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RESEARCH ARTICLE

Intranasal dantrolene nanoparticles inhibit lipopolysaccharide-induced depression and anxiety behavior in mice

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Running head: Intranasal dantrolene nanoparticles inhibit lipopolysaccharide-induced depression and anxiety behavior in mice

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ABSTRACT

This study investigates the therapeutic effectiveness of intranasal dantrolene nanoparticle pretreatment to inhibit lipopolysaccharide (LPS)-induced pathological inflammation, synapse destruction, and depressive and anxiety behavior in mice. B6SJLF1/J adult mice were pretreated with intranasal dantrolene nanoparticles (dantrolene: 5mg/kg), daily, Monday to Friday, 5 days per week, for 4 weeks. Afterwards, mice were treated with a single intraperitoneal injection of LPS (5mg/kg). Behavioral tests for depression and anxiety were performed 24 hours after the one-time LPS injection. Biomarkers for pyroptosis-related inflammation cytokine levels (IL-1β and IL-18) in the blood and brain were measured using enzyme-linked immunosorbent assay (ELISA) and immunoblotting, respectively. Changes in primary protein (NLRP3: NLR family pyrin domain containing 3, Caspase-1, N-GSDMD: N-terminal protein gasdermin D) and synapse protein-related (PSD-95 and synaptin-1) activation of inflammatory pyroptosis in mouse brains were measured using immunoblotting. Results indicated that intranasal dantrolene nanoparticle treatment robustly inhibited LPS-induced increases in depressive and anxiety behavior, LPS-induced pathological elevation of IL-1β and IL-18 levels in the blood and brain, and LPS-induced activation of pyroptosis. Furthermore, intranasal dantrolene nanoparticles significantly decreased PSD-95 and synaptin-1 levels. Intranasal dantrolene nanoparticles have demonstrated neuroprotective effects against inflammation-mediated depression and anxiety

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behaviors and should be studied further as a future effective drug treatment of major depressive or anxiety disorders.

INTRODUCTION

Three hundred million people worldwide suffer from major depressive disorder (MDD)[1]. MDD is a chronic and recurrent disease affecting about 20% of the population and is the global leading cause of suicide[2]. MDD imposes a tremendous psychological burden, as well as significant social repercussions and contributions to other disabilities[3]. The expected direct and indirect costs of MDD are up to $6 trillion in the US alone[4]. An estimated 4% of the global population currently experiences anxiety disorders. In 2019, 301 million individuals reported suffering from an anxiety disorder, with a lifetime prevalence of approximately 34%[5]. MDD and anxiety disorders were twice as prevalent among women[6,7], with overall age-specific rates relatively stable or increasing across the lifespan[8]. Depression and anxiety are the most common psychiatric diseases, chronic diseases with unclear mechanisms of pathology[9], and co-exist frequently.

Current antidepressant treatments suffer from significant limitations, including slow onset of action, high rates of nonresponse, and acute worsening of anxiety[10]. Benzodiazepines are ineffective in some anxiety spectrum disorders and are not recommended for long-term use due to concerns about their potential for abuse, tolerance, and withdrawal[11]. Treatment of MDD faces a high risk of resistance (up to

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30% of patients are unresponsive to first treatment) and relapse (up to 8%)[3,9]. Treatment-resistant anxiety with remission rates may be as low as 25–35%, and relapse rates post-remission may reach 30% within a decade[12]. Thus, there is an urgent need to develop novel approaches to treat depression and anxiety, especially treatment-resistant depression or anxiety, with minimal side effects or organ toxicity.

Ketamine, an N-methyl-d-aspartate (NMDA) glutamate receptor antagonist, was approved in 1970 by the Food and Drug Administration (FDA) for use in children and adults as a general anesthetic. Recent studies indicate that ketamine is effective in treating MDD, especially treatment-resistant depression (TRD)[13,14]. Ketamine is also effective in treating anxiety disorders, even those resistant to traditional treatments[15,16]. While traditional antidepressants can take weeks to months to have an effect, ketamine has a rapid impact on mood and suicidality, with mood changes reported as early as 4 hours after treatment[17]. As an N-methyl-D-aspartate receptor (NMDAR) antagonist, a primary glutamate receptor on the plasma membrane that causes Ca^{2+} influx into the cytosol from the extracellular space, ketamine is a safe and broadly effective drug for treating patients with depression or anxiety disorders, similar to esketamine[18]. This indicates that glutamate-mediated excitotoxicity and its associated disruption of intracellular Ca^{2+} homeostasis play a key role in the pathology of depression and anxiety psychiatric disorders.

Increasing evidence suggests that upstream disruption of intracellular Ca^{2+} homeostasis, together with the associated downstream inflammation and synapse

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dysfunction, plays a critical role in MDD pathologies[19-21] and anxiety disorders[22,23]. Overactivation of the ryanodine receptors (RyR) in MDD and excessive Ca^{2+} release from the endoplasmic reticulum (ER) results in depletion of ER Ca^{2+} and pathological elevation of cytosol and mitochondrial Ca^{2+} concentrations, which is detrimental to synapse function and cell survival[21,24,25]. RyR overactivation was previously demonstrated to increase anxious behavior as well[24]. Upstream Ca^{2+} dysregulation results in mitochondrial dysfunction[26], mitochondrial and cellular oxidative stress[9,27], the activation of inflammasomes, and cell and neuron death via pyroptosis[28-31]. This results in the release of inflammatory cytokines (IL-1β and IL-18) and pathological inflammation-mediated cell death[30,32]. Pathological cytokines, especially pyroptosis-related IL-18, play important roles on inflammation mediated neurodegeneration in psychiatric disorders[33,34]. Gut dysbiosis and associated inflammation further contribute to observed pathologies in depression and anxiety behaviors[35,36]. Thus, a drug that inhibits upstream Ca^{2+} dysregulation, thereby limiting downstream pathological inflammation, programmed cell death by pyroptosis, and synapse destruction, is expected to effectively treat MDD and anxiety disorders.

Dantrolene, a RyR antagonist, is an FDA-approved drug for the treatment of malignant hyperthermia, muscle spasms, and neuroleptic syndrome, with tolerable side effects and occasional liver toxicity at high doses[37]. Dantrolene, given its ability to inhibit upstream critical Ca^{2+} dysregulation, is neuroprotective against many neurodegenerative diseases, including cerebral ischemia[38,39], Huntington's disease[40],

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spinocerebellar ataxia[41], amyotrophic lateral sclerosis[42], and seizures[43,44]. Dantrolene robustly inhibited up to 78% of NMDA-induced elevation of cytosolic Ca^{2+} concentration in cerebral cortical neurons[45], suggesting its high potential to treat MDD, operating with a similar molecular mechanism as ketamine ketamine[46]. These findings suggest an urgent need for further investigation.

In this study, we investigated the therapeutic effectiveness and potential mechanisms of intranasal dantrolene nanoparticles on depression and anxiety behaviors associated with pathological inflammation in adult mice. We induced inflammation via a one-time intraperitoneal injection of LPS. Our results demonstrated that intranasal dantrolene nanoparticle treatment for twelve consecutive weeks robustly inhibited LPS-induced depression and anxiety behaviors in adult mice. These effects were attributed to its robust inhibition of LPS-induced elevation of pyroptosis-related inflammation cytokines IL-1β and IL-18 in the blood and brain, as well as the loss of synapse proteins (PSD-95 and synpatin-1) in the brain. This study indicates that intranasal dantrolene nanoparticles may be an effective treatment for depression and anxiety psychiatric disorders through inhibiting pathological inflammation and synapse destruction in the CNS.

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MATERIALS AND METHODS

Animals

All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Pennsylvania. B6SJLF1/J adult mice were obtained from Jackson Lab. Mice were divided into different cages according to age and gender. There were no more than five mice per cage. Both male and female mice were used in this study. Some mice were excluded based on abnormal appearance, behavior, or body condition following established criteria[47] or as deemed unhealthy by animal facility veterinarian.

Experimental treatment groups

As demonstrated in Supplemental Figure 1, adult male and female mice (5-10 months old) were randomly divided into experimental groups. Littermates were used within each experimental group. Non-littermates with identical genetic background, age, and breeding environment were used among different experimental groups without randomization. The mice were pretreated with either intranasal dantrolene nanoparticles (IN Dan, 5mg/kg) or the empty vehicle (Veh), once a day, 5x/week (Monday through Friday), for four continuous weeks. The purpose of this pretreatment was to optimize therapeutic efficacy and assess potential side effects, given the lack of prior studies on dantrolene as a treatment for depression and anxiety. Control groups received no pretreatment. Dantrolene nanoparticles were freshly prepared before each administration, and an intranasal dantrolene nanoparticle stock solution was made at 5

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mg/ml. The Ryanodex formulation vehicle was made fresh and contained all inactive ingredients in Ryanodex[48,49]. At the end of the 4-week pretreatment period, mice were treated with a one-time intraperitoneal injection of lipopolysaccharides (LPS, 5 mg/kg). Mice in the control group, which did not receive pretreatment, were administered LPS (LPS, 5mg/kg) once as well. The sham control group received no pretreatment or treatment. 24 hours following the one-time LPS treatment, behavioral tests for depression and anxiety behaviors were performed on all mice, with the following order tests ranked for increasing stress: OPT, EPMT, TST, and FST. Both the animal behavior investigators and the data analysts were unaware of the animal grouping information, which effectively avoided investigator bias. Afterwards, all mice were euthanized, and the blood and brain were harvested to examine inflammation, programmed cell death via pyroptosis, and synapse destruction.

Intranasal dantrolene nanoparticle or vehicle administration

Dantrolene (Sigma, St Louis, MO) was dissolved in the Ryanodex Formulation Vehicle (RFV: 125 mg mannitol, 25 mg polysorbate 80, 4 mg povidone K12 in 5 mL of sterile water, with the pH adjusted to 10.3), similar to our previous publications[49,50]. For intranasal administration, the final concentration of dantrolene was 5 mg/mL. Mice were held and fixed by the scruff of their necks with one hand. On the other hand, mice were administered 1μL/gram body weight of dantrolene nanoparticles in the RFV, or the empty vehicle (e.g., a mouse weighing 20 g would be given 20 μL of solution, equivalent to dantrolene dosing at 5 mg/kg). The solution was slowly delivered directly into the

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mouse’s nose. Care was taken to ensure that mice were minimally stressed and that the solution remained in the nasal cavity and did not enter the stomach or lungs.

