



Delta Opioid Receptor Agonist Potential Use in Mitigating Opioid Withdrawal Symptoms

Citation

Montelli, Tressa. 2026. Delta Opioid Receptor Agonist Potential Use in Mitigating Opioid Withdrawal Symptoms. Masters Thesis, Harvard University Division of Continuing Education.

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Delta Opioid Receptor Agonist Potential Use in Mitigating Opioid Withdrawal Symptoms

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A Thesis in the Field of Biology
for the Degree of Master of Liberal Arts in Extension Studies

Harvard University

February 2026

Abstract

The opioid epidemic continues to pose one of the most urgent public health crises worldwide, with relapse frequently fueled by affective symptoms of withdrawal, anxiety, and depression. Current treatments such as methadone and buprenorphine, target mu-opioid receptors (MORs), address cravings and somatic symptoms but provide limited relief for affective symptoms. Delta-opioid receptors (DORs), may offer therapeutic advantages, showing anxiolytic and antidepressant-like effects in preclinical models without the abuse liability of MOR agonists or the dysphoria linked to kappa-opioid receptor (KOR) agonists. This meta-analysis synthesized evidence from 42 controlled preclinical comparisons. Random-effects models indicated that DOR agonists produced large reductions in anxiety-like behaviors (Hedges' $g = 1.40$, 95% CI [0.98, 1.82]) and depression-like behaviors ($g = 1.72$, 95% CI [0.85, 2.59]). In withdrawal models, DOR agonists significantly reduced withdrawal-induced anxiety ($g = 1.85$, 95% CI [1.08, 2.61]) and moderately improved withdrawal-induced depression ($g = 0.76$, 95% CI [0.14, 1.37]). Comparative analyses indicated that DOR agonists have substantial advantages over MOR agonists ($g = 2.68$, 95% CI [1.45, 3.90]) and exhibit favorable profiles relative to KOR agonists, particularly in terms of analgesia and adverse effect outcomes. These findings highlight DOR agonists as promising candidates for addressing both the emotional and physiological dimensions of opioid withdrawal. Although heterogeneity and limited chronic models remain challenges, this analysis establishes a strong foundation for translational research into DOR-targeted therapies for opioid use disorder.

Dedication

For my brother, Christopher Montelli.

Your life, your struggle, and your memory are at the heart of this work.

This thesis is dedicated to you and to everyone who didn't get the chance to recover. May this research be a small step towards the healing you and so many others deserved.

Acknowledgments

I would like to express my gratitude to Professor James R. Morris, who served not only as my research advisor but also as my research director. His guidance, expertise, and support were instrumental throughout every stage of this thesis, and his mentorship ensured that I remained focused on the central aims of my work.

I am also grateful to Sandra Yungblut for her assistance with screening studies. Her careful work and attention to detail made an important contribution to this project.

Finally, I would like to thank my girlfriend, Emanuela Beale, for her unwavering support and encouragement over the past three years. Her help with data extraction was immeasurable, but even more important was her constant understanding and belief in me, which gave me the strength and motivation to complete this work.

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Chapter I.

Introduction

The ongoing opioid epidemic continues to devastate communities worldwide, highlighting the pressing need for improved understanding and management of opioid use disorder (OUD). As the crisis evolves, addressing its biological underpinnings and exploring new innovative therapeutic approaches remains crucial to mitigating its widespread impact. While opioids have been used for pain relief since as early as 3,400 BCE, their high potential for addiction and dependence has fueled an epidemic with devastating consequences (VYDRA, 2020). In fact, in its earliest recorded use, it was referred to as the “Joy plant” due to its pleasurable effects (VYDRA, 2020). Opioids act by binding to specific receptors in the brain, mu-opioid receptors (MORs), delta-opioid receptors (DORs), and kappa-opioid receptors (KORs), which are distributed across key regions of the central nervous system (Pasternak, 2011). This interaction suppresses pain signals, dampens emotional distress, and triggers the release of dopamine, a neurotransmitter associated with reward and pleasure (Pellissier et al., 2018). While this mechanism underpins the effectiveness of opioids in treating pain, it also drives their addictive potential by hijacking the brain’s natural reward system, leading to compulsive drug-seeking behavior.

OUD is characterized by physical dependence, tolerance, and withdrawal symptoms, often accompanied by significant psychological distress (American Psychiatric Association, 2013). The impact of this disorder is staggering, with opioid-related deaths accounting for 76% of overdose fatalities in the United States between

2011 and 2021 (Centers for Disease Control and Prevention, 2023). These numbers reflect not only the scale of the crisis but also its accelerating nature, as opioid-related deaths have increased by 259% over this decade alone (Spencer et al., 2024). Despite increased awareness and harm reduction strategies, such as the distribution of naloxone to reverse overdoses and public health campaigns to combat stigma, the number of opioid-related deaths continues to rise. This suggests that current efforts, while lifesaving in critical moments, are ultimately insufficient in addressing the underlying mechanisms of addiction and relapse. Current medication-assisted treatment (MAT) options, including buprenorphine and methadone, have been developed to manage withdrawal and cravings by targeting MORs, helping individuals stabilize their condition (Dripps & Jutkiewicz, 2018). However, these treatments often fall short in preventing relapse, particularly because they primarily address the physical dimensions of addiction rather than the emotional and psychological challenges that are central to long-term recovery. Relapse rates remain alarmingly high, estimated at 91% following detoxification (National Center for Drug Abuse Statistics, 2019). This highlights the limitations of current treatment paradigms, which inadequately address the complex interplay of physical dependence, psychological distress, and the dysregulated reward systems caused by chronic opioid use.

Neurobiology of the Reward System and Opioid Effects

To understand the behavioral and neurochemical drivers of OUD, it is essential to examine the brain's reward pathway, which is a complex network of neurons and regions dedicated to processing pleasure, motivation, and reinforcement. Key components of this system include the ventral tegmental area (VTA), nucleus accumbens (NAc), prefrontal cortex (PFC), amygdala, and hippocampus (Roese et al., 2017). The NAc, located in the

ventral striatum, is associated with reward, motivation, pleasure, and learning, and it also influences motor functions related to pursuing pleasurable experiences (Roese et al., 2017). The PFC is critical in the processes of decision-making, planning, and evaluating preferences or aversions, while the amygdala regulates emotions, and the hippocampus manages memory formation and retrieval (Roese et al., 2017). The primary neurotransmitters involved in this pathway are dopamine, often called the “feel good” neurotransmitter, gamma-aminobutyric acid (GABA), an inhibitory neurotransmitter, and glutamate, an excitatory neurotransmitter (Roese et al., 2017). The system operates as follows: the VTA receives input from various stimuli, generating and releasing dopamine to the NAc, PFC, amygdala, and hippocampus (Roese et al., 2017). In natural pleasurable conditions, the PFC, amygdala, and hippocampus release glutamate, which signals back to the NAc (Klawonn & Malenka, 2019). The NAc processes these glutamate signals and sends GABA to the VTA, instructing it to stop dopamine production (Klawonn & Malenka, 2019). This feedback loop between the NAc and VTA is a crucial mechanism that regulates the intensity of pleasure derived from stimuli (Klawonn & Malenka, 2019). Overall, this reward system plays a pivotal role in driving behaviors that are essential for survival. However, it is also vulnerable to manipulation and dysregulation, which can potentially lead to maladaptive outcomes.

The critical alteration in the reward system arises from the interaction between the NAc and the VTA (Arias-Carrión et al., 2010). These regions are connected via synapses, with the postsynaptic neurons in the NAc containing numerous dopamine receptors (Roese et al., 2017). When the presynaptic neurons in the VTA release dopamine into the synaptic cleft, these dopamine receptors on the NAc neurons absorb the dopamine

(Klawonn & Malenka, 2019). The presynaptic neurons in the VTA are also equipped with dopamine transporters that facilitate dopamine reuptake, adding another layer of regulation to this complex process (Klawonn & Malenka, 2019). Once dopamine is cleared from the synaptic cleft, GABAergic interneurons in the NAc become more active, releasing GABA toward the VTA (Klawonn & Malenka, 2019). This GABA release inhibits further dopamine production, preventing overstimulation of the reward system and ensuring that only significant rewards trigger a strong response (Roese et al., 2017). Factors such as sleep deprivation, exercise, stress, chronic pain, sugar intake, overeating, and addictive substance use can influence this delicate balance (Klawonn & Malenka, 2019).

However, opioids disrupt this finely tuned system. Shortly after opioid use, the drugs bind to their receptors, primarily MORs, which are highly concentrated in the VTA (Turton & Lungford-Hughs, 2016). Activation of these receptors inhibits the release of GABA, which normally suppresses the activity of dopaminergic neurons (Turton & Lungford-Hughs, 2016). This inhibition leads to a “disinhibition” effect, causing a surge in dopamine neuron activity (Turton & Lungford-Hughs, 2016). As a result, dopamine levels increase significantly in the VTA, leading to elevated dopamine release to the NAc, hippocampus, amygdala, and prefrontal cortex (Klawonn & Malenka, 2019). This unregulated surge of dopamine in the NAc and related areas produces intense euphoria that reinforces opioid use and strengthens associative memories tied to the drug (Turton & Lungford-Hughs, 2016).

Mechanisms of Tolerance, Dependence, and Withdrawal

Another challenge in the addictive nature of opioids is the development of tolerance. Tolerance refers to a diminished effect with repeated use of a substance, requiring escalating higher doses to achieve the same level of euphoria (McQuay, 1999). A crucial aspect of understanding why opioids are so dangerous lies in the uneven rates at which tolerance develops. Tolerance to the euphoric effects of opioids builds quickly, while tolerance to their effects on respiration develops much more slowly (Turton, Lungford-Hughs, 2016). This disparity creates a dangerous situation: users require increasingly larger doses to experience the desired euphoria, but these higher doses amplify the suppression of respiratory function (Turton, Lungford-Hughs, 2016). This imbalance significantly increases the risk of overdose and death. Tolerance to opioids primarily occurs through receptor desensitization (Allouche, Noble, & Marie, 2014). When opioids repeatedly bind to MORs, these receptors become overstimulated (Allouche et al., 2014). In response, the body attempts to protect itself by reducing the sensitivity of its receptors (Allouche et al., 2014). This process involves phosphorylation, a chemical modification where phosphate groups are added to the receptors (Allouche et al., 2014). Phosphorylated receptors are then removed from the cell surface or downregulated, rendering them unresponsive to opioids (Allouche et al., 2014). Once removed, the receptors may either be recycled and returned to the surface after dephosphorylation or degraded entirely, decreasing the number of functional receptors available (Allouche et al., 2014). With fewer active receptors, the opioids' effects are diminished because they have fewer targets to interact with (Allouche et al., 2014). Tolerance to the euphoric effects of opioids develops more rapidly due to a parallel

desensitization process that affects dopamine receptors in the NAc, leading to a twofold increase in downregulated receptors involved in achieving opioid-induced euphoria (Browne et al., 2023). This creates two distinct mechanisms of tolerance that specifically impact the euphoric sensations associated with opioid use.

In addition to tolerance, repeated opioid use leads to dependence, a distinct but closely related phenomenon. Dependence can be conceptualized in two ways: psychological and physical. Psychological dependence refers to the state in which a person requires the drug to function or feel normal (World Health Organization, 1994). Physical dependence, on the other hand, describes the body's physiological adaptation to the persistent presence of the drug, which also takes part in the psychological dependence (World Health Organization, 1994). As opioids are used repeatedly, the brain initiates compensatory mechanisms to counteract their effects and preserve homeostasis (Bradbury, 1995). These adaptations, again, primarily involve the brain's reward circuitry. As previously described, chronic opioid exposure leads to the downregulation of receptors and dampens the neurotransmitter signaling, which shifts the brain's baseline functioning over time (Kosten et al., 2002). As a result, natural rewards, such as food and social interaction, become less satisfying, and opioids become the primary source of pleasure and motivation (Koob et al., 2010). However, this is not the only mechanism contributing to the development of physical dependence. Another critical molecular pathway involved the suppression of cyclic adenosine monophosphate (cAMP), a key second messenger responsible for facilitating neuronal activation (Nestler et al., 1997). Opioids inhibit the production of cAMP by binding to opioid receptors, which block adenylyl cyclase's ability to synthesize cAMP, a process that contributes to the drug's

sedative and analgesic effects (Nestler et al., 1997). Over time, however, the brain compensates by increasing the production or sensitivity of adenylyl cyclase in an effort to restore cAMP levels (Nestler et al., 1997). While this adaptation also contributes to the development of tolerance, it is a central driver of physical dependence, as the presence of opioids becomes necessary to suppress the now overactive cAMP system and prevent neuronal hyperexcitability (Nestler et al., 1997). These neuroadaptations set the stage for the emergence of withdrawal symptoms, which are unpleasant and often debilitating responses that occur when opioid use is reduced or abruptly discontinued.

Opioid Withdrawal and Treatment Gaps

When opioid use is reduced or abruptly stopped, the neuroadaptations that underlie physical dependence give rise to withdrawal symptoms. Without the drug to suppress the overactive cAMP pathway and other compensatory mechanisms, the nervous system becomes destabilized and overactive (Shah & Huecker, 2018). This results in a surge of neuronal activity, manifesting in a range of physiological and behavioral symptoms, including anxiety, depression, agitation, muscle aches, gastrointestinal distress, and autonomic dysregulation, such as sweating, dilated pupils, and an elevated heart rate (Kosten & George, 2002). These symptoms indicate the body's effort to restore homeostasis when the drug it has depended on for balance is no longer present (Shah & Huecker, 2018). Although physical withdrawal symptoms, primarily caused by acute autonomic imbalances and rebound effects like heightened cAMP activity, tend to resolve within a week, emotional disturbances such as anxiety and depression often persist for weeks or even months (Shah & Huecker, 2018).

The persistent emotional symptoms of opioid withdrawal, such as depression and anxiety, are rooted in long-lasting neuroadaptations within the brain circuits, most notably the mesolimbic reward system and the hypothalamic-pituitary-adrenal (HPA) axis. Chronic opioid exposure suppresses dopamine synthesis and receptor responsiveness in the NAc and PFC, which, as previously discussed, are regions critical for motivation, reward processing, and mood regulation (Volkow et al., 2003). Even after cessation of opioid use, these systems remain hypoactive, resulting in persistent anhedonia, diminished motivation, and emotional dysregulation that outlast the physical withdrawal phase (Shah & Huecker, 2018). At the same time, the HPA axis, which typically helps coordinate the body's response to stress, becomes chronically hyperactive during and after opioid withdrawal (Shah & Huecker, 2018). Elevated levels of corticotropin-releasing factor (CRF) in the amygdala and hypothalamus drive heightened stress sensitivity (Koob & Le Moal, 2005). These stress-related adaptations can go on for months, if not longer, contributing to a state of protracted withdrawal (Shah & Huecker, 2018). The combination of an underactive reward system and an overactive stress system creates a neurobiological environment in which emotional distress is sustained, significantly increasing the risk of relapse and complicating long-term recovery.

