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# (R)-2,5-Dimethoxy-4-Iodoamphetamine [(R)-Doi] Influences Coping Strategies to an Escapable Social Stress

Kevin Thomas Krupp
*University of South Dakota*, Kruppkt01@gmail.com

# (*R*)-2,5-DIMETHOXY-4-IODOAMPHETAMINE [(*R*)-DOI] INFLUENCES COPING STRATEGIES TO AN ESCAPABLE SOCIAL STRESS

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Kevin T. Krupp

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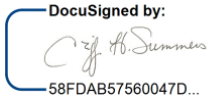


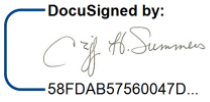

Chairperson, Advisor: Cliff H. Summers, Ph.D., Nolop Distinguished Professor


Kenneth J. Renner, PhD., Professor Emeritus


Lisa M. McFadden, Ph.D., Assistant Professor

# ABSTRACT

Depressive mood disorders are a leading cause of disability worldwide and pharmacological treatments for these disorders are inadequate, requiring new compounds with greater efficacy be investigated. The etiology of depression is heterogeneous; however, it is well established that stress exposure, and the proinflammatory effects of stress have a major role. Psychedelic compounds have rapid and long-lasting anxiolytic and antidepressive effects in humans and animal models of stress induced affective behavior. However, it is not completely understood how these compounds produce such rapid effects. We investigated whether the psychedelic compound (*R*)-2,5-dimethoxy-4-iodoamphetamine [(*R*)-DOI], a selective 5-$HT_{2A}$ partial agonist with potent anti-inflammatory properties, influences stress-related behavior in mice exposed to repeated social aggression, and if these behavioral changes are related to the anti-inflammatory properties of this compound. Animals were subjected to the Stress Alternatives Model (SAM), an escapable social stress paradigm where animals develop either passive coping strategies like remaining in the SAM arena (Stay) with a social aggressor, or active stress coping strategies that involve utilizing the escape holes (Escape) to avoid aggression. Mice expressing these behavioral phenotypes display behaviors like those in other social aggression models that separate animals into stress-susceptible or stress-resilient groups, which have been shown to have distinct inflammatory responses to social stress. These results show that Stay and Escape animals have heightened blood plasma concentrations of the inflammatory cytokine tumor necrosis factor-α (TNF-α) compared to unstressed control mice. Additionally, these results suggest that a single administration of (*R*)-DOI to Stay animals in low doses, can increase active stress coping strategies such as increasing attention to the escape route, promoting escape behavior, and reducing conflict freezing in the SAM. In Escape animals, the middle-dose of (*R*)-DOI shifted behavior in a way that suggests it may have had acute anxiogenic effects in certain behavioral measures.


Thesis Advisor 


Cliff H. Summers, Ph.D., Nolop Distinguished Professor

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## 1. Introduction

Depressive mood disorders are a leading cause of disability worldwide, affecting an estimated 322 million individuals [1,2]. Depression includes a variety of somatic, affective and cognitive symptoms [3], but commonly includes sadness, loss of pleasure (anhedonia), lack of energy and motivation, or changes in appetite. However, individuals display a variety of these symptoms to different extents [4]. Investigations into the pathophysiology responsible for the development depressive mood disorders have not completely resolved the mechanisms involved. However, evidence suggests that reoccurring stress is a major risk factor and can cause disruptions in several biological systems that contribute to the development and persistence of depression symptoms, including dysfunction in monoaminergic systems, neuroplasticity, neurogenesis and inflammation [4].

First-line antidepressants like selective serotonin reuptake inhibitors or tricyclic antidepressants are inadequate, because they require 4-8 weeks of daily administration, can create dependence, and produce side effects [5]. Moreover, these medications only effectively treat about 60% of patients, with one third of individuals deemed treatment-resistant, failing to respond to two or more of these medications [6-8]. These treatments also underserve individuals with depression associated with end-stage cancer [9-12]. While Ketamine has been reported to have rapid-anti-depressive effects [13], these changes only last 2-3 weeks, and this compound has potential for abuse [14]. Studies have shown anti-depressive behavioral effects of ketamine may be maintained for longer durations with weekly administration [15]. These shortcomings have generated interest in exploring other pharmacological tools that may provide more efficient and efficacious treatment for these disorders. Recent research suggests psychedelic drugs produce rapid and long-lasting

therapeutic relief for anxious and depressive mood disorders [9,10,12,16-26] with one or a few spaced administrations. However, the biological mechanisms responsible for these actions are still mostly unknown.

## 2. Stress and Depression

Chronic unpredictable and/or unavoidable stress increase the risk of developing depression and other affective disorders in humans and rodents [27-31] through elevated neurocircuit activation and sustained stress hormone exposure [27,32-34]. Not only do stressful life events impact the onset of depression, but they can also influence severity of symptoms, remission and relapse [3].

Physiological responses to stressors involve changes and coordination between many systems including neuroendocrine, autonomic and the immune system to produce adaptive coping strategies in response to threat and restore homeostasis. Stress-induced activation of the hypothalamic-pituitary-adrenal (HPA) axis promotes the release of corticotropin-releasing hormone (CRH) in the anterior pituitary, stimulating the release of adrenocorticotrophic hormone (ACTH) from corticotropes, which then acts at the adrenal cortex to release glucocorticoids (GC) into circulation. Additionally, stress-induced activation of the HPA axis stimulates the sympathetic nervous system (SNS) which also results in the release of epinephrine (Epi) and norepinephrine (NE) into circulation. Together, these systems result in increased heart rate, breakdown of glucose, and muscle tone to provide greater energy availability to better respond to threating stimuli with fight/flight responses.

A subset of individuals with depression (20 – 80%) display some degree of HPA-axis hyperactivity, as they tend to have higher plasma concentrations of cortisol [35]. Release of cortisol into circulation following stress exposure plays an important role in maintaining homeostasis. One important role of GCs are to reduce the inflammatory response [36] by acting

on glucocorticoid receptors to induce apoptosis in proinflammatory cells such as monocytes, macrophages and T lymphocytes [37], while preventing apoptosis in other tissues by suppressing nuclear factor-κB (NF-κB) signaling [38]. However, prolonged and frequent exposure to stressors can induce GC resistance, resulting in a decreased sensitivity of cells to GCs, reducing their anti-inflammatory capabilities and leading to prolonged pro-inflammatory responses [39].

## 3. Inflammation and Depression

Inflammatory responses have a critical role in stress responses and the etiology of depression. Inflammatory cytokines are released in response to physical and psychological stressors in the periphery and central nervous system (CNS), where they act on receptors altering behavioral and physiological responses to stress. Physiological responses to stress such as activation of the HPA-axis and SNS also relay stress related information to the immune system.

The SNS communicates directly with peripheral immune organs like lymphatic tissues, lymph nodes, bone marrow, and the spleen [40,41]. Release of NE directly into these immune organs, as well as into general circulation plays a critical role in the communication of these systems and is directly involved in the initiation of proinflammatory signaling that occurs with exposure to prolonged/repeated stress. For example, exposure to repeated social stress promotes the release of NE in bone marrow, which stimulates the production and release of myeloid cells like monocytes into the blood [42]. Over time, repeated stress exposure can shift the phenotype of these peripherally released monocytes into a more immature "inflammatory" form, which can be trafficked throughout the body to promote inflammation [43], including the brain, where they contribute to the development of anxious symptoms [40,44]. Peripheral inflammatory markers access the brain through several mechanisms including circumventricular organs, cytokine transport molecules, and activation of the vagal nerve afferents. Some of these inflammatory

markers can also increase the activation of the HPA-axis, like tumor necrosis factor alpha (TNF-α), interleukin 1-β (IL-1β) [45], which likely contributes to their role in stress disorders.

Humans with depression have been shown to exhibit inflammation and stress, resulting in increased plasma concentrations of several proinflammatory cytokines like TNF-α, IL-1β, and interleukin-6 (IL-6) [46]. Positive correlations between the severity of depressive symptoms and concentration of circulating inflammatory markers (IL-6 and IL-1β) are suggestive of functional relationships [46]. Meta-analyses show that humans with major depressive disorder and anxiety express higher levels of TNF-α and/or IL-6 in plasma [47-50]. Additionally, individuals with treatment resistant depression that did not respond to SSRIs also have higher plasma concentrations of IL-6 and TNF-α [51]. Furthermore, socioeconomic status influences the severity and duration of IL-6 signaling seen in response to stress. While there weren't any differences between baseline IL-6 levels when low socioeconomic status subjects were compared to controls , stress increased circulating IL-6 levels in all participants. However, for individuals in the low socioeconomic status group, IL-6 levels continue to increase 75 minutes – 2 hours after a stressor, while IL-6 levels in individuals in the high socioeconomic status group stabilized 75 minutes following stress [52]. Together, this evidence suggests that at least a subset of individuals with depression have higher levels of circulating inflammatory cytokines, and that these cytokines levels may be associated with the severity of symptoms experienced, and treatment resistant depression.

Other evidence supporting the role of inflammation in depression is observed from administration of cytokines such as interferon-α (IFN-α), in humans, which is used to treat various infectious diseases and cancer. Many patients who receive this treatment develop depressive behavioral symptoms [46]. Administration of IFN-α potently induces

proinflammatory cytokines such as IL-6, IL-1β, and TNF-α [53], and the severity of depressive symptoms experienced from IFN-α positively correlated with plasma concentrations of TNF-α and IL-6, but not waking concentrations of plasma cortisol [54]. Additionally, administration of IL-1β has been found to produce various dysfunctional behaviors associated with depression such as impaired social interaction, anorexia and anhedonia [55]. Infliximab, a TNF-α antagonist has been tested for its anti-depressive efficacy in individuals with treatment resistant depression. Results from this study indicated that Infliximab may not be a generalized anti-depressive treatment, as it only relieved symptoms of depression in patients with higher baseline concentrations of TNF-α [56]. However, administration of Infliximab to rodents can reduce behavioral symptoms of anxiety and depression exposed to chronic mild stress [57].

Animal models also support findings that inflammatory responses play a role in acute stress responses, as well as stress-induced affective behavior. In rodents, multiple types of psychological stressors (restraint-stress, open-field, or social isolation) increase concentrations of proinflammatory cytokines such as IL-1β and TNF-α in the periphery and key brain regions involved in emotional regulation. Rodents exposed to an inescapable stress showed increases in circulating TNF-α and interleukin-17A [58]. Other studies have shown similar results, with rodents exposed to a variety of stressors like chronic restraint stress [59], or electric foot shock [60] having higher plasma concentrations of IL-6 . Furthermore, plasma concentrations of IL-6 increase with repeated social defeat [61] and have been shown to be related to social stress susceptibility and resilience in this measure. Stress-susceptible animals displayed higher peripheral levels of IL-6 shortly after a stressor compared to those that were stress-resilient [62]; however administration of IL-6 antibodies prevented this effect. Increased plasma concentrations of IL-6 due to stress exposure appear to be dependent on HPA-axis activity, as adrenalectomy

has been shown to block the stress-induced upregulation of IL-6 [60]. Moreover, chronic stress induced over expression of plasma IL-6 triggered depressive behavior in rodents that was not attenuated by treatment with SSRIs, but was reduced with administration of IL-6 antibodies [63]. [64].

Other animal studies have distinguished differences in the proinflammatory response of physical and psychological stressors [65]. Animals were assigned to two groups, "intruders" received repeated social defeat, or "witness", which were forced to observe the social defeat of the intruders but were not physically attacked. Six days after the final social defeat, animals were introduced to a contextual re-exposure and sacrificed. In intruders, there was a significant increase in proinflammatory cytokines in blood plasma (IL-1β, IL-13, and TNF-α) as well as the anti-inflammatory cytokine IL-10 compared to control animals, but this was not observed in witness animals [65]. This is important, as it shows that different types of stressors produce distinct inflammatory responses and suggests directed social stress produces greater proinflammatory responses compared to a stress that is indirect, and only psychological.

In addition to changes in circulating levels of proinflammatory cytokines, proinflammatory cytokine protein and mRNA expression increase in brain regions that have a critical role in the expression and learning of fear related behaviors. For example, injection of complete Freund's adjuvant (CFA) into the hind paw of mice increases the expression of TNF-α in the basolateral amygdala (BLA) and produces anxiety-like behaviors. Infusion of infliximab into the BLA reversed the anxiety behavior promoted by this protocol [66]. Increased concentration of both TNF-α mRNA and protein in the medial prefrontal cortex (mPFC) have also been observed following social defeat [67,68]. Animals that were susceptible to social defeat showed a dramatic increase in TNF-α in both the prelimbic (PrL) and infralimbic (IL) subregions of the rodent mPFC compared to social stress resilient animals and cage controls [68]. Moreover, virally mediated

overexpression of the GPCR for sphingosine 1-phosphate (S1P), sphingosine-1phosphate receptor 3 (S1PR3), in the mPFC of stress susceptible animals reduced vulnerability to social defeat via reducing TNF-α in the mPFC. Alternatively, mPFC knockdown of S1PR3 promotes susceptibility and increased expression of TNF-α in the mPFC [68]. Other stress models using rodents have shown that stress susceptible and resilient animals also show differences in inflammatory markers in monoaminergic brain centers like the locus coeruleus and dorsal raphe nucleus [55]. Collectively, the expression of proinflammatory markers appears to be important during stress responses.