Forced swimming test (FST)

Depression behavior was assessed using the FST, as previously described in other publications[51]. In each experimental condition, mice were placed in a clear glass tank (25 cm height, 10 cm diameter, 15 cm water depth), with the water’s temperature set to 23 ± 2°C. The total test duration was 6 minutes: 2 minutes for adaptation, followed by 4 minutes of measured and recorded immobility time. Immobility occurred when the mouse ceased floundering and floated passively in an equilibrium state. The greater the immobility time, the more severe the depression behavior was considered.

Tail suspension test (TST)

Depression behavior was assessed using the tail suspension test, as described previously, with the following modifications[52]. Specially manufactured tail suspension boxes made of cardboard, with dimensions of 42 cm (height) x 18 cm (width) x 30 cm (depth) were utilized. To prevent mice from observing or interacting with each other, each mouse was suspended within its own three-walled rectangular box. Animals were acclimated to the testing environment for at least one hour prior to the test. Each mouse was suspended in the middle of the box. The width and depth of the box were sufficiently sized to prevent the mouse from contacting the walls. In this setting, the approximate distance between the mouse's nose and the apparatus floor was 20-25 cm. A plastic suspension bar, with dimensions of 50 cm (height) X 40 cm (depth), was

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positioned at the top of the box and used to suspend the tail of each mouse. At the bottom of each box, paper towels were placed to collect feces and urine. A dark grey box was used for albino-colored mice, while a white colored box was used for mice of other coat colors. Prior to evaluation, the tail of each mouse was securely attached to the suspension bar, which was able to withstand the mouse's weight. A video camera was placed in position to record the TST session. The total duration of the test was 6 minutes. The paper towel was replaced after each trial. After all sessions were finished, Any-maze software (Stoelting, USA) was used to analyze collected data.

During behavioral analysis, the mobility time of each mouse was measured and subtracted from 360 seconds, yielding the immobility time. The greater the immobility time, the more severe the depressive behavior was considered.

Open field test (OFT)

The OFT was performed to evaluate anxiety behavior as described previously in our other publications[53]. Each mouse was individually placed into the OFT apparatus (dimensions of 44 × 44 × 44 cm^3), facing the wall. A camera was placed above the apparatus in dim light and locomotor activity was recorded for 6 minutes. The anxiety behavior of each mouse was measured using the total distance traveled, number of center entries, immobility time, and time spent in the central zone. After each test, the apparatus was cleaned with 75% ethanol. Any-maze software (Stoelting USA) was used to analyze collected data. The less the central zone distance and mean speed, the

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greater the anxiety behavior was considered. The greater the immobility time, the more severe the anxiety behavior was considered.

Elevated plus maze test (EPMT)

Anxiety behavior was assessed using EPMT, as described previously in our other publications[54]. Mice were placed in the center of an elevated plus-maze (dimensions of 33 cm (length) x 5 cm (width) x 25 cm (height) for the closed arms), under dim lighting. Behavior was observed and recorded for 5 minutes. Any-maze software (Stoelting USA) was used to analyze the collected data. Time spent in the closed arms, time spent in the open arms, and the number of explorations of open arms were measured and recorded. The greater the time spent in the closed arms, the more severe the anxiety behavior was considered.

Euthanasia and tissue collection

Mice were euthanized after completion of all behavioral tests. As described previously[49], mice were deeply anesthetized with 2–4% isoflurane delivered through a nose cone, with the concentration adjusted according to toe pinch response. The skin of each mouse was prepared**,** and an incision was made to open the chest and expose the heart. Blood was collected from the heart using a 27G needle and syringe for the serum study. Blood was centrifuged at 1400 rpm at 4°C for 30 min, and the supernatant was collected and frozen at –80°C. The mice were euthanized by transcardial perfusion and exsanguinated with cold phosphate-buffered saline.

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Measurements of serum concentration of IL-1β and IL-18

Following company instructions, serum IL-1β and IL-18 cytokines were measured using an ELISA kit . as described previously for the measurement of S100β[55,56]. All reagents and serum samples were thawed to room temperature (18 - 25 °C) before use. All standards and samples were run at least in duplicate. 100 µL of each standard and sample was added to the appropriate wells. After the wells were covered, the standards were incubated for 2.5 hours at room temperature, while the samples were incubated overnight at 4 °C with gentle shaking. The solution was discarded and washed 4 times with 1X Wash Solution. Each well was then filled with Wash Buffer (300 µL) using a multi-channel pipette or auto washer After the last wash, remaining Wash Buffer was removed by aspirating or decanting. The plate was inverted and blotted against clean paper towels. 100 µL of 1x prepared Detection Antibody was then added to each well. Wells were covered and incubated for 1 hour at room temperature with gentle shaking. The solution was then discarded and the procedure outlined in step 3 was repeated. 100 µL of the prepared Streptavidin solution was added to each well. The wells were covered and incubated for 45 minutes at room temperature with gentle shaking. The solution was discarded, and the wash was repeated as outlined in step 3. 100 µL of TMB One-Step Substrate Reagent (Item H) was then added to each well. The wells were covered and incubated for 30 minutes at room temperature in the dark with gentle shaking. 50 µL of Stop Solution (Item I) was added to each well, whereupon the absorbance was then immediately read at 450 nm by a synergy H1 plate reader

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(Agilent, CA, USA). The mouse IL-1β and lL-18 ELISA Kits were purchased from SIGMA(RAB0275-1KT/RAB08100-1KT).

Immunoblotting (Western Blot)

As previously described in our other publications[57], whole brain tissues were extracted by homogenization using cold RIPA buffer (#9806S, Cell Technology, USA), supplemented with protease inhibitor cocktails (P8340 Roche). Brain homogenates were rocked at 4°C for 90 minutes and then centrifuged at 14,000 rpm (Brushless Microcentrifuge, Denville 260D) for 20 minutes at 4°C to remove cell debris. After collecting the supernatant, protein concentration was measured using a BCA protein assay kit (Pierce, Rockford, IL 61101 USA). To briefly describe, equal amounts of protein (20μg/lane) were loaded onto 4-20% gel electrophoresis of mini-protein TGX precast (Cat. #4561094, BIO-RAD) and transferred to polyvinylidene difluoride (PVDF) membranes (Immobilon-P, MERK Millipore, Ireland) using a wet electrotransfer system (BIO-RAD, USA). The PVDF membrane was blocked with 5% BSA (Sigma-Aldrich) for 1 hour and subsequently incubated overnight at 4°C with primary antibodies targeting NLRP3, cleaved caspase-1, GSDMD-NT, IL-1β, lL-18, PSD95, Synapsin-1, and GAPDH (Table 1). After incubation with HRP-conjugated secondary antibodies (anti-mouse IgG1 and anti-rabbit IgG), the membranes were washed three times for 10 minutes each with TBST (Tris-buffered saline containing 0.2% Tween-20). Protein bands were detected using ECL Western Blotting Detection Reagents (Cytiva,

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Amersham, UK) and quantified with ImageJ software (NIH, Bethesda, MD, USA) by two independent investigators. The results were then averaged for each mouse.

Statistical analysis

All data were represented as mean ± one standard deviation (Means±SD). Statistical analysis was conducted using GraphPad Prism (Version 9.3.1, CA, USA). Comparisons of more than two groups were conducted using one-way ANOVA with Tukey's multiple comparison test. The sample size (N) was estimated to be around 15 mice per group for adequate power analysis based on our previous similar studies[49,58]. Normal distribution tests were performed for all data. Two-sided p-values were reported. $P<0.05$ was considered statistically significant.

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RESULTS

Intranasal dantrolene nanoparticle pretreatment significantly inhibited LPS-induced helplessness and anxiety-related behaviors.

The FST and TST are commonly used to evaluate helplessness and depression behaviors in mice, as well as to compare drug efficacy and side effects[23,59,60]. In both the FST (Fig. 1A) and the TST (Fig. 1B), LPS-induced experimental groups exhibited significantly higher immobility times (indicating helplessness or depressive behaviors), increasing 89% (60.11±42.24 vs. 113.3±52.34) and 90% (98.16±63.99 vs. 186.6±54.98), respectively. However, intranasal dantrolene nanoparticle pretreatment robustly inhibited these increases in immobility time, diminishing by 41% in the FST

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(113.3±52.34 vs. 66.88±36.83) and 46% in the TST (186.6±54.98 vs. 101.5±40.22). In contrast, the intranasal dantrolene nanoparticle vehicle control condition did not demonstrate these same inhibitory effects on helpless behaviors. The elevated plus maze test (EPMT) and open field test (OPT) are commonly used to evaluate anxiety-related behaviors in mice and to test drug efficacy in treating anxiety behaviors[23,61,62]. In both the EPMT (Fig. 1C) and OFT (Fig. 1D), LPS administration significantly increased depression and anxiety behaviors, as indicated by a 46% increase in the time spent in the closed arms of the EPMT (162.2±56.03 vs. 237.0±44.18) and a 122% increase in observed immobility time in the OFT (85.06±47.89 vs. 188.9±63.97). Intranasal pretreatment with dantrolene nanoparticles significantly attenuated these behavioral alterations, reducing time in the closed arms by 29% (237.0±44.18 vs. 168.8±51.44) and immobility time by 40% (188.9±63.97 vs. 113.9±44.61). Conversely, the vehicle-treated controls did not exhibit these protective effects. In the open field test (Supplementary Fig. 2), reduced central zone distance and locomotor speed reflected increased anxiety behavior. LPS administration significantly decreased mean speed relative to untreated controls but did not alter central zone distance. Intranasal dantrolene nanoparticle pretreatment partially reversed the LPS-induced reduction in mean speed and central zone distance.

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Intranasal dantrolene nanoparticle pretreatment significantly inhibited LPS-induced helplessness and anxiety behaviors primarily in female mice.

