comparable levels of freezing during the 3-shock CFC training (Fig. 4B). During context re-exposure, both groups of mice froze equally (Fig. 4C–D). Moreover, both groups of mice exhibited a comparable amount of immobility time during days 1 and 2 of the FST (Fig. 4E–G). These data suggest that (*R,S*)-ketamine is not effective as a prophylactic when PIC is administered following a 3-shock CFC stressor.

4. Discussion

Here, we sought to determine if (*R,S*)-ketamine could act as a prophylactic against inflammatory-induced vulnerability to a fear stressor. (*R,S*)-ketamine was effective as a prophylactic for attenuating learned fear in the O111:B4 and O127:B8 serotypes of LPS. (*R,S*)-ketamine was also effective as a prophylactic for decreasing stress-induced depressive-like behavior in the O111:B4 and O127:B8 serotypes of LPS. Both of these effects were limited to administration of 1.0 mg/kg, but not 0.83 mg/kg of serotypes O111:B4 and O127:B8. (*R,S*)-ketamine was not effective against either stress phenotype following PIC administration. These data suggest that prophylactic (*R,S*)-ketamine may protect against inflammation-induced stress phenotypes following an inflammatory challenge, but is ineffective against a viral challenge.

Weight loss is a significant side effect of interferon and other pro-

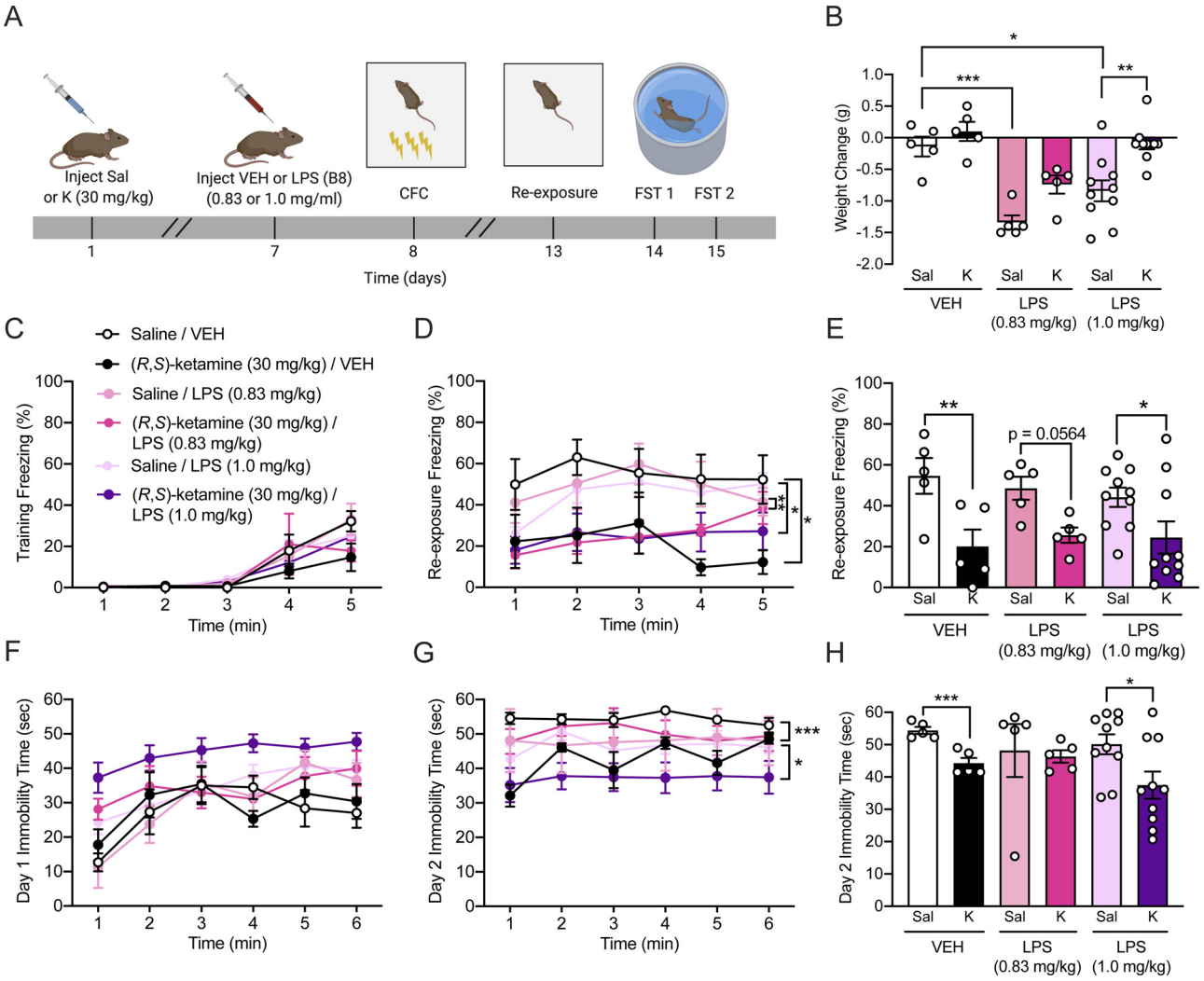

Fig. 2. (*R,S*)-ketamine is efficacious as a prophylactic when administered prior to a single injection of LPS serotype O127:B8 in mice. (**A**) Experimental design. (**B**) There was a significant effect of LPS and of Drug, but not a significant interaction on weight loss. Mice injected with both LPS at 0.83 mg/kg and 1.0 mg/kg lost significantly more weight when compared with mice injected with VEH. (**C**) All groups of mice exhibited comparable levels of freezing during the 3-shock CFC training. (**D**) During context re-exposure, there was a significant effect of Drug, but not of LPS or an interaction. (**E**) In VEH-injected mice, (*R,S*)-ketamine