## 4. Serotonergic Psychedelics

Psychedelic drugs have recently gained attention for their potential to provide more effective treatment for depressive disorders [69]. Psychedelics are psychoactive substances that produce temporary changes in sensory and emotional perception, mood, and cognition [70] through their activity at the serotonin 2A receptor (5-$HT_{2A}$). Examples of psychedelic drugs include lysergic acid diethylamide (LSD), psilocybin, mescaline, and dimethyltryptamine (DMT). In rodent models, psychedelics also produce transient behavioral effects, such as the head twitch response [71,72]. Evidence suggests these compounds have been used by humans for at least 5 millennia [73]. However, it was not until 1943 that western science began seriously investigating these compounds, after Albert Hoffman discovered the psychoactive effects of LSD by accidently ingesting trace quantities when synthesizing it, and later confirming the effects by self-administration [74,75]. Following this discovery, LSD was initially used a model for psychosis, but many clinical scientists also investigated the potential for using these drugs to treat various conditions neuropsychiatric conditions. From the early 1950's to mid 1960's, over 1000 scientific studies were published investigating the use of psychedelics as treatments for conditions like

addiction, and existential crisis associated with terminal illness [75,76]. However, following passage of the Controlled Substances Act in 1970, virtually all research investigating psychedelic drugs was halted until the early 1990s.

The subjective behavioral effects that occur from the administration of psychedelics are dynamic, and perceptual and hallucinatory alterations progress through stages over time following administration [77]. The intensity and length of the experience are largely influenced by dose, route of administration, and which particular psychedelic substance is being administered [77]. Tests to measure the subjective effects that psychedelics produce in humans include questionnaires like Altered States of Consciousness (ACS), and the Mystical Experience Questionnaire (MEQ). These self-report questionnaires include measures that assess positive or pleasurable experiences that can occur, including states of heightened mood (such as bliss, self-dissolution, feelings of unity, and transcendence of space and time), measures of negative experiences (including panic or anxiety, catatonia, and cognitive impairment), as well as measures of the degree of alterations in sensory processing (including hallucinations, illusions, and synesthesia) [77]. Experiences of anxiety or panic after the administration of psychedelics are usually transient and associated with the fear of losing control [78], but can be mitigated by trained psychotherapists present during the administration of these drugs in clinical settings [78,79]. Interestingly, the quality of subjective experiences that occur from administration psilocybin are potentially related to their long-term therapeutic outcomes. Experiencing higher levels of pleasurable states (Oceanic Boundlessness) and lower anxious states (Dread of Ego Dissolution) are predictive of more positive long term therapeutic outcomes [80], suggesting that the therapeutic effects of psychedelics are not only mediated by their pharmacological actions, but they may be experience dependent as well [80]. Furthermore it has been observed that mood is a predictor in the experience produced from

administration of psychedelics [79]. Feelings of calmness before administration of psychedelic drugs was positively correlated with experiences of pleasurable states (Oceanic Boundlessness) [77], corroborating more recent evidence suggesting that both current mood and experiences of psychological distress in the 4 weeks before the administration of psilocybin was more important for predicting responses to psilocybin than factors regarding personality [78]. More pleasant experiences on psilocybin were strongly associated experiencing less psychological problems in the weeks before drug administration, and being in an emotionally excited and active state shortly before drug administration [78].

In animal models, administration of serotonergic psychedelics produce a variety of acute behavioral effects. In rodents, these compounds can produce the head twitch response (HTR), ear scratching and wet-dog shakes [81]. However, other non-psychedelic drugs produce HTR behavior including 5-HT infusions directly into the brain, or other drugs that increase the release of 5-HT in the CNS like 5-$HT_{1A}$ receptor antagonists, or cannabinoid-1 receptor ($CB_1$) receptor antagonists [81].

Aside from their acute behavioral effects, serotonergic psychedelics cause transient increases in heart rate and blood pressure [19]. In addition, psilocybin increases plasma concentrations of cortisol, ACTH, and thyroid-stimulating hormone (TSH) 60 minutes after administration, when subjective effects are at their peak [82]. Similarly, plasma concentrations of ACTH, corticosterone, and oxytocin are increased 30 minutes after ip injections of DOI (5 mg/kg) in mice [83]. Psychedelics are also generally safe compounds with low toxicity and little potential for abuse, as they do not have reinforcing properties, and repeated administration produces rapid tolerance [84]. Since the beginning of the 21$^{st}$ century, there has been a renewed interest in using psychedelics in conjunction with psychotherapy to treat a variety of disorders [70] including alcohol and nicotine

addiction [24,85-87], obsessive compulsive disorder [88,89], anxiety and depression associated with end of life-diagnoses [9-12,90], and treatment resistant depression [21,91].

The mechanisms by which serotonergic psychedelics generate rapid and enduring antidepressant effects [9,12,22,92-94] for individuals with treatment-resistant anxiety/depression (up to 6 months) [21,23] are poorly understood, however, evidence suggests their activity at 5-$HT_{2A}$ receptors is a key component of how they produce their acute behavioral effects [69,95]. Pre-administration of a 5-$HT_{2A}$ receptor antagonist blocks the subjective effects of psilocybin [96] and LSD [97] in humans, as well as the HTR in rodents [72]. Additionally, there is a correlation between the potency of psychedelics in humans and their capacity to elicit the HTR in rodents [98]

One hypothesis as to why psychedelic compounds elicit their behavioral effects while other 5-$HT_{2A}$ receptor agonists do not, is due to functional selectivity or biased signaling [99]. This phenomenon suggests canonical and non-canonical intracellular pathways can be activated by G-protein coupled receptors (GPCR) to different extents, depending on the specific ligand that binds to them. Different ligands engage unique protein residues in the binding pockets of GPCRs, which results in distinct conformational shapes of receptor-ligand complexes, allowing the receptor to engage different transducer-coupled states. This hypothesis is supported by a study which produced the visualizable crystalline structure of the LSD-bound 5-$HT_{2A}$ receptor [100], showing that different 5-$HT_{2A}$ receptor ligands engage partially distinct proteins in the binding pocket of this receptor.

Psychedelic drugs action at 5-$HT_{2A}$ receptors may also contribute to their rapid and long-term therapeutic properties as well, but this is less understood. Chronic stress has been shown to diminish neural functioning by causing apical dendritic atrophy and loss of functional apical spines in layer V projection neurons (PNs) of the medial prefrontal cortex (mPFC) [33,34,101-

103]. These structural responses to stress appear to be mediated by stress-induced alterations in brain-derived neurotrophic factor (BDNF) [104,105] and cortical feedback to the amygdala, leading to improper regulation of emotional interpretation and learning. Similar structural damages and reduced serum levels of BDNF have also been found in humans with major depressive disorder [106-108]. One of the leading hypotheses as to how psychedelics produce such rapid and long-lasting anti-depressive effects is that they promote apical dendrite and spine growth *in vitro* in cultured cortical neurons, and *in vivo* in rodent layer V PNs in the mPFC. These changes in neuroplasticity require 5-$HT_{2A}$ activation and subsequent recruitment of BDNF and mammalian target of rapamycin (mTOR) [20,109,110].

Another mechanism that may contribute to the rapid anxiolytic or antidepressive effects of psychedelics that is less understood, are their anti-inflammatory properties. In a study that assessed the anti-inflammatory potency of different psychedelic compounds in rat model of allergic asthma, these compounds exhibited distinct anti-inflammatory capabilities [111]. Several psychedelics were assessed for their anti-inflammatory potency in reducing airway hyperresponsiveness. Interestingly, in this model, psychedelics that produced anti-depressive effects in humans like DMT showed no efficacy in reducing inflammation, while LSD was shown to have partial anti-inflammatory efficacy, and psilocin, the active metabolize of psilocybin, and (*R*)-DOI had full efficacy. In this model, inhaled or subcutaneously injected (*R*)-DOI also reduced the gene expression of several proinflammatory markers in whole lung samples, including TNF-α, IL-6, and IL-1β [111]. These anti-inflammatory properties were dependent on activation of 5-$HT_{2A}$ specifically; blocking 5-$HT_{2B}$ or 5-$HT_{2C}$ receptors had no impact on anti-inflammatory effects, and there were no anti-inflammatory effects seen in 5-$HT_{2A}^{-/-}$ receptor knockout mice. Astoundingly, the $EC_{50}$ dose for anti-inflammatory effects of (*R*)-DOI

in this study was found to be 0.003 mg/kg, which is about 30 times less than what is required to induce behavioral effects (HTR) [111]. This suggests that therapeutic properties of this compound may be observed even at doses that are significantly lower than what would be required to produce behavioral changes. These results confirmed the findings that (*R*)-DOI has therapeutic effects at extremely low doses in treating multiple inflammatory disorders in the periphery when administered systemically [112-115]. Continuous delivery of (*R*)-DOI at extremely low concentrations through an osmotic minipump to achieve a steady plasma concentration of 0.5 ng/mL (1.5 μg/hr), showed significant therapeutic efficacy in reducing mRNA levels of proinflammatory cytokines in vascular tissue and circulating levels of cholesterol, as well as normalizing glucose homeostasis promoted by a high fat "western" diet in apolipoprotein E (ApoE) $^{-/-}$ knockout mice [114]. Administered intraperitoneally, (*R*)-DOI at a dose as low as 0.01 mg/kg blocks the proinflammatory effects of systemic TNF-α administration in several tissue types [113]. Previous research demonstrates that (*R*)-DOI potently inhibits the increase in circulating IL-6 resulting from systemic administration of TNF-α in whole animals, at doses as low as 0.01 mg/kg. The anti-inflammatory effects of (*R*)-DOI were also shown to be specifically dependent its action at 5-$HT_{2A}$ receptors [113]. While these studies show that (*R*)-DOI has potent anti-inflammatory actions, there is no research regarding whether these properties can alleviate stress induced affective behaviors. In addition, inflammatory biomarkers have been measured after administration of other psychedelic compounds in humans like ayahuasca, which contains dimethyltryptamine (DMT). In this study there was no effect of ayahuasca on IL-6 levels, but there was a reduction in C-reactive protein levels compared to control treated individuals [17]. Another study examining the effects of inhaled 5-methoxy-N,N-

dimethyltryptamineamine (5-MeO-DMT) found that IL-6 levels were reduced shortly after administration, but these changes weren't correlated to changes in mental health measured [116].

A class of compounds known as halogenated phenethylamines, which includes DOI, have long lasting psychedelic effects in humans, causing difficulties sleeping and hallucinogenic effects lasting more than 24 hours at higher doses [117]. The structure of DOI contains a chiral center that allows it to exist in one of two enantiomers; (*R*)-(-)-DOI and (S)-(+)-DOI [72]. Compared to the racemate (±) or (+) enantiomer, (*R*)-DOI has been shown has a higher affinity for rat cortical 5-$HT_{2A}$ receptors [118,119] and in human recombinant 5-$HT_{2A}$ and 5-$HT_{2C}$ receptors [120]; as well as lower affinity for 5-$HT_{2B}$ receptors [120], making it a more selective 5-$HT_{2A}$ agonist. A recent study in mice [71], showed half-life of DOI (1 mg/kg) in blood is shorter than that compared to the forebrain, suggesting that there is an accumulation of this drug in the brain. Moreover, (*R*)-DOI is also more behaviorally potent in mice (ear-scratch response) [121], rats (drug discrimination) [122], and humans (psychedelic effects, subjective) [117,123].

Activation of 5-$HT_{2A}$ receptors is a key mechanism in how psychedelics mediate their acute behavioral effects. Further, there is evidence that 5-$HT_{2A}$ targeting is responsible for the long-lasting therapeutic effects of psychedelics. Therefore, studying a compound with high selectivity for 5-$HT_{2A}$ like (*R*)-DOI can help support whether the therapeutic behavioral effect of psychedelics is primarily mediated through 5-$HT_{2A}$ activity.

*4.1. 5-Hydroxytrypatamine-2A (5-$HT_{2A}$) Receptor*

The 5-Hydroxytryptamine-2A (5-$HT_{2A}$) receptor is a class A seven transmembrane G protein-coupled receptor (GPCR) that belongs to the 5-$HT_2$ family of receptors, which also includes 5-$HT_{2B}$, and 5-$HT_{2C}$ receptors. It is most widely expressed 5-HT receptor in mammals [124], found in many tissues and cells in the periphery and central nervous system (CNS) [125].

The canonical intracellular signaling pathway promoted by 5-$HT_{2A}$ receptor activation involves dissociation of the $G_{q\alpha}$ from $G_{\beta\gamma}$ subunits which phosphorylates phospholipase C (PLC), in turn, promotes the release of diacylglycerol (DAG) and inositol trisphosphate ($IP_3$). From there, DAG activates protein kinase C (PKC) activity, and $IP_3$ promotes intracellular calcium ($Ca^{2+}$) release into the cytoplasm from the endoplasmic reticulum (ER) [125,126].

In the periphery, 5-$HT_{2A}$ receptors are found in platelets, acting to facilitate platelet aggregation, in blood vessels, monocytes, the vagus nerve [127], smooth muscle cells, as well as the gut and stomach [128]. Additionally, 5-$HT_{2A}$ receptors can impact immune responses in tissues and cells associated with the immune system; such as the spleen, thymus and lymphocytes [129], where 5-HT acts to mainly promote proinflammatory effects [115].

In the central nervous system, 5-$HT_{2A}$ receptors have been found to be expressed in many regions and implicated in a variety of mood disorders. In rodents, 5-$HT_{2A}$ is expressed in glutamatergic and γ-Aminobutyric acid (GABA) neurons in many brain regions that are implicated in anxious and depressive disorders like the amygdala [130], hippocampus [131,132], prefrontal cortex [133] and monoaminergic brainstem regions like the dorsal and median raphe nucleus, ventral tegmental area and locus coeruleus [134]. Although it's role is still not well understood, it has also been observed that the brain region with the highest expression of 5-$HT_{2A}$ is the claustrum [135].