Sex differences in the neuroprotective effects of intranasal dantrolene nanoparticles on helplessness and anxiety behaviors were then evaluated. As illustrated in Fig. 2, dantrolene nanoparticle treatment, but not the vehicle control, significantly reduced helplessness behaviors in female mice, as measured by the FST (Fig. 2A) and TST (Fig. 2B); no such effects were observed in males. For anxiety behaviors, dantrolene nanoparticles attenuated LPS-induced anxiety in males as assessed by the EPMT (Fig. 2C), but the most robust effects were observed in females, where reductions were evident in both the EPMT (Fig. 2C) and OFT (Fig. 2D).

Intranasal dantrolene nanoparticles significantly inhibited LPS-induced pathological elevation of pyroptosis-related inflammation cytokines in blood and brains.

Compared with untreated controls, a single intraperitoneal injection of LPS (5 mg/kg) significantly increased blood IL-1β concentrations by 146% (136.4±29.31 vs. 335.6±41.12; Fig. 3A) and IL-18 concentrations by 67% (233.1±17.20 vs. 390.2±47.65; Fig. 3B). Intranasal dantrolene nanoparticle treatment, but not the vehicle control, significantly attenuated these increases, reducing IL-1β levels by 46% (335.6±41.12 vs. 183.2±70.05) and IL-18 levels by 23% (390.2±47.65 vs. 299.2±45.18; Fig. 3B). Moreover, dantrolene nanoparticles significantly suppressed LPS-induced elevations of

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IL-1β and IL-18 protein levels in both the blood (Fig. 3A, B) and brain tissue (Fig. 3C, D; Supplementary Figs. 3 and 4).

Intranasal dantrolene nanoparticles modulate LPS-induced programmed cell death by pyroptosis in the mouse brain.

Relative to untreated controls, LPS administration elevated expression of pyroptosis-related proteins (NLRP3, caspase-1, and N-terminal GSDMD) in mouse brain tissue, with a significant increase in GSDMD. Intranasal dantrolene nanoparticles significantly inhibited the LPS-induced elevation of caspase-1 (P20), a marker of programmed cell death through pyroptosis and apoptosis[65,66] (Fig. 4C, D), and reduced pyroptosis pathway activation, with a significant effect on cleaved caspase-1 (Fig. 4).

Intranasal dantrolene nanoparticles significantly inhibited LPS-induced synapse protein loss in mouse brains.

Compared with untreated controls, LPS administration significantly reduced synaptic protein levels of PSD-95 by 45% (1.322±0.0118 vs. 0.726±0.123; Fig. 5A, C) and synapsin-1 by 44% (0.926±0.0335 vs. 0.520±0.0851; Fig. 5B, D) in mouse brains. Four weeks of intranasal pretreatment with dantrolene nanoparticles, but not the vehicle control, significantly attenuated these reductions, restoring PSD-95 by 77% (0.726±0.123 vs. 1.286±0.2538; Fig. 5A, C) and synapsin-1 by 76% (0.520±0.0851 vs. 1.021±0.1467; Fig. 5B, D).

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DISCUSSION

There is an urgent need for novel therapeutic interventions to prevent or treat MDD[3,63] and anxiety[5] psychiatric disorders, especially in cases of drug-resistant or recurrent depression and anxiety[5,12,64]. In this study, intranasal dantrolene nanoparticles were shown to ameliorate helplessness and anxiety behaviors in adult mice. This effect was evidenced by the inhibition of pathological elevations of inflammatory cytokine levels (IL-1β and IL-18) in the blood and the pyroptosis biomarker caspase-1 (P20), as well as the loss of synaptic proteins in the brain. Considering that upstream Ca^{2+} dysregulation plays a critical role in the pathology of depression and anxiety, supported by the clinical efficacy of ketamine and esketamine in treatment-resistant cases[10,12], these findings suggest that dantrolene, or related compounds, may represent a new class of therapeutics. These would be aimed at correcting upstream Ca^{2+} dysregulation and its downstream consequences, including pathological inflammation and pyroptosis, in psychiatric disorders.

Increasing studies suggest that inflammation plays a critical role in the pathophysiology of MDD and anxiety, making it an attractive therapeutic target[65-68]. Intracellular Ca^{2+} dysregulation has been proposed as an upstream driver of mitochondrial dysfunction, oxidative stress, and inflammation in MDD[21,69,70] and anxiety[34,71]**.** Notably, inhibition of Ca^{2+} dysregulation via suppression of RyR overactivation has been shown to reduce inflammation in Gulf War illness, a condition associated with depression and dementia[24]. Dantrolene, a clinically available RyR

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antagonist, has previously demonstrated anti-inflammatory effects in diabetes[72,73], sepsis[74,75] , and COVID-19 infection[76,77]. However, its ability to inhibit Ca^{2+} dysregulation and related inflammation in the context of depression and anxiety had not been investigated prior to this study.

Several animal models have been developed to study depression and anxiety behaviors, based on proposed neurobiological mechanisms such as altered neurotransmission, hypothalamic–pituitary–adrenal (HPA) axis dysregulation under chronic stress, inflammation, reduced neuroplasticity, and network dysfunction [78-80]. Among these, induction of inflammation with lipopolysaccharide (LPS) has become a widely used approach to model depression and anxiety and to evaluate the efficacy of potential treatments[33,69,81-84]. Intraperitoneal (IP) administration is the most common method, given its ease and reproducibility, though effective dosing and regimens vary. A single high dose of LPS (5 mg/kg) has been shown to reliably induce inflammation and depressive behaviors[69]. Consistent with these findings, our study confirmed that one-time IP LPS administration (5 mg/kg) produced robust and reproducible inflammation, as well as depression and anxiety behaviors in adult mice, providing a suitable model for evaluating the therapeutic efficacy of dantrolene.

Using this model, we demonstrated that intranasal dantrolene nanoparticle pretreatment robustly attenuated LPS-induced inflammation and associated helplessness and anxiety behaviors. In addition to its previously reported protective effects against memory loss in various AD animal models[49,85-87], intranasal dantrolene

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nanoparticles also significantly inhibited neuroinflammation, pyroptosis-related cytokines (IL-1β and IL-18), and synaptic protein loss, highlighting a role for inflammation and pyroptosis in both cognitive dysfunction and psychiatric disorders. These findings suggest that targeting inflammation may represent a unifying therapeutic strategy for treating both memory impairment and psychiatric symptoms in conditions such as Alzheimer's disease. Importantly, our data indicates that intranasal dantrolene nanoparticles could be developed as a novel therapeutic approach for major depressive disorder (MDD) and anxiety, potentially through inhibition of pathological inflammation.

MDD affects women at twice the rate of men[88,89]. Proposed mechanisms underlying this sex difference include the influence of estrogen[90], alterations in serotonin and tryptophan metabolism[91], and differential stress responses[89]. An important finding in this study is that intranasal dantrolene nanoparticles were more effective in ameliorating helplessness and anxiety behaviors in female mice than in male mice. This observation aligns with the higher prevalence of MDD in females and suggests that dantrolene may target key pathophysiological processes contributing to sex differences in depression and anxiety. The more pronounced inhibition of inflammation observed in female mice further supports the possibility that dantrolene acts by suppressing upstream Ca^{2+} dysregulation, thereby mitigating downstream pathological processes, including inflammation, pyroptosis, and synaptic protein loss. Although the precise mechanisms remain to be elucidated, these findings highlight a potential sex-specific therapeutic benefit of intranasal dantrolene nanoparticles.

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Intranasal drug delivery, particularly in a nanoparticle formulation, offers a promising strategy to bypass the blood-brain barrier (BBB) and enhance central nervous system penetration with minimal peripheral toxicity[92-94]. Our previous work demonstrated that intranasal dantrolene nanoparticles achieve greater therapeutic efficacy in the brain compared with oral and subcutaneous forms of administration, with minimal or no side effects following chronic use[49,50,58]. Notably, intranasal dantrolene nanoparticles significantly ameliorated memory loss in 5XFAD mice, supporting their potential as a disease-modifying therapy[49]. RyR overactivation and the resulting Ca^{2+} dysregulation have been identified as upstream pathological events that drive mitochondrial dysfunction[95], oxidative stress[96], pathological inflammation[97], and pyroptotic neuronal death[98]. These processes contribute to the development of depression and anxiety disorders[30,68,99]. In the present study, we demonstrated that inhibition of RyR overactivation with dantrolene robustly attenuated pathological inflammation, prevented synaptic protein loss, and ameliorated depressive and anxiety behaviors in mice. These findings reinforce the concept that upstream Ca^{2+} dysregulation and downstream inflammation and synaptic damage are critical drivers of psychiatric pathology. Similar to esketamine[18,100], intranasal dantrolene nanoparticles hold promise as a potential disease-modifying treatment for depression and anxiety and warrant further investigation in clinical studies.

Since depression and anxiety are chronic disorders, effective treatments must be suitable for long-term use with minimal side effects or organ toxicity. A key advantage of

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utilizing intranasal dantrolene nanoparticles, compared with oral or intravenous administration, is their significant increase in the brain-to-blood concentration ratio of dantrolene[49,50], particularly in aged mice[58]. This enhanced CNS delivery improves therapeutic efficacy while reducing systemic exposure and minimizing adverse effects[49]. In our previous studies, chronic intranasal administration of dantrolene nanoparticles for up to 10 months in adult 5XFAD mice did not cause detectable side effects on nasal structure or olfactory function[49,101], liver structure[49] or function, or muscle function[48,49,85]. These findings suggest that intranasal dantrolene nanoparticles are well-tolerated with chronic use in animal models. Nonetheless, further studies are needed to evaluate the long-term safety and tolerability of chronic dantrolene treatment in human patients.

