The Effect of Opioid Blockade on Pain in Healthy Humans

A Systematic Review and Meta-Analysis

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The Effect of Opioid Blockade on Pain in Healthy Humans: A Systematic Review and Meta-Analysis

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Abstract

Background: Although several observations indicate that endogenous opioids regulate pain and support analgesia, pharmacological blockade of opioid activity does not consistently worsen pain in experiments. Insufficient elimination of opioid responses is one possible reason for the discrepancy between studies, but this has not been investigated. It is also unclear whether endogenous opioids selectively modulate pain under specific conditions. *Objective:* To establish the impact of complete opioid blockade on pain outcomes and clarify in which instances pain regulation is opioid dependent, we conducted a systematic review and meta-analysis of experimental pain studies in healthy humans. *Methods:* We searched Web of Science, Scopus, PubMed and EMBASE on October 7, 2020. Eligible studies were at least double-blind, randomized, and placebo-controlled, used physiological pain interventions, and administered a centrally active μ-opioid antagonist. Quality assessments of each study and funnel plots were used to evaluate risk of bias. To compare treatment effects on pain responses, we calculated Hedges' *g* for individual outcomes and used a three-level random effects meta-analysis to estimate the summary effect. This work was an independent project. Data collection was performed by the thesis author and the thesis presents preliminary results. *Results:* A total of 60 studies (n = 2011) were included in the analyses. Most studies measured pain intensity and used the antagonist drug naloxone and thermal pain stimulation. Overall, experimental pain responses were significantly higher with full μ-opioid blockade compared to conditions where participants received a pharmacologically inert substance (Hedges' *g* [95% CI] = 0.23 [0.10, 0.36]), but there was considerable heterogeneity present (I^2 = 77.2%). While pain intensity, tolerance and threshold worsened with opioid blockade, pain unpleasantness was not significantly altered. There were no substantial differences between brief and prolonged pain stimuli. *Conclusion:* Complete blockade of endogenous opioids appears to produce a small increase in pain sensitivity in experimental pain models in healthy humans. However, due to reporting bias in the literature, the size of this effect may be overstated. Further investigation is needed to assess the reasons for the observed variability.

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1 The Effect of Opioid Antagonism on Pain in Healthy Humans: A Systematic Review and Meta-Analysis

Endogenous opioids, notably endorphins, are commonly regarded as the body's own pain-killers (Michigan Medicine - University of Michigan, 2021). These peptides were discovered after receptors for powerful opioid analgesics, such as morphine, had been localized in the brain (Hughes et al., 1975; Pert & Snyder, 1973). The name "endorphin" is a portmanteau, as endorphins were found to be endogenous ligands for the receptors on which morphine acts (Cox, 2020). Today, a whole anatomical system of opioid receptors and ligands has been identified. A large body of evidence appears to support the hypothesis that endogenous opioids possess the same pain-relieving properties as exogenous opioids. However, as Watkins and Mayer noted as early as 1982, "the demonstration of a well-defined neural system capable of potently blocking pain transmission suggests, but by no means proves, that the function of this system is to modulate the perceived intensity of noxious stimuli" (p. 1185).

In an effort to establish the function of endogenous pain relief, a multitude of experimental pain studies have been conducted over the past several decades. These studies typically attempt to block the effects of endogenous opioids (through the use of antagonist drugs) to infer their role in human pain perception. Interestingly, inhibiting the activity of endogenous opioids has not consistently been demonstrated to produce deficits in the body's capability to reduce pain (Werner et al., 2015). The purpose of this meta-analysis is to systematically review and synthesize evidence from available research on this topic, with the intent of clarifying whether there is currently a valid scientific basis for asserting that endogenous opioids play an important part in relieving pain in healthy humans. Thus, the meta-analysis will address the following question: Does pharmacological blockade of endogenous opioid signaling lead to increased pain compared to pharmacologically inert treatments in healthy humans, and if so, under which conditions?

1.1 The Experience of Pain

1.1.1 Definition and Terminology

Pain is defined by the International Association for the Study of Pain (IASP) as "an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage." This definition was recently updated to account more clearly for the inherent subjectivity of pain perception (pain as a personal experience), and to

clarify that a lack of verbal communication regarding the experience of pain does not negate its presence (Raja et al., 2020).

The IASP emphasizes that nociception does not necessarily equate pain, stating that pain "cannot be inferred solely from activity in sensory neurons" (Raja et al., 2020). However, afferent input from specialized sensory neurons tasked with detecting damaging or potentially damaging stimuli, i.e., nociceptors, is a central part of adaptive pain perception. In essence, nociceptive pain functions as an internal alarm system designed to signal potential threats and damage to the organism. It represents the "first line of defense" against environmental stimuli that threaten to disrupt the body's integrity, by sensing such stimuli so that the individual can act accordingly to avoid injury (Woolf & Ma, 2007). In that way, pain can also be seen as an emotion that motivates the individual to modify behavior in order to maintain homeostasis. Pain then comprises both "a sensation and a behavioral drive with reflexive autonomic adjustments" (e.g., withdrawal) and an affective motivation (Craig, 2003, p. 304).

Traditionally, pain has been divided into two categories: Nociceptive and neuropathic pain. Nociceptive pain is defined by the IASP as "pain that arises from actual or threatened damage to non-neural tissue and is due to the activation of nociceptors", while neuropathic pain is defined as "pain caused by a lesion or disease of the somatosensory nervous system" (*IASP Terminology*, n.d.). As such, the latter is usually chronic. Recently, nociplastic pain was added as a third category to describe chronic pain conditions that are characterized by evidence of altered nociceptive processing (Kosek et al., 2016). It is defined as "pain that arises from altered nociception despite no clear evidence of actual or threatened tissue damage causing the activation of peripheral nociceptors or evidence for disease or lesion of the somatosensory system causing the pain" (*IASP Terminology*, n.d.).

1.1.2 Pain Anatomy and Physiology

Generally, acute pain arises from nociception, i.e., "the neural process of encoding and processing noxious stimuli" (Dubin & Patapoutian, 2010, p. 3761). Such stimuli are detected by nociceptors, which are free nerve endings that normally have a high activation threshold, and only respond to potential or actual tissue damage (Voscopoulos & Lema, 2010; Woolf & Ma, 2007). Tissue damage lowers this threshold. This sensitization may generate hyperalgesia (increased pain from noxious stimuli or lowered pain threshold), allodynia (pain in response to normally non-noxious stimulation), and potential spontaneous (i.e., not caused by external stimuli) nociceptive activation (Dahl & Rinvik, 2010; Millan, 1999). In chronic pain, these phenomena can persist after the original tissue injury has been healed due to peripheral and

central sensitization, representing a form of neuronal plasticity (Sandkühler, 2013). In some forms of chronic pain, however, such as neuropathic and cancer pain, allodynia or spontaneous pain may arise from irreparable nerve damage or structural reorganization of nerves, respectively, in combination with central plasticity (Devor, 2013; Mantyh, 2013). In contrast to acute pain, chronic pain is of longer duration and represents maladaptive pathological changes in the nociceptive system (Grichnik & Ferrante, 1991; Woolf & Ma, 2007). Conversely, acute pain signals injury and prompts the individual to protect damaged areas to allow for healing (i.e., it serves a protective function). Acute pain may provoke reflexive motor withdrawal and a 'flight' response to improve chances of successfully removing oneself from a harmful situation or stimulus (Millan, 1999). In addition to eliciting behavioral motivations, pain teaches individuals how to predict dangerous situations in the future (Navratilova & Porreca, 2014).