## 5. Conclusions

Shortcomings of current anti-depressive treatments require more efficacious pharmacological tools should be investigated. While the pathophysiology of depressive disorders is complex and involves many biological systems, it is well established that stress and inflammation play a major role in the development of these disorders. Psychedelic compounds have been found to exert rapid

and long-lasting antidepressive behavioral outcomes in humans and animal models, but it's unclear whether the anti-inflammatory properties some of these drugs contribute to their potential therapeutic behavioral impacts. Evidence suggests that systemic administration of (*R*)-DOI has potent anti-inflammatory activity in a variety of peripheral tissues, and has greater anti-inflammatory potency compared to most other psychedelics tested. However, there are no clinical studies examining the antidepressive potential of (*R*)-DOI in humans, and most animal behavioral studies examining this compound assessed the acute behavioral effects this drug, not whether it produces anxiolytic or antidepressive effects after acute behavioral effects have subsided. In this study we designed experiments that aimed to better understand how (*R*)-DOI impacts stress coping strategies when administered systemically when faced with repeated escapable social stress, whether these behavioral changes are related to the anti-inflammatory effects of this compound.

Measuring these markers will help us understand how circulating inflammatory cytokines are influenced by social aggression in the Stress Alternatives Model (SAM), and whether animals that learn active coping strategies (Escape) have reduced levels of certain proinflammatory cytokines compared to those that learn passive coping strategies (Stay). Additionally, we tested whether a single subcutaneous administration of (*R*)-DOI reduces the levels of inflammatory markers like TNF-α, and if these potential anti-inflammatory effects correlate with changes in stress coping behavior. As dendritic arborization and synaptogenesis promoted by DOI similar to other serotonergic psychedelics [20,109,110], we hypothesized that (*R*)-DOI would have similar antidepressive efficacy [22,24,136,137]. Additionally, this compound is also a potent anti-inflammatory agent, which may contribute to its therapeutic mechanism in treating stress related behavior [113,115].

## Chapter 2

Single Administration of a Psychedelic [(*R*)-DOI] Influences Coping Strategies to an Escapable Social Stress

### 1. Introduction

Depressive mood disorders impact an estimated 322 million individuals worldwide [1,2], and first line antidepressive treatments like reuptake inhibitors for serotonin (5-HT) and/or norepinephrine, or tricyclic antidepressants are among the most prescribed medications in the United States [138]. However, for many, these treatments are inadequate, requiring 4-8 weeks of daily administration, creating dependence, and producing side effects [5]. These medications only effectively treat 60% of patients, while one third are treatment-resistant (fail to respond to two or more first line antidepressants), and even more, 23% of suicide victims are on an antidepressant at the time of death [6-8]. While ketamine has recently gained attention for its rapid antidepressive efficacy, its therapeutic effects are short lasting (2-3 weeks), and has potential for abuse due to its reinforcing qualities [14]. Given the limitations of current antidepressant medications, it is imperative that new drugs with greater efficacy and fewer side effects are tested and made available.

While behavioral and physiological responses to stress are adaptive, exposure to stress is an independent risk factor for the development depressive and anxious mood disorders [139]. Chronic unpredictable and/or unavoidable stressors increase the risk of developing depression and other affective disorders in humans and rodent models [27-31], partly through elevated neurocircuit activation and sustained stress hormone exposure [27,32-34]. Additionally, different types of stressors (i.e., social vs. non-social) can elicit unique behavioral and physiological responses [140-142]. In humans, social stress is the most common form of stress encountered,

and is perceived as being more intense compared to other stressors [143]. Many rodent models have shown that acute responses to social stress promote physiological changes consistent with humans exposed to a psychosocial stress [139]. Nonetheless, there is considerable variation between individuals in terms of whether a specific stressor will lead to psychopathologies, as some individuals are more susceptible to stress than others. It's been suggested that coping strategies in response to a stressor are related to susceptibility. Active coping strategies, defined as behavioral responses which minimize physical, psychological or social harm, are also related to stress resiliency [139].

Inflammatory responses to stress also have a critical role in depression and stress susceptibility. Proinflammatory cytokines are released in response to physical and psychological stressors in the periphery and central nervous system (CNS), where they act on receptors altering behavioral and physiological stress responses. Some of these proinflammatory cytokines including tumor necrosis factor- α (TNF-α) and interleukin-1β (IL-1β) [45] increase activation of the hypothalamic pituitary adrenal Axis (HPA-axis)., which likely contributes to their role in stress disorders. Humans with depression have shown increased plasma concentrations of TNF-α, IL- 1β, and IL-6 [46]. In rodents, different types of stressors increase concentrations of proinflammatory cytokines like IL-1β and TNF-α in the periphery and brain regions important for emotional regulation [46,58]. Even more, administration of IL-1β in rodents promotes impaired social interaction, anorexia and anhedonia [55], which are common symptoms experienced by humans with depression. Inflammatory responses may also be related to differences in stress susceptibility and what coping strategies an individual uses, as susceptible and resilient animals show differences in the expression of proinflammatory markers in brain regions associated with stress responses [55].

Classic serotonergic hallucinogens (psychedelics) exert acute, powerful behavioral effects in humans such altered sensory and emotional perception, cognition, and sense of self [70]. In rodents these substances also induce acute behavioral changes such as the head twitch response [71,72] . Recent research demonstrates that psychedelics may be a potentially superior alternative to current antidepressants, as some of these substances like psilocybin (prodrug of psilocin) produce rapid and long-lasting antidepressive effects in clinical models of depression [9,11,12,18,21-23,26,80,93,94,144] and may provide therapeutic relief longer [21] than ketamine [136,145], while circumventing toxic and addictive risks [146-148]. Evidence suggests a key mechanisms by which serotonergic psychedelics exert their acute behavioral effects, is through biased agonism of the 5-hydroxytryptamine 2A (5-$HT_{2A}$) receptor, as systemic pre-administration of a 5-$HT_{2A}$ receptor antagonist has been shown to block the acute behavioral effects these drugs produce in humans [96,97] and rodents [72]. The potential of these compounds as antidepressants requires a thorough investigation into the neurophysiological circuits and specific intracellular signaling mechanisms that are involved in their rapid therapeutic behavioral effects. Specific molecular mechanisms and dosage effects must be analyzed for psychedelic drugs to yield a potentially therapeutic outcome.

The halogenated phenethylamine 2,5-dimethoxy-4-iodoamphetamine (DOI) is a serotonergic psychedelic that may have antidepressive potential, but remains untested for its therapeutic value. However, DOI has a more specific pharmacological profile than other psychedelics, primarily acting on 5-$HT_{2A/2B/2C}$ receptors, with the highest selectivity for 5-$HT_{2A}$ receptors. Other psychedelics like psilocin and DMT act on other receptor subtypes like the 5-$HT_{1A}$, making it more difficult to discern their mechanism of action. Recent research demonstrates that (*R*)-DOI has potent anti-inflammatory effects, which may also make it a good candidate for an

effective antidepressant. There are a number of reports indicating (*R*)-DOI has therapeutic effects at extremely low doses in treating multiple inflammatory disorders in the periphery when administered systemically to rodents [112-115], reducing circulating TNF-α and IL-6 at a doses as low as 0.01 mg/kg [113,149]. The anti-inflammatory effects of (*R*)-DOI were also shown to be dependent its action at the 5-$HT_{2A}$ receptor [113].

The goal of this study was to how a single administration of (*R*)-DOI (0.3, 0.03, 0.015 mg/kg) influences stress coping strategies in response to escapable, repeated social aggression using the Stress Alternatives Model (SAM). In this behavioral paradigm, animals that exhibit more passive coping strategies (Stay), display stress-induced dysfunctional behaviors and physiological changes consistent with affective disorders in humans, while animals with more active coping strategies (Escape) do not [150-153]. We also investigated whether behavioral phenotypes in this model have differences in circulating inflammatory cytokines, and whether behavioral changes observed with treatment of (*R*)-DOI relate to its anti-inflammatory properties.

## 2. Methods

### *2.1. Animals*

All mice were maintained on a 12:12 light-dark cycle (lights on at 6 a.m.) and given *ab libitum* access to food and water throughout all experiments. All behavioral experiments were done during the animals' active phase (scotophase). Male (C57BL/6NHsd) mice between 6-8 weeks (Envigo, Indianapolis, IN; (N=103), weighing (21-25g), were group housed (4-5 animals/cage) upon arrival for a 5-day acclimation period. A separate cohort of retired male breeder Hsd:ICR (CD1) mice (Envigo) were housed in the same way, and used to provide aggression to the C57BL/6N mice in the Stress Alternatives Model (SAM). Mice were then separated into their own individual cages (4-5 days) prior to the start of behavioral experiments.

After being separated into individual cages, animals were handled, and had their body weight and food consumption monitored daily until the end of experimentation. Behavioral experiments were performed in a way that minimized suffering, and the number of animals used. Mice (N=7) were excluded from behavioral analysis if three criteria were met. One, the C57BL6/N mouse was in the first cohort that experienced high heat and humidity. Two, a CD1 utilized one of the escape routes on any day when a C57BL/6N was in the SAM. Three, an animal did not display typical phenotype stability that we have observed in previous SAM experiments.

The experiments described were designed in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) and approved by the Institutional Animal Care and Use Committee of the University of South Dakota.

### *2.2. Drug & Drug Administration*

On drug administration day (*R*)-DOI hydrochloride (Sigma Aldrich, St. Louis, MO) was dissolved into saline to achieve the desired concentrations [high-dose (0.3), middle-dose (0.03), low-dose (0.015 mg/kg)] or vehicle (saline) to be given as 0.1 mL injections. Immediately following social interaction on day 2 of the SAM, animals were given subcutaneous injections, then placed directly into their home cage.

### *2.3. Design of behavioral experiments*

Behavioral experiments/measures were performed during the animals' active cycle (scotophase) under red light (~700 nm λ) and video recorded using a GoPro (Hero 3 or Hero 7) for later behavioral analysis. Three cohorts of C57BL/6NHsd mice were ordered for this experiment. Cage control animals were not exposed to any stressor so they could be used as a stress control to compare measurements in weight, food intake, and TNF-α plasma levels. The

first cohort consisted of a total of 40 animals. Cage controls (N=6 mice), vehicle-treated animals (N=16 mice) and (*R*)-DOI (0.3 mg/kg) treated (N=18 mice). The second cohort of 23 mice had cage controls (N=3), vehicle-treated animals (N=5) and (*R*)-DOI (0.03 mg/kg) animals (N=15). The third cohort of 40 animals had cage controls (N=4), vehicle-treated (N=7), (*R*)-DOI (0.015 mg/kg) (N=20), and (*R*)-DOI (0.3 mg/kg) (N=7) and (*R*)-DOI (0.03 mg/kg) (N=2).

All animals were subjected to 5 minutes in the Stress Alternatives Model (SAM) for 4 days in a row. Before social interaction begins in the SAM each day, mice were exposed to a fear conditioning paradigm where the conditioned stimulus (2500 Hz tone played at 75 dB) was played right before animals were exposed to the unconditioned stimulus of social aggression by the CD1 aggressor. On Day 5, mice were tested for their fear conditioning response to the CS tone. Afterwards animals were anesthetized with isoflurane (5% at 1.0 min/L for ~2 min) then rapidly decapitated to collect trunk blood and brains for later analysis.

While all cohorts underwent the same experimental design, the first cohort experienced high temperature and humidity which received the high-dose of (*R*)-DOI (0.3 mg/kg). Between Day 2 and 3 of the SAM for this first cohort (after treatment), the air conditioning in the facility broke. This caused an increase in the temperature and humidity throughout the animal housing facility including the housing room and where behavioral experiments are done. Additionally, there was also coolant that was leaking from the ceiling of the facility for several days after the incident occurred which had a strong odor. Due to the high heat and humidity, several dehumidifiers and small air conditioners were placed in the housing and experiment rooms to try and mitigate the higher temperature and humidity, however these also provided a consistent white noise in the background that is usually not present during SAM experiments.

*2.3.1 Stress Alternatives Model (SAM)*

The SAM apparatus (Fig. 1) is a clear rectangular box (91 cm x 22 cm x 30 cm) covered with white opaque paper on the outside. Two curved, white opaque dividers are used to separate the two safety areas (10 cm x 22 cm x 30 cm) from the main open field of the SAM (71 cm, 22 cm, 30 cm), where social interaction takes place. The curved dividers each have a small escape hole which only the smaller C57BL/6N mice can fit through to escape social aggression. At the beginning of each day of the SAM, prior to social interaction, C57BL/6N mice are exposed to a fear conditioning paradigm in the SAM. An opaque cylinder (diameter = 15 cm, width = 20 cm) is placed in the center of the open field of the SAM, which the C57BL/6N mouse is then placed into, providing a barrier to separate the C57BL/6N mouse from the aggressive CD1 mouse which is already in the SAM open field. The fear conditioning paradigm consists of a 30 second acclimation period, followed by a 2500 Hz tone played at 75 dB (CS) for 5 seconds, followed by a 10 second post-tone period (trace period), after which the cylinder is lifted, exposing the C57BL/6N mouse to the aggressive CD1 mouse in the open field of the SAM.
Immediately following day 2 of the SAM, animals received subcutaneous (sc*)* administration of (*R*)-DOI (0.3, 0.03, 0.015 mg/kg) or saline before being placed into their home cages. This provided the opportunity to test if administration of (*R*)-DOI changes stress coping strategies in Stay and Escape animals after the phenotype had been determined.

When C57BL/6N mice received aggression that appears potentially life threatening from an aggressive CD1 mouse, a perforated divider (15 cm wide x 20 cm high) was temporarily placed on top of the CD1 mouse to interrupt the aggressive bout. After 5 minutes both mice were removed from the SAM and placed back into their home cages. In instances where a C57BL/6N mouse escaped the SAM, they were left in the safety area for the remainder of the 5 minutes, and a clear perforated sheet of plastic was placed in front of the escape route.

All behaviors were recorded in the SAM using a GoPro (Hero 3 & Hero 7) and videos were stored onto an external hard drive so behavioral analysis could be done later. Behaviors from these videos were measured using ANY-maze Video Tracking Software (Version 6.0, Stoelting Co, Wood Dale, IL) or scored manually by individuals that were blind to treatment groups. In between each trial in the SAM, the apparatus was thoroughly cleaned by wiping it down with 70% ethanol and disinfectant wipes, and then dried with fresh paper towels.