This study has the following limitations: 1) The mice used were 5–10 months old, an age range considered adult; however, future studies should examine different age groups, particularly aged mice, given the high prevalence of psychiatric disorders in older populations. The use of littermates within each experimental group may bias the observations in behavioral testing. 2) We were unable to measure cytosolic versus mitochondrial Ca^{2+} levels in brain tissue due to technical challenges. Nevertheless, prior studies have demonstrated that dantrolene inhibits LPS- or AD-related gene mutation-induced RyR overactivation and Ca^{2+} dysregulation in cell culture models[102,103]. 3) We did not measure reactive oxygen species (ROS) concentrations in the brain, which are typically upstream drivers of NLRP3 inflammasome activation[104]. 4) Although intranasal dantrolene nanoparticles robustly and significantly inhibited LPS-induced pathological

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inflammation, as well as depression and anxiety behaviors, their effects on programmed cell death by pyroptosis could not be consistently confirmed, underscoring the need for further mechanistic studies of dantrolene's neuroprotective actions. 5) We did not investigate dose-response effects of dantrolene on depression and anxiety behaviors. Because physiological Ca^{2+} release from the ER is essential for cellular function, excessive inhibition could be detrimental. Determining the optimal dose of intranasal dantrolene nanoparticles for psychiatric disorders is therefore critical before translation to clinical use. 6) Biomarker measurements were performed in whole-brain tissue rather than in specific regions, which may have masked region-specific alterations and reduced sensitivity to detect localized changes relevant to disease processes.

In conclusion, this study demonstrates that intranasal dantrolene nanoparticles significantly ameliorated inflammation-induced depression and anxiety behaviors in adult mice. These neuroprotective effects were associated with suppression of pathological cytokine elevation in the blood and preservation of synaptic proteins in the brain. Taken together, our findings highlight the potential of intranasal dantrolene nanoparticles as a novel therapeutic strategy for psychiatric disorders, warranting further preclinical and clinical investigation.

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performed in the lab of Dr. Huafeng Wei and should be attributed to the Department of Anesthesiology, University of Pennsylvania. We appreciate technical support from Rebecca Chae from Rowan University, New Jersey, U.S.A. and Kyulee Kim from UPENN, Pennsylvania, U.S.A.

Author contributions

H.W. conceived and designed the study. J.L., Y.L, P.B. J.G, L.S.L, Y.Y., G.L, H.W. conducted experiments, acquired and analyzed the data, J.L., Y.L, P.B. J.G, L.S.L, Y.Y., G.L, H.W. analyzed data and contributed to manuscript preparation. H.W. wrote the manuscript. All the authors reviewed and approved the final manuscript.

Conflicts of interest

Drs. Huafeng Wei and Ge Liang are listed as inventors of patents entitled “Intranasal Administration of Dantrolene for Treatment of Alzheimer’s Disease” in multiple countries by The Trustees of the University of Pennsylvania. Dr. Huafeng Wei is listed as the inventor of the USA patents application repurposing intranasal dantrolene nanoparticles to treat neurodegenerative diseases and psychiatric disorders.

Ethics approval and consent to participate

All methods were performed in accordance with the relevant guidelines and regulations. This study was reviewed and approved by the Institutional Animal Care and Use

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Committee (IACUC) at the University of Pennsylvania, under approval number 805897.

Informed consent was obtained from all participants prior to their inclusion in the study.

Data Availability

Data available upon request.

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REFERENCES

1. Herrman H, Kieling C, McGorry P, Horton R, Sargent J, Patel V: Reducing the global burden of depression: a Lancet-World Psychiatric Association Commission. Lancet 2019; 393: e42-e43
2. Rikhani K, Vas C, Jha MK: Approach to Diagnosis and Management of Treatment-Resistant Depression. Psychiatr Clin North Am 2023; 46: 247-259
3. Belge JB, Sabbe ACF, Sabbe B: An update on pharmacotherapy for recurrent depression in 2022. Expert Opin Pharmacother 2023: 1-8
4. Gururajan A, Reif A, Cryan JF, Slattery DA: The future of rodent models in depression research. Nat Rev Neurosci 2019; 20: 686-701
5. Szuhany KL, Simon NM: Anxiety Disorders: A Review. JAMA 2022; 328: 2431-2445
6. Jarkas DA, Villeneuve AH, Daneshmend AZB, Villeneuve PJ, McQuaid RJ: Sex differences in the inflammation-depression link: A systematic review and meta-analysis. Brain Behav Immun 2024; 121: 257-268
7. Altemus M, Sarvaiya N, Neill Epperson C: Sex differences in anxiety and depression clinical perspectives. Front Neuroendocrinol 2014; 35: 320-30
8. Somers JM, Goldner EM, Waraich P, Hsu L: Prevalence and incidence studies of anxiety disorders: a systematic review of the literature. Can J Psychiatry 2006; 51: 100-13
9. Ait Tayeb AEK, Poinsignon V, Chappell K, Bouligand J, Becquemont L, Verstuyft C: Major Depressive Disorder and Oxidative Stress: A Review of Peripheral and Genetic Biomarkers According to Clinical Characteristics and Disease Stages. Antioxidants (Basel) 2023; 12
10. Paganin W, Signorini S, Leccese V, Sciarretta A: [Treatment-resistant depression. From classification to new therapies.]. Riv Psichiatr 2022; 57: 258-272
11. Robertson S, Peacock EE, Scott R: Benzodiazepine Use Disorder: Common Questions and Answers. Am Fam Physician 2023; 108: 260-266
12. Tabaac BJ, et al.: Psychedelic Therapy: A Primer for Primary Care Clinicians-Historical Perspective and Overview. Am J Ther 2024; 31: e97-e103
13. Ren L: The mechanistic basis for the rapid antidepressant-like effects of ketamine: From neural circuits to molecular pathways. Prog Neuropsychopharmacol Biol Psychiatry 2024; 129: 110910
14. Costi S: Ketamine for Major Depressive Disorder. Curr Top Behav Neurosci 2023
15. Bruton AM, Wesemann DG, Machingo TA, Majak G, Johnstone JM, Marshall RD: Ketamine for mood disorders, anxiety, and suicidality in children and adolescents: a systematic review. Eur Child Adolesc Psychiatry 2024
16. Tully JL, Dahlen AD, Haggarty CJ, Schioth HB, Brooks S: Ketamine treatment for refractory anxiety: A systematic review. Br J Clin Pharmacol 2022; 88: 4412-4426
17. Matveychuk D, et al.: Ketamine as an antidepressant: overview of its mechanisms of action and potential predictive biomarkers. Ther Adv Psychopharmacol 2020; 10: 2045125320916657
18. Rosso G, et al.: Esketamine Treatment Trajectory of Patients with Treatment-Resistant Depression in the Mid and Long-Term Run: Data from REAL-ESK Study Group. Curr Neuropharmacol 2025; 23: e1570159X337670
19. Deutschenbaur L, et al.: Role of calcium, glutamate and NMDA in major depression and therapeutic application. Prog Neuropsychopharmacol Biol Psychiatry 2016; 64: 325-33

ARTICLE IN PRESS

20. Fries GR, Saldana VA, Finnstein J, Rein T: Molecular pathways of major depressive disorder converge on the synapse. Mol Psychiatry 2023; 28: 284-297
21. Garrosa-Jimenez J, et al.: Intracellular calcium and inflammatory markers, mediated by purinergic stimulation, are differentially regulated in monocytes of patients with major depressive disorder. Neurosci Lett 2021; 765: 136275
22. Valiati FE, et al.: Inflammation and damage-associated molecular patterns in major psychiatric disorders. Trends Psychiatry Psychother 2022
23. Wang Y, et al.: Mitochondrial calcium uniporter knockdown in hippocampal neurons alleviates anxious and depressive behavior in the 3XTG Alzheimer's disease mouse model. Brain Res 2024; 1840: 149060
24. Phillips KF, Santos E, Blair RE, Deshpande LS: Targeting Intracellular Calcium Stores Alleviates Neurological Morbidities in a DFP-Based Rat Model of Gulf War Illness. Toxicol Sci 2019; 169: 567-578
25. Cardon I, et al.: Mitochondrial and Cellular Function in Fibroblasts, Induced Neurons, and Astrocytes Derived from Case Study Patients: Insights into Major Depression as a Mitochondria-Associated Disease. Int J Mol Sci 2024; 25
26. Song Y, et al.: Mitochondrial dysfunction: A fatal blow in depression. Biomed Pharmacother 2023; 167: 115652
27. Lindqvist D, et al: Oxidative stress, inflammation and treatment response in major depression. Psychoneuroendocrinology 2017; 76: 197-205
28. Zhou XY, et al.: Mitochondrial health, NLRP3 inflammasome activation, and white matter integrity in adolescent mood disorders: A pilot study. J Affect Disord 2023; 340: 149-159
29. Cao H, et al.: Hesperidin may improve depressive symptoms by binding NLRP3 and influencing the pyroptosis pathway in a rat model. Eur J Pharmacol 2023; 952: 175670
30. Li S, et al.: NLRP3/caspase-1/GSDMD-mediated pyroptosis exerts a crucial role in astrocyte pathological injury in mouse model of depression. JCI Insight 2021; 6
31. Simon MS, et al.: Monocyte mitochondrial dysfunction, inflammaging, and inflammatory pyroptosis in major depression. Prog Neuropsychopharmacol Biol Psychiatry 2021; 111: 110391
32. Kaufmann FN, et al.: NLRP3 inflammasome-driven pathways in depression: Clinical and preclinical findings. Brain Behav Immun 2017; 64: 367-383
33. Vogelzangs N, de Jonge P, Smit JH, Bahn S, Penninx BW: Cytokine production capacity in depression and anxiety. Transl Psychiatry 2016; 6: e825
34. Zhang LM, et al.: IL-18BP Alleviates Anxiety-Like Behavior Induced by Traumatic Stress via Inhibition of the IL-18R-NLRP3 Signaling Pathway in a Mouse Model of Hemorrhagic Shock and Resuscitation. Mol Neurobiol 2023; 60: 382-394
35. Bhatt S, et al.: Role of Brain-Gut-Microbiota Axis in Depression: Emerging Therapeutic Avenues. CNS Neurol Disord Drug Targets 2023; 22: 276-288
36. Anand N, Gorantla VR, Chidambaram SB: The Role of Gut Dysbiosis in the Pathophysiology of Neuropsychiatric Disorders. Cells 2022; 12
37. Ratto D, Joyner RW: Dantrolene, StatPearls. Treasure Island (FL), 2020
38. Nakayama R, Yano T, Ushijima K, Abe E, Terasaki H: Effects of dantrolene on extracellular glutamate concentration and neuronal death in the rat hippocampal CA1 region subjected to transient ischemia. Anesthesiology 2002; 96: 705-10