significantly attenuated learned fear when compared with saline. (*R,S*)-ketamine significantly attenuated learned fear in LPS-injected mice administered 1.0 mg/kg but not 0.083 mg/kg of serotype O127:B8 when compared with saline. (**F**) All groups of mice had comparable amount of immobility time during day 1 of the FST. (**G-H**) During day 2 of the FST, there was a significant effect of Drug, but there was no effect of LPS and no significant interaction of Time x Drug and Time x Drug x LPS treatment. (*R,S*)-ketamine significantly decreased immobility time when compared with saline in VEH-injected mice. (*R,S*)-ketamine significantly decreased immobility time when compared with saline in LPS-injected mice administered 1.0 mg/kg of serotype O127:B8. (n = 5–10 male 129S6/SvEv mice per group). Error bars represent ± SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Sal, saline; K, (*R,S*)-ketamine; VEH, vehicle; LPS, lipopolysaccharide; CFC, contextual fear conditioning; FST, forced swim test; sec, seconds; min, minutes.

inflammatory drugs, and there are currently limited treatments available [35]. Additionally, weight alterations are often a measure of depression in a clinical population [36]. Here, we report that LPS administration increases weight loss and that (*R,S*)-ketamine is effective against LPS-induced weight loss in the O127:B8 strain, but not in the O111:B4 strain of LPS. Future studies will be necessary to determine if (*R,S*)-ketamine is effective against other stressor-induced weight alterations or against varying doses of LPS. Of note, we previously reported that social defeat (SD) mice experience significantly more weight loss when compared with control (Ctrl) mice [22]. Antidepressant (post SD), but not prophylactic (before SD) (*R,S*)-ketamine attenuated this weight loss in SD mice [22]. These data suggest that (*R,S*)-ketamine may be useful for protecting against stress-induced weight changes, but more comprehensive studies will be needed for inflammation-induced weight changes.