Withdrawal symptoms present one of the most significant challenges to recovery from OUD. While the physical effects, such as muscle pain and gastrointestinal distress, are often very intense, it is the persistent psychological symptoms, including severe anxiety, depression, and dysphoria, that many individuals find the most distressing (Pergolizzi et al., 2020). These emotional disturbances not only impair quality of life during recovery but are also a primary driver of relapse (Kadam et al., 2017). Current

pharmacotherapies, such as methadone and buprenorphine, primarily target MORs to reduce physical symptoms and curb drug cravings but are less effective at addressing the emotional dimensions of withdrawal (Adams et al., 2021). This gap in treatment underscores the need for novel approaches that can comprehensively address both the physiological and psychological aspects of recovery and reduce relapse risk.

While much of the focus in OUD research has been on MORs, the role of DORs offers a promising avenue for addressing the psychological challenges of withdrawal. Unlike MORs, which are concentrated in regions associated with reward and euphoria, DORs are primarily localized in brain areas involved in mood regulation, such as the amygdala and hippocampus (Ware & Dunn, 2023). Studies have shown that genetic knockout (KO) of DORs in mice leads to heightened anxiety and depressive-like behaviors, highlighting their critical role in emotional stability (Filliol et al., 2000). Importantly, research indicates that DOR agonists can alleviate anxiety and depression without inducing addictive behaviors. For example, preclinical studies using conditioned place preference (CPP) and drug self-administration (SA) models have demonstrated that DOR agonists do not produce the same addictive responses as MOR agonists (Suzuki et al., 1996). These findings suggest that targeting DORs could address the psychological burden of withdrawal while minimizing the risk of new dependencies.

DOR-Mediated Neural Mechanisms of Mood and Stress Regulation

Beyond the behavioral evidence for DOR involvement in anxiety and depression, a growing body of research has clarified how DORs modulate the neural circuits and molecular pathways that underlie mood and stress regulation. DORs are abundantly expressed in key limbic and cortical structures that are central to emotional processing,

including the amygdala, hippocampus, and medial prefrontal cortex (Jutkiewicz, 2006). These receptors influence mood by modulating neurotransmitter activity, synaptic plasticity, and neuroendocrine signaling.

The amygdala governs fear and anxiety by detecting potential threats and activating the HPA axis through its projections to the hypothalamus, resulting in the release of stress hormones, such as cortisol (Ulrich-Lai & Herman, 2009). Within this circuitry, DOR activation has been shown to reduce anxiety-related behaviors by dampening excitatory neurotransmission in the amygdala (Reyes et al., 2017). Selective DOR agonists, such as KNT-127, have been shown to facilitate fear extinction and reduce conditioned fear responses via signaling in the basolateral amygdala (Saitoh et al., 2018). The hippocampus, another critical region in stress regulation, contributes to mood by exerting negative feedback control over the HPA axis, thereby inhibiting further cortisol release during and after stress (Kim et al., 2015). DOR activation in the hippocampus enhances this regulatory function, promoting emotional stability and resilience (Saitoh et al., 2018). Additionally, because chronic stress suppresses hippocampal neurogenesis, DOR agonists have shown promise in restoring neuroplasticity and producing antidepressant-like effects (Miyuki et al., 2018). Blocking DORs with naltrindole reduces hippocampal BDNF expression, whereas KNT-127 has been found to restore neurogenesis and reduce stress-induced inflammation and neuronal damage (Miyuki et al., 2018; Miyuki et al., 2021).

The medial prefrontal cortex (mPFC) and the HPA axis serve distinct yet complementary functions in the regulation of mood and stress. The mPFC plays a critical role in top-down regulation of emotional responses, particularly through inhibitory

control over limbic regions such as the amygdala. Within this region, delta opioid receptors (DORs) are primarily located on GABAergic interneurons, where their activation leads to the disinhibition of cortical pyramidal neurons via the PI3K–Akt–mTORC1 signaling pathway, resulting in rapid antidepressant-like effects (Hiranita et al., 2024). This mechanism parallels the action of fast-acting antidepressants such as ketamine, which also enhance synaptic plasticity and cortical output. In parallel, the HPA axis governs the endocrine response to stress by modulating the release of glucocorticoids, including corticosterone and aldosterone. Chronic dysregulation of this axis is associated with heightened stress sensitivity and depressive symptomatology. While activation of kappa opioid receptors (KORs) is known to stimulate the HPA axis, leading to increased stress hormone release, DOR activation appears to exert a buffering effect. Antagonism of DORs with naltrindole has been shown to enhance stress-induced elevations in corticosterone and aldosterone, whereas DOR agonists normalize HPA activity under chronic stress conditions, reducing neuroendocrine overactivation (Miyuki et al., 2018; Miyuki et al., 2021).

Taken together, these findings underscore the critical role of DORs in modulating the neural substrate of mood and stress regulation. Through their influence on neurotransmission, neuroplasticity, and the HPA axis, DORs support emotional resilience and counteract the neural and hormonal disruptions associated with chronic stress, a common symptom in opioid withdrawal. DOR's widespread expression in the amygdala, hippocampus, and medial prefrontal cortex positions them as promising modulators of affective circuits. Importantly, DOR agonists have demonstrated the ability to engage these mechanisms without the abuse potential or dysphoric effects associated with other

opioid receptor subtypes. This positions them as compelling therapeutic targets for addressing the emotional and neurobiological challenges of opioid withdrawal. However, despite growing interest, findings across the literature remain fragmented. Synthesizing this evidence is crucial for clarifying the extent and consistency of these mechanisms and for systematically evaluating the therapeutic potential of DOR agonists.

Evaluating the Therapeutic Distinctions Between DOR, MOR, and KOR agonists

Although mu (MOR), delta (DOR), and kappa (KOR) opioid receptors are structurally related, they differ significantly in their behavioral effects and therapeutic profiles. These differences are especially relevant in the context of treating opioid withdrawal and co-occurring affective symptoms. MOR agonists like methadone and buprenorphine are the primary pharmacological treatments for opioid use disorder due to their ability to relieve pain and suppress withdrawal. However, their efficacy is limited by high abuse potential, respiratory depression risk, and inadequate impact on anxiety and depression (Adams et al., 2021; Dripps & Jutkiewicz, 2018). KOR agonists provide analgesia and may reduce drug-seeking, but are often accompanied by dysphoria, anxiety, and cognitive blunting due to their suppression of mesolimbic dopamine activity (Bruchas et al., 2010). These adverse effects have restricted their clinical use. DOR agonists offer a distinct profile, combining analgesic, anxiolytic, and antidepressant-like effects without the reinforcing or dysphoric outcomes associated with MOR and KOR agonists (Jutkiewicz, 2006; Saitoh et al., 2018). Their expression in mood-related brain regions and engagement of neuroplasticity-promoting pathways support their potential for addressing both the somatic and emotional aspects of withdrawal (Miyuki et al., 2021; Hiranita et al., 2024).

These therapeutic distinctions suggest that DOR agonists may fill a critical gap in current treatment strategies by targeting the affective dimension of withdrawal without introducing new dependency risks. Clarifying these differences through systematic analysis is essential to evaluating the full therapeutic potential of DOR-targeted interventions.

DOR Agonists in Models of Substance Withdrawal

Withdrawal from opioids and other addictive substances often involves not only physical discomfort but also intense psychological symptoms, such as anxiety, depression, and dysphoria, which are strong predictors of relapse (Kadam et al., 2017). While treatments like buprenorphine and methadone address many somatic symptoms, they often fall short in treating these affective dimensions. Emerging research suggests that delta opioid receptor (DOR) agonists may offer a more holistic approach by targeting both physical and emotional aspects of withdrawal.

Although studies directly examining DOR agonists in opioid withdrawal models remain limited, recent preclinical evidence is promising. For instance, KNT-127, a DOR agonist, was shown to reduce anxiety-like behavior and improve social deficits in mice undergoing naloxone-precipitated morphine withdrawal, without inducing rewarding effects or sedation (Moriya et al., 2023). Additional studies have demonstrated similar benefits in models of cocaine and ethanol withdrawal. In one example, SNC80 attenuated anxiety-like behavior and reversed anhedonia in a rodent model of cocaine withdrawal (Perrine et al., 2008). These findings indicate that DOR agonists can mitigate both emotional and somatic withdrawal symptoms with minimal abuse liability. While further research is needed to assess their efficacy specifically in opioid withdrawal, synthesizing

findings from related substance models may offer the necessary rationale for expanding their therapeutic application.

Specific Aims, Goals, and Hypothesis

Opioid use disorder (OUD) is marked by high rates of relapse, driven in part by the affective symptoms of withdrawal, namely anxiety, depression, and dysphoria. While current treatments such as buprenorphine and methadone provide relief from somatic symptoms by acting on mu-opioid receptors (MORs), they often fail to address these emotional dimensions adequately. Delta-opioid receptors (DORs), by contrast, are distributed in brain regions involved in affective regulation and have demonstrated anxiolytic and antidepressant-like effects without producing reward or sedation in preclinical models. However, findings across the literature remain fragmented, and the therapeutic potential of DOR agonists in mitigating withdrawal-associated mood disturbances has yet to be synthesized.

The overarching goal of this meta-analysis is to evaluate the efficacy of DOR agonists in reducing affective symptoms associated with substance withdrawal, with a focus on opioid withdrawal models. This work aims to clarify DOR-mediated mechanisms, examine their relevance across various substances, and assess their therapeutic distinction from MOR and KOR pathways. Based on previous literature, it was hypothesized that delta-opioid receptor (DOR) agonists would alleviate anxiety- and depression-like symptoms associated with opioid withdrawal. The following specific aims will guide this investigation:

Specific Aim 1: Determine whether DOR agonists reduce anxiety- and depression-like behaviors.

- Methods: Synthesize data from preclinical studies using validated behavioral assays, such as the elevated plus maze, open-field test, forced swim test, and social interaction paradigms.
- Expected results: It was anticipated that DOR agonists would significantly reduce anxiety- and depression-like behaviors, indicating a potential role in mitigating affective symptoms that contribute to relapse risk.

Specific Aim 2: Evaluate the effects of DOR agonists in models of opioid and other substance withdrawal.

- Methods: Results from animal models of morphine, ethanol, and cocaine withdrawal were aggregated to assess both somatic withdrawal signs and affective outcomes following DOR agonist administration.
- Expected results: DOR agonists were expected to attenuate withdrawal severity across substances, reducing both physical symptoms and negative affective states, thereby demonstrating translational relevance for opioid use disorder treatment.

Aim 3: Compare the behavioral and neurobiological effects of DOR agonists with MOR and KOR agonists.

- Methods: A comparative meta-analysis was conducted across studies that directly evaluated DOR, MOR, and KOR agonists on behavioral measures and neurobiological markers.
- Expected results: It was anticipated that DOR agonists would demonstrate a unique therapeutic profile characterized by reduced abuse liability relative to MOR agonists and fewer dysphoric or anxiogenic effects

relative to KOR agonists, highlighting their potential advantages within the opioid receptor system.

Through these aims, this meta-analysis seeks to bridge critical gaps in the literature and inform the development of new treatments for opioid withdrawal that address both physiological and psychological components of recovery.

Definition of terms

“Agonist(s)”: a substance that initiates a physiological response when combined with a receptor, usually excitatory

“Antagonist(s)”: a biological structure or chemical agent that interferes with the physiological action of another substance, usually inhibitory

“Conditioned Place Preference (CPP)”: a behavioral experiment used to measure the rewarding or aversive effects of a stimulus, such as a drug, by associating it with a specific environment and observing the subject’s preference for that environment

“Delta Opioid Receptor (DOR)”: A protein receptor in the nervous system that binds opioids and regulates pain and mood (Pasternak, 2011)

“Drug Self-Administration (SA)”: a behavioral experiment in which subjects, typically animals, are trained to perform an action (like pressing a lever) to administer a drug to themselves, used to study drug reinforcement and addiction

“Euphoria”: an intense feeling of happiness, pleasure, or well-being, often experienced as a result of certain activities, substances, or brain chemical releases

“Gamma- aminobutyric acid (GABA)”: a neurotransmitter in the brain that inhibits nerve activity, helping reduce stress, promote relaxation, and maintain balance in the nervous system

“Genetic Knockout (KO)”: a technique in which a specific gene is completely disabled or removed in an organism to study the gene’s function by observing the resulting effects

“Kappa Opioid Receptor (KOR)”: a protein receptor in the nervous system that binds to opioid-like molecules, playing a role in pain relief, stress response, mood regulation, and addiction

“Medication-Assisted Treatment (MAT)”: the use of FDA-approved medications, along with counseling and behavioral therapies, to treat opioid addiction and support long-term recovery

“Mu Opioid Receptor (MOR)”: A protein receptor in the nervous system that binds to opioids and is primarily responsible for pain relief, feelings of euphoria, and side effects like respiratory depression and dependence (Pasternak, 2011)

“Nucleus Accumbens (NAc)”: a brain region involved in reward, motivation and pleasure, playing a central role in the brain’s reward system

“Opioids”: a class of drugs that act on opioid receptors in the brain to relieve pain. Including prescription painkillers like oxycodone and morphine, as well as illegal drugs like heroin. Opioids pose a high risk of addiction, tolerance, and overdose

“Opioid use disorder (OUD)”: A chronic condition characterized by the compulsive use of opioids despite negative consequences (DSM-5, 2013)

“Receptor Desensitization”: when a cell’s receptors become less responsive to a substance, like a drug or hormone, after prolonged or repeated exposure, reducing the substance’s effect over time

“Receptor Downregulation”: a process where the number of receptors on a cell’s surface decreases in response to prolonged or excessive stimulation, reducing the cell’s sensitivity to a substance

“Tolerance”: a loss of effect following repeated use such that a higher dose is required for equivalent effect (McQuay, 1999)

“Ventral tegmental area (VTA)”: a part of the brain located in the midbrain that plays a key role in reward, motivation, and addiction by releasing dopamine

“Withdrawal”: the physical and psychological symptoms that occur when a person reduces or stops using a substance, such as opioids, after developing dependence

Chapter II.

Methods

This meta-analysis synthesized preclinical research on delta opioid receptor (DOR) agonists to assess their effects on anxiety and depression like behaviors, stress-related neurobiological markers, and withdrawal outcomes. Additionally, compared the efficacy of DOR agonists to mu (MOR) and kappa (KOR) opioid receptor agonists across these domains. The goal of this study was to provide an integrated synthesis of behavioral and neurobiological findings that inform the therapeutic potential of DOR agonists for substance withdrawal, especially in the context of opioid use.