ARTICLE IN PRESS

ARTICLE IN PRESS

39. Wei HF, Perry DC: Dantrolene is cytoprotective in two models of neuronal cell death. Journal of Neurochemistry 1996; 67: 2390-2398
40. Chen X, Wu J, Lvovskaya S, Herndon E, Supnet C, Bezprozvanny I: Dantrolene is neuroprotective in Huntington's disease transgenic mouse model. Mol Neurodegener 2011; 6: 81
41. Chen X, et al. Deranged calcium signaling and neurodegeneration in spinocerebellar ataxia type 3. J Neurosci 2008; 28: 12713-24
42. Rothstein JD, Kuncl RW: Neuroprotective strategies in a model of chronic glutamate-mediated motor neuron toxicity. J Neurochem 1995; 65: 643-51
43. Royero PX, et al.: Ryanodine receptors drive neuronal loss and regulate synaptic proteins during epileptogenesis. Exp Neurol 2020; 327: 113213
44. Keshavarz M, Fotouhi M, Rasti A: Dantrolene: A Selective Ryanodine Receptor Antagonist, Protects Against Pentylenetetrazole-Induced Seizure in Mice. Acta Med Iran 2016; 54: 555-561
45. Frandsen A, Schousboe A: Mobilization of dantrolene-sensitive intracellular calcium pools is involved in the cytotoxicity induced by quisqualate and N-methyl-D-aspartate but not by 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionate and kainate in cultured cerebral cortical neurons. Proc Natl Acad Sci U S A. 1992; 89: 2590-2594
46. Chen T, et al.: Molecular mechanisms of rapid-acting antidepressants: New perspectives for developing antidepressants. Pharmacol Res 2023; 194: 106837
47. Burkholder T, Foltz C, Karlsson E, Linton CG, Smith JM: Health Evaluation of Experimental Laboratory Mice. Curr Protoc Mouse Biol 2012; 2: 145-165
48. Vera R, et al.: Effects of intranasal dantrolene nanoparticles on brain concentration and behavior in PS19 tau transgenic mice. Res Sq 2023
49. Shi Y, et al.: Intranasal Dantrolene as a Disease-Modifying Drug in Alzheimer 5XFAD Mice. J Alzheimers Dis 2020; 76: 1375-1389
50. Wang J, et al.: Intranasal administration of dantrolene increased brain concentration and duration. PLoS One 2020; 15: e0229156
51. Ueno H, et al.: Effect of simultaneous testing of two mice in the tail suspension test and forced swim test. Sci Rep 2022; 12: 9224
52. Can A, Dao DT, Terrillion CE, Piantadosi SC, Bhat S, Gould TD: The tail suspension test. J Vis Exp 2012: e3769
53. Jones SK, McCarthy DM, Vied C, Stanwood GD, Schatschneider C, Bhide PG: Transgenerational transmission of aspartame-induced anxiety and changes in glutamate-GABA signaling and gene expression in the amygdala. Proc Natl Acad Sci U S A 2022; 119: e2213120119
54. Cho WH, et al.: Hippocampal astrocytes modulate anxiety-like behavior. Nat Commun 2022; 13: 6536
55. Joseph DJ, Liu C, Peng J, Liang G, Wei H: Isoflurane mediated neuropathological and cognitive impairments in the triple transgenic Alzheimer's mouse model are associated with hippocampal synaptic deficits in an age-dependent manner. PLoS One 2019; 14: e0223509
56. Liang G, Ward C, Peng J, Zhao YF, Huang BS, Wei HF: Isoflurane Causes Greater Neurodegeneration Than an Equivalent Exposure of Sevoflurane in the Developing Brain of Neonatal Mice. Anesthesiology 2010; 112: 1325-1334

ARTICLE IN PRESS

ARTICLE IN PRESS

57. Wang Y, Liang G, Liang S, Mund R, Shi Y, Wei H: Dantrolene Ameliorates Impaired Neurogenesis and Synaptogenesis in Induced Pluripotent Stem Cell Lines Derived from Patients with Alzheimer's Disease. Anesthesiology 2020; 132: 1062-1079
58. Vera R, et al.: Effects of Intranasal Dantrolene Nanoparticles on Brain Concentration and Behavior in PS19 Tau Transgenic Mice. J Alzheimers Dis 2024
59. Liu QF, et al.: Co-Treatment with the Herbal Medicine SIP3 and Donepezil Improves Memory and Depression in the Mouse Model of Alzheimer's Disease. Curr Alzheimer Res 2022; 19: 246-263
60. Amada N, et al.: Synergistic anti-depressive effect of combination treatment of Brexpiprazole and selective serotonin reuptake inhibitors on forced swimming test in mice. Neuropsychopharmacol Rep 2023; 43: 132-136
61. Varkonyi D, et al.: Investigation of Anxiety- and Depressive-like Symptoms in 4- and 8-Month-Old Male Triple Transgenic Mouse Models of Alzheimer's Disease. Int J Mol Sci 2022; 23
62. Pentkowski NS, Rogge-Obando KK, Donaldson TN, Bouquin SJ, Clark BJ: Anxiety and Alzheimer's disease: Behavioral analysis and neural basis in rodent models of Alzheimer's-related neuropathology. Neurosci Biobehav Rev 2021; 127: 647-658
63. Brady LS, Lisanby SH, Gordon JA: New directions in psychiatric drug development: promising therapeutics in the pipeline. Expert Opin Drug Discov 2023: 1-16
64. Jha MK, Mathew SJ: Pharmacotherapies for Treatment-Resistant Depression: How Antipsychotics Fit in the Rapidly Evolving Therapeutic Landscape. Am J Psychiatry 2023; 180: 190-199
65. Han Q, et al.: Microglial NLRP3 inflammasome-mediated neuroinflammation and therapeutic strategies in depression. Neural Regen Res 2024; 19: 1890-1898
66. Su WJ, Hu T, Jiang CL: Cool the Inflamed Brain: A Novel Anti-inflammatory Strategy for the Treatment of Major Depressive Disorder. Curr Neuropharmacol 2024; 22: 810-842
67. Chin Fatt CR, Mayes TL, Trivedi MH: Immune Dysregulation in Treatment-Resistant Depression: Precision Approaches to Treatment Selection and Development of Novel Treatments. Psychiatr Clin North Am 2023; 46: 403-413
68. Guo B, Zhang M, Hao W, Wang Y, Zhang T, Liu C: Neuroinflammation mechanisms of neuromodulation therapies for anxiety and depression. Transl Psychiatry 2023; 13: 5
69. Arioz BI, et al.: Melatonin Attenuates LPS-Induced Acute Depressive-Like Behaviors and Microglial NLRP3 Inflammasome Activation Through the SIRT1/Nrf2 Pathway. Front Immunol 2019; 10: 1511
70. Resende R, et al.: Mitochondria, endoplasmic reticulum and innate immune dysfunction in mood disorders: Do Mitochondria-Associated Membranes (MAMs) play a role? Biochim Biophys Acta Mol Basis Dis 2020; 1866: 165752
71. Zhou GQ, et al.: Intestinal microbiota via NLRP3 inflammasome dependent neuronal pyroptosis mediates anxiety-like behaviour in mice exposed to 3.5 GHz radiofrequency radiation. Sci Total Environ 2024; 927: 172391
72. Clark AL, et al.: Targeting Cellular Calcium Homeostasis to Prevent Cytokine-Mediated Beta Cell Death. Sci Rep 2017; 7: 5611
73. Johnson JD, et al.: RyR2 and calpain-10 delineate a novel apoptosis pathway in pancreatic islets. J Biol Chem 2004; 279: 24794-802

ARTICLE IN PRESS

ARTICLE IN PRESS

74. Fischer DR, et al.: Dantrolene reduces serum TNFalpha and corticosterone levels and muscle calcium, calpain gene expression, and protein breakdown in septic rats. Shock 2001; 15: 200-7
75. Hotchkiss RS, Karl IE: Dantrolene ameliorates the metabolic hallmarks of sepsis in rats and improves survival in a mouse model of endotoxemia. Proc Natl Acad Sci U S A. 1994; 91: 3039-3043
76. Al-Kuraishy HM, Al-Gareeb AI, El-Bouseary MM, Alexiou A, Papadakis M, Batiha GE: Calpain/calpstatin pathway in COVID-19: Role of dantrolene. J Cell Mol Med 2024; 28: e18005
77. Alkazmi L, Al-Kuraishy HM, Al-Gareeb AI, El-Bouseary MM, Ahmed EA, Batiha GE: Dantrolene and ryanodine receptors in COVID-19:The daunting taskand neglected warden. Clin Exp Pharmacol Physiol 2023
78. Villas Boas GR, et al.: Molecular aspects of depression: A review from neurobiology to treatment. Eur J Pharmacol 2019; 851: 99-121
79. Dean J, Keshavan M: The neurobiology of depression: An integrated view. Asian J Psychiatr 2017; 27: 101-111
80. Sapolsky RM: Psychiatric distress in animals versus animal models of psychiatric distress. Nat Neurosci 2016; 19: 1387-1389
81. Mahdirejei HA, Peeri M, Azarbayjani MA, Fattahi Masrour F: Fluoxetine combined with swimming exercise synergistically reduces lipopolysaccharide-induced depressive-like behavior by normalizing the HPA axis and brain inflammation in mice. Pharmacol Biochem Behav 2023; 232: 173640
82. Zhuo R, et al.: Cinnamic Acid Improved Lipopolysaccharide-Induced Depressive-Like Behaviors by Inhibiting Neuroinflammation and Oxidative Stress in Mice. Pharmacology 2022; 107: 281-289
83. Li W, et al.: Ibrutinib alleviates LPS-induced neuroinflammation and synaptic defects in a mouse model of depression. Brain Behav Immun 2021; 92: 10-24
84. Li W, et al.: TRPV4 inhibitor HC067047 produces antidepressant-like effect in LPS-induced depression mouse model. Neuropharmacology 2021; 201: 108834
85. Peng J, et al.: Dantrolene ameliorates cognitive decline and neuropathology in Alzheimer triple transgenic mice. Neurosci Lett 2012; 516: 274-9
86. Chakroborty S, et al: Stabilizing ER Ca2+ channel function as an early preventative strategy for Alzheimer's disease. PLoS One 2012; 7: e52056
87. Oules B, et al.: Ryanodine receptor blockade reduces amyloid-beta load and memory impairments in Tg2576 mouse model of Alzheimer disease. J Neurosci 2012; 32: 11820-34
88. Shors TJ, Millon EM, Chang HY, Olson RL, Alderman BL: Do sex differences in rumination explain sex differences in depression? J Neurosci Res 2017; 95: 711-718
89. Fernandez-Guasti A, Fiedler JL, Herrera L, Handa RJ: Sex, stress, and mood disorders: at the intersection of adrenal and gonadal hormones. Horm Metab Res 2012; 44: 607-18
90. Iqbal J, Huang GD, Xue YX, Yang M, Jia XJ: Role of estrogen in sex differences in memory, emotion and neuropsychiatric disorders. Mol Biol Rep 2024; 51: 415
91. Songtachalert T, Roomruangwong C, Carvalho AF, Bourin M, Maes M: Anxiety Disorders: Sex Differences in Serotonin and Tryptophan Metabolism. Curr Top Med Chem 2018; 18: 1704-1715
92. Maher R, Moreno-Borrallo A, Jindal D, Mai BT, Ruiz-Hernandez E, Harkin A: Intranasal Polymeric and Lipid-Based Nanocarriers for CNS Drug Delivery. Pharmaceutics 2023; 15
93. Fonseca LC, et al.: Intranasal drug delivery for treatment of Alzheimer's disease. Drug Deliv Transl Res 2021; 11: 411-425