The dose selectivity of (*R,S*)-ketamine efficacy for both strains is consistent with previous findings reporting functional differences between specific LPS doses and serotypes [37]. Specifically, by using four different *E. coli* LPS serotypes and doses (O111:B4, O55:B5, O127:B8, and O128:B12), Migale and colleagues demonstrated functional disparity in LPS serotype activation of inflammatory pathways in mouse uteri and brain [37]. This study suggests that specific LPS serotypes activate different transcriptional inflammatory pathways, leading to different protein expression; for example, IL-1β is significantly increased by the O111:B4 serotype treatment. However, both O127:B8 and O111:B4 serotypes increase inflammatory protein expression such as Tumor necrosis factor (TNF-α), IL-6, Interleukin 8 (IL-8), and Matrix metalloproteinase-10 (MMP10), which is consistent with the similar effects induced by prophylactic (*R,S*)-ketamine in mice injected with these two strains of LPS [37]. Moreover, Dogan and colleagues analyzed the effect of O55:B5, O127:B8, and O111:B4 strains of LPS on body temperature in rats and report that different strains induce dose- and

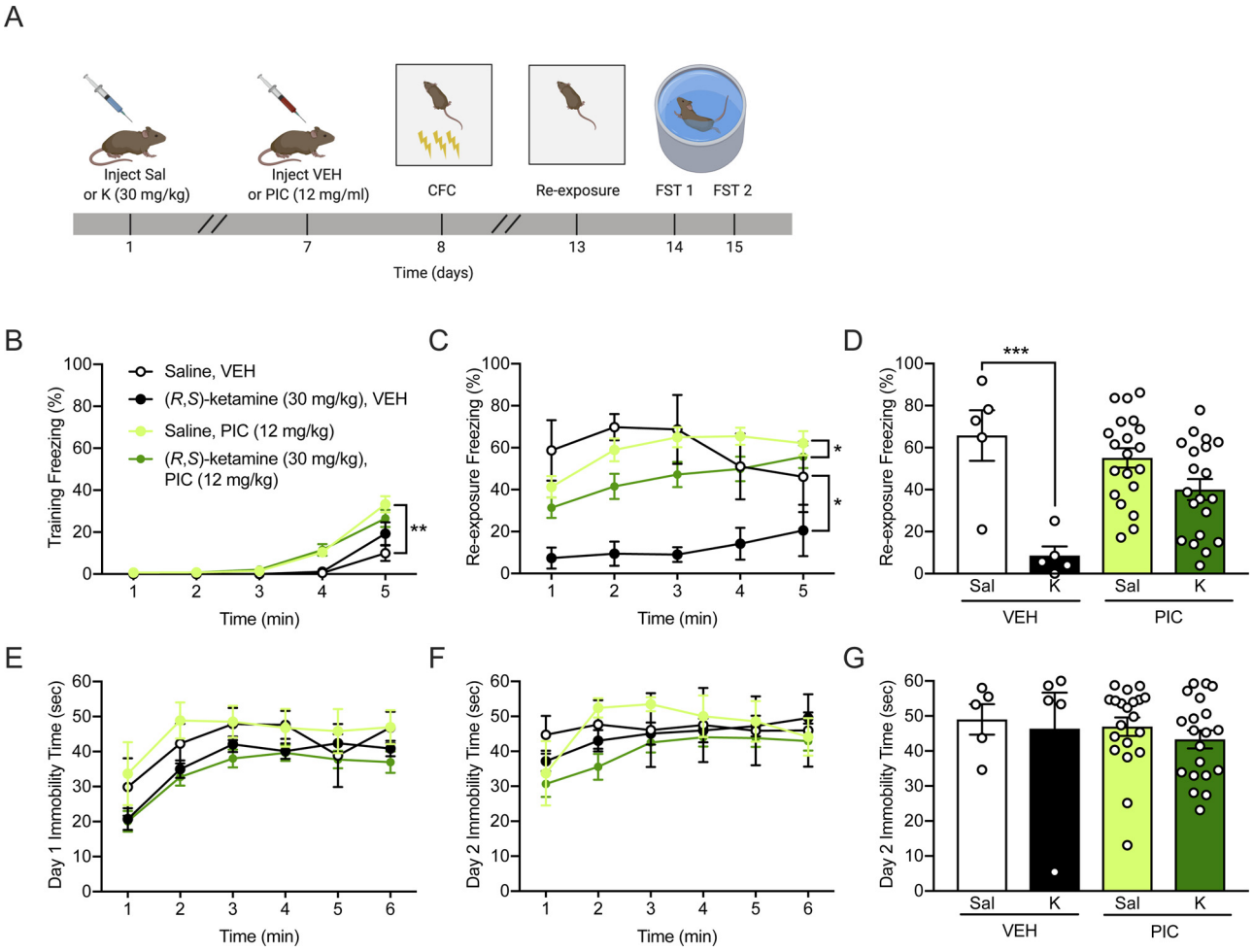

Fig. 3. (*R,S*)-ketamine is not efficacious as a prophylactic when administered prior to PIC, a viral mimetic, in male mice. (**A**) Experimental design. (**B**) During the 3-shock CFC training, there was a significant effect of PIC, but not of Drug or an interaction on fear encoding. In saline-treated mice, PIC increased freezing when compared with VEH. (**C-D**) During context re-exposure, there was a significant effect of PIC, Drug, and an interaction. For average freezing, (*R,S*)-ketamine significantly reduced fear when compared with saline in VEH-injected mice. (*R,S*)-ketamine did not reduce fear when compared with saline in PIC-injected mice. (**E**) On day 1 of the FST, there was a significant effect of PIC, but not a significant effect of Drug or an