Nociceptors can be found in skin, muscle, joints, and visceral organs, and are present with higher density in some areas than others (Voscopoulos & Lema, 2010). They can be activated by chemical, mechanical, thermal, and electrical noxious stimuli (Dahl & Rinvik, 2010; Mouraux et al., 2010). Some are also activated by ischemia or hypoxia and increases in pressure (Millan, 1999). Nociceptors are either specific to a certain stimulus type or, most commonly, polymodal (Voscopoulos & Lema, 2010). Once activated, nociceptors project to the dorsal horn of the spinal cord via afferent fibers, where they synapse with central neurons that project via the medulla, mesencephalon and thalamus to the cortex (Dubin & Patapoutian, 2010; McMahon, 2013). The somatosensory cortex is thought to play a role in the sensory-discriminative aspect of pain, while the anterior cingulate cortex drives affective-cognitive aspects (Millan, 1999). Nociceptor axons are either thinly myelinated A-δ fibers or unmyelinated C-fibers. A-δ fibers have a larger diameter, which in conjunction with the myelin sheath allows them to conduct pain faster than the more numerous C-fibers – at approximately 5-30 m/s compared to 0,4-1,4 m/s (Craig, 2003). Thus, A-δ fibers produce the perception of sharp, momentary and easily localized pain (*first pain*), while neuronal transmission via C-fibers gives rise to burning, slow and diffuse pain (*second pain;* Craig, 2003; National Research Council, 2009). Muscular pain, however, typically presents as aching, cramp-like and dull even initially, despite being meditated by both A-δ fibers and C-fibers (Millan, 1999).

In addition to transmitting pain signals more slowly to the spinal cord, C-fibers have higher activation thresholds in healthy (non-sensitized) tissue (Voscopoulos & Lema, 2010). Consequently, less intense noxious stimuli produce the initial feeling of sharp pain through

activation of A-δ fibers, while higher stimulus intensity is required to generate the subsequent dull, longer lasting pain produced by C-fiber activation. It has been shown that by selectively blocking A-δ fibers or C-fibers, first or second pain sensations can be extinguished (Purves et al., 2001). 'Silent' C-fiber nociceptors also exist – these receptors are normally unresponsive to noxious stimuli but become sensitized and easily activated in the presence of inflammatory mediators that are released as a consequence of tissue injury. 'Silent' nociceptors are associated with heightened spontaneous activity (National Research Council, 2009).

Two primary groups of A-δ nociceptors exist; these can be identified by a difference in responsiveness to noxious heat and how they are influenced by tissue damage (Julius & Basbaum, 2001). Generally, A-δ nociceptors respond to heat or mechanical stimulation (or a combination of the two), but some also respond to noxious cold (Dubin & Patapoutian, 2010). C-fiber nociceptors respond to chemical stimuli, such as capsaicin, in addition to noxious thermal and mechanical stimuli. The majority are polymodal and respond to all three modalities. Compared to A-δ nociceptors, C-fiber nociceptors have more widely distributed branches in skin, which increases the size of their receptive field and hinders clear localization of noxious stimuli (Dubin & Patapoutian, 2010).

1.1.3 Pain as a Non-Linear Experience

In sum, different nociceptors encode and transmit different types of noxious stimuli, which they detect above varying sensory thresholds and transmit to the spinal cord at dissimilar firing rates (Voscopoulos & Lema, 2010). It follows that pain is not a uniform response to any kind of nociceptive activation. Rather, the quality and intensity of perceived pain is influenced by a multitude of factors, with nociceptor activation representing only one moderating variable. Importantly, many contextual and psychological factors, such as attention, expectations, contextual learning cues and concurrent pain, also play a role in shaping pain perception (Heinricher & Fields, 2013). Whereas nociceptor activation determines the sensory characteristics of pain, the resulting pain experience is influenced by context, including affective, emotional and cognitive factors (Navratilova & Porreca, 2014). As a result, the same sensory input can produce vastly different experiences of pain within an individual (Bingel & Tracey, 2008).

Psychological factors such as mood and emotional state are well known determinants in shaping the pain experience, as well as individual abilities to cope with pain (Tracey & Mantyh, 2007). Anxiety related to pain magnifies perceived pain intensity and lowers pain thresholds, while fear unrelated to pain, perhaps analogously to threatening situations, increases tolerance to pain and decreases reactivity to painful stimuli (McCracken et al., 1992;

Ploghaus et al., 2001; Rhudy & Meagher, 2000). Negative mood-induction can also increase pain perception (Bair et al., 2003; Villemure & Bushnell, 2002). Pain catastrophizing is another psychological construct that is associated with increased pain sensitivity (Tracy, 2017). In contrast, reward cues typically reduce pain perception (Dum & Herz, 1984; Leknes & Tracey, 2008; Navratilova & Porreca, 2014).

Pain perception is also influenced by the physiological condition of the body, determined by factors such as sleep, temperature, blood pressure and hormone levels (Craig, 2003; Schrimpf et al., 2015). Sleep deprivation has been found to induce hyperalgesia across multiple nociceptive modalities, an effect that may be due to a transient disturbance of the descending inhibitory control system (Stefan Lautenbacher et al., 2006; Schuh-Hofer et al., 2013). In women, variations in estrogen levels are associated with μ-opioid neurotransmission, and these variations correlate with pain ratings (Smith et al., 2006).

Moreover, expectation is a powerful determinant of pain. Multiple studies have provided evidence that positive expectation of pain relief can produce analgesic effects in humans by suppressing activity in several pain processing regions and engaging brainstem mechanisms that support descending inhibition of pain (Tracey & Mantyh, 2007). Paralleling opioid analgesia, placebo analgesia is associated with endogenous opioid activity in μ-opioid receptors in several cortical and subcortical brain regions (Scott et al., 2008; Zubieta et al., 2005). Correspondingly, blockade of endogenous opioids can reduce placebo responses (Amanzio & Benedetti, 1999; Benedetti, 1996; Eippert et al., 2009; Levine et al., 1978). Conditioning through the combination of contextual treatment cues and the experience of pain relief (e.g., from analgesic drugs) can also influence placebo effects (Wager & Fields, 2013).

Fields' motivation-decision model of pain suggests that pain will be downregulated whenever a competing reward or punisher is more important for survival, and that both rewards and threats can inhibit upcoming nociceptive information via the descending inhibitory system of the brainstem (Fields, 2004). For instance, the use of a concurrent stressor during pain induction can induce hypoalgesia, and some evidence suggests that stress-induced analgesia is mediated in part by endogenous opioids (Bodnar et al., 1980; Butler & Finn, 2009). Similarly, pain relief is rewarding, and thus analgesic expectations can activate motivational, opioid-mediated circuits (Leknes & Tracey, 2008; Navratilova & Porreca, 2014).

Altogether, the many factors involved in influencing the perception of pain point to a non-linear relationship between nociceptor activation and the pain experience (Navratilova & Porreca, 2014). Pain as a concept is not purely the product of sensory input, but is learned by

individuals through their life experiences (Raja et al., 2020). Recently, it has been proposed that pain might be best thought of in terms of predictive processing, i.e., that the brain uses a probabilistic model akin to Bayes theorem to generate perception. This perspective suggests that pain sensations are based on continuously updated hypotheses about the state of the world and the body, rather than an "objective" sensory reality (Ongaro & Kaptchuk, 2019). By constantly generating and revising these hypotheses, the brain attempts to optimize its representation of the world according to probability in order to minimize misperceptions (Hohwy, 2013). From the perspective of predictive processing, "we feel pain because we predict that we are in pain, based on an integration of sensory inputs, prior experience, and contextual cues" (Ongaro & Kaptchuk, 2019, p. 2).