Video recordings of the SAM were analyzed using ANY-maze software (Version 6.0). Behavioral analysis during the SAM began after the FC cylinder was lifted, until the end of the 5-minute interaction, or until the C57BL/6N mouse escaped. Animals were separated into two phenotype groups based on their behavior in the SAM by the end of day 2. Mice that stayed in the open field of the arena and did not utilize the escape route by the end of day 2 were Stay mice, while Escape mice used the escape routes in the SAM to avoid the social aggressor by the end of day 2. Since animals were separated into these phenotypic groups before treatment, behavioral measurements could be done across phenotypic groups, and comparing animals to themselves across days. Behaviors that were recorded include latency to escape, freezing, conflict freezing, attention to the escape route, and number of jumps. Attention to the escape route is the number of seconds an animal spends investigating either of the escape routes (within 3 cm of the escape hole) while in the open field of the SAM. Conflict freezing in the SAM is defined as the amount of time an individual spends in the open field of the SAM not moving for more than 1 second. Latency to escape is measured as the time in seconds it takes for an animal to go through one of the escape routes. If an animal doesn't escape by the end of the 5-minute interaction, they are assigned a latency to escape time of 300 seconds. Previous SAM

experiments have found that the time it takes an animal to escape declines over time once it has escaped once, suggesting that spatial and/or social learning is occurring [152-154].

*2.3.1.1. SAM Validation*

The SAM is a tool for assessing stress-related behavior that may be appropriately described as anxious and depressive behaviors, and as such has been progressively subjected to accepted validation testing, using the specific criteria for Construct, Predictive, and Face Validities [155]. Through incorporation of social defeat elements as well as active avoidance, the SAM construct incorporates elements of fear and anxiety [156-159], social stress and depression [160,161], but also alleviation of these stress-related outcomes through Escape, while being both ecologically and ethologically relevant [151,162,163] and maintaining similarities to relevant human disorders [164,165], suggesting a degree of Construct Validity. Predictive validation of the SAM has been demonstrated through the induction of behavioral changes, including phenotype reversal, using known anxiolytic, antidepressive, or anxiogenic drugs (NPS, antalarmin, and yohimbine) [152-154]. Also, SAM has been used in conjunction with, and produces comparable results to, the Social Interaction/Preference (SIP) Test [150,166], which has been validated as translationally and predictively reliable in demonstrating the effectiveness of pharmacotherapies used to treat anxiety (benzodiazepines) and depression (SSRIs) [167-171]. Furthermore, elevated glucocorticoid levels demonstrate an enhanced physiological stress response in animals encountering social aggression in the SAM, with the Stay phenotype expressing the greatest increase [150-154]. With respect to Face Validity, SAM exposure results in behavioral outcomes, largely examples of startle (Conflict Startle), behavioral inhibition (FC freezing, Conflict freezing), and social avoidance (Escape, Escape Latency, and SIP test), that reflect those seen in human anxiety and depression.

*2.4. Fear Conditioning (FC) paradigm*

Fear conditioning training and testing of C57BL/6N mice was done in an opaque cylinder before social interaction and aggression (unconditioned stimulus, US) occurs in the SAM, as well as on day 5, without exposure to the US. Freezing behavior of C57BL/6N mice was measured in an opaque cylinder in response to the conditioned stimulus (CS) tone, which was paired to the US of the cylinder being lifted to expose the animal to the SAM open field and CD1 aggressor. Once placed into the opaque cylinder, C57BL/6N mice were given a 30 second acclimation period before the exposure to the CS tone for 5 seconds, followed by a 10 second trace period before the opaque cylinder was lifted (US). After day 5 FC took place, animals were briefly anesthetized using Isoflurane (5% at 1.0 min/L for ~2 min) and rapidly decapitated to collect blood plasma and brains, which were then rapidly frozen and stored in -80°C freezers until analysis.

Behavioral analysis of FC was done using ANY-maze (Version 6.0) software. Contextual freezing behavior was measured as time spent freezing in the FC cylinder during the 30 second acclimation period before exposure to the CS tone. Cued freezing behavior was measured as time spent freezing after the begging of the 5 second CS tone, until the end of the 10 second trace period when the cylinder was being removed from the SAM open field (US).

*2.5. Home cage Mobility*

Immediately following day 3 of the SAM, animals were put back into their home cage, then brought from the behavioral experiment room to their housing room. and their cage was placed on a table so that home cage mobility could be assessed to determine whether (*R*)-DOI at any dose impacted locomotion. Animals were video recorded using a GoPro (Hero 7 or Hero 3) for 5 minutes and then placed back into their housing rack.

Behavioral analysis of home cage mobility was performed using ANY-maze (Version 6.0) software. Locomotion in the home cage was measured as the distance individuals traveled in their home cage (meters/second).

*2.6 Blood Plasma Analysis*

Following testing on day 5, trunk blood was collected from each animal in heparinized tubes and centrifuged for 10 minutes to separate blood plasma. After separating plasma, samples were immediately collected then placed on dry ice until they could be stored in a -80 °C freezer. Plasma concentrations of 9 inflammatory markers were quantified using Mouse Cytokine 9-Plex ELISA Kits (PBL Assay Science, Piscataway, NJ; Cat. No. 52500-1) [172]. Plasma samples were shipped to PBL Assay Science in dry ice to be quantified. This kit provides a standard for every cytokine that will be measured interleukin-1α (IL-1α), interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-10 (IL-10), chemokine ligand 5 (RANTES), tumor necrosis factor-α (TNF-α), interferon-α (IFN-α), interferon-β (IFN-β), and interferon-γ (IFN-γ). The format of the assay is that of a sandwich ELISA with chemiluminescent output that utilizes a 7-point standard curve for running standards at varying diluted concentrations to ensure accuracy.

*2.7 Statistical Analysis*

All experimental designs and statistical analyses were based on *a priori* hypotheses. For conditions that changed over 4 days of SAM social interaction, we compared outcomes using a two-way repeated measures ANOVA ([R]-DOI x behavioral phenotype x day of SAM interaction design), where phenotype was either Stay or Escape. In addition, two-way ANOVA ([R]-DOI drug x Phenotype design) was utilized to determine subcutaneous (sc) (*R*)-DOI administration (including 3 doses: 0.015, 0.03, 0.3 mg/kg) relative to the expression of behavioral phenotypes (Stay vs Escape; Phenotype Effects) and Phenotype by Conditioning

(Interaction Effects). To compare changes occurring within a treatment group across SAM interaction days, a one-way repeated measures ANOVA ([R]-DOI drug x day of SAM interaction design) was performed. While the statistics for behavior did not include cage controls; these controls were necessary for interpretation of hormonal corticosterone levels, because samples from SAM treatments were compared to baseline levels determined by the mean [B] value of home-cage control animals. Therefore home-cage controls were added for specific one-way ANOVA comparisons. Comparison of locomotion in the home cage after drug treatment was also accomplished by one-way ANOVA. Comparisons between two treatments (Vehicle, [R]-DOI) within a given phenotype (Escape or Stay) were analyzed by Student's t-tests. To determine differences in percentage of escape, a chi-square statistical analysis was performed, where the previous days were utilized as expected values.

Each animal provided only a singular sample for multiple measures, for all analyses. Five assumptions of parametric statistics were applied to the data, which were transformed when necessary. Analysis with both non-parametric and parametric statistics (previously mentioned) were performed along with examination for multiple comparisons using the Holm-Sidak method, and when the statistical analyses match, as they do for the data herein, we report the parametric results without α adjustment [173-178]. Significant effects between groups for one-way analyses were examined with Student–Newman–Keuls post-hoc analyses (to minimize Type I error) and Duncan's Multiple Range Test (to minimize Type II error).

## 3. Results

### *3.1 Animal Weights*

To determine if (*R*)-DOI administration produced any change in animal weights compared to non-stress cage controls of vehicle-treated animals, we monitored animal weights throughout the experiments. Comparison of all treatment groups revealed there was an effect of treatment (Fig. 3; $F_{4,91} = 4.8$, $P < 0.001$). Direct comparisons between these groups showed that unstressed cage control animals had significantly lower weight gain compared to vehicle-treated ($t_{35} = 4.7$, $P < 0.001$), high-dose (*R*)-DOI ($t_{33} = 2.7$, $P \leq 0.012$), middle-dose (*R*)-DOI ($t_{28} = 3.7$, $P < 0.001$), and low-dose (*R*)-DOI ($t_{31} = 2.5$, $P \leq 0.019$) treated animals. Additionally, vehicle-treated animals had significantly higher weight gain compared to animals treated with the high-dose of (*R*)-DOI ($t_{44} = 2.5$, $P \leq 0.015$)

### *3.2 Food Consumption*

Food consumption was monitored throughout experiments to determine whether administration of (*R*)-DOI had an impact on food consumption compared with vehicle-treated animals (Fig. 4). However, there were not meaningfully significant differences in food consumption between treatment groups.

### *3.3. Home cage Mobility*

To determine whether any of the doses of (*R*)-DOI produced acute increases in locomotion in response to social aggression the day following administration, we measured home cage mobility immediately following the SAM on Day 3 (Fig. 5). Comparing all treatment groups together ($F_{3, 74} = 3.7$, $P \leq 0.016$) showed that animals that received the lowest dose of (*R*)-DOI (0.015 mg/kg) has significantly higher home cage locomotion following Day 3 of the SAM compared to

vehicle-treated animals ($t_{40} = 2.8$, $P \leq 0.009$) as well as animals that received the high- (0.3 mg/kg) ($t_{40} = 2.8$, $P \leq 0.009$), and middle-dose of (*R*)-DOI (0.03 mg/kg) ($t_{32} = 2.5$, $P \leq 0.016$) .

*3.4 Behavior in the Stress Alternatives Model (SAM) apparatus*

*3.4.1. Escape*

In previous experiments using the SAM [150,152,153] , Stay and Escape animals consistently displayed stable phenotypic behaviors after day 2 through day 4 of the SAM, unless animals are given some pharmacological intervention. In this experiment, this was the case for Escape animals, however, on day 4, there were some vehicle-treated Stay animals that escaped (3 animals;18%) although this change was not significant ($\chi^2$: $F_1 = 1.5$, $p \geq 0.217$). However, animals treated with the low-dose of (*R*)-DOI had a significantly higher proportion or animals that escaped on Day 3 compared to vehicle-treated Stay animals (Fig. 6; 40% increase; $\chi^2$: $F_1 = 2.8$, $p \leq 0.029$). While there were no differences among treatment groups on Day 4, animals that received the middle-dose (40% increase; $\chi^2$: $F_1 = 4.3$, $p \leq 0.039$) and low-dose (50% increase; $\chi^2$: $F_1 = 4.3$, $p \leq 0.039$) of (*R*)-DOI had a higher proportion of animals that escaped compared to themselves on Day 2.

*3.4.2. Latency to Escape*

Comparison of latency to escape between vehicle-treated Stay and Escape animals revealed a phenotypic effect (Fig. 7A; Phenotype Effect: $F_{1,60} = 126.9$, $P < 0.001$; Day Effect: $F_{3,60} = 10.4$, $P < 0.001$; Interaction Effect: $F_{3,60} = 9.2$, $P < 0.001$; Stay vs Escape: Day 1, $t_{22} = 3.1$, $P \leq 0.006$; Day 2, $t_{22} = 8.8$, $P < 0.001$; Day 3, $t_{22} = 7.5$, $P < 0.001$; Day 4, $t_{22} = 6.5$, $P < 0.001$). Like other SAM experiments, Escape animals learned to utilize the escape routes faster over time which is thought to reflect learning ($F_{3,33} = 11.1$, $P < 0.001$; Escape Day 1 vs Day 2: $t_{22} = 3.7$, $P < 0.001$; Escape Day 1 vs Day 3: $t_{22} = 3.2$, $P \leq 0.004$; Escape Day 1 vs Day 4: $t_{22} = 5.4$, $P < 0.001$).

Previous results from our laboratory have shown that latency to escape in Stay animals can be reduced with the administration of drugs that reduce stress-related affective behaviors [150,153,179]. We compared vehicle control to all three doses of (*R*)-DOI (Fig. 7B; Treatment Effect: $F_{3, 117} = 1.1$, $P \geq 0.378$; Time Effect: $F_{3, 117} = 11$, $P < 0.001$; Interaction Effect: $F_{9,117} = 1.2$, $P \geq .297$) anticipating that some of the doses of (*R*)-DOI would reduce latency to escape on Day 3 and 4 compared to vehicle controls. However, there was no effect of treatment on latency to escape for day 3 ($F_{3,39} = 1.9$, $P \geq 0.152$) or day 4 ($F_{3,39} = 0.4$, $P \geq 0.399$). Based on *a priori* hypothesis we compared vehicle control Stay animals to all doses of (*R*)-DOI on days 3 and 4. Both the middle- ($t_{20} = 2.1$, $P \leq 0.05$) and low-dose ($t_{20} = 2.6$, $P \leq 0.019$) significantly reduced latency to escape compared to vehicle controls on day 3, but no treatment effect was seen on day 4.

Previous results from our laboratory show that Escape animals escape faster from the SAM with time, which is thought to reflect learning [150,153,179]. Our results from this experiment show that for escape animals, all treatments showed a learning response, with Days 2, 3, and 4 escape times being significantly lower than day 1, except for the middle-dose on day 3 ($t_6 = 1.4$, $P \geq 0.224$). We compared vehicle control to all three doses of (*R*)-DOI (Fig. 7C; Treatment Effect: $F_{3,102} = 0.6$, $P \geq 0.613$; Time Effect: $F_{3,102} = 37.5$, $P < 0.001$; Interaction Effect: $F_{9,102} = 0.8$, $P \geq 0.604$) anticipating that treatment with some of the doses would change latency to escape on days 3 and 4 compared to vehicle controls. However, there was no effect of treatment on day 3 ($F_{3,36} = 1.7$, $P \geq 0.175$) or day 4 ($F_{3,36} = 1.2$, $P \geq 0.328$). Based on *a priori* hypothesis we compared vehicle control to all doses of (*R*)-DOI on days 3 and 4. Only the lowest dose influenced latency to escape compared to vehicle controls on Day 4 ($t_{20} = 2.4$, $P \leq 0.024$).