interaction. (**F-G**) During day 2 of the FST, all groups of mice exhibited a comparable amount of immobility time, but there was a significant interaction of Time x PIC. (n = 5–20 male 129S6/SvEv mice per group). Error bars represent ± SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Sal, saline; K, (*R,S*)-ketamine; VEH, vehicle; PIC, polyinosinic-polycytidylic acid; CFC, contextual fear conditioning; FST, forced swim test; sec, seconds; min, minutes.

serotype-specific changes [38]. In summary, these data indicate a dose- and serotype-specific activation of inflammatory pathways that might be related to structural differences between the LPS strains which could translate into variable phenotypic responses [37].

Here, we report that (*R,S*)-ketamine is effective against LPS, but is not effective against PIC. Of note, the pharmacokinetics of LPS and PIC are significantly different. LPS is a stable amphipathic molecule that rapidly passes from the peritoneal cavity into the bloodstream [39,40], and as a consequence, can activate innate immune cells throughout the body. Conversely, PIC is a large, charged molecule that is rapidly degraded by ubiquitous RNases in the body fluids [41]; thus, when injected intraperitoneally, it does not reach the circulation and its cerebral effects are mediated by blood-borne inflammatory factors [42]. In line with these pharmacokinetic and pharmacodynamic differences, significant dissimilarities between LPS- and PIC-induced behavioral effects have been reported. For example, PIC was found to be less potent in inducing anorexia and lethargy and less effective as a pyrogen than LPS in rats [43]. Moreover, at a transcriptome level, LPS and PIC were found to regulate a different set of genes [44]. Therefore, future studies will be necessary to determine if (*R,S*)-ketamine is effective against a range of peripheral inflammatory stressors such as Interleukin 1 (IL-1) [45], TNF-α [46], or interferon alpha (IFN-α) [47].