1.1.4 Modulation of Pain in Ascending and Descending Pathways

Modulation of the pain experience occurs in both ascending and descending pathways. In bottom-up, afferent nociceptive signaling, acute pain registered by dorsal horn nociceptors is transmitted to the brain via two major pathways, although several less prominent ones exist. The spinothalamic tract carries the bulk of the signals via the thalamus to the somatosensory cortex and other areas of the cortex, while the spinoparabrachial pathway ascends through the parabrachial area in the brain stem to the ventral medial nucleus of the hypothalamus and the central nucleus of the amygdala (Hunt & Mantyh, 2001). The spinothalamic pathway provides information about the location and intensity of the painful stimulus, while the spinoparabrachial pathway contributes to the affective component of the pain experience (Basbaum et al., 2009). However, many cortical areas are involved in the processing of different aspects of pain; the areas that are most commonly activated include the primary and secondary somatosensory cortex, the insular cortex, the anterior cingulate cortex, and the prefrontal cortex (Apkarian et al., 2005).

While all nociceptors project to the dorsal horn of the spinal cord and the brainstem, nociceptive afferents connect differently to central circuits. A-δ nociceptors and C-fiber nociceptors project to laminae I and V and laminae I and II of the dorsal horn, respectively (Dubin & Patapoutian, 2010). In the dorsal horn, A-δ nociceptors synapse directly onto spinothalamic neurons, while C-fiber nociceptors mainly synapse with local interneurons before entering the spinothalamic tract (Dahl & Rinvik, 2010). This allows for varying degrees of modulation of nociceptive signals in ascending pathways, as interneurons in the dorsal horn can have inhibitory or excitatory effects on the transmission of these signals (Dubin & Patapoutian, 2010). Prior to reaching the spinal cord, afferent nociceptive signals can be modulated through local effects on nociceptor nerve endings by a wide range of

mediating substances, including endogenous opioids, glutamate, prostaglandins, noradrenaline, ATP and other mediators (Pace et al., 2018). Opioid peptides are also synthesized by in the spinal cord by dorsal horn interneurons (Julius & Basbaum, 2001).

Modulation of nociceptive signaling largely occurs through descending inhibitory and facilitating pathways (Dubin & Patapoutian, 2010). Descending modulatory control is mainly carried out by a specific network, notably comprised by the periaqueductal gray (PAG) and rostral ventromedial medulla (RVM). The PAG-RVM system has both anti-nociceptive and pro-nociceptive effects on pain signaling in the dorsal horn, either inhibiting or enhancing nociceptive transmission through RVM output (Heinricher & Fields, 2013). Through connections to the PAG and RVM, ascending pain pathways can activate the descending feedback system, allowing for regulation of afferent pain signals in the spinal cord (Basbaum et al., 2009). In this way, the PAG-RVM system can integrate bottom-up noxious information and top-down signaling to modulate the pain experience. Many pharmacological agents are involved in pain modulation in these brainstem areas; however, the PAG and RVM have been deemed central to opioid analgesia in particular (Heinricher & Fields, 2013). Cortical and subcortical areas, especially the anterior cingulate cortex, amygdala, hypothalamus, and prefrontal cortex transmit direct and indirect input to descending inhibitory pathways, thereby modulating afferent pain transmission in the dorsal horn (Fields, 2004; Heinricher et al., 2009; Leknes & Tracey, 2008). Thus, top-down modulation of pain contributes to shaping the pain experience by activating descending pathways that can either inhibit or facilitate nociceptive transmission (Navratilova & Porreca, 2014).

From a functional and evolutionary perspective, the ability to regulate nociceptive transmission and thereby perceived pain is very important. Pain is, in essence, a warning signal that serves to protect the individual from stimuli and situations that may cause functional impairment or shorten the life span. Thus, it is clearly adaptive to learn which situations cause pain and strive to avoid those. Central modulation of pain contributes to reinforce these associations and promote adaptive behavioral patterns. For instance, the anticipation of and anxiety about pain is known to exacerbate the experienced pain – a highly adaptive response in terms of harm avoidance (Tracey & Mantyh, 2007). Furthermore, threatening situations reliably produce antinociception in animals and humans (Heinricher & Fields, 2013). Tissue damage, on the other hand, is associated with facilitation of nociceptive signaling in descending pathways (Bingel & Tracey, 2008). Both modulatory responses are in general beneficial, as they allow for protection of the individual through fight or flight and promotion of healing, respectively. The bidirectional modulation of pain thus serves to

"facilitate the execution of the selected action and inhibit conflicting motivations" (Navratilova & Porreca, 2014, p. 3).

1.2 The Study of Pain

1.2.1 Experimental Pain Models of Acute and Clinical Pain

To understand the effects and mechanisms of pain in humans, a number of experimental pain models have been developed, with the aim of activating different nociceptors, comparing different tissues, and activating specific pathways (Staahl & Drewes, 2004). Common methods of pain-induction are heat, cold, ischemia, electrical and chemical stimulation, mechanical pressure, and exercise (Gracely, 2013; Staahl & Drewes, 2004). Experimental pain models are used to examine sensory and perceptual mechanisms of perception and response to pain, as well acute and chronic pain states (Edens & Gil, 1995).

Heat pain is usually evoked by contact or radiant heat, for instance by a contact thermode or infrared light source. This is one of the most frequently used methods in experimental pain research (Gracely, 2013). Rapid skin heating activates A-δ nociceptors followed by C-fiber nociceptors, while slow heating bypasses the former and preferentially activates C-fibers, thus separating first and second pain (Staahl & Drewes, 2004). With increasingly high temperatures, polymodal nociceptors, high-threshold mechanoreceptors, and cold receptors are also activated. A potential confounding factor with this method is that thermode application can also activate low-threshold non-nociceptive sensory neurons, which have an inhibitory effect on pain transmission (Le Bars et al., 2001). Experimentally, contact heat generally produces lower unpleasantness ratings compared to other pain-induction methods, and is thus perhaps best suited to study the sensory-discriminatory dimension of pain (Rainville et al., 1992). However, repeated cutaneous thermal stimulation can induce central phenomena such as hyperalgesia, allodynia and temporal summation of pain, making it a useful method to study the underlying mechanisms of clinical pain characterized by abnormalities in pain processing, such as neuropathic and inflammatory pain, migraine, and fibromyalgia (Latremoliere & Woolf, 2009; Staahl & Drewes, 2004).

Cold stimuli can also be applied using contact stimulators. Perhaps more commonly, however, the cold pressor test is used. This method involves immersion of a limb in very cold water, and evokes high levels of quickly increasing pain, which can only be tolerated for a few minutes by most people (Gracely, 2013). Cold pressor pain is probably evoked by A-δ and C-fiber nociceptors in cutaneous veins (Staahl & Drewes, 2004). Compared to briefer stimuli, the continuous cold pressor test, involving both skin and deeper structures of the arm,

is rated as more unpleasant and may be more analogous to chronic pain states (Latremoliere & Woolf, 2009). It is sometimes used in conjunction with other methods (e.g., phasic heat pain) as a conditioning stimulus to study conditioned pain modulation (inhibitory nociceptive modulation where "pain inhibits pain"). Impairments in conditioned pain modulation has been found in multiple idiopathic pain syndromes, as well as chronic pain in general, and decreased inhibitory efficiency has been proposed as a mechanism for the development of persistent pain (Szikszay et al., 2020; Yarnitsky, 2010).

Ischemic pain is induced by obstructing blood flow in an arm through the use of a tourniquet, while performing an isometric or isotonic hand exercise. This method can also be used as an experimental stressor, and produces severe, continuous pain that increases over time (Gracely, 2013). It effectively produces muscle pain, as well as pressure pain at the cuff site. Ischemic pain, like heat pain, also involves activation of non-nociceptive nerves, which can inhibit pain mechanisms (Staahl & Drewes, 2004). Unpleasantness ratings of ischemic pain are high; thus, its affective component is similar to chronic pain (Latremoliere & Woolf, 2009). The mechanism of ischemic pain has been described as more closely related to clinical pain than that of various other noxious stimuli (Maurset et al., 1991). Ischemic pain is also observed clinically in certain acute and chronic conditions (Anitescu, 2018).