*3.4.3. Conflict Freezing in the SAM*

Comparison of the amount of time spent freezing in the SAM between vehicle-treated Stay and Escape animals showed there was an effect of phenotype, similar to other SAM experiments (Fig. 8A; Phenotype Effect: $F_{1,65} = 32.6$, $P < 0.001$; Day Effect: $F_{3,65} = 1.2$, $P \geq 0.329$; Interaction Effect: $F_{3,65} = 0.9$, $P \leq 0.45$; Stay vs Escape: Day 1, $t_{22} = 2.9$, $P \leq 0.008$; Day 2, $t_{22} = 4.1$, $P < 0.001$; Day 3, $t_{21} = 4.2$, $P < 0.001$; Day 4, $t_{22} = 2.6$, $P \leq 0.017$).

Previous experiments from our laboratory have shown that conflict freezing increases over the 4 days of the SAM in Stay treated animals [150,153,179]. We compared vehicle control to all three doses of (*R*)-DOI (Fig. 8B; Treatment Effect: $F_{3,116} = 1.5$, $P \geq 0.226$; Time Effect: $F_{3,116} = 11.4$, $P < 0.001$; Interaction Effect: $F_{9,116} = 2.1$, $P \leq 0.031$) anticipating that some of the treatments may reduce freezing responses after treatment (Day 3 and 4). While there were no effects of treatment on Day 3 ($F_{3,38} = 0.7$, $P \geq 0.561$), conflict freezing was reduced on Day 4 with treatment of (*R*)-DOI ($F_{3,39} = 3.1$, $P \leq 0.037$). While treatment with the high- (0.3 mg/kg; $t_{21} = 0.4$, $P \geq 0.721$) and low- (0.015 mg/kg; $t_{20} = 1.6$, $P \geq 0.123$) dose of (*R*)-DOI did not reduce conflict freezing on day 4 compared to vehicle-treated Stay animals, the middle-dose significantly reduced freezing ($t_{20} = 2.6$, $P \leq 0.018$). The middle-dose of (*R*)-DOI also significantly lowered Day 4 conflict freezing compared to the high-dose ($t_{19} = 2.6$, $P \leq 0.018$).

We also compared whether conflict freezing in vehicle controls was different from any of the doses of (*R*)-DOI administered in Escape animals (Fig. 8C; Treatment Effect: $F_{3,108} = 0.8$, $P \geq 0.521$; Time Effect: $F_{3,108} = 1.4$, $P \geq 0.248$; Interaction Effect: $F_{9,108} = 1.9$, $P \geq 0.71$) expecting there to be an effect of treatment on day 3 or 4. However there was no effect of treatment on day 3 ($F_{3,36} = 2.2$, $P \geq 0.109$) or day 4 ($F_{3,36} = 2.4$, $P \geq 0.086$). Based on *a priori* hypotheses, we compared vehicle control escape animals to those treated with all doses of (*R*)-DOI on days 3 and 4. On day 3, animals that received the high-dose of (*R*)-DOI spent significantly less time

freezing in the SAM compared to vehicle controls ($t_{21} = 2.1$, $P \leq 0.046$), as well as animals given the middle-dose ($t_{16} = 2.2$, $P \leq 0.041$). Animals treated with the low-dose of (*R*)-DOI spent significantly less time freezing in the SAM on day 4 compared to vehicle controls ($t_{20} = 2.7$, $P \leq 0.015$) as well as the group that received the middle-dose of (*R*)-DOI ($t_{15} = 2.4$, $P \leq 0.03$).

*3.4.4. Attention to the Escape Route*

Comparison of attention to the escape route between Stay and Escape vehicle-treated animals resulted in significant phenotypic effects over time (Fig. 9A; Phenotype Effect: $F_{1,65} = 18.8$, $P < 0.001$; Day Effect: $F_{3,65} = 0.5$, $P \geq 0.691$; Interaction Effect: $F_{3,65} = 1.0$, $P \geq 0.395$; Stay vs Escape: Day 1, $t_{22} = 2.9$, $P \leq 0.008$; Day 2, $t_{22} = 4.4$, $P < 0.001$; Day 3, $t_{21} = 3.2$, $P \leq 0.005$; Day 4, $t_{22} = 2.6$, $P \leq 0.017$). As with previous experiments, Escape mice exhibited more attention to the escape hole [150,179].

Previous SAM experimentation from our laboratory have shown that time spent attentive to the escape route is a motivational behavior [150,179]. We compared stay vehicle control animals to those treated with all doses of (*R*)-DOI (Fig. 9B; Treatment Effect: $F_{3,115} = 6.1$, $P \leq 0.002$; Time Effect: $F_{3,115} = 1$, $P \geq .376$; Interaction Effect: $F_{9,115} = 1$, $P \geq 0.478$) anticipating that treatment at any dose may have changed attention to the escape hole.

There were effects of treatment on both day 3 ($F_{3,38} = 4.2$, $P \leq 0.011$) and day 4 ($F_{3,39} = 3$, $P \leq 0.043$). On Day 3, Stay animals given the low-dose of (*R*)-DOI spent significantly more time attentive to the escape holes compared to vehicle-treated animals ($t_{19} = 2.7$, $P \leq 0.016$), and those given the high-dose ($t_{19} = 2.7$, $P \leq 0.013$) of (*R*)-DOI. On Day 4, Stay animals treated with the low-dose of (*R*)-DOI also spent significantly more time investigating the escape routes compared to those given vehicle ($t_{20} = 3.7$, $P < 0.001$), the high-dose of (*R*)-DOI ($t_{19} = 2.6$, $P \leq 0.01$) and the middle-dose ($t_{18} = 2.5$, $P \leq 0.013$).

We made the same comparisons amongst escape vehicle-treated animals with those treated with all doses of (*R*)-DOI (Fig. 9C; Treatment Effect: $F_{3,108} = 1.1$, $P \geq 0.343$; Time Effect: $F_{3,108} = 1$, $P \geq 0.382$; Interaction Effect: $F_{9,108} = 1.3$, $P \geq 0.242$) anticipating treatment with (R)-DOI would reduce fear conditioning responses, however, there were no differences in time spent attentive to the escape hole among any treatments from vehicle control on day 3 or 4.

*3.4.5. Fear Conditioning*

Fear conditioning responses were assessed in mice on test day (day 5), as previous experiments have showed that both Stay and Escape mice show enhanced freezing to the CS+ [150,153,179]. While there were no differences between freezing behavior in Stay and Escape animals as previous experiments have shown [150,179], both Stay ($t_{11} = 3.5$, $P \leq 0.005$) and Escape ($t_{11} = 3.3$, $P \leq 0.007$) animals froze significantly more post-tone compared to pre-tone (Fig. 10A).

In Stay animals, we compared vehicle controls to all three doses of (*R*)-DOI anticipating that treatment may reduce the fear conditioning responses. While all animals froze more post-tone compared to pre-tone, there were no differences between any of the treatment groups (Fig. 10B; Treatment Effect: $F_{3,39} = 1.2$, $P \geq 0.321$; CS+ Effect: $F_{1,39} = 49.6$, $P < 0.001$; Interaction Effect: $F_{3,39} = 1.0$, $P \geq 0.41$; Vehicle Pre vs. Post Tone: $t_{11} = 4.4$, $P < 0.001$; (0.3 mg/kg) Pre vs. Post Tone: $t_{10} = 4.4$, $P < 0.001$; (0.03 mg/kg) Pre vs. Post Tone: $t_9 = 2.1$, $P \leq 0.046$; (0.015 mg/kg) Pre vs. Post Tone: $t_9 = 3.3$, $P \leq 0.002$). In Escape animals there was also an effect of the CS+ on freezing behavior (Treatment Effect: $F_{3,36} = 2.8$, $P \geq 0.838$; CS+ Effect: $F_{1,36} = 16.9$, $P < 0.001$; Interaction Effect: $F_{3,36} = 0.57$, $P \geq 0.639$). However, this effect was only seen for vehicle-treated Escape animals (Fig. 10C; $t_{11} = 3.3$, $P \leq 0.007$).

*3.5. Plasma Cytokine Concentration.*

*3.5.1. Tumor Necrosis Factor alpha (TNF-α)*

In other rodent model, social stress produces increased peripheral levels of TNF-α in mice with passive coping strategies compared to those that developed active coping strategies [180]. To test whether Stay and Escape animals in the SAM follow a similar trend, we analyzed the plasma concentrations of TNF-α in cage control (N=6) animals compared to vehicle-treated Stay (N=8) and Escape (N=5) animals (Fig. 11A; Kruskal Wallis One Way ANOVA; H = 5.039, 2 df, P = 0.081). Based on *a priori* hypotheses, we then directly compared cage controls to vehicle-treated Stay (Mann-Whitney t-test; U-statistic = 39.5; T = 29.5, P = 0.043) and Escape animals (Mann-Whitney t-test; U-statistic = 5.0; T = 40, P = 0.082). There was no significant difference in plasma TNF-α concentrations between vehicle-treated Stay and Escape animals (Mann-Whitney t-test; U-statistic = 19; T = 36, P = 0.943). These results suggest acts of social aggression promotes increased TNF-α in plasma, such that vehicle-treated Stay animals have significantly higher levels of plasma TNF-α, and vehicle-treated Escape animals have a moderate increase compared to cage control animals.

Next, we wanted to determine if treatment with (*R*)-DOI might reduce circulating levels of TNF-α in Stay and Escape animals promoted by social stress, 3 days after administration. Comparing cage control animals to each Stay treatment group showed there was an effect of treatment on circulating TNF-α levels (Kruskal Wallis One Way ANOVA; H=11.104, 4 df, P = 0.025). Direct comparisons of each Stay treatment group to cage controls showed that vehicle-treated (Fig. 11B; Mann-Whitney t-test; U-statistic=39.5; T=29.5, P = 0.043), high-dose-treated (N=8; Mann-Whitney t-test; U-statistic=44.5; T=24.5, P = 0.005 and middle-dose-treated (N=6; Mann-Whitney t-test; U-statistic=34; T=23, P = 0.009) Stay animals had significantly higher concentrations of plasma TNF-α compared to unstressed cage controls. However, there was no

significant difference between low-dose-treated (N=4) Stay animals and cage controls (Mann-Whitney t-test; U-statistic = 4; T = 30, P = 0.114), or any other groups.

Comparison of every Escape treatment group to cage controls also revealed a trend towards a significant effect of treatment on plasma TNF-α levels (Fig. 11C; Kruskal Wallis One Way ANOVA; H=8.272, 4 df, P = 0.082). Direct comparison of cage controls to each Escape treatment group show that vehicle-treated animals (N = 5; Mann-Whitney t-test; U-statistic=5.0; T=40, P = 0.082) had a trend towards an increase in TNF-α compared to unstress cage controls, while middle-dose-treated Escape animals had a significant increase compared to cage controls (N = 4; Mann-Whitney t-test; U-statistic=2.0; T=32, P = 0.038). However, cage controls were not different compared to high-dose-treated (N = 3; Mann-Whitney t-test; U-statistic=4.5; T=19.5, P = 0.262) or low-dose-treated (N = 6; Mann-Whitney t-test; U-statistic=13.0; T=32, P = 0.792) Escape animals. There was also a trend towards a significant difference between middle-dose-treated Escape animals and low-dose-treated Escape animals (Mann-Whitney t-test; U-statistic=2.0; T=28, P = 0.063), but not between any other treatment groups.

## 4. Discussion

Pharmacological treatments for depression have been limited, until recently, to serotonin and other monoamine reuptake inhibitors [181], which produce limited efficacy and satisfaction among patients [182]. The range of treatment has recently been broadened by current research on ketamine [13,15,183,184], orexin drugs [150,179] and psychedelics [9,12,16,21,22,24,70,84,93,183,185]. Serotonergic psychedelic compounds have been shown to produce rapid and long-lasting anti-depressive effects in clinical trials [12,18,21,23,116], and in rodent models [20,91,137,183,186]. Here, we show that subcutaneous administration (sc) of (*R*)-DOI, a psychedelic with potent anti-inflammatory properties [115], rapidly reduces passive coping strategies to a repeated social stress and promotes active coping strategies. Specifically, the lowest dose of (*R*)-DOI (0.015 mg/kg) administered reduced conflict-related

freezing during social interaction, increased motivation to escape (increased attention to the escape route) and reduced latency to escape, in Stay mice. Additionally, we show that in the SAM paradigm, vehicle-treated Stay and Escape animals have higher circulating plasma levels of TNF-α compared to unstressed cage control animals.

Susceptibility to developing depression has been shown to be related to behavioral coping strategies in response to stress, with passive coping strategies being more associated with depressive disorders compared to those with active coping strategies [187-189]. There have also been similar findings in rodents [55], with rats displaying passive coping strategies in response to social stress showing behavioral and endocrine changes similar to those seen in humans with depression, while rats with active coping strategies do not [55]. Stay and Escape phenotypes in the SAM have shown related behavioral and endocrine responses as well [153]. Social stress-induced behaviors in the SAM apparatus are easily identifiable as either active (Escape, attention to the escape route) or passive (Stay, conflict freezing) coping strategies [152,153]. Previous research that included anxiolytic/antidepressive ($CRF_1$ antagonist antalarmin; $Orx_2$ agonists [$Ala^{11}$, D-$Leu^{15}$]-Orexin B or YNT 185; $Orx_1$ antagonist SB-674042) or anxiogenic/pro-depressive (NE $\alpha_2$ antagonist Yohimbine; $Orx_2$ receptor antagonist, MK-1064) drugs, suggest that reversal of active coping behaviors indicates increased anxious and/or depressive behaviors, and reversal of passive to become active coping strategies indicates relief from stress-related anxious and depressive behavioral responsiveness [150,153,179]. Thus, the effects of sc (*R*)-DOI treatment after day 2 of the SAM social interaction trials indicates rapid effects that promote active coping strategies over passive coping strategies, suggesting this compound has anxiolytic or antidepressive properties.