ARTICLE IN PRESS

94. Hanson LR, Frey WH, 2nd: Intranasal delivery bypasses the blood-brain barrier to target therapeutic agents to the central nervous system and treat neurodegenerative disease. BMC Neurosci 2008; 9 Suppl 3: S5
95. Garbincius JF, Elrod JW: Mitochondrial calcium exchange in physiology and disease. Physiol Rev 2022; 102: 893-992
96. Gleitze S, et al.: Ryanodine Receptor Mediated Calcium Release Contributes to Ferroptosis Induced in Primary Hippocampal Neurons by GPX4 Inhibition. Antioxidants (Basel) 2023; 12
97. Triantafilou K, Hughes TR, Triantafilou M, Morgan BP: The complement membrane attack complex triggers intracellular Ca2+ fluxes leading to NLRP3 inflammasome activation. J Cell Sci 2013; 126: 2903-13
98. Wongpun J, et al.: The potential effects of festidinol treatment against the NLRP3 inflammasome and pyroptosis in D-galactose and aluminum chloride-induced Alzheimer's-like pathology in mouse brain. Int Immunopharmacol 2023; 119: 110181
99. Wan T, Li X, Fu M, Gao X, Li P, Guo W: NLRP3-Dependent Pyroptosis: A Candidate Therapeutic Target for Depression. Front Cell Neurosci 2022; 16: 863426
100. Fountoulakis KN, Saitis A, Schatzberg AF: Esketamine Treatment for Depression in Adults: A PRISMA Systematic Review and Meta-Analysis. Am J Psychiatry 2025: appiajp20240515
101. Jiang B, Shi Y, Abou MB, Xu L, Liang G, Wei H: Effects of chronic intranasal dantrolene on nasal mucosa morphology in mice. Eur Rev Med Pharmacol Sci 2022; 26: 198-203
102. Hasko G, Szabo C, Nemeth ZH, Lendvai B, Vizi ES: Modulation by dantrolene of endotoxin-induced interleukin-10, tumour necrosis factor-alpha and nitric oxide production in vivo and in vitro. Br J Pharmacol 1998; 124: 1099-106
103. Zhang IX, et al.: ER stress increases expression of intracellular calcium channel RyR1 to modify Ca(2+) homeostasis in pancreatic beta cells. J Biol Chem 2023; 299: 105065
104. Abais JM, Xia M, Zhang Y, Boini KM, Li PL: Redox regulation of NLRP3 inflammasomes: ROS as trigger or effector? Antioxid Redox Signal 2015; 22: 1111-29

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Table 1. Antibodies used for Western Blot

	Cat. and Manufacture	Dilution
Primary antibody		
Anti- NLRP3	68102-1-Ig; Proteintech	1:2 000
Anti-Human procaspase-1/ cleaved caspase-1 p20	#2225S, Cell signaling Technology,USA	1:1 000
Anti- Cleaved GSDMD	#36425S; Cell signaling Technology, USA	1:1 000
Anti-IL-1β	31202S, Cell signaling Technology, USA	1:1 000
Anti-IL-18	10663-1-AP; Proteintech	1:3 000
Anti-PSD95	#2507; Cell signaling Technology, USA	1:1 000
Anti-Synapsin-1	#5297; Cell signaling Technology, USA	1:1 000
GAPDH	MA5–15738, Thermo Fisher Scientific	1:10 000
Secondary antibody		
Anti-Mouse IgG1; HRP - linked	# PA1-74421, Thermo Fisher Scientific	1:10 000
Anti-rabbit IgG, HRP-linked	#7074; Cell signaling Technology, USA	1:1 000

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Main figure legends/titles

Figure 1. Intranasal dantrolene nanoparticles significantly reduced LPS-induced acute helplessness and anxiety-related behaviors. Adult B6SJLF1/J mice were treated with intranasal dantrolene nanoparticles (IN-DAN) or vehicle (VEH) for 28 days, followed by a single i.p. injection of lipopolysaccharide (LPS) (IN-DAN+LPS). Control mice either had no treatment (No Tx), only LPS (LPS), or VEH+LPS. The forced swim test (FST, A), tail suspension test (TST, B), elevated plus maze (EPMT, C) and open field test (OFT, D) were performed 24 hours after the LPS injection. Increased mobility time indicates helplessness or anxiety-related behaviors (A, B, D). Increased time in the closed arm indicates anxiety (C). N=18-19 mice, Mean±95%CI, One-Way ANOVA followed by Tukey post hoc test. *P<0.05, **P<0.01, ****P<0.0001

Figure 2. Intranasal dantrolene nanoparticles reduced LPS-induced acute helplessness and anxiety-related behaviors in more female than male mice. The sex of the adult B6SJLF1/J mice, pretreated with intranasal dantrolene nanoparticles (IN-DAN) or vehicle (VEH) for 28 days, followed by a single i.p. injection of LPS (IN-DAN+LPS), was examined. No treatment controls (No Tx), LPS only (LPS), or VEH+LPS. The forced swimming test (FST, A), tail suspension test (TST, B), elevated plus maze (EPMT, C), and open field test (OFT, D) were performed 24 hours after the LPS injection. N=8-10 mice, Mean±95%CI, Two-Way ANOVA followed by Tukey post hoc test. *P<0.05,**P<0.01, ****P<0.0001

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Figure 3. Intranasal dantrolene significantly inhibited lipopolysaccharide-induced pathological elevation of IL-18 and IL-1β cytokines in the blood and brains. B6SJLF1/J adult mice were pretreated with intranasal dantrolene nanoparticles (Dan, 5mg/kg) or vehicle (Veh) control daily, Monday to Friday, for continuous 4 weeks. Mice were then treated with one-time IP injection of lipopolysaccharide (LPS, 5 mg/kg). Mice in control (No Tx) group received no treatments. IL1β in blood (A) and brain (C, E) and IL18 in blood (B) and brains (D, F) were measured using ELISA assay kit (A, B) or immunoblotting (C-F)., N=18-20 mice for blood measurement (A, B) and N=4 brains for immunoblotting (C-F), Means±95% CI, One-Way ANOVA followed by Tukey post hoc test. **P<0.01, ****P<0.0001, respectively.

Figure 4. Effects of intranasal dantrolene nanoparticles on lipopolysaccharide-induced programmed cell death by pyroptosis in the brains. B6SJLF1/J adult mice (5-10 months old) were pretreated with intranasal dantrolene nanoparticles (Dan, 5mg/kg) or vehicle (Veh) daily, Monday to Friday, for continuous 4 weeks. Mice were then treated with a one-time IP injection of lipopolysaccharide (LPS, 5 mg/kg). Mice in control (No Tx) group received no treatments. Representative western blot (A, C, E) and statistical analysis (B, D, F) determined the protein levels of critical regulatory proteins of pyroptosis pathway (A, B, NLRP3; C, D, Active caspase-1 (P-20), E, F, N terminal GSDMD (GSDMD-NT) in brains. N=4 different mice brain in each group. GAPDH as

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loading control. Means±95%CI, One-Way ANOVA followed by Tukey post hoc test. * P<0.05.

Figure 5. Intranasal dantrolene nanoparticles significantly inhibited lipopolysaccharide-induced synapse protein loss in brains. B6SJLF1/J adult mice were pretreated with intranasal dantrolene nanoparticles (Dan, 5mg/kg) or vehicle (Veh) daily, Monday to Friday, for continuous 4 weeks. Mice were then treated with one-time IP injection of lipopolysaccharide (LPS, 5 mg/kg). Mice in control (No Tx) group received no treatments. Western blot measured changes of synapse proteins of PSD-95 (A, C) and Synapsin-1 (B, D) in the brains. GAPDH as loading control. N=4 different mice in each group, Means±95%CI, One-Way ANOVA followed by Tukey post hoc test. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, respectively.