Inclusion Criteria

To be included in this meta-analysis, studies must have met the following criteria:

- Population
 - Preclinical studies involving animal models of any sex
 - Subjects exposed to opioids, ethanol, cocaine, or other substances associated with withdrawal, or undergoing tests for affective behaviors or adverse effects
- Intervention
 - Experimental administration of DOR agonists by any route

- DOR-related intervention or measurement must be explicitly reported and measurement must be directly relevant to the experimental condition
- Comparators
 - Studies must include a control group (e.g., vehicle, no treatment, wild type) or active comparator (e.g., MOR or KOR agonist)
- Outcomes
 - Eligible studies must report at least one of the following:
 - Behavioral outcomes: anxiety-like (e.g., EPM, OFT) or depression-like behaviors (e.g., FST, SPT)
 - Withdrawal-related measures: Somatic withdrawal symptoms (e.g., wet dog shakes, jumping), conditioned place aversion/preference, or neuroadaptations due to substance discontinuation
 - Adverse effect measures: Respiratory depression, motor coordination and abuse liability
 - Outcomes must include extractable group-level data (means, SD/SEM, sample size) or visual data suitable for data extraction (e.g., graphs)
- Study design
 - Randomized or controlled experimental designs
 - Peer-reviewed publications, dissertations, and full-text reports in English

- Time frame
 - Studies published between 1993 and 2025

Exclusion criteria

Studies were excluded based on the following:

- Human, in vitro, or computational models
- Lack of a control or comparator group
- No clear use of a DOR agonist as part of the intervention
- Outcomes unrelated to affective behavior, neurobiological stress markers, substance adverse effects or substance withdrawal
- Reviews, commentaries, protocols, conference abstracts, or non-English texts without full access

Conflicts in study selection were resolved through discussion or consensus between reviewers.

Search strategy

A comprehensive literature search was conducted across the following databases: PubMed, Google Scholar, Web of Science, PsycINFO, and Cochrane Library. Search terms combined keywords and MeSH terms related to DOR agonists and relevant outcomes. Examples included:

- “Delta opioid receptor” (AND, OR) “anxiety” (AND, OR) “depression”
- “DOR agonist” (AND, OR) “stress response”
- “DOR agonist” (AND, OR) “withdrawal”

- “DOR agonist” (AND, OR) “MOR agonist” (AND, OR) “KOR agonist”

Boolean operators (AND, OR) were used to construct search strings appropriate for each database.

Screening and Selection Process

All records were imported into Covidence for screening and duplicate removal. Two researchers independently reviewed titles and abstracts. Full texts were assessed for eligibility using pre-defined criteria. Conflicts were resolved via discussion or by involving a third-party reviewer. The selection process followed PRISMA guidelines and was documented with a PRISMA flow chart.

Data Extraction

Data were extracted into a structured spreadsheet that included:

- Study metadata (author, year, species)
- Intervention characteristics (agonist type, dose, route, duration)
- Comparator details (e.g., vehicle, MOR/KOR agonist)
- Outcome category (behavioral, neurobiological, withdrawal-related)
- Raw or estimated group-level data (mean, SD/SEM, sample size)
- Outcome domain aligned to the corresponding search aim
- Study quality indicators (e.g., randomization, blinding)

Multiple entries were made per study if multiple experimental groups or outcome domains were reported.

Quality Assessment

Risk of bias was evaluated using a modified SYRCLE Risk of Bias tool. The following domains were assessed:

- Randomization and allocation concealment
- Blinding of outcome assessors
- Consistency of intervention administration and reporting
- Incomplete outcome data
- Selective reporting

Each study was rated as having low, unclear, or high risk of bias per domain.

Sensitivity analyses were performed by excluding studies with high overall bias to assess the robustness of the results.

Statistical Analysis

- Effect size metrics:
 - Standardized mean differences (SMDs) were calculated for continuous outcomes
 - Odds ratios (ORs) or risk ratios (RRs) were computed for dichotomous outcomes
 - 95% confidence intervals (CIs) were reported for all effect sizes
- Meta-Analysis approach:
 - Random-effects models (DerSimonian and Laird method) were used to account for between-study heterogeneity
 - Fixed-effects models were tested in sensitivity analyses

- Heterogeneity assessment:
 - Quantified using the I^2 statistic (low <25%, moderate 25-50%, high >50%) and Cochran's Q test ($p < 0.10$)
 - Subgroup analyses were performed by agonist drug, behavioral domain, and substance model (e.g., opioid, ethanol).
- Software
 - All analyses were conducted in R using the “metafor” and “meta” packages, ensuring transparency and reproducibility

Specific Aims Data

Specific Aim 1: Investigate whether DOR agonists reduce anxiety and depression-like behaviors in animal models

- Selection criteria: Included studies employed DOR agonists in animal models using validated behavioral assays for anxiety and depression, such as the elevated plus maze, open field test, light-dark box, forced swim test, and tail suspension test.
- Data extracted: Behavioral scores (e.g., time in open arms, immobility duration), drug dose and timing, stress exposure (if any), and control group performance.
- Analysis: Standardized mean differences (Hedges' g) were calculated to compare treatment vs. control. Analyses were stratified by behavioral domain (anxiety vs. depression).

Specific Aim 2: Evaluate the effects of DOR agonists in models of substance withdrawal

- Selection criteria: Studies that administered DOR agonists to rodents undergoing opioid, ethanol, or cocaine withdrawal, either spontaneously or following naloxone-precipitated withdrawal.
- Data extracted: Withdrawal symptom scores (e.g., jumping, teeth chattering, ptosis), affective behaviors during withdrawal, time course of withdrawal and drug administration.
- Analysis: Hedges' g calculated for withdrawal symptom reduction. Subgroup analysis by substance type and withdrawal model (precipitated vs. spontaneous).

Specific Aim 3: Compare DOR agonist effects with other opioid receptor types (MOR/KOR) across behavioral, affective, and safety domains

- Selection criteria: Studies directly comparing DOR agonists with MOR or KOR agonists in the same behavioral, neurobiological, or substance withdrawal model.
- Data extracted: Behavioral or neurobiological outcomes across treatment groups (DOR vs. MOR/KOR), including affective symptoms, reward sensitivity/addictive potential, tolerance, and lethality
- Analysis: Comparative effect sizes computed for each domain. Where possible, within-study contrasts were prioritized to reduce cross-study heterogeneity and emphasize direct receptor-type comparisons.

Chapter III

Results

The following results are organized according to the three specific aims of this meta-analysis. Specific Aim 1 examines the effects of delta opioid receptor (DOR) agonists on affective behaviors, including anxiety-like and depression-like outcomes, in preclinical models. Specific Aim 2 evaluates the influence of DOR agonists in models of substance withdrawal, with analyses focused on anxiety, depression, and drug-association outcomes. Specific Aim 3 compares the behavioral and neurobiological effects of DOR agonists to those of μ -opioid receptor (MOR) and κ -opioid receptor (KOR) agonists across domains such as analgesia, adverse effects, and withdrawal behaviors. Results for each aim are presented with corresponding forest plots, subgroup analyses, and sensitivity models, alongside tables summarizing pooled effect sizes, heterogeneity estimates, and moderator tests.

Specific Aim 1: Behavioral Effects of DOR Agonists

The results for Specific Aim 1 are presented in two sections: anxiety-like behaviors and depression-like behaviors. Findings for anxiety-like behaviors are discussed first, followed by depression-like behaviors, and concluding with an overall summary of Specific Aim 1 results.

Anxiety-like behaviors

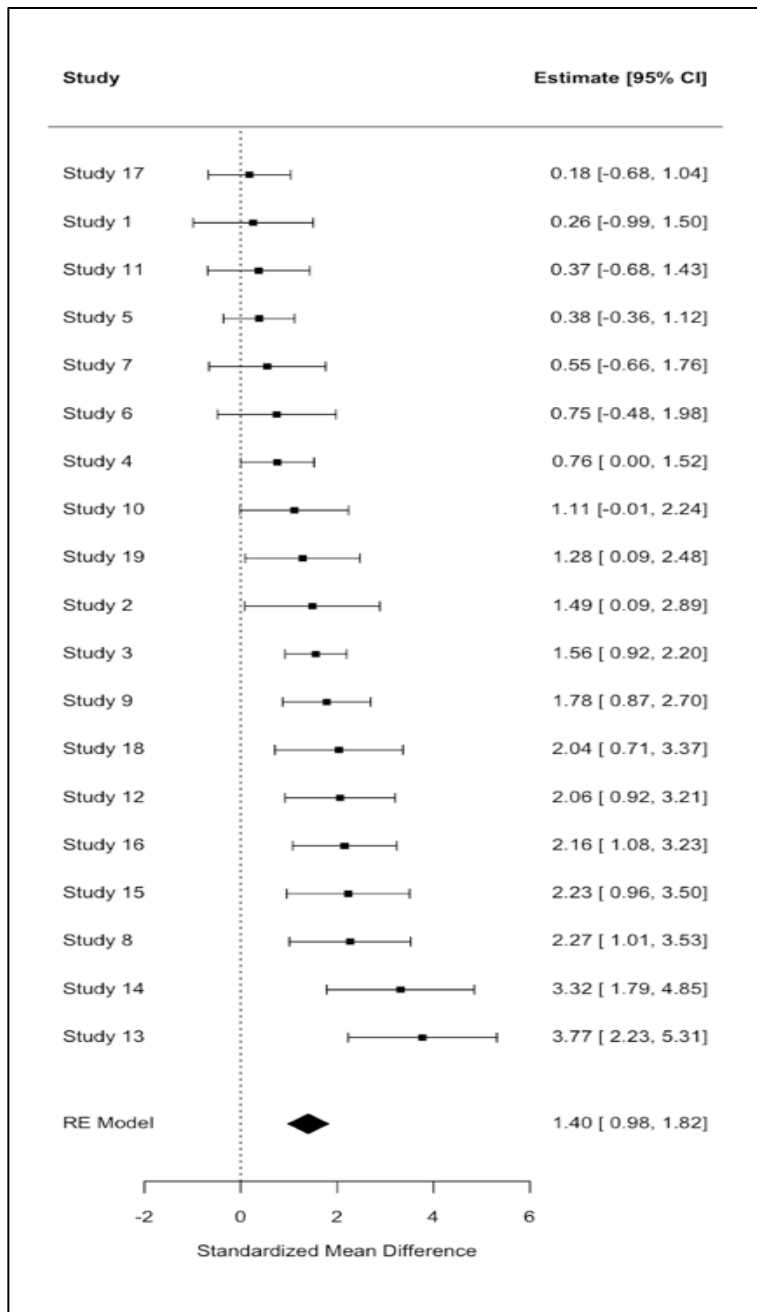


Figure 1a. Anxiety overall forest plot

Forest plot showing the overall effects of DOR agonists on anxiety-like behavior across 19 comparisons. Each square represents the standardized mean difference (SMD) for an individual study, with the size of the square proportional to its weight in the meta-analysis. Horizontal lines indicate 95% confidence intervals. The diamond at the bottom depicts the pooled random-effects estimate (SMD = 1.40, 95% CI [0.98, 1.82]), indicating a significant overall reduction in anxiety-like behavior.

Short	Full	Short	Full
Study 1	Ambrose, Lanci, L. M., Sterling, R. C., & Van Bockstaele, E. J. (2010). Cocaine withdrawal-induced anxiety in females: Impact of circulating estrogen and potential use of delta-opioid receptor agonists for treatment. <i>Journal of neuroscience research</i> , 88(4), 816-824.	Study 11	Solati, J. (2011). Dorsal hippocampal N-methyl-d-aspartate glutamatergic and $\text{G}\ddot{\text{Y}}$ -opioidergic systems modulate anxiety behaviors in rats in a noninteractive manner. <i>The Kaohsiung Journal of Medical Sciences</i> , 27(11), 485-493.
Study 2	Ambrose, Lanci, L. M., Sterling, R. C., & Van Bockstaele, E. J. (2010). Cocaine withdrawal-induced anxiety in females: Impact of circulating estrogen and potential use of delta-opioid receptor agonists for treatment. <i>Journal of neuroscience research</i> , 88(4), 816-824.	Study 12	Sugiyama, A., Yamada, M., Furue, H., Gotoh, L., Saitoh, A., Nagase, H., ... & Yamada, M. (2019). Systemic administration of a delta opioid receptor agonist, KNT-127, facilitates extinction learning of fear memory in rats. <i>Journal of Pharmacological Sciences</i> , 139(3), 174-179.
Study 3	Richards, E. M., Mathews, D. C., Luckenbaugh, D. A., Ionescu, D. F., Machado-Vieira, R., Niciu, M. J., ... & Zarate Jr, C. A. (2016). A randomized, placebo-controlled pilot trial of the delta opioid receptor agonist AZD2327 in anxious depression. <i>Psychopharmacology</i> , 233(6), 1119-1130.	Study 13	Sugiyama, A., Yamada, M., Furue, H., Gotoh, L., Saitoh, A., Nagase, H., ... & Yamada, M. (2019). Systemic administration of a delta opioid receptor agonist, KNT-127, facilitates extinction learning of fear memory in rats. <i>Journal of Pharmacological Sciences</i> , 139(3), 174-179.
Study 4	Kawaminami, A., Yamada, D., Yanagisawa, S., Shirakata, M., Iio, K., Nagase, H., & Saitoh, A. (2022). Selective $\text{G}\ddot{\text{Y}}$ -opioid receptor agonist, KNT-127, facilitates contextual fear extinction via infralimbic cortex and amygdala in mice. <i>Frontiers in Behavioral Neuroscience</i> , 16, 808232.	Study 14	Sugiyama, A., Yamada, M., Saitoh, A., Nagase, H., Oka, J. I., & Yamada, M. (2018). Administration of a delta opioid receptor agonist KNT-127 to the basolateral amygdala has robust anxiolytic-like effects in rats. <i>Psychopharmacology</i> , 235(10), 2947-2955.
Study 5	Kawaminami, A., Yamada, D., Yanagisawa, S., Shirakata, M., Iio, K., Nagase, H., & Saitoh, A. (2022). Selective $\text{G}\ddot{\text{Y}}$ -opioid receptor agonist, KNT-127, facilitates contextual fear extinction via infralimbic cortex and amygdala in mice. <i>Frontiers in Behavioral Neuroscience</i> , 16, 808232.	Study 15	Sugiyama, A., Yamada, M., Saitoh, A., Nagase, H., Oka, J. I., & Yamada, M. (2018). Administration of a delta opioid receptor agonist KNT-127 to the basolateral amygdala has robust anxiolytic-like effects in rats. <i>Psychopharmacology</i> , 235(10), 2947-2955.
Study 6	Kawaminami, A., Yamada, D., Yoshioka, T., Hatakeyama, A., Nishida, M., Kajino, K., ... & Saitoh, A. (2024). The delta opioid receptor agonist KNT-127 relieves innate anxiety-like behavior in mice by suppressing transmission from the prelimbic cortex to basolateral amygdala. <i>Neuropsychopharmacology Reports</i> , 44(1), 256-261.	Study 16	Sugiyama, A., Yamada, M., Saitoh, A., Nagase, H., Oka, J. I., & Yamada, M. (2018). Administration of a delta opioid receptor agonist KNT-127 to the basolateral amygdala has robust anxiolytic-like effects in rats. <i>Psychopharmacology</i> , 235(10), 2947-2955.
Study 7	Kawaminami, A., Yamada, D., Yoshioka, T., Hatakeyama, A., Nishida, M., Kajino, K., ... & Saitoh, A. (2024). The delta opioid receptor agonist KNT-127 relieves innate anxiety-like behavior in mice by suppressing transmission from the prelimbic cortex to basolateral amygdala. <i>Neuropsychopharmacology Reports</i> , 44(1), 256-261.	Study 17	Sugiyama, A., Yamada, M., Saitoh, A., Nagase, H., Oka, J. I., & Yamada, M. (2018). Administration of a delta opioid receptor agonist KNT-127 to the basolateral amygdala has robust anxiolytic-like effects in rats. <i>Psychopharmacology</i> , 235(10), 2947-2955.
Study 8	Randall-Thompson, J. F., Pescatore, K. A., & Unterwald, E. M. (2010). A role for delta opioid receptors in the central nucleus of the amygdala in anxiety-like behaviors. <i>Psychopharmacology</i> , 212(4), 585-595.	Study 18	Sugiyama, A., Yamada, M., Saitoh, A., Nagase, H., Oka, J. I., & Yamada, M. (2018). Administration of a delta opioid receptor agonist KNT-127 to the basolateral amygdala has robust anxiolytic-like effects in rats. <i>Psychopharmacology</i> , 235(10), 2947-2955.
Study 9	Richards, E. M., Mathews, D. C., Luckenbaugh, D. A., Ionescu, D. F., Machado-Vieira, R., Niciu, M. J., ... & Zarate Jr, C. A. (2016). A randomized, placebo-controlled pilot trial of the delta opioid receptor agonist AZD2327 in anxious depression. <i>Psychopharmacology</i> , 233(6), 1119-1130.	Study 19	Sugiyama, A., Yamada, M., Saitoh, A., Nagase, H., Oka, J. I., & Yamada, M. (2018). Administration of a delta opioid receptor agonist KNT-127 to the basolateral amygdala has robust anxiolytic-like effects in rats. <i>Psychopharmacology</i> , 235(10), 2947-2955.
Study 10	Solati, J. (2011). Dorsal hippocampal N-methyl-d-aspartate glutamatergic and $\text{G}\ddot{\text{Y}}$ -opioidergic systems modulate anxiety behaviors in rats in a noninteractive manner. <i>The Kaohsiung Journal of Medical Sciences</i> , 27(11), 485-493.		