Electrical stimulation causes transient pain that is only present during stimulus administration (Staahl & Drewes, 2004). It can be applied to skin, muscle, teeth, and the stomach or intestines (Gracely, 2013). Stimulus currents are often gradually increased within experiments and can be delivered in the form of trains of very short pulses. Electrical cutaneous stimulation non-selectively activates multiple types of primary afferent nerves, producing not only pain, but a variety of sensations (Le Bars et al., 2001). As a result, electrical stimulation on skin may be felt as discomfort rather than pain (Edens & Gil, 1995). Electrical stimulation of dental pulp, on the other hand, has been cited as an ideal pain stimulus (Gracely, 2013). As with thermal heat, repetitive electrical stimulation of the skin can be used to induce phenomena of central sensitization, and this method thus permits mechanistic study of pain disorders where central pain processing is altered (Latremoliere & Woolf, 2009; Staahl & Drewes, 2004).

Chemical stimulation involves the administration of an algogenic substance, such as mustard oil, nerve growth factor, intramuscular hypertonic saline, or topical or intradermal capsaicin (the pungent ingredient of chili pepper). These chemicals can be applied both internally and externally on the body, e.g., to skin (intact, punctured or blistered), nasal, esophagal, gastric or intestinal mucosa, eyes, or teeth, and can thus be used to study visceral

pain (Gracely, 2013; Ness & Gebhart, 1990). Compared to other pain induction methods, chemical stimulation is slow, with long latency times to effect onset (Le Bars et al., 2001; Staahl & Drewes, 2004). Capsaicin is commonly used to induce skin pain and secondary hyperalgesia, but can also block nociceptor activation (Gracely, 2013; Staahl & Drewes, 2004). Intradermal capsaicin injections produce a burning sensation and sometimes itch through binding to TRPV1-receptors on polymodal C-fiber nociceptors and mechano-heat A-δ nociceptors, and subsequently causes thermal and mechanical hyperalgesia (Frias & Merighi, 2016). Capsaicin is often used in conjunction with other stimulation methods to study phenomena such as primary heat hyperalgesia and secondary mechanical hyperalgesia or allodynia, which are conditions of central sensitization usually only found in persistent clinical pain (Frias & Merighi, 2016; Gracely, 2013). Chemical pain is probably the best model for acute clinical pain, as the resulting pain is prolonged, progressive, highly unpleasant, and inescapable in character (Le Bars et al., 2001). Stimuli such as capsaicin or intramuscular injections of hypertonic saline mimic many central features of clinical pain syndromes (Gracely, 2013). Infusion of exogenous algogenic substances into muscles can also be used as a method of eliciting muscle hyperalgesia, and is thought to mimic the inflammatory effect and lowered pain threshold in various musculoskeletal disorders (Staahl & Drewes, 2004)

Mechanical pressure is a widely used method where pain is induced from deformation of the skin, for instance by pinching and pinprick, using stimuli such as needles, von Frey hairs, and pressure algometers. These stimuli can elicit pain over a range of intensities and duration (Gracely, 2013; Staahl & Drewes, 2004). Sharp or punctuate pressure predominantly activates A-δ nociceptors, whereas pain from blunt pressure is C-fiber mediated (Treede et al., 2002). However, pressure stimulation may also recruit mechanoreceptors and the sensation is thus not specifically nociceptive (Staahl & Drewes, 2004). Pressure pain can be used as the test stimulus in assessments of conditioned pain modulation, and can thus be used to study conditions where pain inhibition may be altered (Szikszay et al., 2020). Many clinical syndromes are characterized by enhanced sensitivity to blunt pressure (e.g., myofascial pain, temporomandibular disorder, and tension-type headache), which may be due to peripheral or central sensitization (Treede et al., 2002).

Finally, exercise-induced pain can be evoked through intense and repetitive muscular contraction (Astokorki & Mauger, 2017). This type of muscle pain is perceived as aching, diffuse and cramp-like, and may share some pathogenetic features with ischemic pain (Staahl & Drewes, 2004). Delayed-onset muscle soreness is viewed as comparable model for acute

mild to moderate musculoskeletal disorders such as back pain (Szikszay et al., 2020). Exercise can also be used to study exercise-induced hypoalgesia, a phenomenon involving descending inhibition of pain. As imbalanced descending control is one proposed mechanism for the transition from acute to chronic pain, investigating exercise-induced hypoalgesia may provide knowledge regarding this shift (Vaegter et al., 2014).

1.3 The Endogenous Opioid System

1.3.1 Anatomical Distribution

The endogenous opioid system is a set of receptors and ligands that are present at both supraspinal and various peripheral sites, "particularly in circuits involved in pain modulation, reward, responses to stress, and autonomic control" (Benarroch, 2012, p. 807). Opioid-synthesizing neurons and receptors can be found in both the central and the peripheral nervous system, the gastrointestinal tract, and in multiple cell types in the immune system (Plein & Rittner, 2018; Toubia & Khalife, 2019). In the central nervous system, opioid receptors are widely distributed and are present in different concentrations in the cerebral cortex, limbic system, basal ganglia, brainstem, dorsal horn, and dorsal root ganglion (Benarroch, 2012). The receptors of the endogenous opioid system constitute four major classes: μ-opioid receptors, δ-opioid receptors, κ-opioid receptors, and nociceptin opioid receptors. These G-protein coupled receptors are structurally very similar, but encoded by four separate genes (*Oprm1*, *Oprd1*, *Oprk1*, *Oprl1*). Four major types of ligands bind to opioid receptors: β-endorphins, enkephalins, dynorphins, and nociceptin/orphanin FQ (Corder et al., 2018). Colloquially, endogenous opioids are commonly referred to as endorphins (endogenous neuropeptides that have morphine-like effects; Sprouse-Blum et al., 2010). These groups can be further divided into various subclasses, with varying affinities for the different types of opioid receptors. In general, endorphins bind preferentially to μ-opioid receptors, and enkephalins and dynorphins tend to bind to δ-opioid receptors and κ-opioid receptors, respectively. This division is not absolute, however, since each peptide has limited selectivity. Most also have some binding activity at other receptor subtypes (Li et al., 2012). For instance, enkephalins also act as potent μ-opioid receptor agonists (Castro et al., 2021). μ-opioid receptors in particular are present in areas of the brainstem thought to be central to inhibitory pain modulation and analgesia, i.e., the PAG and RVM (Heinricher & Fields, 2013). In animals, the μ-opioid receptor is the most abundant receptor type in the brainstem (relative to other opioid receptors), as well as in the thalamus and amygdala (Benarroch, 2012). The distribution of μ-opioid receptors in the human brain is illustrated in Figure 1.