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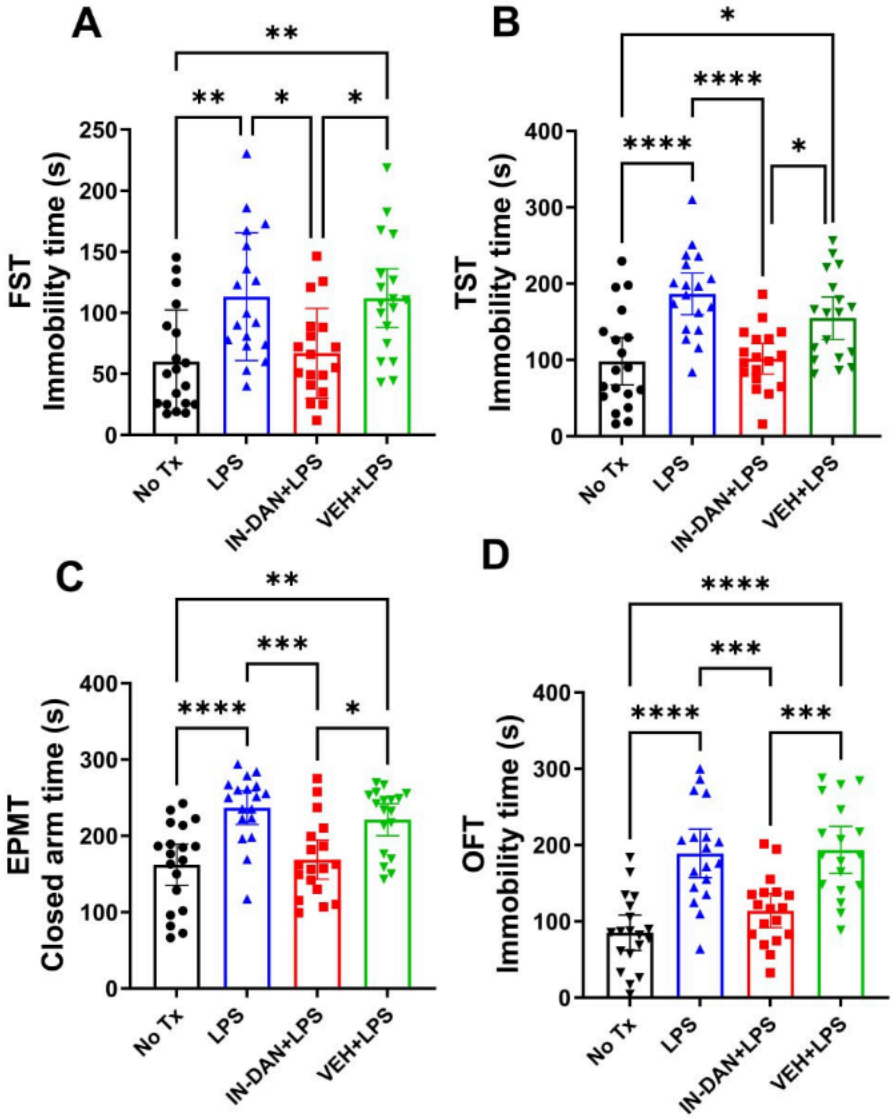
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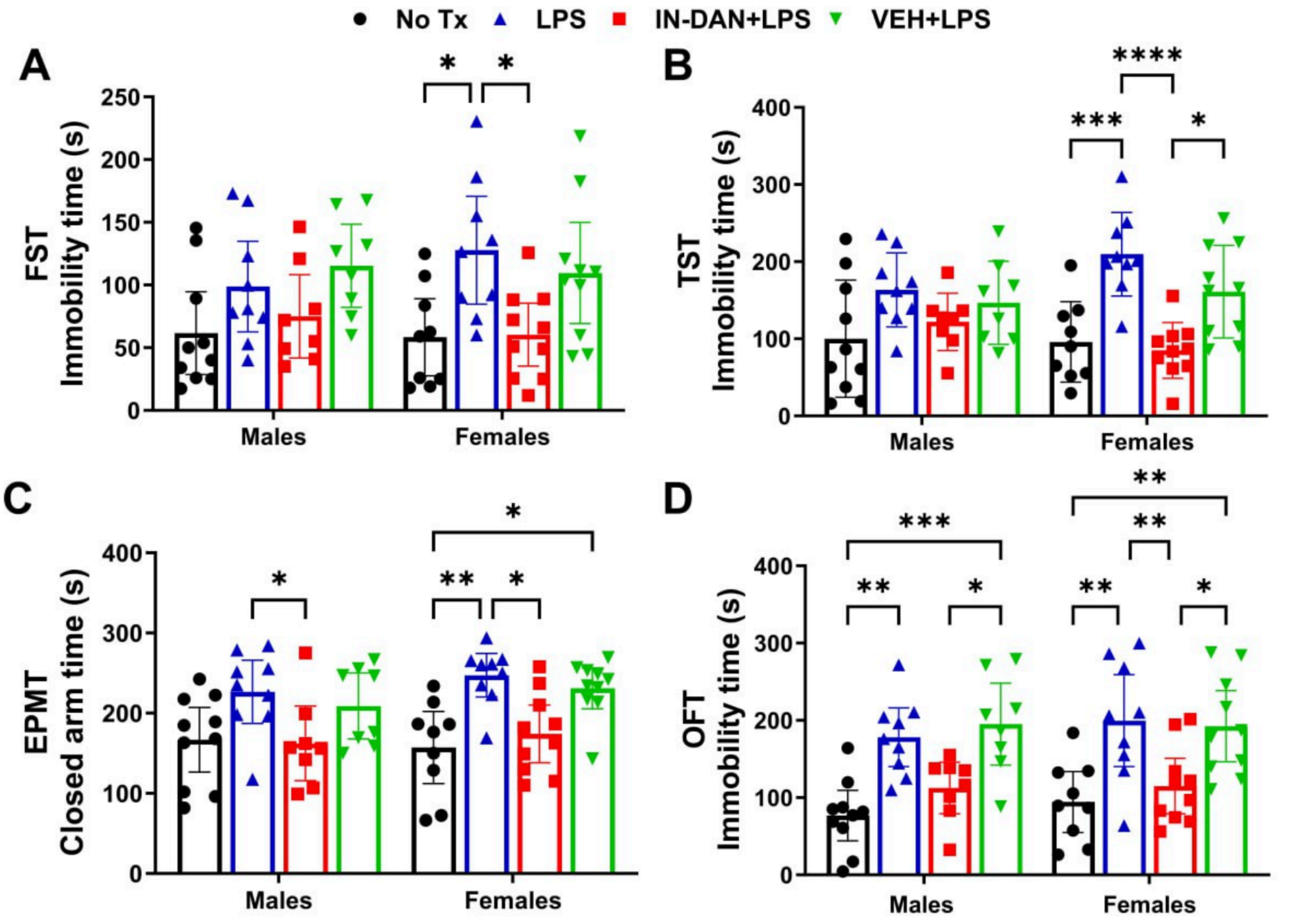
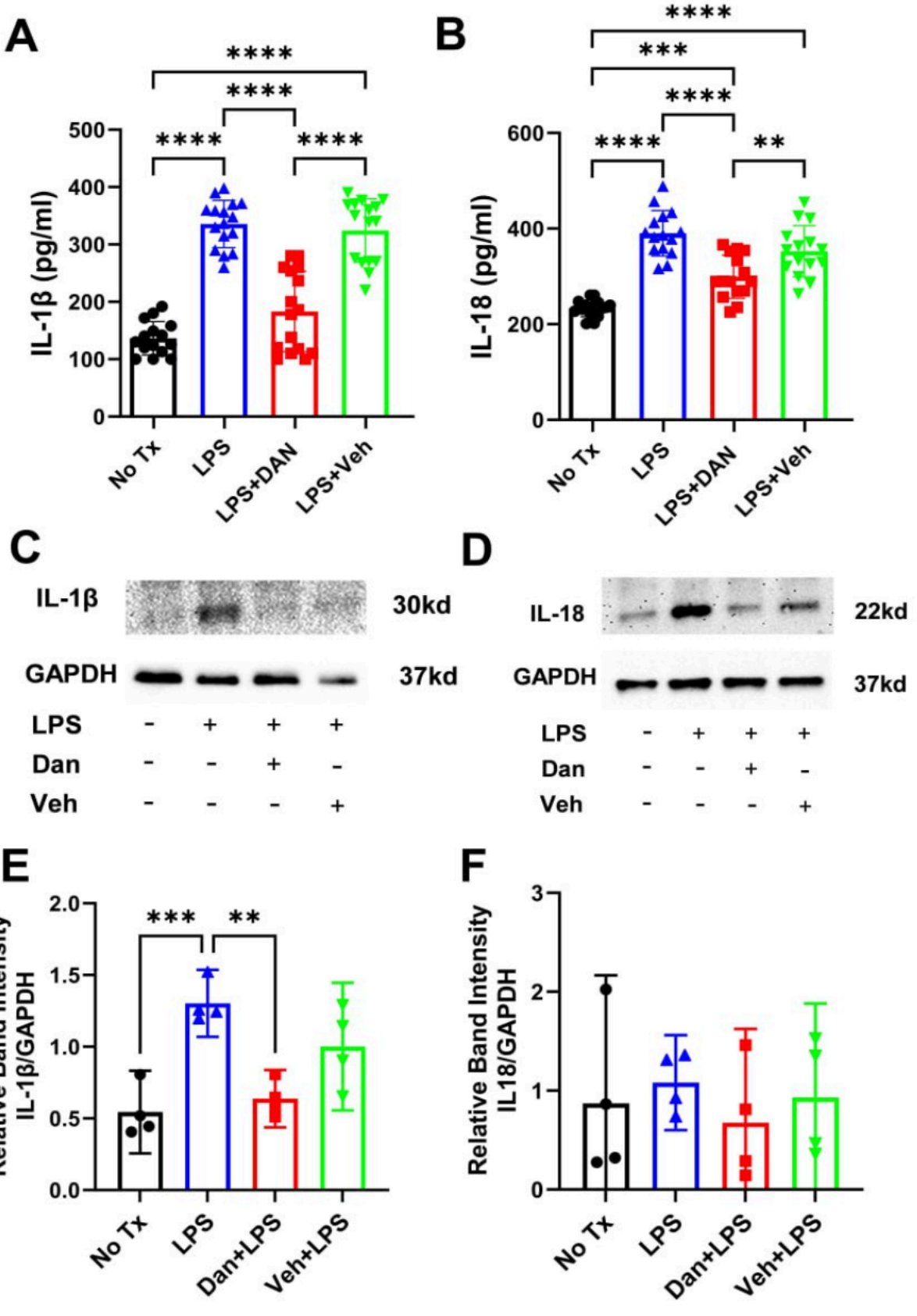
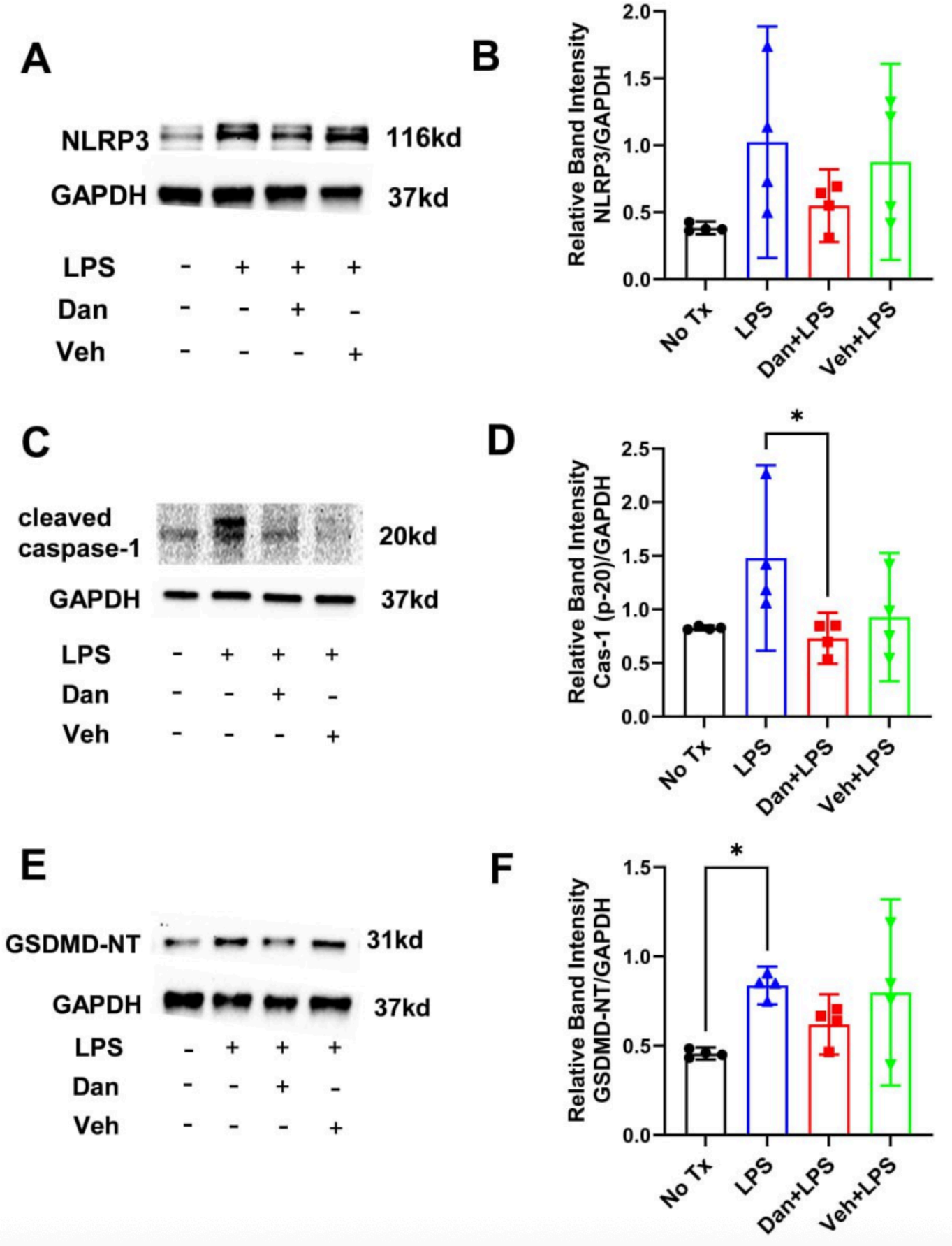
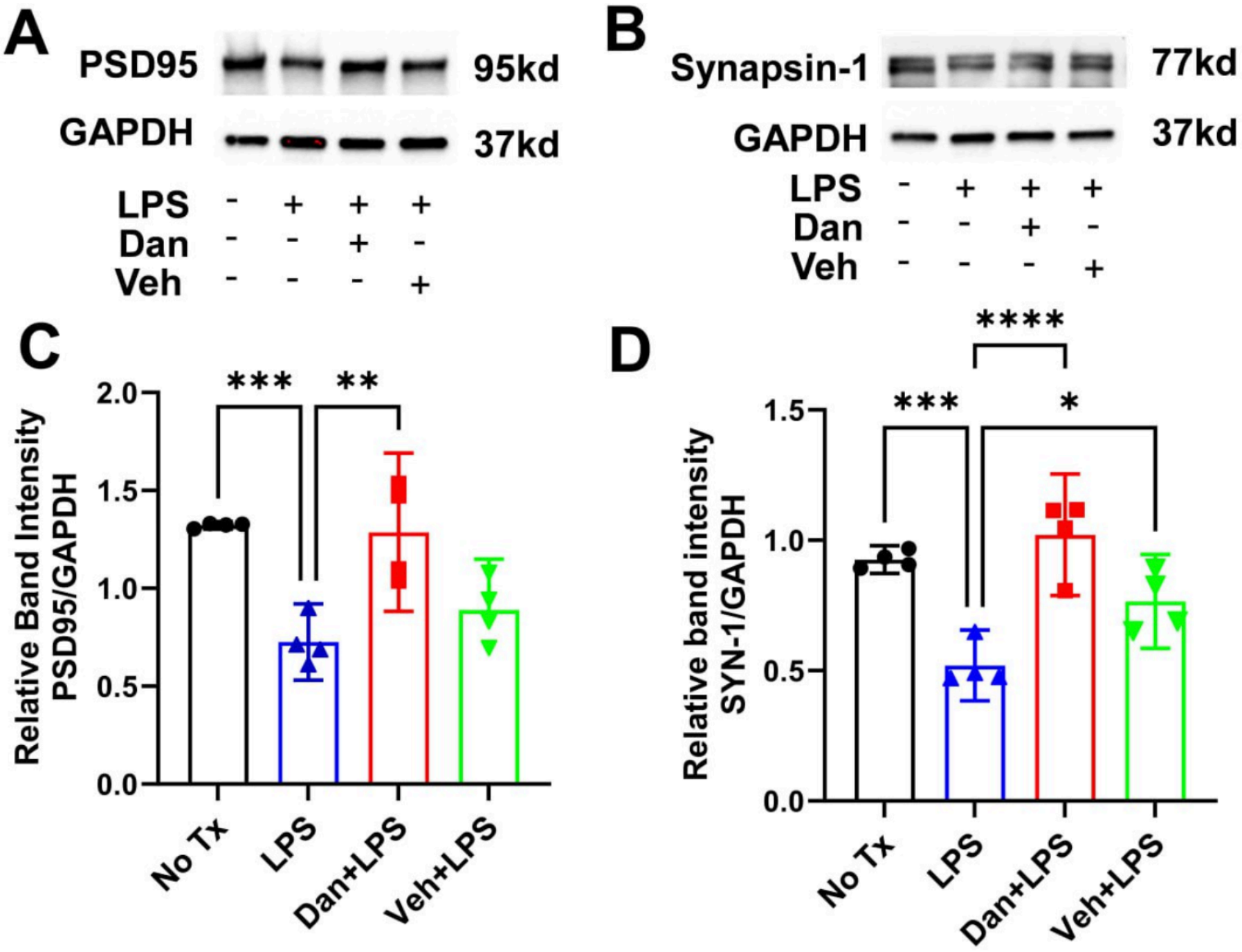
C
EPMT
Closed arm time (s)
400
300
200
100
0
**
***
****
*
No Tx
LPS
IN-DAN+LPS
VEH+LPS
D
OFT
Immobility time (s)
400
300
200
100
0
****
***
****
***
No Tx
LPS
IN-DAN+LPS
VEH+LPS