Figure 1b. Key for short-cited studies in Figure 1a

Table 1. Anxiety-like behaviors analysis

Random-effects model: Anxiety-like behaviors								
k	Pooled SMD [95% CI]	SE	z (p)	Tau ²	I ² %	H ²	Q(df), p	Prediction Interval
19	1.4 [0.98, 1.82]	0.215	6.51 ($<.0001$)	0.552	66.1	2.95	50.38 (18), $<.0001$	[-0.11, 2.92]

Results of the random-effects meta-analysis model for anxiety-like behaviors, showing the pooled standardized mean difference (SMD), standard error (SE), test of overall effect, heterogeneity estimates, and prediction interval ($k = 19$).

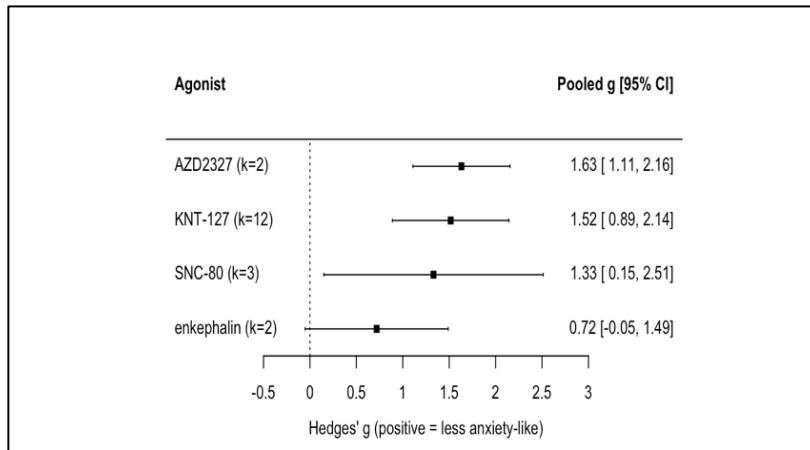


Figure 2. Subgroup analysis by DOR agonist

Subgroup analysis by DOR agonist, showing pooled effect sizes (Hedges' g) with 95% confidence intervals. Squares represent standardized mean differences, with square size proportional to study weight; horizontal lines represent confidence intervals. Positive values indicate reduced anxiety-like behavior.

Table 2. Anxiety subgroup by agonist random-effects meta-analysis

Anxiety Subgroup Analysis by DOR Agonist				
agonist	k	SMD_CI	I ² _pct	p_text
AZD2327	2	1.63 [1.11, 2.16]	0	< .0001
enkephalin	2	0.72 [-0.05, 1.49]	0	0.067
KNT-127	12	1.52 [0.89, 2.14]	74.6	< .0001
SNC-80	3	1.33 [0.15, 2.51]	59.7	0.027

Results of the subgroup random-effects meta-analysis for anxiety-like behaviors by DOR agonist, showing pooled standardized mean differences (SMD) with 95% confidence intervals, heterogeneity (I^2), and p-values

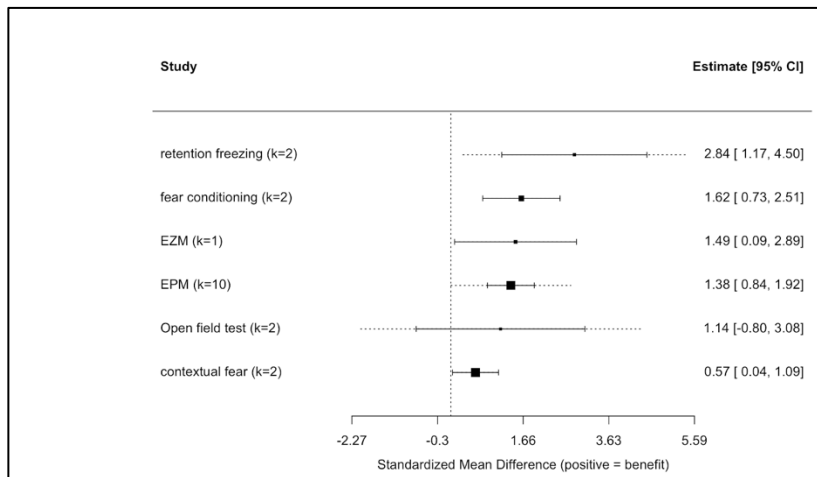


Figure 3. Subgroup analysis by assay

Subgroup analysis by behavioral assay, showing pooled effect sizes with 95% confidence intervals. Squares represent standardized mean differences, with square size proportional to study weight; horizontal lines represent confidence intervals. Positive values indicate reduced anxiety-like behavior.

Table 3. Anxiety Subgroup by assay random effects

Anxiety Subgroup Results by Assay				
test	k	SMD CI	I ² pct	p text
contextual fear	2	0.57 [0.04, 1.09]	0	0.036
EPM	10	1.38 [0.84, 1.92]	58.1	< .0001
EZM	1	1.49 [0.09, 2.89]	0	0.038
fear conditioning	2	1.62 [0.73, 2.51]	0	0
Open field test	2	1.14 [-0.8, 3.08]	87.4	0.248
retention freezing	2	2.84 [1.17, 4.5]	67.2	0.001

Results of the subgroup random-effects meta-analysis for anxiety-like behaviors by assay, showing pooled standardized mean differences (SMD) with 95% confidence intervals, heterogeneity (I²), and p-values for each test type

Table 4. Anxiety subgroup analysis by dose (systemic and Intra-BLA).

Anxiety Subgroup Analysis by Dose (systemic)				
dose bin	k	SMD CI	I ² pct	p text
[3,3.00000001]	4	2.09 [1.32, 2.85]	57.7	< .0001
(3.00000001,5]	3	1.33 [0.15, 2.51]	59.7	0.027
(5,10]	2	0.65 [-0.21, 1.51]	0	0.14
By Dose (intra-BLA)				
ug bin	k	SMD CI	I ² pct	p text
All 0.01g doses	6	1.77 [0.9, 2.65]	69.4	< .0001

Results of the subgroup random-effects meta-analysis for anxiety-like behaviors by dose, separated by systemic and intra-BLA administration routes. Table reports pooled standardized mean differences (SMD) with 95% confidence intervals, heterogeneity (I²), and p-values

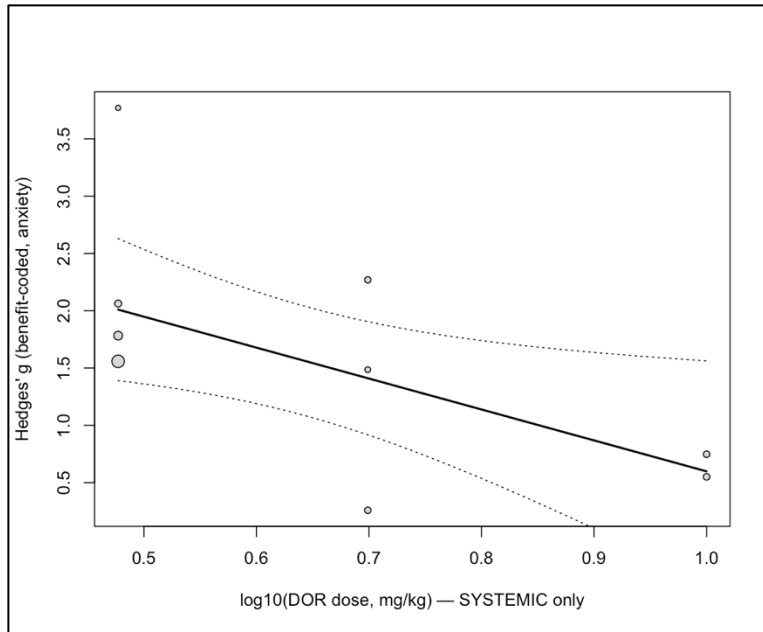


Figure 4. Bubble plot of subgroup analysis by dose (systemic)

Systemic administration: log-transformed dose (mg/kg) plotted against effect size (Hedges' g). Circles represent individual study comparisons, solid lines indicate the meta-regression slope, and dotted lines represent 95% confidence intervals

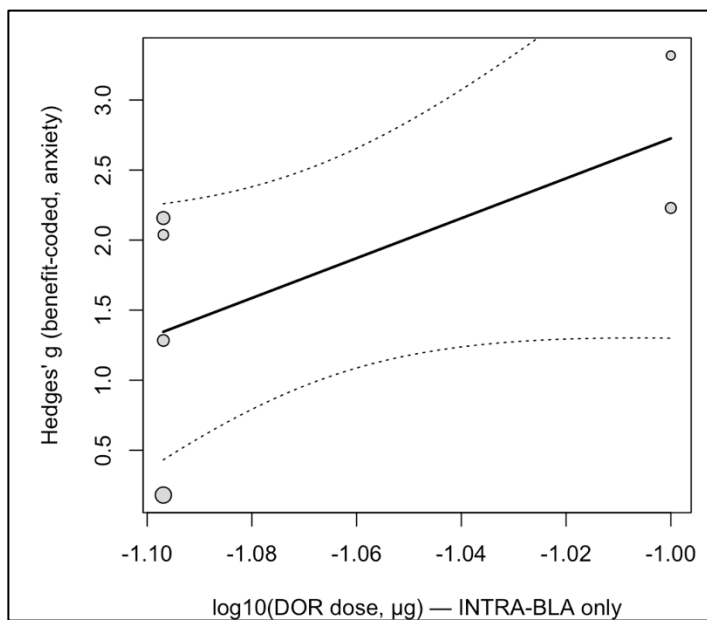


Figure 5. Bubble plot of anxiety subgroup analysis by dose (intra-BLA)

Intra-BLA administration: log-transformed dose (μg) plotted against effect size. Circles represent individual study comparisons, solid lines indicate the meta-regression slope, and dotted lines represent 95% confidence intervals

Across $k = 19$ comparisons, delta-opioid manipulations produced a large anxiolytic effect (Hedges' $g = 1.40$, $SE = 0.22$; 95% CI 0.98–1.82; $z = 6.51$; $p < .0001$), with moderate–high heterogeneity ($Q(18) = 50.38$, $p < .0001$; $I^2 = 66.07\%$; $\tau^2 = 0.5519$) (See Figure 1 & Table 1). By agonist, subgroup estimates were AZD2327 ($k = 2$), $g = 1.63$ [1.11, 2.16], $I^2 = 0\%$; KNT-127 ($k = 12$), $g = 1.52$ [0.89, 2.14], $I^2 = 74.6\%$; SNC-80 ($k = 3$), $g = 1.33$ [0.15, 2.51], $I^2 = 59.7\%$; and enkephalin ($k = 2$), $g = 0.72$ [−0.05, 1.49], $I^2 = 0\%$ (See figure 2 & table 2). A mixed-effects model with agonist as a categorical moderator (CR2 cluster-robust inference) did not indicate overall differences (omnibus $p \approx .084$); relative to AZD2327, enkephalins were smaller ($\Delta g \approx -0.93$, $p = .0028$), while KNT-127 ($\Delta g \approx -0.17$, $p = .701$) and SNC-80 ($\Delta g \approx -0.33$, $p = .708$) were not different. By assay, subgroup estimates were EPM ($k = 10$), $g = 1.38$ [0.84, 1.92], $I^2 = 58.1\%$; fear conditioning ($k = 2$), $g = 1.62$ [0.73, 2.51], $I^2 = 0\%$; contextual fear ($k = 2$), $g = 0.57$ [0.04, 1.09], $I^2 = 0\%$; retention freezing ($k = 2$), $g = 2.84$ [1.17, 4.50], $I^2 = 67.2\%$; open field test ($k = 2$), $g = 1.14$ [−0.80, 3.08], $I^2 = 87.4\%$; and EZM ($k = 1$), $g = 1.49$ [0.09, 2.89]. A test-moderator model explained $R^2 = 12.56\%$ of heterogeneity ($QM(5) = 6.72$, $p = .242$); the retention freezing coefficient was individually significant ($\beta = 2.22$, $p = .012$) (See figure 3 & table 3). Dose analyses separated by route showed, for systemic mg/kg studies ($k = 9$), a significant negative association with $\log_{10}(\text{dose})$ (intercept $g = 3.30$, $p < .0001$; slope $\beta = -2.70$, 95% CI −5.06 to −0.34, $p = .0249$; residual $I^2 = 38.5\%$; $R^2 = 58.4\%$) (See figure 4 & table 4). For intra-BLA μg studies ($k = 6$), the $\log_{10}(\text{dose})$ slope was not significant ($\beta = 14.24$, $p = .110$) with residual $I^2 = 61.5\%$ (See figure 5 & table 4).