In this study, we focused on fear and depressive-like behaviors, rather than anxiety-like behavior, as our previous work has shown that (*R,S*)-ketamine is effective at attenuating learned fear and protecting against depressive-like behavior, but is not effective against anxiety-like behavior when administered 1 week before a fear stressor. However, LPS and PIC have been successfully used to establish animal models for some of the most common and debilitating neuropsychiatric disorders, including depression and anxiety [11]. LPS was previously found to decrease social exploration in the social interaction test [48] and to increase anxiety-like behavior in the elevated plus maze (EPM) in rats [49]. PIC has been reported to induce depressive- and anxiety-like behaviors 24 h following administration, as measured by the sucrose preference test (SPT) and the open field (OF) test, respectively [50]. Therefore, it remains to be determined if (*R,S*)-ketamine can additionally attenuate stress-induced anxiety-like behavior following an inflammatory stressor such as LPS or PIC.

Previous studies demonstrate that LPS alone can induce behavioral deficits in the FST and SPT in C57BL/6 mice, but this has not yet been tested in 129S6/SvEv mice [10,12]. 129S6/SvEv mice are our model of choice since this strain is vulnerable to stress, unlike the C57BL/6 mice which are often resilient to stress [51]. However, previous studies assay depressive-like behavior 24 h after an LPS injection unlike our current