While inflammatory cytokines play a role in the pathophysiology of affective mood disorders [40], it is unclear whether the anti-inflammatory properties of psychedelics [111] contribute to their potential therapeutic behavioral effects. This study aimed to understand whether male mice that adopt passive coping strategies in response to social aggression (Stay) show differences in circulating proinflammatory cytokines compared to those that adopt active coping strategies (Escape). Additionally, we wanted to

know whether the potent anti-inflammatory properties of (*R*)-DOI were correlated with specific coping behaviors.

One important distinction of this study, is that it captures the behavioral changes that result from administration of (*R*)-DOI that occur the acute behavioral effects (HTR) have subsided [71]. For example, in studies that assessed how DOI administration influences impulsive behaviors, behavioral measures were taken 10-30 minutes after drug administration [190-192], which is when peak HTR responses occur, as well as blood and brain concentration of the drug is highest [71]. Studies demonstrating the rapid and sustained antidepressive behavioral effects of other psychedelics in humans [12,16] and rodents [91,186] assess behavioral changes after the acute behavioral effects (hallucinatory response in humans; HTR response in rodents) of the drugs have worn off, which to our knowledge, has not been assessed with (*R*)-DOI.

The results here support the concept that administration of serotonergic psychedelic compounds produce rapid anxiolytic and antidepressive behavioral responses not only in humans [22], but in experimental animal models of mood disorders [137,186]. Clinical studies using psilocybin require trained therapists to be present with patients when the drug is given, as the doses used are large enough to produce psychedelic subjective effects. However, these results suggest lower doses of (*R*)-DOI have greater efficacy to treat anxious or depressive disorders compared to higher doses, as only dose of (*R*)-DOI administered in this study that is high enough to produce HTR [98], and to be behaviorally detected by rodents [193] was the high-dose (0.3 mg/kg), which was the least efficacious. Additionally, there has been an effort to synthesize novel compounds that produce the rapid and long lasting antidepressive effects of psychedelics, but do not produce psychedelic subjective effects [194]. Based on the results here, the doses of (*R*)-DOI required to elicit antidepressive behavioral effects, may be much lower than that which is required to produce these effects. While more preclinical and clinical studies are required, these results confirm others findings [111], that (*R*)-DOI may have rapid therapeutic properties at doses that are lower than that which are required to elicit subjective psychedelic effects. Additionally, these results suggest that the potent anti-inflammatory properties of (*R*)-DOI may be related to its antidepressive

behavioral effects, although further studies are required to determine whether (*R*)-DOI administration reduces inflammatory markers in specific stress related brain regions like the amygdala or medial prefrontal cortex [68].

## 5. Conclusion

Results reported here indicate that a single administration of low doses of (*R*)-DOI, which are lower than that which is required to elicit acute behavioral responses (HTR), rapidly promotes active coping strategies in response to an escapable social stress. This supports the findings of others that administration serotonergic psychedelics have rapid antidepressive properties, and that (*R*)-DOI has therapeutic properties at doses lower than that which are behaviorally detected in rodents or produce the HTR. These findings also show that exposure to social stress in the SAM for 4 days increases circulating plasma levels of TNF-α, consistent with other rodent models of social stress. In this study, Stay (susceptible) animals had significantly higher concentrations of plasma TNF-α compared to unstressed cage control animals, while Escape (resilient) animals only showed a trend for having higher plasma concentrations compared to unstressed cage controls. Additionally, the rapid antidepressive behavioral effects of (R)-DOI may be related to its potent anti-inflammatory properties, as administration of the lowest dose of (*R*)-DOI (0.015 mg/kg) prevented increased circulating levels of TNF-α promoted by social stress.

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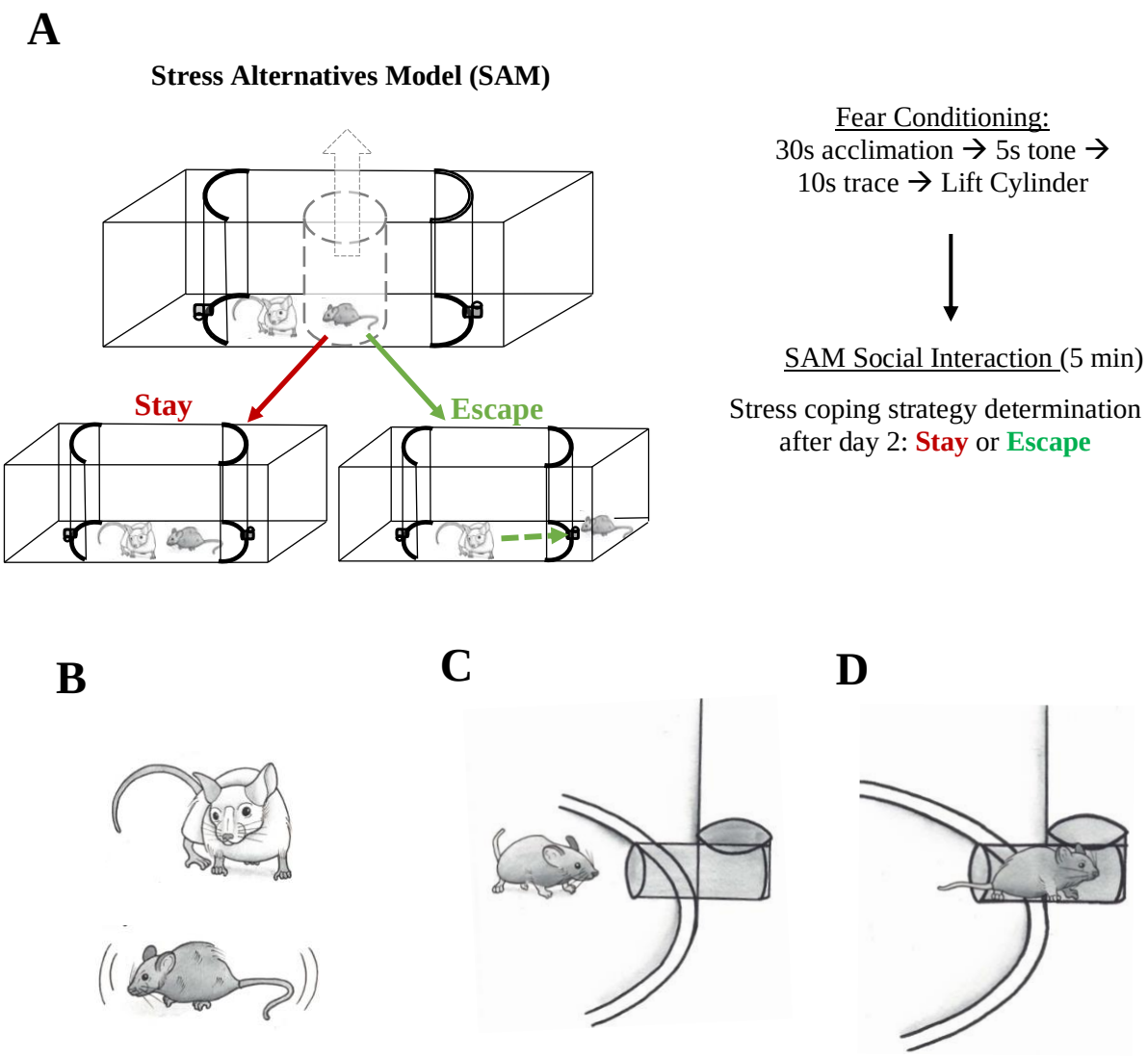


**Figure 1. A)** Representation of the Stress Alternatives Model (SAM) apparatus and protocol. C57BL/6N mice are subjected to a fear conditioning paradigm in an opaque cylinder placed in the open field of the SAM before facing social aggression from the CD1 on all 4 SAM days. Small escape holes present on both sides of the SAM can be used by the C57BL/6N to escape aggression by the larger CD1 mouse. By the end of day 2, C57BL/6 mice acquire one of two stress coping strategies, active **(Escape)** or passive **(Stay)** that persist for days 3 & 4. **B)** Illustration representing conflict freezing behavior in the SAM, which occurs predominately in Stay animals. **C)** Representation of attention to the escape hole in the SAM, and **D)** actual escape behavior, which are observed in Escape animals.

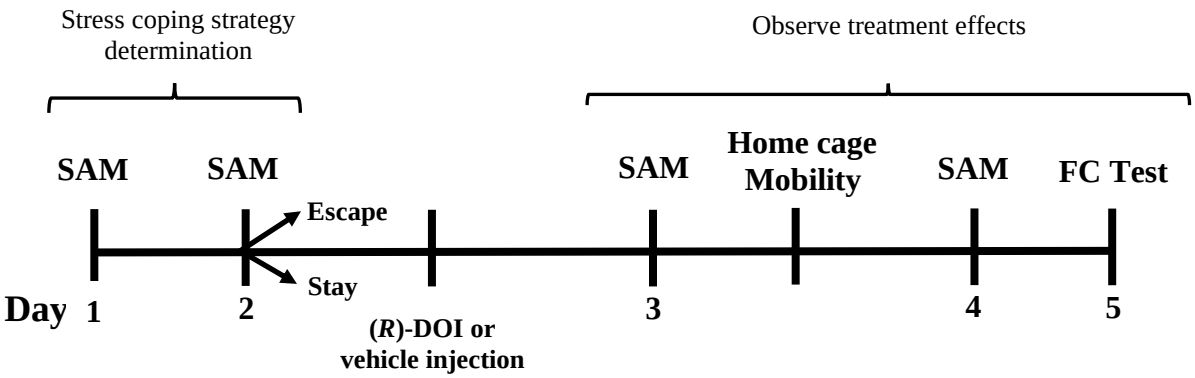


**Figure 2.** Experimental design for project. C57BL/6N mice receive social aggression in the SAM on days 1 and 2 to determine whether individual C57BL/6N mice use passive (Stay) or active (Escape) coping strategies. Immediately following the SAM on day 2, C57BL/6N mice receive a single subcutaneous (sc) administration of (R)-DOI (0.3, 0.03, or 0.015 mg/kg) or vehicle (saline) and are then immediately placed back into their home cages. Days 3 and 4 of the SAM to determine how treatment impact stress coping strategies and related behaviors. Following the SAM on day 3, animals are placed back into their home cage to assess home-cage mobility. On day 5, C57BL/6N mice are tested for fear conditioning (FC) responses (freezing) in the SAM without the unconditioned stimulus. Afterwards, animals are briefly anesthetized and rapidly decapitated to collect blood and brain samples.

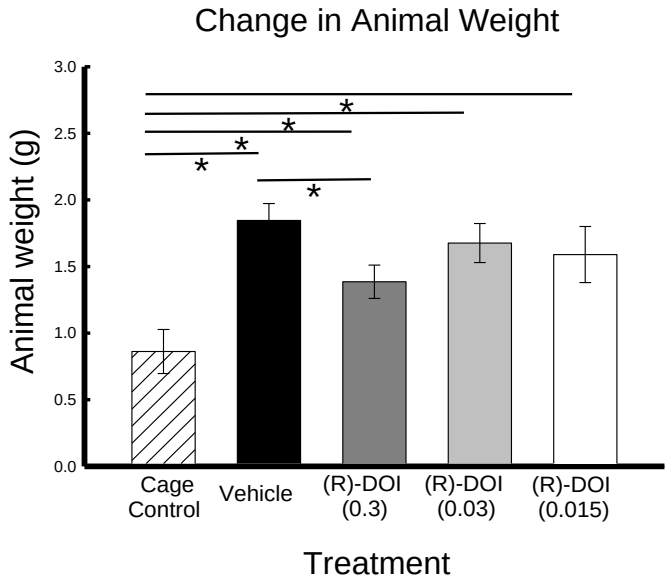


**Figure 3.** Animal weights were monitored throughout the experiments. All treatment groups [vehicle-treated, N=24; high-dose-treated (0.3 mg/kg), N=22; middle-dose-treated (0.03 mg/kg), N=17; low dose-treated (0.015 mg/kg), N=20] were significantly higher compared to cage controls animals (N=13). The only treatment group that was different compared to vehicle-treated animals were animals treated with the high-dose of (*R*)-DOI (0.3 mg/kg). Bars represent ± SEM. * = $p < 0.05$.

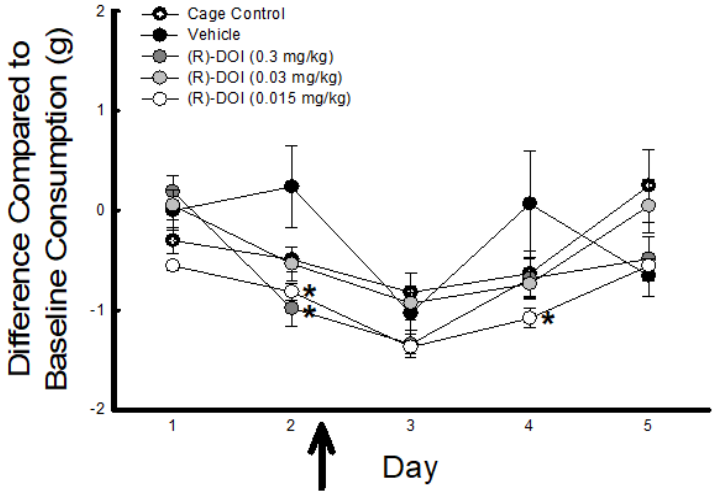


**Figure 4.** Food weights were monitored throughout the experiments to assess whether treatment with any of the doses of (*R*)-DOI changed food consumption, however, no statistically significant differences were observed. Bars represent ± SEM. * = $p < 0.05$ compared to vehicle-treated animals.