Depression-like behaviors

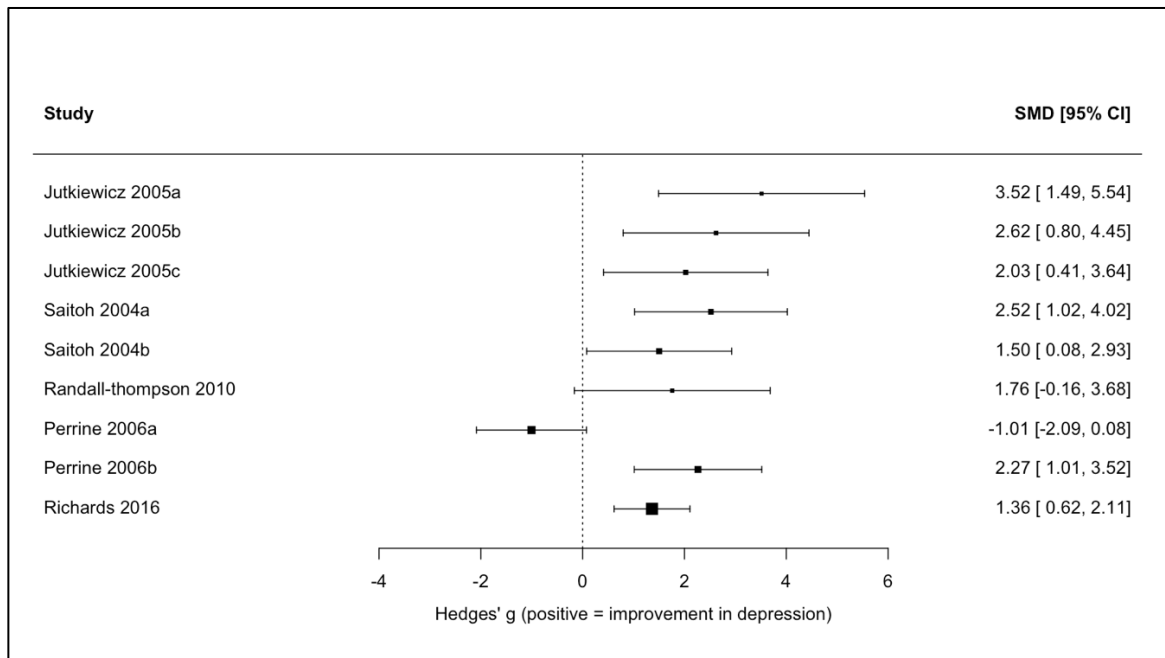


Figure 6. Forest plot of DOR agonist effects on depression-like behaviors in animals

Forest plot of individual study effect sizes (Hedges' g) with 95% confidence intervals for DOR agonist effects on depression-like behaviors in animal models. Squares represent standardized mean differences, with square size proportional to study weight, and horizontal lines indicate confidence intervals. Positive values reflect improvement in depression-like behaviors.

Table 5. Random-effects model results for depression-like behaviors

Depression Random-Effects Results								
k	Pooled SMD [95% CI]	SE	z (p)	Tau ²	I ² %	H ²	Q(df), p	Prediction Interval
9	1.72 [0.85, 2.59]	0.444	3.87 (0.000)	1.207	72.2	3.59	29.64 (df=8), 0.000	[-0.6, 4.04]

Results of the random-effects meta-analysis model for depression-like behaviors, showing the pooled standardized mean difference (SMD), standard error (SE), test of overall effect, heterogeneity estimates, and prediction interval (k = 9)

Table 6. Depression subgroup analysis by DOR agonist

Depression Subgroup Analysis by DOR Agonist				
agonist	k	SMD_CI	I ² _pct	p_text
SNC-80	7	1.81 [0.68, 2.95]	75.6	0.002
DPDPE	1	1.76 [-0.16, 3.68]	0	0.073
AZD2327	1	1.36 [0.62, 2.11]	0	0

Results of the subgroup random-effects meta-analysis for depression-like behaviors by DOR agonist, showing pooled standardized mean differences (SMD) with 95% confidence intervals, heterogeneity (I^2), and p-values.

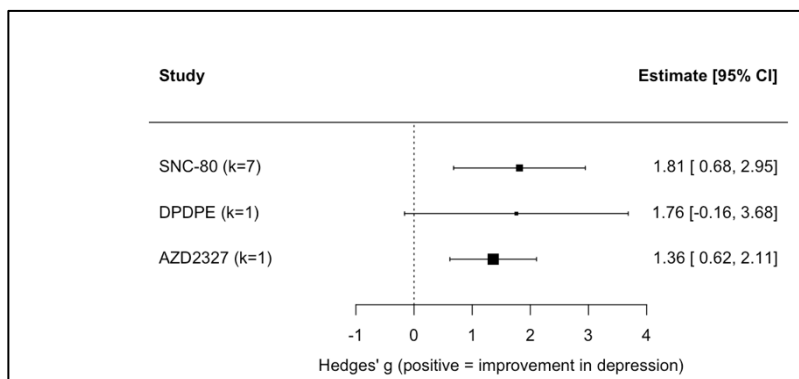


Figure 7. Depression subgroup by assay analysis

Results of the subgroup random-effects meta-analysis for depression-like behaviors by assay, showing pooled standardized mean differences (SMD) with 95% confidence intervals, prediction intervals, heterogeneity (I^2), and p-values.

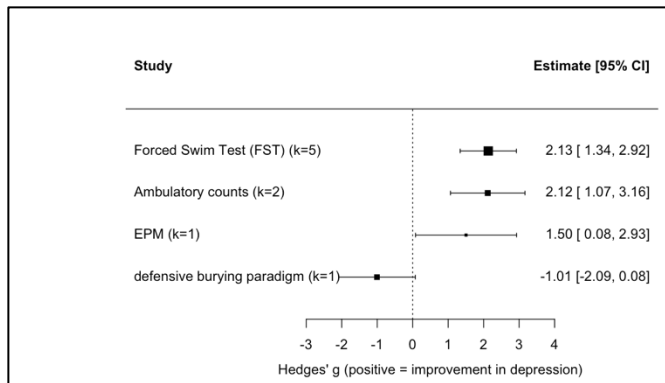


Figure 8. Depression subgroup analysis by assay

Subgroup analysis by behavioral assay. Squares represent standardized mean differences with size proportional to study weight, horizontal lines represent 95% confidence intervals. Positive values indicate improvement in depression-like behaviors.

Table 7. Depression subgroup analysis by dose

Depression Subgroup Analysis by Dose			
Term	Estimate [95% CI]	SE	p (classical)
Intercept (effect at 1 mg/kg)	0.55 [,]	1.865	0.769
log10(dose) per 10 ^{√ó}	1.54 [,]	2.319	0.507

Results of the subgroup random-effects meta-analysis for depression-like behaviors by dose, showing meta-regression estimates with 95% confidence intervals, standard errors (SE), and p-values.

Table 8. Depression subgroup analysis by route

Depression Subgroup Analysis by Route				
route	k	SMD_CI	I ² _pct	p_text
i.p.	1	1.36 [0.62, 2.11]	0	0
i.v.	3	2.61 [1.57, 3.65]	0	< .0001
intra-amygdala	1	1.76 [-0.16, 3.68]	0	0.073
s.c.	4	1.28 [-0.35, 2.91]	84	0.124

Results of the subgroup random-effects meta-analysis for depression-like behaviors by route of administration, showing pooled standardized mean differences (SMD) with 95% confidence intervals, heterogeneity (I^2), and p-values.

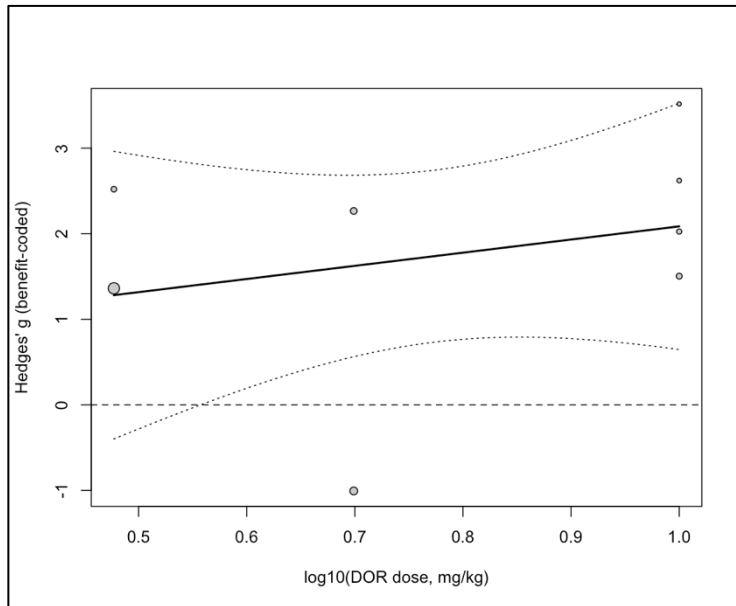


Figure 9. Depression subgroup analysis by dose

Bubble plot of meta-regression examining the association between DOR dose (mg/kg, log-transformed) and effect size. Circles in bubble plots represent individual study comparisons with regression slopes shown by solid lines and 95% confidence intervals by dotted lines. Positive values indicate improvement in depression-like behaviors.

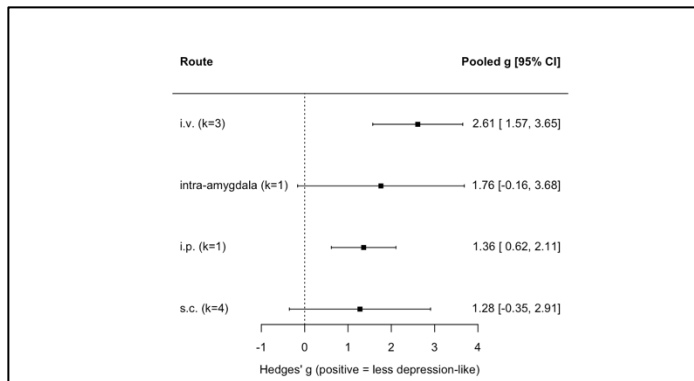


Figure 10. Depression subgroup analysis by route of administration

Subgroup analysis by route of administration. Squares represent standardized mean differences with size proportional to study weight, horizontal lines represent 95% confidence intervals. Positive values indicate improvement in depression-like behaviors.

Across $k = 9$ comparisons, delta-opioid manipulations yielded a large antidepressant-like effect (Hedges' $g = 1.72$, $SE = 0.44$; 95% CI 0.85–2.59; $z = 3.87$; $p = .0001$), with substantial heterogeneity ($Q(8) = 29.64$, $p = .0002$; $I^2 = 72.18\%$; $\tau^2 = 1.207$; 95% PI -0.60 to 4.04) (see figure 6 & table 5). By agonist, subgroup estimates were SNC-80 ($k = 7$) $g = 1.81$ [0.68, 2.95], $I^2 = 75.6\%$; DPDPE ($k = 1$) $g = 1.76$ [-0.16, 3.68]; and AZD2327 ($k = 1$) $g = 1.36$ [0.62, 2.11] (see table 6). A mixed-effects model with agonist as a categorical moderator did not indicate overall differences ($QM(2) = 0.093$, $p = .955$); CR2 cluster-robust contrasts were likewise non-significant (vs. SNC-80: AZD2327 $\Delta g = -0.45$, $p = .557$; DPDPE $\Delta g = -0.05$, $p = .942$) (see figure 7). By assay, pooled effects were large for the Forced Swim Test (FST; $k = 5$) $g = 2.13$ [1.34, 2.92], $I^2 = 35.5\%$, and Ambulatory counts ($k = 2$) $g = 2.12$ [1.07, 3.16], $I^2 = 0\%$; the single EPM study was positive ($g = 1.50$ [0.08, 2.93]), whereas defensive burying was negative and not significant ($k = 1$) $g = -1.01$ [-2.09, 0.08] (see table 7). A test-moderator model showed significant between-test differences ($QM(3) = 15.58$, $p = .0014$), driven by

smaller effects for defensive burying ($\beta = -3.10$, $p = .0012$), and explained $R^2 \approx 83\%$ of heterogeneity (residual $I^2 = 27.3\%$; $\tau^2 = 0.206$) (see figure 8). Dose meta-regression using $\log_{10}(\text{mg/kg})$ showed no dose-response ($k = 8$; slope $\beta = 1.54$, $p = .507$; $R^2 \approx 0\%$; residual $I^2 = 76.9\%$), and adding route (i.v., s.c.) as a moderator did not alter conclusions (QM(3) = 1.52, $p = .678$; CR2 results similar) (see figures 9, 10, tables 8 & 9).

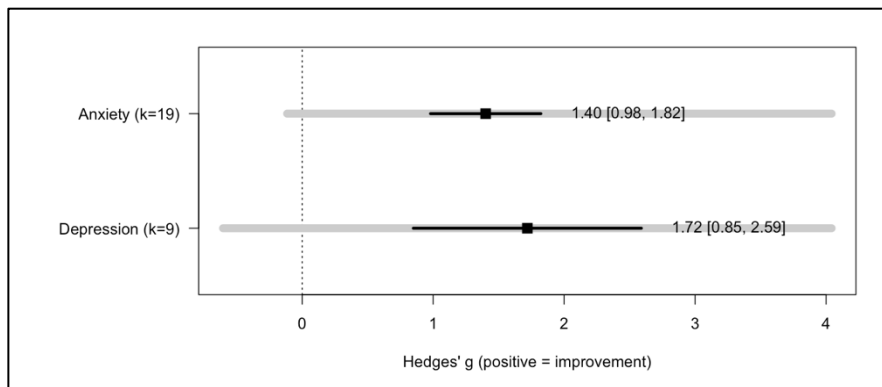


Figure 11. Depression and Anxiety pooled summary

Summary forest plot of pooled effect sizes (Hedges' g with 95% confidence intervals) for anxiety-like ($k = 19$) and depression-like ($k = 9$) behaviors, presented side-by-side for Specific Aim 1. Squares represent standardized mean differences, horizontal lines indicate 95% confidence intervals, and positive values reflect improvement in affective behaviors.