No Tx
LPS
IN-DAN+LPS
VEH+LPS
A
FST
Immobility time (s)
250
200
150
100
50
0
* *
Males
Females
B
TST
Immobility time (s)
400
300
200
100
0
**** *** *
Males
Females
C
EPMT
Closed arm time (s)
400
300
200
100
0
* ** * *
Males
Females
D
OFT
Immobility time (s)
400
300
200
100
0
*** ** * ** ** **
Males
Females


A
IL-1β (pg/ml)
500
400
300
200
100
0
****
****
****
****
No Tx
LPS
LPS+DAN
LPS+Veh
B
IL-18 (pg/ml)
600
400
200
0
****
***
****
****
**
No Tx
LPS
LPS+DAN
LPS+Veh
C
IL-1β
30kd
GAPDH
37kd
LPS - + + +
Dan - - + -
Veh - - - +
D
IL-18
22kd
GAPDH
37kd
LPS - + + +
Dan - - + -
Veh - - - +
E
Relative Band Intensity
IL-1β/GAPDH
2.0
1.5
1.0
0.5
0.0
***
**
No Tx
LPS
Dan+LPS
Veh+LPS
F
Relative Band Intensity
IL18/GAPDH
3
2
1
0
No Tx
LPS
Dan+LPS
Veh+LPS


A
NLRP3
116kd
GAPDH
37kd
LPS - + + +
Dan - - + -
Veh - - - +
B
Relative Band Intensity NLRP3/GAPDH
2.0
1.5
1.0
0.5
0.0
No Tx
LPS
Dan+LPS
Veh+LPS
C
cleaved caspase-1
20kd
GAPDH
37kd
LPS - + + +
Dan - - + -
Veh - - - +
D
*
Relative Band Intensity Cas-1 (p-20)/GAPDH
2.5
2.0
1.5
1.0
0.5
0.0
No Tx
LPS
Dan+LPS
Veh+LPS
E
GSDMD-NT
31kd
GAPDH
37kd
LPS - + + +
Dan - - + -
Veh - - - +
F
*
Relative Band Intensity GSDMD-NT/GAPDH
1.5
1.0
0.5
0.0
No Tx
LPS
Dan+LPS
Veh+LPS


A
PSD95
95kd
GAPDH
37kd
LPS - + + +
Dan - - + -
Veh - - - +
B
Synapsin-1
77kd
GAPDH
37kd
LPS - + + +
Dan - - + -
Veh - - - +
C
Relative Band Intensity
PSD95/GAPDH
2.0
1.5
1.0
0.5
0.0
***
**
No Tx
LPS
Dan+LPS
Veh+LPS
D
Relative band intensity
SYN-1/GAPDH
1.5
1.0
0.5
0.0
****
***
*
No Tx
LPS
Dan+LPS
Veh+LPS