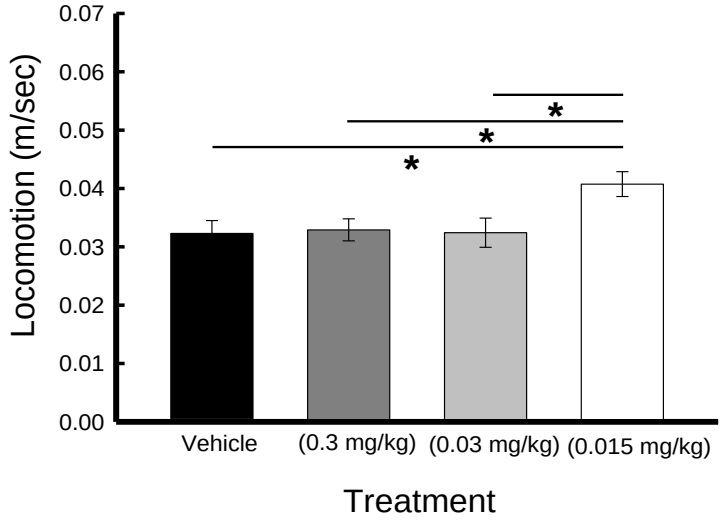


**Figure 5.** Home cage mobility was assessed immediately following treatment with vehicle or (*R*)-DOI. Animals that received the low-dose of (*R*)-DOI (N=20) had significantly higher levels of home cage mobility compared to vehicle-treated (N=24), as well as high-dose-treated (N=22) or middle-dose-treated (N=17) animals. Bars represent ± SEM. * = $p < 0.05$

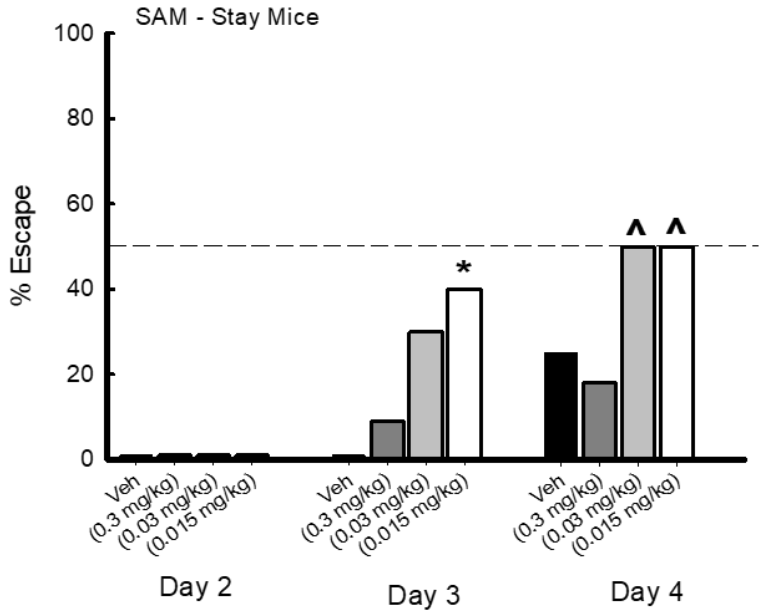


**Figure 6.** Escape behavior in the SAM for Stay animals. On Day 3, animals that were treated with the low-dose of (*R*)-DOI (N=10) had a significantly higher proportion of animals that escaped compared to vehicle-treated Stay mice (N=12). While there were not statistically significant differences between treatment groups on Day 4, Stay mice treated with the middle- (N=10) and low-doses of (*R*)-DOI had a significantly higher percent of animals that escaped compared to themselves on Day 2, however this was not the case for animals treated with vehicle or the high-dose of (*R*)-DOI (N=11). Bars represent ± SEM. * = $p < 0.05$ compared to vehicle, ^ = $p < 0.05$ compared to day 2.

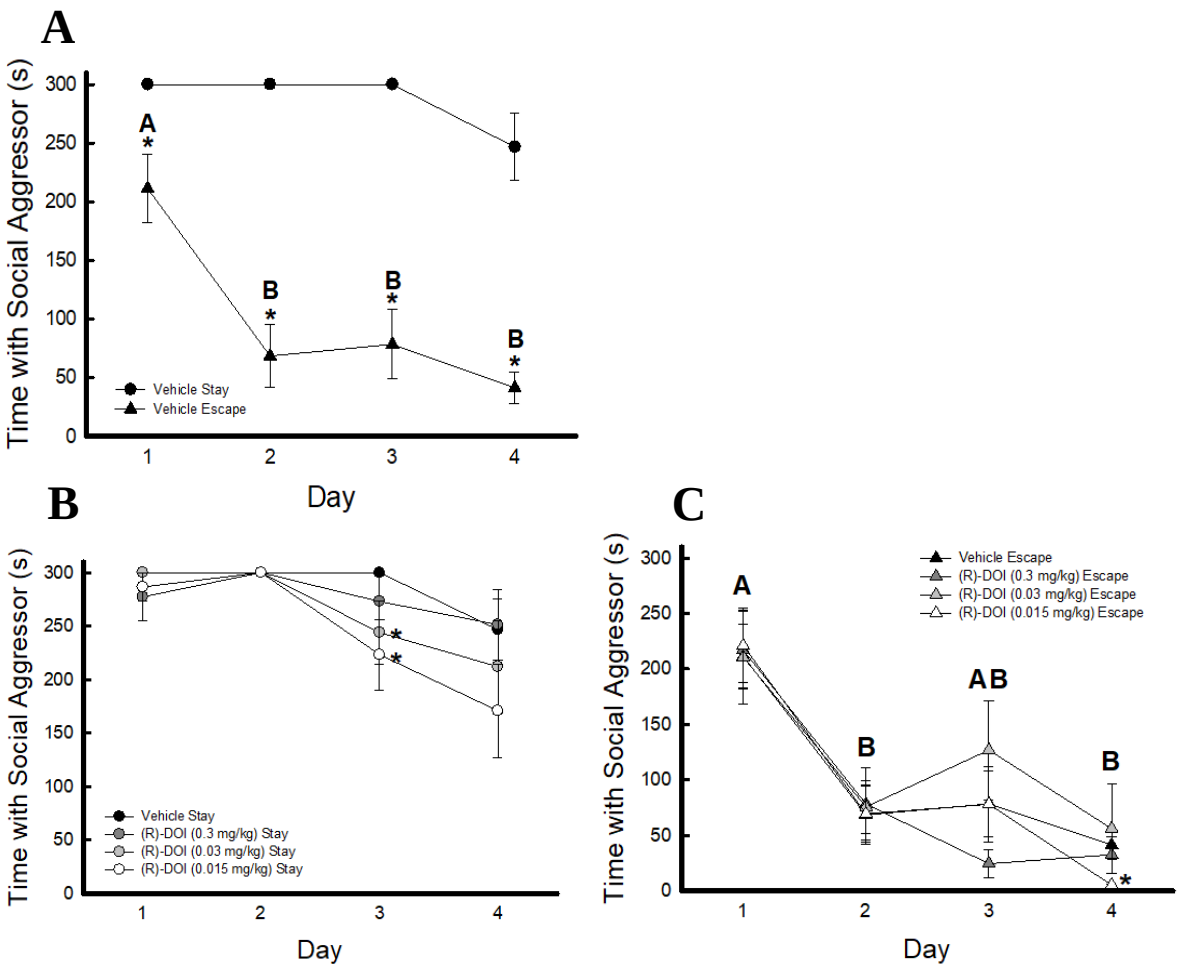


**Figure 7.** Administration of (*R*)-DOI influences latency to escape. A) Like previous SAM experiments, we saw that vehicle-treated Escape animals (N=12) learned to escape faster with time, and that they spent significantly less time in the SAM on all days compared to vehicle-treated Stay animals (N=12). B) Administration of the middle-dose (N=10) and low-dose (N=10) of (*R*)-DOI decreased latency to escape in Stay animals compared to those treated with vehicle on day 3 of the SAM, but not Stay animals given the high-dose (N=11). C) Administration of the low-dose (N=10) of (*R*)-DOI to Escape animals decreased latency to escape on Day 4 compared to vehicle-treated Escape animals, but not those given the high-dose (N=11) or middle-dose (N=7). Additionally, only the Escape animals treated with the middle-dose of (*R*)-DOI did not escape significantly faster on day 3 compared to themsleves on day 1, suggesting this dose may be anxiogenic. Bars represent ± SEM. For A) * = $p < 0.05$ compared to vehicle-treated Stay animals, A = $p < 0.05$ compared to B within Escape animals. For B) * = $p < 0.05$ compared to vehicle-treated Stay animals. For C) * = $p < 0.05$ compared to vehicle-treated Escape animals, A = $p < 0.05$ compared to B within middle-dose-treated Escape animals, AB = not different from A or B.

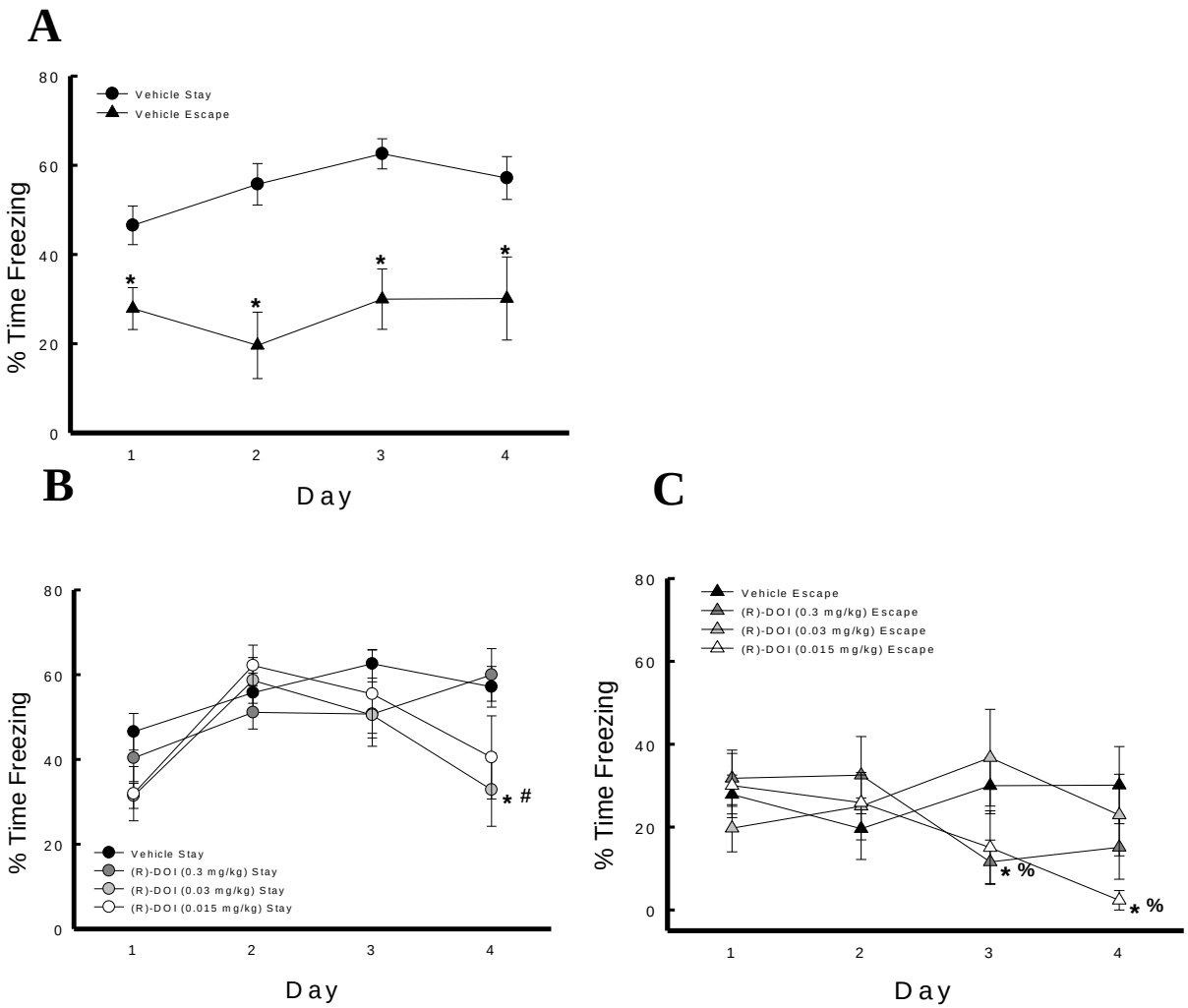


**Figure 8.** Administration of (*R*)-DOI reduces conflict freezing in the SAM. A) Vehicle-treated Stay animals (N=12) spend more time freezing in the SAM on all days compared to vehicle-treated Escape animals (N=12). B) Stay animals administered the middle-dose of (*R*)-DOI (N=10) had significantly reduced conflict freezing in the SAM compared to vehicle and high-dose-treated (N=11) Stay animals, but not those given the low-dose (N=10). C) In Escape animals, treatment with the high-dose of (*R*)-DOI (N=11) significantly reduced conflict freezing compared to vehicle-treated and middle-dose-treated (N=7) animals, but not those given the low-dose (N=10). On day 4, Escape animals treated with the low-dose of (*R*)-DOI spent significantly less time freezing compared to vehicle and middle-dose-treated animals. Bars represent ± SEM. For A) *= $p < 0.05$ compared to vehicle-treated Stay animals. For B) * = $p < 0.05$ compared vehicle-treated Stay animals, # = $p < 0.05$ compared to high-dose (*R*)-DOI treated Stay animals. For C) * $p < 0.05$ compared to vehicle-treated Escape animals, % = $p < 0.05$ compared to middle-dose (*R*)-DOI treated animals.