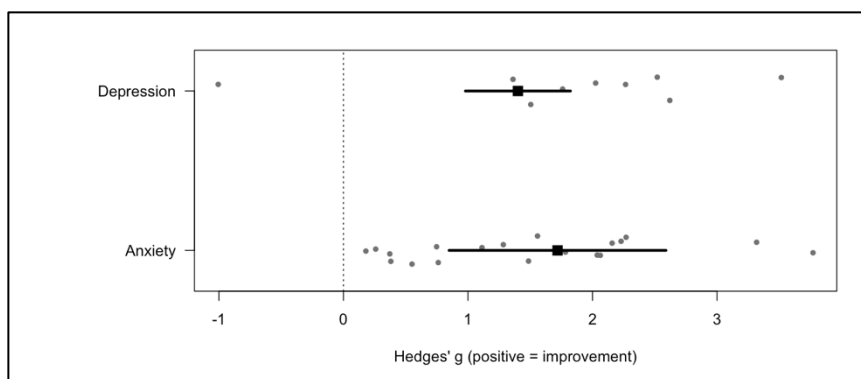


Figure 12. Depression and anxiety dispersion stripe chart

Stripe chart of individual study effect sizes (Hedges' g) for anxiety-like and depression-like behaviors, with pooled standardized mean differences (squares) and 95% confidence intervals (horizontal lines). Each dot represents a study comparison, and positive values reflect improvement in affective behaviors.

Across preclinical comparisons, delta-opioid receptor (DOR) agonists showed strong improvements in affective behaviors. A random-effects meta-analysis revealed a large anxiolytic effect (Hedges' $g = 1.40$, 95% CI [0.98, 1.82], $k = 19$) and a similarly strong antidepressant-like effect (Hedges' $g = 1.72$, 95% CI [0.85, 2.59], $k = 9$), indicating consistent behavioral benefits (Figure 11). Examination of individual study effects further supported these findings, with most comparisons clustering toward positive values, reflecting reliable improvements across assays and compounds (Figure 12). Overall, these results demonstrate that DOR agonists significantly reduce both anxiety-like and depression-like behaviors in preclinical models.

Specific Aim 2: Withdrawal Models

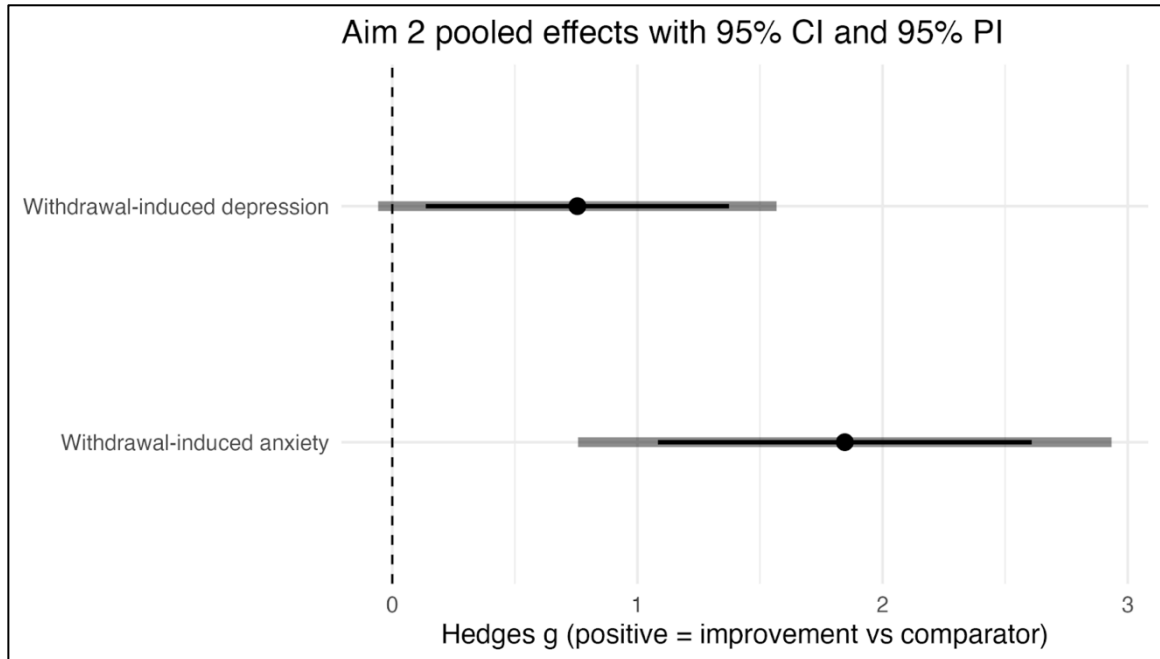


Figure 13. Withdrawal behavior: anxiety and depression

Summary forest plot of pooled effect sizes (Hedges' g) for withdrawal-induced anxiety and depression, showing 95% confidence intervals (black lines) and 95% prediction intervals (gray bars). Positive values reflect improvement compared to comparator groups.

Table 9. Withdrawal behavior: anxiety and depression

Withdrawal-induced Anxiety & Depression									
Outcome	k	Pooled SMD [95% CI]	SE	z (p)	Tau ²	I ² %	H ²	Q(df), p	Prediction Interval
Withdrawal-induced anxiety	5	1.85 [1.08, 2.61]	0.389	4.75 (<0.0001)	0.157	20.74	1.26	4.91 (4), 0.2968	[0.76, 2.93]
Withdrawal-induced depression	4	0.76 [0.14, 1.37]	0.316	2.39 (0.0167)	0.072	18.15	1.22	3.47 (3), 0.3241	[-0.06, 1.57]

Results of the random-effects meta-analysis for withdrawal-induced anxiety and depression, showing pooled standardized mean differences (SMD) with 95% confidence intervals, standard errors (SE), heterogeneity estimates (τ^2 , I^2 , H^2 , Q -test), and prediction intervals.

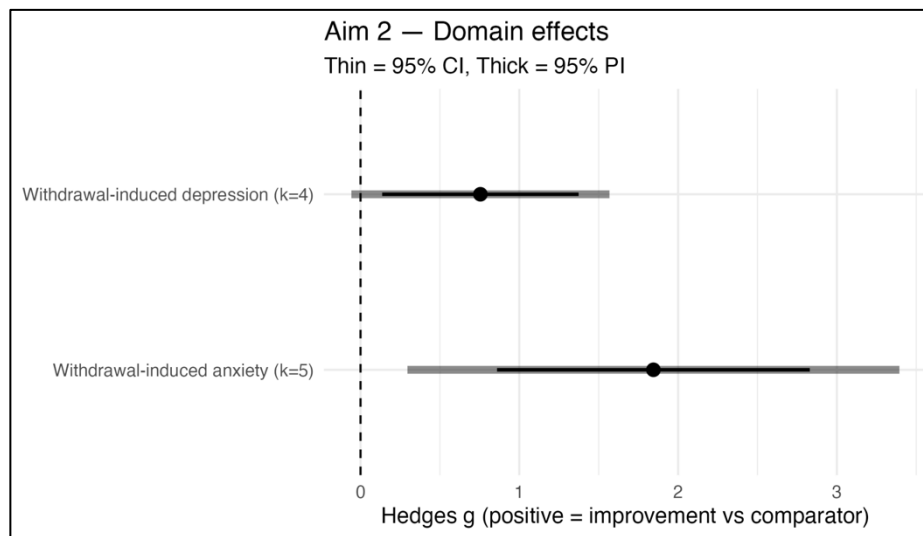


Figure 14. Withdrawal behavior: anxiety and depression

Summary forest plot of pooled effect sizes (Hedges' g) for withdrawal-induced anxiety ($k = 5$) and depression ($k = 4$). Thin lines represent 95% confidence intervals, and thick lines represent 95% prediction intervals. Positive values reflect improvement compared to comparator groups.

Table 10. Withdrawal-induced anxiety and depression random-effects analysis

Domain Differences				
Outcome	k	Pooled SMD [95% CI]	Prediction Interval	z (p)
Withdrawal-induced anxiety	5	1.84 [0.86, 2.83]	[0.30, 3.39]	3.67 (0.0002)
Withdrawal-induced depression	4	0.76 [0.14, 1.37]	[-0.06, 1.57]	2.39 (0.0167)
Difference (Dep ,ái Anx)	NA	-1.51 [-2.57, -0.44]	NA	-2.77 (0.0056)

Results of the random-effects meta-analysis comparing withdrawal-induced anxiety and depression. The table shows pooled standardized mean differences (SMD) with 95% confidence intervals, prediction intervals, and test statistics (z, p), as well as the estimated difference between domains.

Across withdrawal models, DOR agonists improved affective outcomes. For withdrawal-induced anxiety, the random-effects model (REML; $k = 5$) showed a large pooled effect ($g = 1.85$, 95% CI [1.08, 2.61], $z = 4.75$, $p < .0001$) with low heterogeneity ($\tau^2 = 0.16$; $I^2 = 20.74\%$; $Q(4) = 4.91$, $p = .297$) and a 95% prediction interval of [0.76, 2.93] (see figure 13 & table 9). For withdrawal-induced depression, the random-effects model ($k = 4$) indicated a moderate pooled effect ($g = 0.76$, 95% CI [0.14, 1.37], $z = 2.39$, $p = .017$) with low heterogeneity ($\tau^2 = 0.07$; $I^2 = 18.15\%$; $Q(3) = 3.47$, $p = .324$) and a 95% prediction interval of [-0.06, 1.57] (see figure 14 & table 10). A multivariate random-effects model formally testing domain differences ($k = 9$) showed a significant depression–anxiety contrast (Dep-Anx = -1.51, SE = 0.54, 95% CI [-2.57, -0.44]; $QM(1) = 7.68$, $p = 0.0056$) with non-significant residual heterogeneity ($QE(7) = 8.38$, $p = .300$) (see figure 14 & table 10).

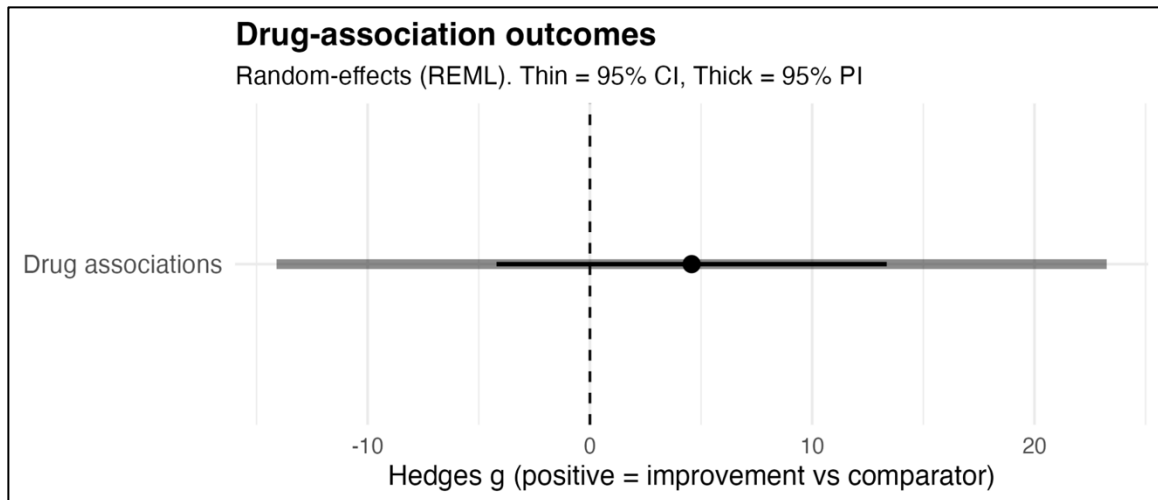


Figure 15. Pooled effect sizes for drug-association outcomes

Summary forest plot of pooled effect size (Hedges' g) for drug-association outcomes under a random-effects model (REML). Thin lines represent 95% confidence intervals, and thick lines represent 95% prediction intervals. Positive values reflect improvement compared to comparator groups.

Table 11. Drug-association outcomes

Drug associations									
Outcome	k	Pooled SMD [95% CI]	SE	z (p)	Tau ²	I ² %	H ²	Q(df), p	Prediction Interval
Other Aim 2	4	4.58 [-4.20, 13.35]	4.478	1.02 (0.3069)	70.701	99.39	163.04	32.45 (3), <0.0001	[-14.10, 23.25]

Results of the random-effects meta-analysis for drug-association outcomes, showing pooled standardized mean differences (SMD) with 95% confidence intervals, standard errors (SE), heterogeneity estimates (τ^2 , I^2 , H^2 , Q-test), and prediction intervals.

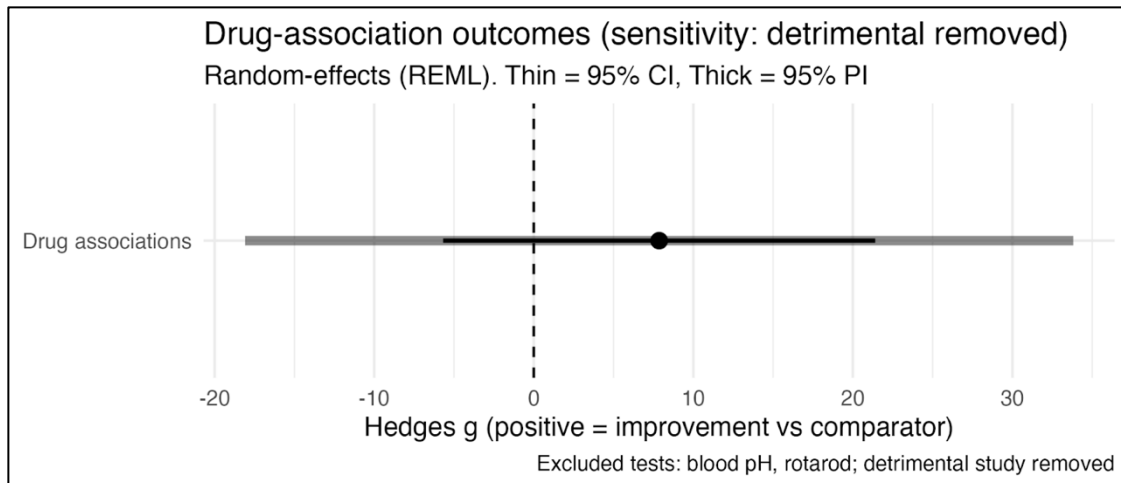


Figure 16. Pooled effect sizes for drug-association post sensitivity analysis

Summary forest plot of pooled effect size (Hedges' g) for drug-association outcomes after sensitivity analysis, excluding blood pH, rotarod, and a detrimental study. Random-effects model (REML) shown with 95% confidence intervals (thin lines) and 95% prediction intervals (thick lines). Positive values reflect improvement compared to comparator groups.

Table 12. Drug-association post sensitivity analysis

Detrimental removed									
Outcome	k	Pooled SMD [95% CI]	SE	z (p)	Tau ²	I ² %	H ²	Q(df), p	Prediction Interval
Other Aim 2	3	7.86 [-5.68, 21.40]	6.908	1.14 (0.2552)	127.597	99.07	107.81	12.08 (2), 0.0024	[-18.09, 33.81]

Results of the random-effects meta-analysis for drug-association outcomes after removal of a detrimental study, showing pooled standardized mean differences (SMD) with 95% confidence intervals, standard errors (SE), heterogeneity estimates (τ^2 , I^2 , H^2 , Q -test), and prediction intervals.