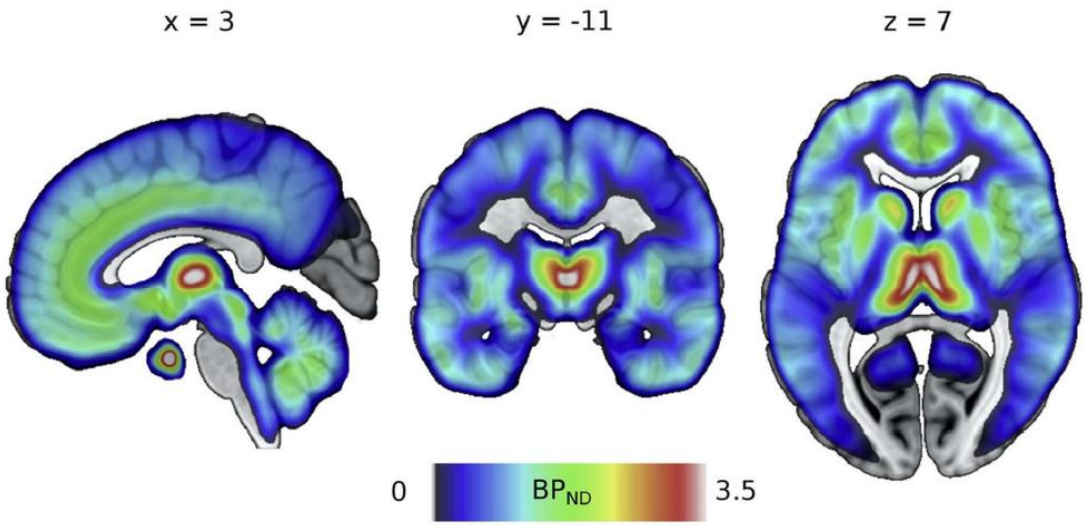

Figure 1. Composite PET image showing the anatomical distribution and density (indicated by [11C]carfentanil radioligand binding potential) of μ-opioid receptors in the healthy human brain. Figure adapted from "The Role of Mu-Opioids for Reward and Threat Processing in Humans: Bridging the Gap from Preclinical to Clinical Opioid Drug Studies" by Meier, I. M., Eikemo, M., & Leknes, S., 2021, *Current Addiction Reports, Volume Articles in Press.* (doi: 10.1007/s40429-021-00366-8). Copyright 2021 by Springer Nature Switzerland AG.

1.3.2 Physiology

The endogenous opioid system contributes to a multitude of physiological functions, including mood regulation, respiration, gastrointestinal motility, modulation of the stress response, and endocrine and immune functions (Le Merrer et al., 2009; Toubia & Khalife, 2019). It also plays an important role in the regulation of well-being and addictive behaviors. Crucially, the opioid system is regarded as central to nociception and analgesia (Le Merrer et al., 2009). Furthermore, opioid neurotransmission is involved in processes such as cardiovascular regulation, thermoregulation, learning and memory, reward, and several neurological and psychiatric disorders (Castro et al., 2021; Khachaturian et al., 1993). With regards to analgesia and pain modulation, β-endorphin and enkephalins are the most central endogenous ligands. Both β-endorphin and met-enkephalin are involved in supraspinal and spinal analgesia, and also act in the peripheral nervous system (Sprouse-Blum et al., 2010). The analgesic effects of β-endorphin are the result of central synaptic transmission and diffusion to distant brain parts through the cerebrospinal fluid; however, β-endorphin can also reduce pain through local release in peripheral tissues (Veening & Barendregt, 2015). Through binding activity at μ-opioid receptors, β-endorphin and met-enkephalin are involved in inhibiting pain signaling at the cellular level. Activation of μ-opioid receptors inhibits presynaptic neurotransmitter release and decreases the excitability of postsynaptic neurons

(Benarroch, 2012). Most opioid analgesics target pain by acting on μ-opioid receptors, as only μ agonists consistently produce potent analgesia (Fields, 2011; Li et al., 2012).

1.3.3 Opioid Agonists

Opium is the basic compound that underlies all opioid agonist drugs – as well as the discovery of the endogenous opioid system – and has probably been used to treat pain for thousands of years (Brownstein, 1993). It contains high concentrations of morphine, codeine, and thebaine, which are used in the synthesis of many opioid drugs. A number of fully synthetic opioid analogs have also been developed in an attempt to create analgesics that lack the addictive properties and abuse potential that traditional opiates possess (Pasternak & Pan, 2013). Morphine is the archetypical opiate analgesic, and the μ (mu)-opioid receptor is named after this ligand. Other μ-opioid receptor agonists that are commonly used in clinical practice include oxycodone, codeine, and fentanyl, as well as partial agonists (ligands that bind to receptors but only produce a partial biological response regardless of dosage) such as buprenorphine (Pathan & Williams, 2012). All μ-opioid agonists reliably produce analgesia that is unparalleled by non-opioid analgesics in both range of efficacy and potency (Fields, 2011). However, while the analgesic effectiveness of these drugs is well documented in the short and medium term, long-term usage (extending beyond eight weeks) has not been proven effective for chronic non-cancer pain (Fields, 2011; Kalso et al., 2004; Noble et al., 2010; Pathan & Williams, 2012). Long term use of μ-opioids may in fact increase pain sensitivity and lead to the development of analgesic tolerance (Chang et al., 2007; Lueptow et al., 2018). Like endogenous μ-opioids, exogenous μ-opioid agonists are thought to induce pain-relief through the activation of descending inhibitory pathways emerging from the PAG, which leads to a decrease in excitatory output to the RVM and subsequent inhibition of nociceptive signaling in the spinal cord (Lueptow et al., 2018). μ-opioid agonists can also directly inhibit neurotransmission in the substantia gelatinosa of the dorsal horn, as well as afferent signaling from peripheral nociceptors (Pathan & Williams, 2012).

1.3.4 Opioid Antagonists

Information about the functions of the endogenous opioid system has been obtained in part through the use of antagonist drugs. β-endorphins, enkephalins and other endogenous opioids are agonists, which implies that they activate the receptors they bind to and produce a tissue response (either full or partial). A tissue response implies that the cell's behavior has been changed by receptor activation. This in turn generates a biological response, such as the release of a neurotransmitter, an increase in blood pressure, muscle relaxation, or a behavioral

response, which can be observed *in vivo* or *in vitro.* In contrast, receptor antagonists are drug molecules that bind to the receptors and do not cause activation, but prevent agonists from binding (Rang et al., 2016). Opioid antagonists thus block the activation of opioid receptors, and accordingly inhibit the occurrence of an opioidergic response. As a result, they can be used to deduce the effect of receptor activation. If the activation of a certain receptor subtype normally produces a specific biological response, blocking the receptors should eliminate that response (by preventing changes in cell function caused by agonist binding activity). Thus, endogenous opioid involvement can be probed by blocking opioid receptors and observing whether changes in biological responses occur. If opioid antagonism causes the complete disappearance of a usual biological response, the effect can be termed opioid dependent. Correspondingly, if opioid antagonism does not alter the usual response, the effect is opioid independent (Eikemo et al., 2021). Both conclusions require complete receptor occupancy by the antagonist, i.e., that opioid agonists are fully prevented from binding. More specifically, the validity of this approach demands irreversible competitive antagonism, where "no change in the antagonist occupancy takes place when the agonist is applied" (Rang et al., 2016, p. 11). Only then is it possible to conclude that the observed effect (or lack thereof) results from the inhibition of endogenous opioids.

1.3.5 Positron Emission Detection Studies to Determine Degree of μ-Opioid Antagonism

Naloxone and naltrexone, the most commonly used opioid antagonists, are both competitive receptor antagonists, with a high affinity for μ-opioid receptors (followed by κ- and δ-opioid receptors; (Niciu & Arias, 2013; Rang et al., 2016). To quantify the necessary doses for complete opioid receptor occupancy in humans, several studies using positron emission tomography (PET) and dual-detection systems (Bice et al., 1986) have been conducted. [11C]carfentanil, a selective μ-opioid receptor agonist, is typically used as a radiotracer (Colasanti et al., 2013).