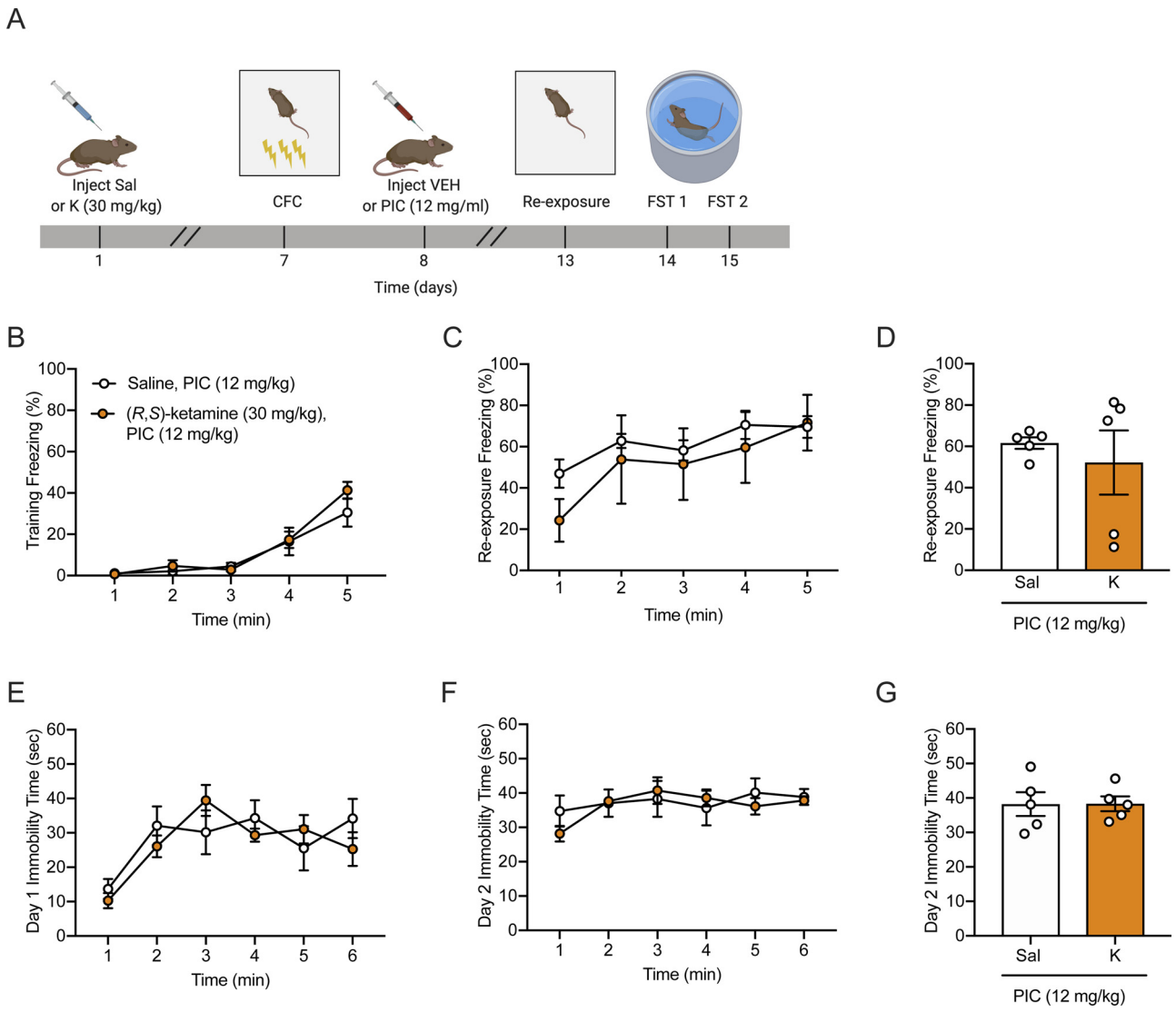

Fig. 4. (*R,S*)-ketamine is not efficacious as a prophylactic when PIC is administered following CFC in male mice. (**A**) Experimental design. (**B**) Both groups of mice exhibited comparable levels of freezing during 3-shock CFC training. (**C-D**) During context re-exposure, both groups of mice froze equally. (**E**) Both groups of mice had comparable immobility time during day 1 of the FST. (**F-G**) Both groups of mice had comparable immobility time during day 2 of the FST. (n = 5 male 129S6/SvEv mice per group). Error bars represent ± SEM. Sal, saline; K, (*R,S*)-ketamine; VEH, vehicle; PIC, polyinosinic-polycytidylic acid; CFC, contextual fear conditioning; FST, forced swim test; sec, seconds; min, minutes.

study, which assayed behavior days after an injection. Therefore, LPS may induced depressive-like behavior if injected more proximately to the behavioral assays. Here, we do not report significant main effects of LPS on behavior, but we report significant main effects of PIC on fear behavior. Therefore, it remains to be determined if LPS and/or PIC can induce behavioral deficits without being paired with a fear-based stressor in 129S6/SvEv at a range of doses. In future experiments, LPS-induced inflammation alone will serve as the stressor and/or LPS-induced vulnerability to other stressors such as SD, chronic corticosterone (CORT), or learned helplessness (LH) will be assayed.

In summary, we report that (*R,S*)-ketamine is effective as a prophylactic for attenuating learned fear in the O111:B4 and O127:B8 strains of LPS. (*R,S*)-ketamine is also effective as a prophylactic for decreasing stress-induced depressive like behavior in the O111:B4 and O127:B8 strains. (*R,S*)-ketamine is not effective against either stress phenotype following PIC administration. Therefore, these data suggest that prophylactic (*R,S*)-ketamine may protect against selective inflammation-induced stress phenotypes following an inflammatory challenge. This work brings to light the need for novel therapeutics for treating depression in patient population with peripheral inflammation.

Financial Disclosures

JCM and CAD are named on provisional and non-provisional patent applications for the prophylactic use of (*R,S*)-ketamine and related compounds against stress-related psychiatric disorders.

Acknowledgements

AM was supported by a Sackler Award from The Sackler Institute for Developmental Psychobiology and a Rotary Global GrantGG1864162. OC was supported by the Summer Program for Under-Represented Students (SPURS) at Columbia University Irving Medical Center (CUIMC). SPURS is supported by an NIHR25NS076445to Andrew Marks. CTL was supported by an NIH DP5 OD017908. JCM was supported by a Neurobiology & Behavior (NB&B) Research Training GrantT32 HD007430-19 and by an NSF Graduate Research Fellowship Program DGE 16-44869. HCH was supported by an NIH 2T32MH015174-40 and an NIHK99AG059953-01A1. CAD was supported by a retention package from the NYSPI.

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