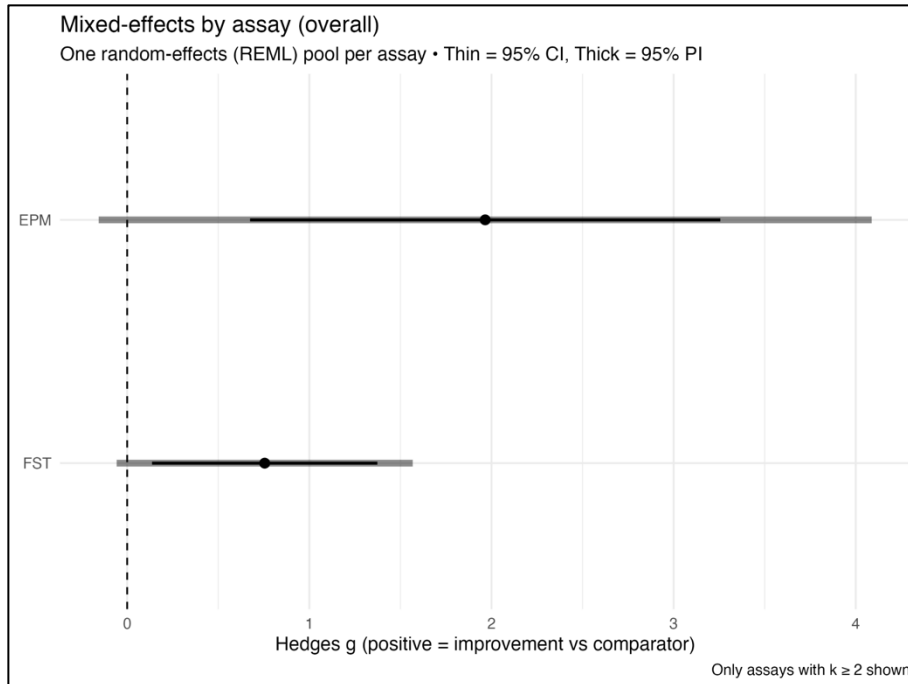


Figure 17. Mixed effects by Assay

Summary forest plot of pooled effect sizes (Hedges' g) for assays with at least two comparisons, including the Elevated Plus Maze (EPM) and Forced Swim Test (FST). Random-effects (REML) estimates are shown with 95% confidence intervals (thin lines) and 95% prediction intervals (thick lines). Positive values indicate improvement compared to comparator groups.

For drug-association outcomes (overall, excluding blood pH and rotarod), the random-effects model (REML; $k = 4$) was imprecise ($g = 4.58$, 95% CI $[-4.20, 13.35]$, $z = 1.02$, $p = .307$) with extreme heterogeneity ($\tau^2 = 70.70$; $I^2 = 99.39\%$; $Q(3) = 32.45$, $p < .0001$) and a wide prediction interval $[-14.10, 23.25]$ (see figure 15 & table 11). A sensitivity model removing the single detrimental study ($k = 3$) remained imprecise ($g = 7.86$, 95% CI $[-5.68, 21.40]$, $z = 1.14$, $p = .255$) with extreme heterogeneity ($\tau^2 = 127.60$; $I^2 = 99.07\%$; $Q(2) = 12.08$, $p = .002$) and prediction interval $[-18.09, 33.81]$ (see figure 16 & table 12). Mixed-effects meta-regression by assay ($k = 9$) did not detect a

significant moderator effect ($QM(3) = 4.10, p = .251$) with modest residual heterogeneity ($\tau^2 = 0.22; I^2 = 36.40\%$) (see figure 17).

Specific Aim 3: Comparison of DOR with MOR and KOR Agonists

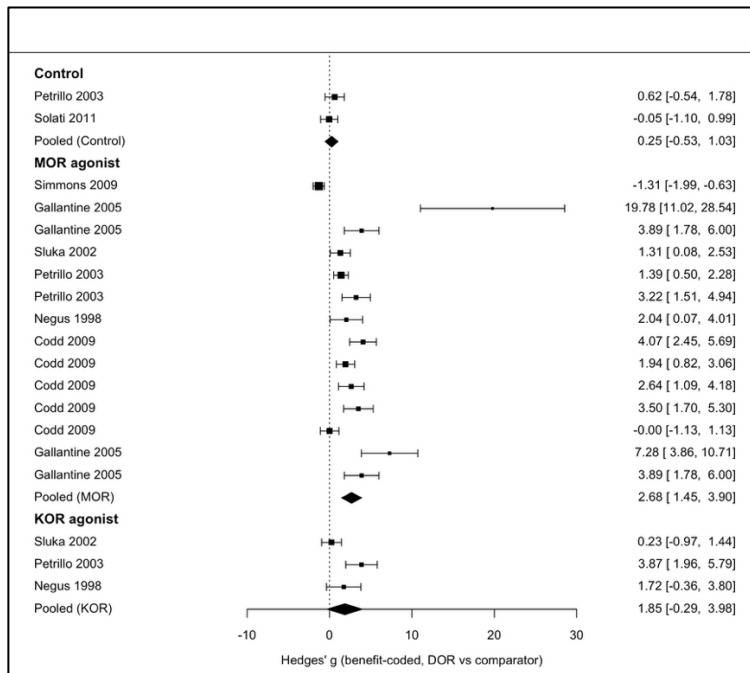


Figure 18. Forest plot comparison of DOR agonist effects to comparators

Forest plot comparing DOR agonist effects to control, MOR agonist, and KOR agonist comparators. Individual study effect sizes (Hedges' g) are shown with 95% confidence intervals (horizontal lines), and diamonds represent pooled effect sizes for each comparator group under a random-effects model. Positive values indicate greater benefit of DOR agonists relative to comparator groups.

Table 13. DOR agonists versus comparator agonists analysis

Dor vs comparator overall									
label	k	g	se	ci.lb	ci.ub	z	p	tau2	I2
DOR vs Control	2	0.25	0.4	-0.53	1.03	0.63	0.53	0	0
DOR vs MOR	14	2.68	0.62	1.45	3.9	4.29	1.79E-05	4.405	89.9
DOR vs KOR	3	1.85	1.09	-0.29	3.98	1.69	0.0904	2.771	78.5

Results of the random-effects meta-analysis comparing DOR agonists to control, MOR agonists, and KOR agonists. Table shows pooled effect sizes (Hedges' g) with 95% confidence intervals, standard errors (SE), test statistics (z, p), heterogeneity estimates (τ^2 , I^2), and number of comparisons (k).

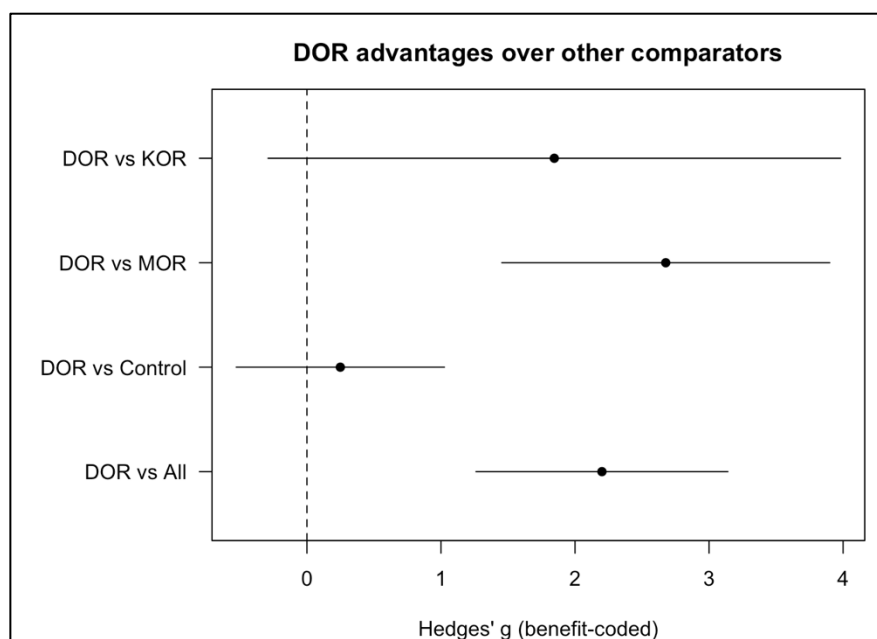


Figure 19. Summary comparator figure

Summary forest plot of pooled effect sizes (Hedges' g) comparing DOR agonists to control, MOR agonists, KOR agonists, and all comparators combined. Points represent standardized mean differences with 95% confidence intervals. Positive values indicate greater benefit of DOR agonists relative to comparator groups.

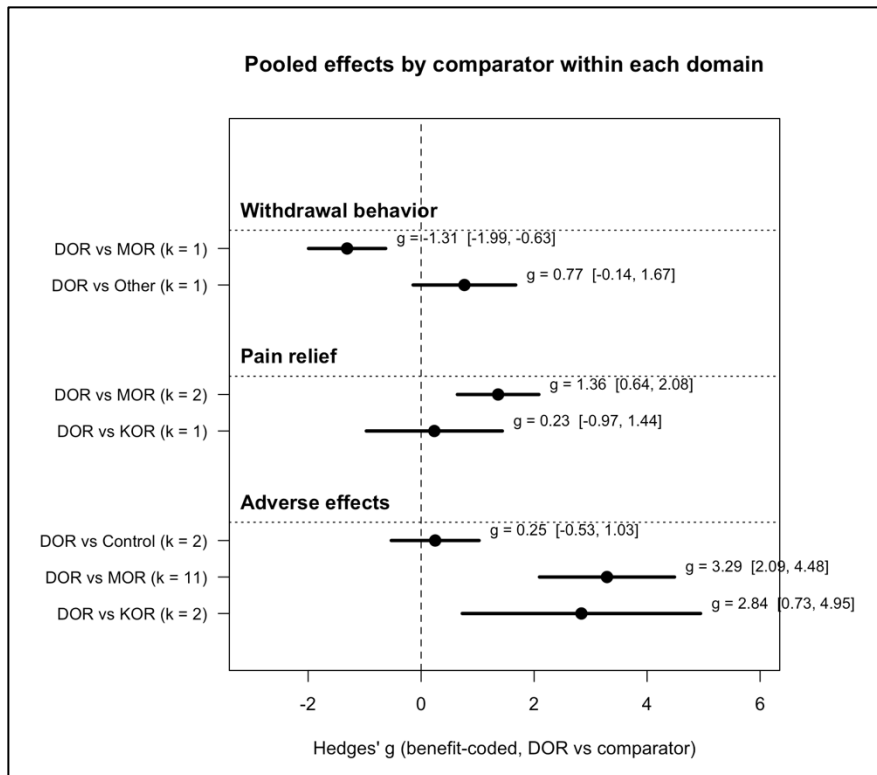


Figure 20. Pooled effects by comparator within each domain

Forest plot of pooled effect sizes (Hedges' g) comparing DOR agonists to control, MOR agonists, KOR agonists, and other comparators across three domains: withdrawal behavior, pain relief, and adverse effects. Points represent standardized mean differences with 95% confidence intervals. Positive values indicate greater benefit of DOR agonists relative to comparator groups.

Table 14. Full model statistics by domain and comparator

Domain by comparator						
Domain	Comparator	k	g	ci.lb	ci.ub	I ²
Adverse effects	Control	2	0.25	-0.53	1.03	0
Adverse effects	MOR	11	3.29	2.09	4.48	78.7
Adverse effects	KOR	2	2.84	0.73	4.95	55
Pain relief	MOR	2	1.36	0.64	2.08	0
Pain relief	KOR	1	0.23	-0.97	1.44	0
Withdrawal behavior	MOR	1	-1.31	-1.99	-0.63	0
Withdrawal behavior	Other	1	0.77	-0.14	1.67	0

Results of the random-effects meta-analysis by domain and comparator. Table shows pooled effect sizes (Hedges' g) with 95% confidence intervals, number of comparisons (k), and heterogeneity (I^2) across domains of adverse effects, pain relief, and withdrawal behavior.

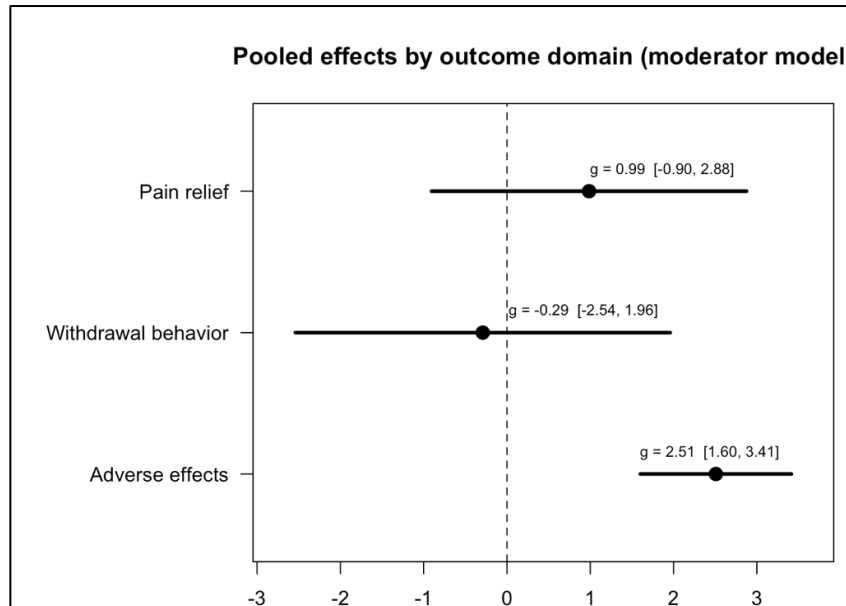


Figure 21. Pooled effects by outcome domain (moderator model)

Forest plot summarizing standardized mean differences (Hedges' g) across domains of pain relief, withdrawal behavior, and adverse effects. Points represent pooled effect estimates, horizontal lines indicate 95% confidence intervals, and effects are coded such that positive values reflect relative benefit of DOR agonists compared to comparators.

Across $k = 19$ comparisons, DOR agonists were evaluated against control, MOR, and KOR comparators (see figure 18 & table 13). Relative to controls ($k = 2$), DOR agonists showed no significant effect (Hedges' $g = 0.25$, 95% CI -0.53 to 1.03 , $p = .53$; $I^2 = 0\%$). Compared to MOR agonists ($k = 14$), DOR agonists produced a large and significant benefit ($g = 2.68$, 95% CI 1.45 – 3.90 , $p < .0001$; $I^2 = 89.9\%$), while comparisons with KOR agonists ($k = 3$) yielded a large but non-significant effect ($g = 1.85$, 95% CI -0.29 – 3.98 , $p = .09$; $I^2 = 78.5\%$). A mixed-effects model including comparator type did not detect significant moderator effects ($QM(2) = 2.47$, $p = .29$; (see figure 19).