Mayberg and Frost (1990) reported that in healthy humans, doses of 0.1-1 mg/kg intravenous naloxone produced near complete inhibition of the binding of a selective μ-opioid receptor agonist, i.e., close to total μ-opioid receptor blockade by the antagonist. Doses of 0.01 mg/kg produced about 65% blockade. Information on the time to peak occupancy and elimination was missing from this study, however. Villemagne et al. (1994) found that 0.01-1 mg/kg intravenous naloxone resulted in approximately 100% receptor occupancy 45-65 minutes post-injection (while 0.001 mg/kg produced circa 40% blockade). A subsequent study investigating the lower end of this dose range reported that 2 mg (~0.03 mg/kg) intravenous naloxone produced 81% receptor occupancy 45-65 minutes after drug

administration. This declined to 47% after ~3 hours, with minimal blockade after ~9 hours (Kim et al., 1997). More recently, intranasal naloxone doses of 2 mg and 4 mg have been shown to produce average peak μ-opioid receptor occupancies of 67% and 85%, respectively. Peak occupancy was seen at 15-80 minutes for the low dose and 12-100 minutes for the high dose (Johansson et al., 2019). Complimenting these findings, a recent pupillometry study found that 93% of the effect of steady state opioid agonism could be reversed within 4 minutes of intravenous naloxone administration (1 mg), which suggests rapid uptake and onset of effects in the central nervous system (Tylleskar et al., 2018). The estimated blockade half-life of naloxone in these studies ranged from 60-120 minutes (Johansson et al., 2019; Kim et al., 1997; Tylleskar et al., 2018).

In studies with naltrexone, high levels of μ-opioid receptor antagonism have been more consistently demonstrated. In one study, an oral dose of 50 mg produced 91% μ-opioid receptor blockade 48 hours after administration in healthy subjects, which decreased to 80% after 72 hours and 46% after 120 hours. After 168 hours blockade was at 30% (Lee et al., 1988). A more recent study in abstinent alcohol-dependent participants found that the standard dose of 50 mg inhibited agonist binding to μ-opioid receptors by 95% after daily administration for four days (Weerts et al., 2008). Subsequently, in an equivalent study design, >90% antagonist occupancy with 50 mg naltrexone has been demonstrated in healthy controls as well. Here, blockade ranged from 93,1% to 98,9% depending on whether subjects were homozygous or heterozygous for the A allele on A118G SNP on the μ-opioid receptor gene (Weerts et al., 2013). Furthermore, Rabiner et al. (2011) found that 50 mg per oral naltrexone resulted in approximately 95% blockade up to eight hours after dosing in healthy males. One study measured μ-opioid receptor blockade 3-144 hours after oral administration of 50 and 100 mg doses of naltrexone, but omitted blockade percentages from the published report (Bednarczyk et al., 2005).

Nalmefene, another non-selective μ-opioid antagonist, has been shown to block 99% of receptors following intravenous administration of a single 1 mg dose 45-65 minutes prior, and maintained 90% blockade at ~3 hours and 96% at ~5 hours post-injection. The corresponding percentages decreased to 52%, 33% and 47% if the dose was 1 microgram. Thus, clearance of nalmefene took longer compared to naloxone. Brain clearance was also seen to be much slower than plasma clearance (Kim et al., 1997). Oral administration of 20 mg nalmefene (single dose) has been shown to generate virtually complete blockade for the first three hours following administration, estimated at around 95% by Kyhl et al. (2016). Simulations suggest that receptor occupancy remains within or above 60-90% for 22-24

hours. Previously, single dose administration of 20 mg per oral nalmefene was shown to result in approximately 100% and 85% μ-opioid receptor occupancies after respectively 3 and 26 hours (Ingman et al., 2005).

As can be seen from these studies, while antagonist doses that block the vast majority of opioid receptors are known, 100% μ-opioid receptor occupancy is generally not demonstrated with the most frequently used antagonists, naloxone and naltrexone. Furthermore, there is some variability in the maximum level of receptor blockade between studies, suggesting individual differences in antagonist efficiency. Time to peak receptor occupancy is also not always reported (e.g., Mayberg & Frost, 1990). Consequently, some uncertainty exists regarding the extent to which the observed effects in antagonist studies can be attributed to the extinction of an opioidergic response. Many studies may only be able to inform of potential opioid fine-tuning or suggest the possibility of an opioid-mediated effect (Eikemo et al., 2021; see Figure 2).

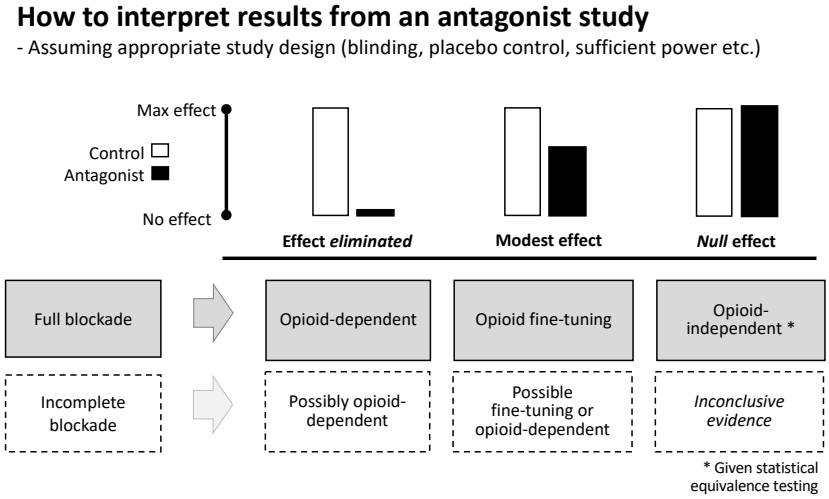

Figure 2. From "Do endogenous opioids mediate or fine-tune human pain relief?" by Eikemo, M., Løseth, G., & Leknes, S., 2021, *PAIN, Volume Articles in Press.* (doi: 10.1097/j.pain.0000000000002286). Copyright 2021 by International Association for the Study of Pain.

1.4 Purpose of the Thesis

1.4.1 Objective

The purpose of this thesis was to reconcile some of the challenges related to dose and the establishment of causal inference in antagonist studies examining the effects of endogenous opioids on pain. The objective was to clarify how (or if) various aspects of the pain experience depends on and is modulated by endogenous opioids.

1.4.2 Investigative Approach

To integrate the available evidence and achieve this goal, the conduction of a systematic review and meta-analysis was considered an appropriate approach. A meta-analysis involves "the statistical synthesis of results from a series of studies" – studies that have been systematically located, usually in the context of a systematic review (Borenstein, 2009, p. xxi). A systematic review "attempts to identify, appraise and synthesize all the empirical evidence that meets pre-specified eligibility criteria to answer a specific research question", using a systematic approach (Cochrane Library, 2021). This type of review often entails a qualitative synthesis of results. A meta-analysis builds on this approach by applying predefined statistical methods to synthesize these results quantitatively, and can accordingly be seen as a quantitative systematic review (Cook et al., 1997). As a statistical method it utilizes effect sizes from individual studies to estimate a summary effect. By combining the results from multiple studies, meta-analyses can estimate the true effect of an intervention more precisely, as well as demonstrate whether such an effect can be seen consistently in all studies or if there is a high degree of variability. Furthermore, meta-analyses can identify how the magnitude of an effect might vary across populations, and whether it is more strongly influenced by some variables than others (Borenstein, 2009). In both meta-analyses and qualitative systematic reviews, criteria for study inclusion and exclusion need to be explicitly stated, in order to ensure transparency and make reproduction of the review possible. Meta-analyses are especially useful when research so far has produced divergent results that are challenging to adequately and accurately combine through the authors' own assessment and interpretation (Borenstein, 2009).