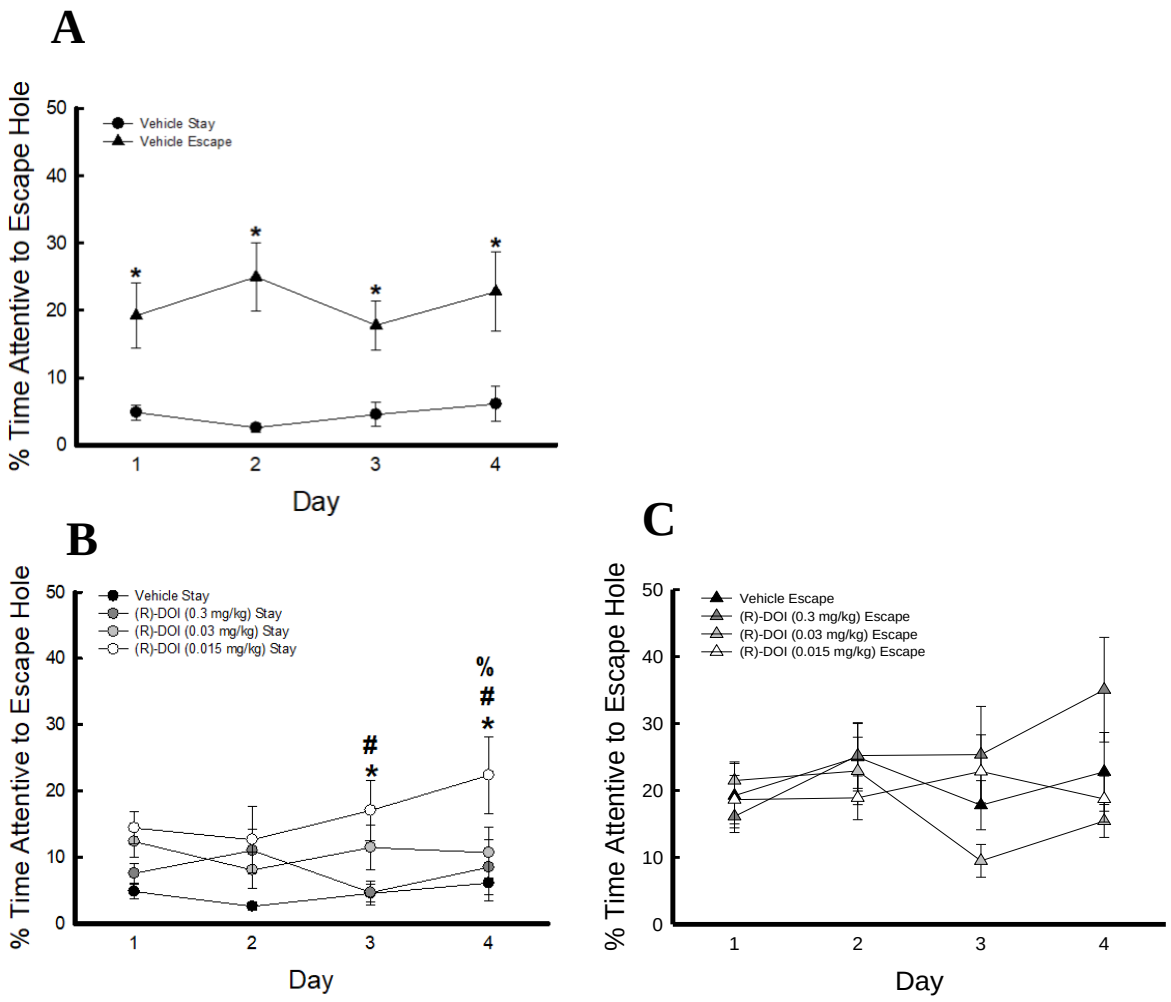


**Figure 9.** Administration of (*R*)-DOI changes motivation to escape in Stay animals. A) Vehicle-treated Escape animals (N=12) spent significantly higher percentage of time investigating the escape holes in the SAM on all days compared to vehicle Stay animals (N=12). B) Stay animals given the low-dose of (*R*)-DOI (N=10) spent significantly more time investigating the escape hole on day 3 of the SAM compared to Stay animals treated with vehicle and the high-dose of (*R*)-DOI (N=11), but not those given the middle-dose (N=10). However, on day 4, low-dose-treated animals spent significantly more time attentive to the escape hole compared to all Stay treatment groups. C) No Escape treatment group had any significant differences in time spent attentive to the escape routes on any day of the SAM compared to one another. Bars represent ± SEM. For A) * = $p < 0.05$ compared to vehicle-treated Stay animals. For B) * = $p < 0.05$ compared to vehicle-treated Stay animals, # = $p < 0.05$ compared to high-dose-treated Stay animals.

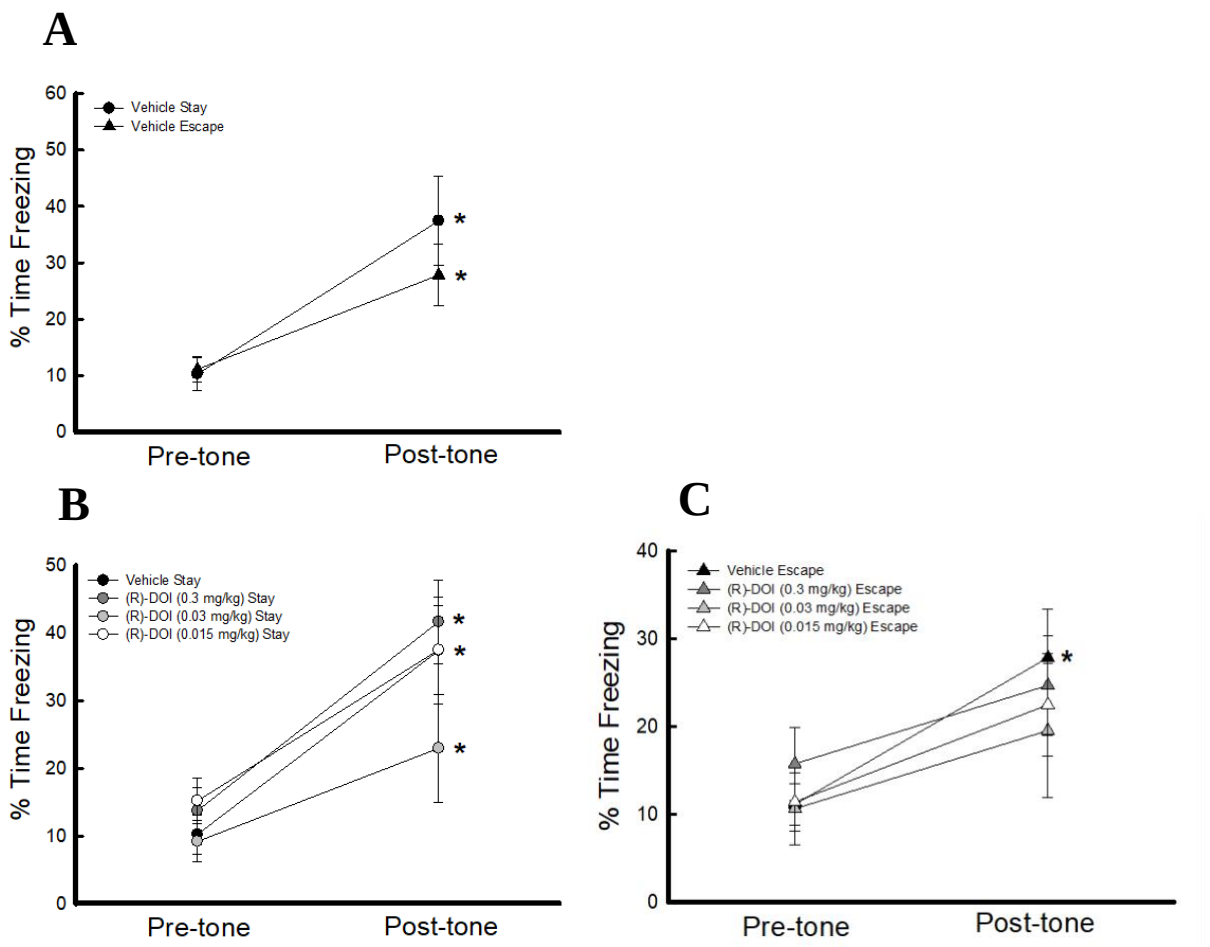


**Figure 10.** Effect of (*R*)-DOI on fear conditioned freezing behavior on day 5 (test day). A) Vehicle-treated Stay (N=12) and Escape (N=12) animals both spent significantly more time freezing after the tone (CS+) compared to before the tone, but there was no difference between phenotypes. B) All Stay animals, regardless of treatment spent significantly more time freezing pre-tone compared to post-tone, however there were no differences in freezing between treatment groups pre-tone and post-tone. C) In Escape animals, only the group that received vehicle froze significantly more post-tone compared to pre-tone, but there was no difference among treatment groups. Bars represent ± SEM. * = $p < 0.05$ comparing pre-tone to post-tone within treatment groups.

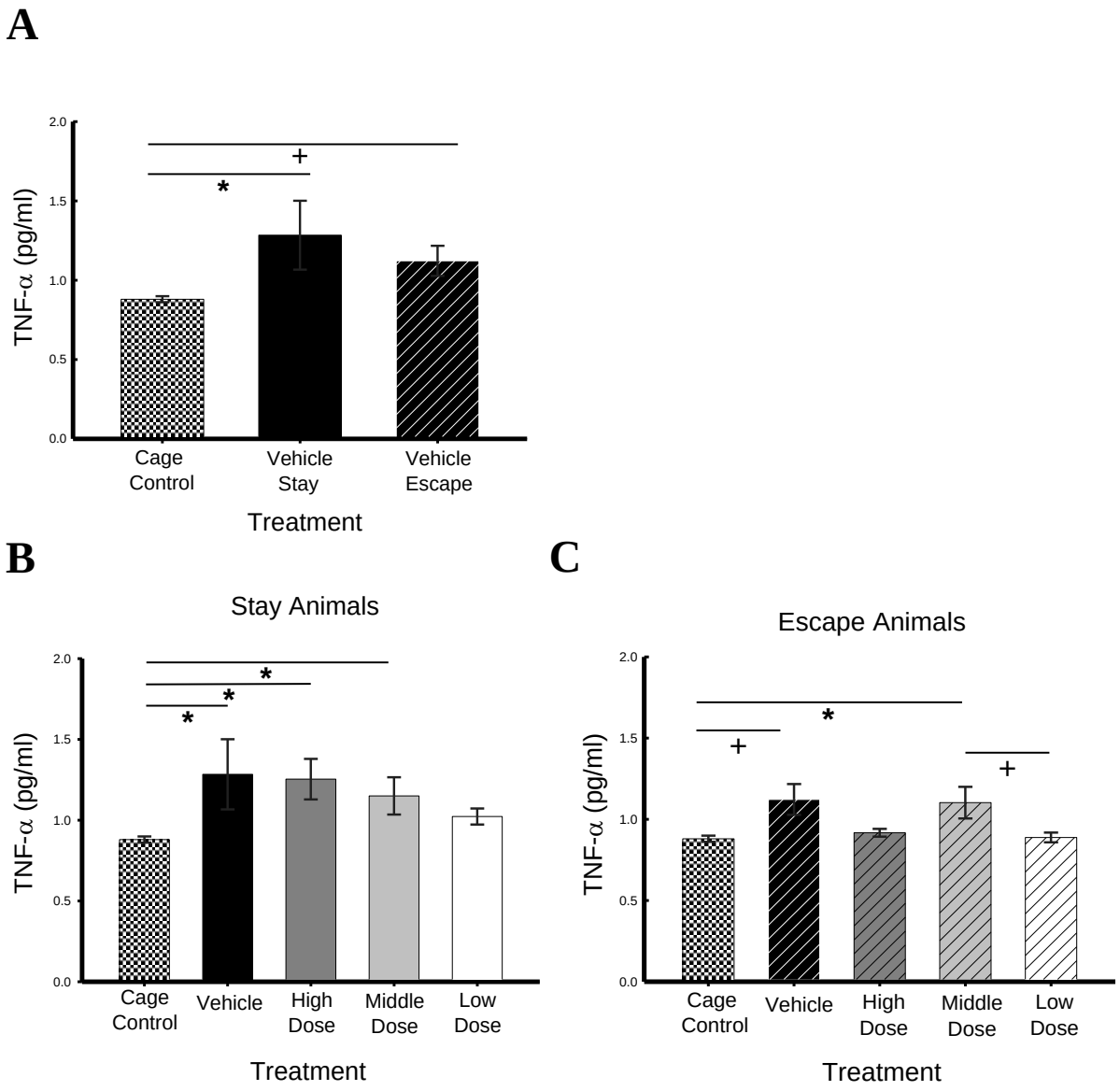


**Figure 11.** Plasma concentrations of TNF-α (pg/ml) of animals after day 5 testing. A) Repeated social stress in the SAM leads to significant increase in plasma TNF-α (pg/ml) in vehicle-treated Stay animals (N=8), and a trend towards an increase in vehicle-treated Escape animals (N=5), compared to unstress cage controls (N=6). B) Stay animals treated with the low-dose of (*R*)-DOI (N=4) do not have increased plasma concentrations of TNF-α compared to unstressed cage control animals, while vehicle-treated, high-dose-treated (N=8), and middle-dose-treated (N=6) do. C) In Escape animals, the only treatment group that had significantly higher plasma concentration of TNF-α compared to unstressed cage controls were those given the middle-dose of (*R*)-DOI (N=4), who also had a trend toward increased TNF-α concentration compared to low-dose-treated (N=5). High-dose-treated Escape animals (N=3) were not different from any Escape treatment group. Bars represent ± SEM. * = $p < 0.05$, + = $p < 0.08$.