By outcome domain, DOR agonists showed a large pooled benefit on adverse effects ($k = 16$, $g = 2.58$, 95% CI 1.57 – 3.58 , $p < .0001$; $I^2 = 83.4\%$), with subgroup estimates indicating stronger effects versus MOR ($k = 11$, $g = 3.29$, 95% CI 2.09 – 4.48) and KOR ($k = 2$, $g = 2.84$, 95% CI 0.73 – 4.95) than control ($k = 2$, $g = 0.25$, 95% CI -0.53 – 1.03 ; see figure 20 & table 14). In withdrawal behavior outcomes ($k = 2$), DOR agonists did not show significant effects ($g = -0.29$, 95% CI -2.33 – 1.74 ; $I^2 = 92.2\%$), and single-study subgroup estimates were inconsistent across comparator types. In pain relief outcomes ($k = 3$), DOR agonists significantly improved analgesia ($g = 1.05$, 95% CI 0.35 – 1.74 , $p = .003$; $I^2 = 18.3\%$), with effects observed versus MOR ($k = 2$, $g = 1.36$, 95% CI 0.64 – 2.08) and a non-significant result versus KOR ($k = 1$, $g = 0.23$, 95% CI -0.97 – 1.44). A mixed-effects model including domain as a moderator indicated significant differences across outcome types ($QM(2) = 6.26$, $p = .044$; (see figure 21).

Results Summary

Across all analyses, DOR agonists demonstrated consistent behavioral benefits in preclinical models. Specific Aim 1 showed robust anxiolytic (Hedges' $g = 1.40$; see Figure 1 & Table 1) and antidepressant-like effects ($g = 1.72$; see Figure 6 & Table 5), with subgroup analyses indicating that these improvements were broadly observed across compounds, behavioral assays, and dosing regimens (Figures 2–5, Tables 2-4; Figures 7-10, Tables 6-9). Specific Aim 2 indicated that DOR agonists attenuate withdrawal-induced anxiety and, to a lesser extent, depression (see Figures 13–14 & Tables 10-11), though results for drug-association outcomes were heterogeneous and imprecise (Figures 15-16 & Tables 12-13). Specific Aim 3 highlighted that, compared with MOR and KOR agonists, DOR agonists generally conferred larger advantages in reducing adverse effects and enhancing analgesia (Figures 18-21; Tables 14-15), while effects on withdrawal behavior were less consistent. Together, these findings provide converging evidence that DOR agonists produce significant improvements in affective and withdrawal-related outcomes, with a distinct therapeutic profile relative to other opioid receptor classes.

Chapter IV

Discussion

The present meta-analysis evaluated the therapeutic potential of delta opioid receptor (DOR) agonists across preclinical models of affective behavior and substance withdrawal. Three specific aims guided this investigation: (1) to determine whether DOR agonists reduce anxiety- and depression-like behaviors, (2) to evaluate their effects in models of withdrawal from opioids and other substances, and (3) to compare their efficacy and side-effect profile relative to mu opioid receptor (MOR) and kappa opioid receptor (KOR) agonists. By integrating results across 42 controlled comparisons, this study provides a comprehensive synthesis of the behavioral and neurobiological impact of DOR agonists. The following discussion interprets these findings in the context of existing literature, highlights translational implications for opioid use disorder (OUD) treatment, and identifies key limitations and directions for future research.

Interpretation of Specific Aim 1 Results

Across assays and compounds, DOR agonists produced large and significant reductions in both anxiety and depression behaviors, highlighting their robust affective benefits in preclinical models. The magnitude of these effects ($g=1.40$ for anxiety and $g=1.72$ for depression) is noteworthy, particularly given the consistency across diverse paradigms such as the elevated plus maze, forced swim test, and contextual fear conditioning. These results align with prior reports that highlight the anxiolytic and

antidepressant potential of DOR agonists, reinforcing their role in modulating limbic and stress-related circuitry (Pasternak, 2011). At the same time, subgroup analyses revealed some variability by agonist type and assay, suggesting that the therapeutic profile of DOR activation may depend on drug-specific properties and the behavioral assay used.

Subgroup analyses provided a further look into the sources of variability underlying these effects. Among individual compounds, AZD2327 and KNT-127 demonstrated the strongest and most consistent anxiolytic benefits, whereas enkephalins showed more modest effects. This highlights potential differences in pharmacokinetics or receptor selectivity. Similarly, there were assay-level differences, particularly robust effects in retention freezing and fear conditioning tasks, while open-field test results were less reliable. Dose-response analyses added a layer of complexity, with systemic administration showing a significant negative association with increasing dose, suggesting an inverted U-shaped relationship that has been commonly observed with DOR agonists. In contrast, intra-BLA administration did not reveal a clear dose-response relationship. These findings indicate that both compound choice and administration parameters critically shape the affective outcomes of DOR activation.

Taken together, the significant pooled effects across assays and compounds suggest that DOR agonists can reliably improve depression- and anxiety-like behaviors, though the strength of these effects does depend on pharmacological and methodological factors. It is important to note that the observed anxiolytic and antidepressant-like properties extended beyond a single assay or compound, pointing to a more generalizable role of DOR activation in regulating mood-related behaviors. These findings are particularly relevant for translational research, as comorbid anxiety and depression

represent significant barriers to recovery in opioid use disorder. By demonstrating a consistent efficacy across preclinical models, these findings strengthen the rationale for further development of DOR-targeted compounds as potential therapeutics for affective symptoms associated with withdrawal and relapse risk.

Interpretation of Specific Aim 2 Results

DOR agonists produced robust effects in withdrawal-related contexts, particularly for anxiety. The meta-analysis showed a significant and consistent improvement in withdrawal-induced anxiety ($g=1.85$), while the impact of withdrawal-induced depression was a bit more modest ($g=0.76$). A formal contrast confirmed that anxiolytic benefits were significantly greater than antidepressant effects, and drug-association outcomes were marked by extreme heterogeneity, which limited more concrete conclusions.

The differential impact of DOR agonists on anxiety versus expression is consistent with neurobiological evidence that withdrawal states preferentially activate stress and fear-related circuits, such as the extended amygdala, where DOR signaling plays a regulatory role (Kosten & George, 2002). The comparatively weaker effects on depression-like behaviors may reflect differences in assay sensitivity or in the chronicity of withdrawal models. Meanwhile, the instability of drug-association outcomes suggests that these paradigms may be more variable and less reliable indicators of DOR agonist efficacy, possibly due to task-specific confounds or the small number of available studies.

Together, these findings indicate that DOR agonists may be particularly effective for alleviating acute withdrawal-induced anxiety, a core feature of opioid and other substance withdrawal syndromes that contribute strongly to relapse risk. The moderate benefits observed for depressive symptoms, although less consistent, further suggest that

DOR agonists could support stabilizing mood during withdrawal, though additional studies are needed. Addressing the variability in drug-association assays is an important step to take in clarifying the broader impact of DOR activation on withdrawal-related behaviors.

Interpretation of Specific Aim 3 Results

When compared directly to other opioid receptor agonists, DOR agonists demonstrated a distinct pattern of effects. Relative to controls, they did not produce significant differences; however, when compared to MOR agonists, DOR agonists showed a considerable advantage ($g = 2.68$). Against KOR agonists, effects were large in magnitude but did not reach statistical significance. At the domain level, DOR agonists provided substantial benefits for adverse effect outcomes and significant improvements in analgesia, though effects on withdrawal behaviors were inconsistent.

These results reinforce the idea that DOR agonists occupy a unique therapeutic space among opioid receptor ligands. MOR agonists remain the gold standard for analgesia but are limited by high abuse liability, tolerance, and adverse effects such as respiratory depression (Adams et al., 2021; Dripps & Jutkiewicz, 2018). KOR agonists, while less rewarding, are often associated with dysphoria and cognitive disruption. By contrast, DOR agonists in this analysis combined affective benefits with analgesic efficacy and a comparatively favorable adverse effect profile. The lack of significant findings in some domains, particularly withdrawal behavior, likely reflects the small number of available studies and variability in comparator conditions.

These findings suggest that DOR agonists offer therapeutic advantages over traditional MOR- and KOR-targeted treatments by providing analgesia and affective

improvements with fewer liabilities. This comparative benefit highlights DOR activation as a promising strategy for developing next-generation therapeutics for pain and substance use disorders. However, the limited sample size and high heterogeneity in some comparisons underscore the need for direct preclinical and clinical studies to establish the relative benefits and risks of DOR agonists more clearly.

Summary Interpretation of Results

Across all three aims, there is a consistent trend that shows DOR agonists reliably reduce anxiety- and depression-like behaviors, show meaningful benefits in withdrawal-related contexts, particularly anxiety, and demonstrate advantages over MOR and KOR agonists in several outcome domains. While variability was observed across compounds, assays, and dosing paradigms, the overall pattern supports the conclusion that DOR activation confers robust affective and analgesic benefits with a more favorable adverse effect profile. These findings highlight DOR agonists as promising candidates for addressing both the emotional and physical dimensions of opioid withdrawal, while also suggesting a broader therapeutic role in affective disorders.

Study Limitations

Several limitations of this meta-analysis should be acknowledged. First, moderate to high heterogeneity was observed in several models, indicating variability in study designs, compounds tested, and behavioral assays. This heterogeneity reduces the precision of pooled estimates, suggesting that the effects of DOR agonists may be context dependent. Second, the number of studies available for certain compounds (e.g., AZD2327, enkephalins) and for some behavioral paradigms (e.g., defensive burying) was

limited, which reduced the strength of subgroup comparisons and increased the risk of unstable estimates. Third, most included studies used acute or short-term dosing, which may not fully capture the chronic or relapsing nature of substance use disorders. Fourth, potential publication bias cannot be ruled out, as null and negative results may be underrepresented in the literature. Finally, while this review synthesized results from a wide range of preclinical models, generalization to humans remains uncertain, and translational validity depends on future clinical testing.

Study Implications

Future research should address the gaps and uncertainties identified in this analysis. Standardizing behavioral assays and outcome measures across laboratories would reduce heterogeneity and improve comparability. Expanding studies to include chronic dosing and relapse paradigms is essential to capture the long-term dynamics of substance use and withdrawal. Direct comparisons of DOR agonists with MOR and KOR agonists are also necessary to clarify their relative efficacy and safety, particularly in terms of analgesic and adverse effect profiles. Additionally, greater attention to dose-response relationships and administration routes will help refine our understanding of the conditions under which DOR activation is most effective. Finally, translational studies, including biomarker-based approaches and early-phase clinical trials, will be critical for determining whether the promising preclinical findings extend to human populations and can inform novel treatment strategies for opioid use disorder and comorbid affective symptoms.

Conclusion

This meta-analysis provides strong evidence that DOR agonists produce robust improvements in affective behaviors, particularly anxiety, and offer meaningful benefits in withdrawal-related contexts. Compared to MOR and KOR agonists, DOR agonists showed distinct advantages, combining affective and analgesic efficacy with a potentially more favorable adverse effect profile. While heterogeneity, small sample sizes, and limited chronic models highlight the need for further research, the overall findings point to DOR activation as a promising pharmacological strategy for addressing the emotional and physical challenges of opioid withdrawal. By integrating results across diverse preclinical paradigms, this study establishes a foundation for future translational work aimed at developing DOR-targeted therapies for substance use disorders and related affective conditions.

Appendix

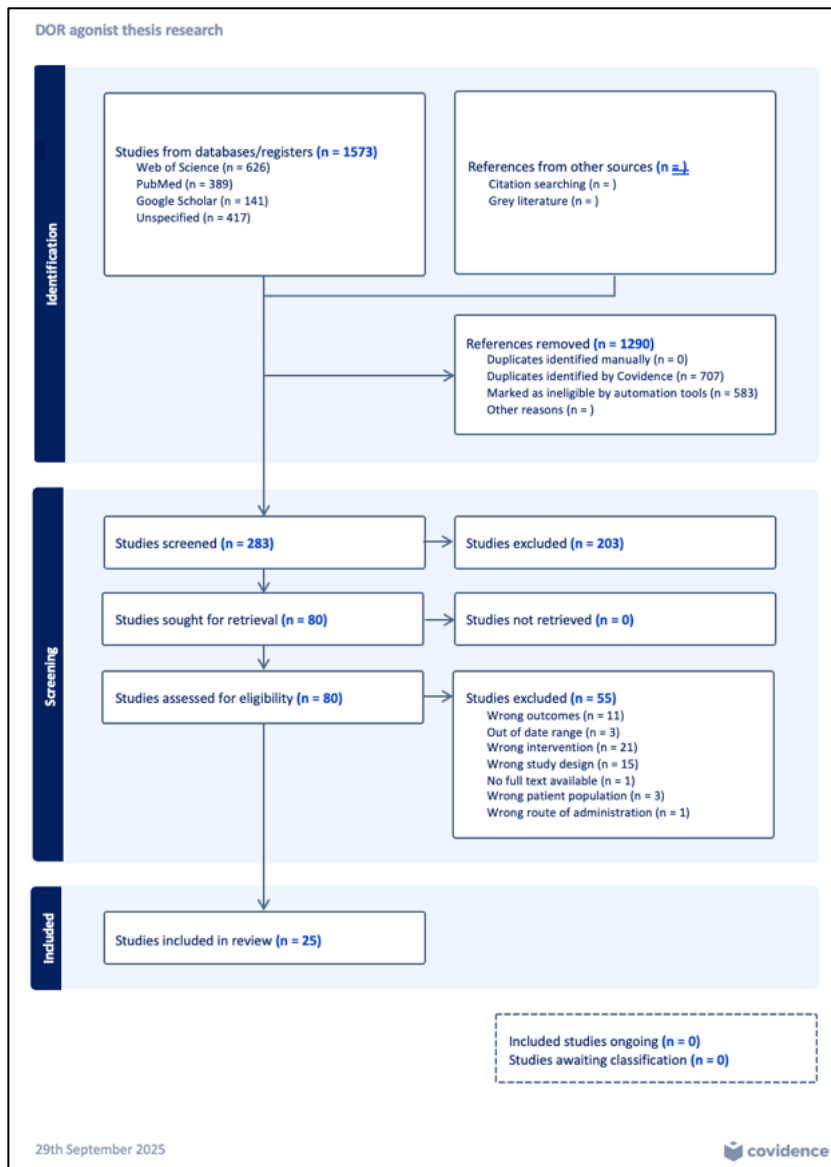


Figure 22. Covidence PRISMA flow chart

Flow diagram illustrating the systematic review process conducted in Covidence. Records identified through database searching and other sources were screened for eligibility, with duplicates removed prior to screening. Studies were excluded at the title/abstract and full-text review stages according to predefined inclusion and exclusion criteria. The final number of studies included in the meta-analysis is shown at the bottom of the diagram

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