In the case of the present meta-analysis, a previously conducted systematic review by Werner et al. (2015) inspired the project. This review assessed and summarized experimental pain studies that investigated the effect of opioid antagonism on pain sensitization and inhibition. The authors concluded that blockade of opioid receptors "appears to have a demonstrable and relatively reliable effect in stress-induced analgesia … and repetitive transcranial magnetic stimulation", but that it produced a "variable and unreliable effect" in all of the other pain models (Werner et al., 2015, p. 31). This conclusion was based on a qualitative synthesis of results in which studies that reported significant findings (in favor of antagonist effects) were counted as support for an endogenous opioid-mediated mechanism for attenuation of pain. There are limitations associated with this approach, as it is mainly based on the assessment of *p*-values (whether they are significant or not), which does not provide information about the magnitude of the effect (Borenstein, 2009). Importantly,

Werner et al. did not evaluate studies based to the achieved level of μ-opioid receptor blockade, i.e., whether the study participants were given a sufficient antagonist dose to completely inhibit central μ-opioid receptors (although the dose-issue was noted; Werner et al., 2015, p. 27). As a result, it cannot be concluded from the review whether the observed lack of a stable and reliable antagonist effect across studies is truly attributable to a lack of endogenous opioid involvement in experimental pain – opioidergic responses might simply not have been adequately blocked. The present systematic review and meta-analysis was conducted with the intent of resolving this issue. The scope of the present meta-analysis is also wider than that of Werner et al.

1.4.3 The Present Meta-Analysis

In order to clarify the conditions under which pain is modulated by endogenous opioids, the current meta-analysis aimed to include all available studies investigating experimental pain in healthy humans, with the primary condition that opioid antagonism be compared to no blockade. A variety of studies, some not explicitly concerned with our investigational focus, were thus included in this review. These studies were divided into categories to permit evaluation of how different variables affect pain. Specifically, studies that employed adequate antagonist doses according to our estimations were grouped to allow for investigation of how endogenous opioid involvement might vary between measures of pain perception, duration of pain stimuli, modalities, and depending on to which area of the body noxious stimulation was applied. Considering the importance of psychological variables and context in the experience of pain, we also examined the effect of additional (non-drug) interventions on pain, in order to find out whether conditioning effects – causing either sensitization or pain relief – could be blocked, irrespective of potential antagonist effects on pain. The primary outcome of this meta-analysis was the effect of complete blockade of μ-opioid receptor on pain intensity and pain tolerance. As a secondary outcome, the effect of complete μ-opioid receptor blockade on pain threshold and pain unpleasantness was examined.

1.4.4 Hypotheses

We hypothesized that:

1. Measures of pain perception would indicate more pain following administration of an opioid antagonist compared to the placebo condition, provided that the dose induced full μ-opioid receptor blockade.

2. There would be a positive dose-response relationship between opioid antagonism and pain, such that a higher degree of blockade would induce a greater increase in pain perception.

Confirming these hypotheses would provide support for a role of endogenous μ-opioids in the downregulation of experimental pain. In contrast, lack of support would indicate a less essential function of endogenous opioids in pain relief. The conclusion would also rely on the size of the summary effect. In general, effect sizes of around 0.2, 0.5, and 0.8 are considered small (but not trivial), medium, and large, respectively (Cohen, 1988).

2 Methods

2.1 Protocol and Registration

A protocol for this systematic review and meta-analysis is available at the Open Science Framework (OSF) Registries (**https://osf.io/g8m6w**). Preregistration was done during data extraction, but prior to data analysis. The review followed PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (Page et al., 2021). A completed PRISMA checklist can be found in Appendix B.

2.2 Eligibility Criteria

To be eligible for inclusion, studies needed to contain a sample of healthy, non-patient participants. Acceptable subject descriptors were “controls”, “normal controls”, “healthy controls”, “volunteers”, “normal volunteers”, “healthy volunteers”, “students”, “young adults”, “healthy adults”, “healthy subjects”, “pain-free subjects”, and similar terms indicating good or average health status. Patient samples and subjects described as having a medical condition were excluded. There was no lower limit for sample size, as we wanted to include as many studies and participants as possible in the meta-analysis.

It was required that studies follow a randomized, placebo-controlled, double-blind (or triple-blind) design. Eligible studies were those in which experimental pain testing was performed under the influence of an opioid antagonist, and correspondingly with the use of a pharmacologically inert substance (placebo). Opioid antagonists were required to be centrally active and have a primary affinity for μ-opioid receptors. Examples include naloxone and naltrexone (as well as nalmefene, samidorphan, nalorphine, levallorphan, nalodeine and the newer compound GSK1521498). All routes of administration and dosages were included. In the placebo condition, the use of an inactive substance of identical presentation (placebo drug) was required. Thus, subjects receiving an opioid antagonist must be compared to a control group receiving a sham pharmacological treatment, e.g., intravenous saline (vehicle) infusions, and placebo capsules or topical creams. Both repeated-measures and between-groups study designs were acceptable.

To avoid confounding with regards to drug effects, each study must have a pure opioid antagonist condition, i.e., no co-administration of other study drugs. This requirement also applied to the placebo condition. Consequently, studies in which an opioid agonist or any other drug had been administered prior to the antagonist or placebo were excluded. We did not count dietary supplements, such as fish oil, as drugs.

Importantly, studies must employ experimental pain interventions, which we defined as procedures that induce pain in subjects who were not in pain previously and who are otherwise healthy. Pain must be provoked in an experimental setting by means of physiological stimulation, i.e., with chemical, electrical, mechanical, pharmacological, and thermal stimuli (either used alone or in combination). Examples of permissible pain-provoking techniques were subcutaneous injection of capsaicin, electric shocks, pinprick stimulation, heat stimulation, ischemic pain tasks, and the cold pressor test. Studies assessing acute pain, e.g., from intense exercise or dental procedures, were also eligible. Data from chronic pain populations were not included; neither were data where pain was induced via psychological stimuli.

Furthermore, we required that pain measurements be direct verbal reports or behavioral signals rather than physiological correlates. Studies in which pain was assessed indirectly, for instance through recordings of nociceptive flexion reflexes, were excluded.

Finally, eligible studies must measure the effect of opioid antagonism on pain perception, as indicated through reports of pain intensity, tolerance, unpleasantness, and threshold. We did not require that studies have the same investigational focus as this review in order to be included. Thus, studies might be investigating outcomes separate from or in addition to the effects of endogenous opioid blockade on pain perception. Other non-drug interventions might be employed in addition to the treatment of interest (antagonist or inactive comparator drug) to induce sensitization or hypoalgesia. Examples included stress induction, placebo treatments (such as sham medications or verbal suggestions), and reward, among other interventions. Studies must measure antagonist effects on changes in pain perception produced by such conditioning stimuli.

2.3 Literature Search

The databases Web of Science, Scopus, PubMed and EMBASE (via Ovid) were searched on October 7, 2020. This combination of databases likely provides adequate of the available literature (Bramer et al., 2017). The search aimed to identify original, randomized, placebo-controlled studies using experimental pain models and opioid antagonism, and was open to all publication languages. Review articles were excluded from the search. There were no restrictions with regards to time of publication.

The full search strategy for each database is available in the review protocol (**https://osf.io/g8m6w**). The search combination included synonymous terms to locate studies with the following constituents:

1) Centrally active μ-opioid antagonists

2) Placebo-control
3) Blinding and randomization
4) Human participants
5) Pain

Search results were imported to Mendeley, in which automatic deduplication and merging of close duplicates was performed. This method has been recommended for this purpose, and yields a low number of false negatives and false positives (Kwon et al., 2015). Additional duplicates were then removed manually in EndNote by a review team member based on similarities in title, author, year of publication, journal, volume, issues, pages, and abstract.

2.4 Study Selection

Two reviewers independently screened all study records according to the established eligibility criteria. Both were blinded to the other's decisions. Studies were sequentially assessed for eligibility and the final study selection was entered into an Excel spreadsheet. The study selection for this thesis was based on the thesis author's evaluation of eligibility. However, the second reviewer's evaluation was highly concordant, with 96.6% agreement.

2.5 Data Collection

Data was extracted independently by three reviewers. The thesis author extracted data from most studies and the other reviewers extracted data from approximately 8%. The extracted data was stored in an Excel spreadsheet. Records were allocated according to preference but were generally assessed alphabetically. A standardized form was used for data extraction. This was based on a piloted version which was tested in a semirandom sample (n = 13) of the selected studies, in line with the recommendation from the Cochrane Handbook for Systematic Reviews of Interventions (Higgins et al., 2021).

The following data items were extracted from the included studies:

1. Record information: Author(s), year of publication, title, name of journal, volume, issue, pages, study name if applicable, country of origin.
2. Sample information: Total number of participants, proportion of females and males, mean participant age with standard deviation, participant descriptor, group name or label.
3. Drug information: Name of antagonist drug, dose, route of administration, waiting activity during drug absorption, study design (between-subjects or within-subjects), intersession interval (if applicable).

4. Pain induction information: Time of pain induction relative to drug administration, stimulus or task, body part tested, duration of pain stimulation, number of noxious stimulations, whether stimulus intensity was calibrated or identical for all participants, time of calibration relative to drug administration (if applicable), target pain level of calibration (if applicable).
5. Pain assessment information: Time of pain assessment relative to drug administration, pain measurement category (intensity, tolerance, threshold, unpleasantness), format of pain measurement and reported scores (e.g., NRS, VAS, degrees C, seconds, minutes, percentage, AUC), interpretation of reported scores (higher scores indicate more pain, or lower scores indicate more pain).
6. Conditioning stimulus information (if applicable): Stimulus description, timing of conditioning stimulus.
7. Outcome data per treatment condition: Number of participants, mean pain assessment score before drug with standard deviation, mean pain score after drug with standard deviation, mean change in pain assessment scores with standard deviation.
8. Mood assessment information: Whether mood measurement occurred, name of scale or questionnaire used.

No selections were made in the different outcome domains; all relevant results were recorded if available. The data items were entered as described in the individual articles, with the exception of pain stimulus or task names, which were partly standardized to allow for more straightforward analysis. A simplification was made for studies using a within-subjects design: In cases where the intersession interval varied for participants, the reported time between sessions was recorded as the minimum number of days (e.g., an intersession interval of 2-4 days was recorded as “2 days”). The lower threshold was recorded to permit screening for residual drug effects with greater sensitivity.

For articles where some or all of the data was reported in figures, the online tool WebPlotDigitizer (Rohatgi, 2020) was used to extract figure data. This program has been shown to produce results with high validity and intercoder reliability (Drevon et al., 2017) and was therefore judged to be a satisfactory option when numbers were not otherwise reported. Missing data was left out of the preliminary analyses. Where possible, the relevant study investigators were contacted with a request for missing data and/or necessary methodological details. This included relevant unpublished data identified in the search, e.g., via conference posters. Study authors were contacted through email after all records had been assessed. We also attempted to confirm the extracted figure data by requesting accurate numbers from the

authors. One follow-up email was sent approximately two weeks later in cases where we did not receive a response.

2.6 Synthesis of Results

The primary outcome of the meta-analysis was the difference in measures of pain intensity and pain tolerance between the two drug conditions (opioid antagonist versus inert substance). Secondary outcomes were pain unpleasantness and pain threshold. We also analyzed the overall effect across pain measurement categories.

Differences in pain perception between treatment groups for the individual studies were synthesized via the calculation of Hedges' *g* (standardized mean difference with bias correction). In cases where pain assessment scores were available both as post-drug means and change scores, we utilized change scores in order to control for potential differences between groups at baseline. For studies where within-subject correlation coefficients were not reported or available upon request, the value was arbitrarily set to 0.5 to allow for calculation of Hedges' *g*. A sensitivity analysis was performed to investigate whether findings were robust across different correlation coefficients. Hedges' *g* for individual studies were adjusted to correct for differences in the direction of the scale by multiplying values for which a lower score indicated more pain with -1. Thus, for all analyses, a positive value for Hedges' *g* signified more pain (as indicated by higher ratings of pain intensity and unpleasantness, and lower ratings of pain tolerance and threshold) in the antagonist conditions compared to the control conditions, whereas a negative value indicated less pain (as indicated by lower ratings of pain intensity and unpleasantness, and higher ratings of pain tolerance and threshold).

A random effects model was judged to be appropriate due to the variability in stimulus modalities and antagonists tested (including dosage), as well as the relative heterogeneity of the participant groups (Borenstein, 2009). Data was analyzed in R (R Core Team, 2020) by a review team member using the metafor package (Viechtbauer, 2010). Multiple outcomes were extracted from each study where available. To account for dependencies in the data, we used a three-level model which added a random effect at the study-level (Thompson et al., 2001). This approach generates precise estimates without requiring an estimate of the correlation between outcomes (Moeyaert et al., 2017). Results were visually displayed in orchard plots; a practical alternative to forest plots for meta-analyses containing large numbers of effect sizes (Nakagawa et al., 2021).

2.6.1 Statistical Heterogeneity

Heterogeneity was assessed using Cochran's *Q* test, the I^2 index, T^2 and *T*. Heterogeneity implies that the size of the true effects differs between studies, i.e., that variation in study outcomes is not simply due to chance but reflects real underlying differences (Higgins & Thompson, 2002). Cochran's *Q* test was used to evaluate the null hypothesis that all studies share a common effect and that observed differences are due to within-study error. A significant *Q* statistic indicates that the observed variance is due to differences in true effects between studies rather than sampling error alone (Borenstein, 2009). I^2 estimates the percentage of this variance that stems from real differences between studies and is not due to chance. If I^2 is 0%, chance alone explains the variability. On the other hand, an I^2 of 50% means that 50% of the observed variation in effect sizes cannot be attributed to chance, and therefore must be explained by other factors (West et al., 2010). Between-studies variance of the true effects was estimated using T^2. *T* is the standard deviation of the summary effect and was used to describe the distribution of true effects around the mean (Borenstein, 2009).

2.6.2 Statistical Significance

To assess the statistical significance of individual study results and the effect estimates for each analysis, 95% confidence intervals (CI) were calculated.

We did not adjust for multiple testing. General recommendations for this do not exist currently, as there is no simple and completely satisfactory solution to the issue of multiplicity in systematic reviews (Bender et al., 2008; Higgins et al., 2021).

2.7 Subgroup Analyses

Pre-registered meta-regression and subgroup analyses were used to investigate potential sources of heterogeneity.

To assess variability in antagonist effects resulting from different dosage levels, studies were grouped based on the estimated percentage of μ-opioid receptors that were blocked at peak antagonist concentration during pain testing. These estimates were arrived at through the synthesis of results from a composite of PET-imaging studies (see Introduction), which together indicated the level of receptor blockade at different time points. We defined full blockade as >90% μ-opioid receptor occupancy, in accordance with central PET studies which categorized this level of receptor inhibition as essentially complete (Mayberg & Frost, 1990; Weerts et al., 2008). Medium and minimal blockade was defined as >20-90% and 0-20% μ-opioid receptor occupancy, respectively. Only studies where both pain induction and

pain measurements were completed within the window of maximum antagonist concentration were included in the full blockade group. If testing was performed outside of this window, studies were included in a lower category, i.e., with less blockade. The possibility of a dose-response relationship between opioid antagonism and level of pain was investigated using meta-regression. Further subgroup analyses were constrained to results categorized as >90% μ-opioid receptor blockade.

The second subgroup analysis examined antagonist effects on different facets of pain, by grouping pain recordings according to which category of pain perception was measured (pain intensity, pain tolerance, pain unpleasantness, or pain threshold). If measures such as allodynia, hyperalgesia, or analgesia were reported, these were allocated to the category they corresponded to (e.g., if allodynic thresholds were measured, the category used in the analysis was pain threshold).

To examine differences in antagonist effects on pain stimulation of different duration, pain stimuli were divided into brief stimuli (duration of up to one minute) and prolonged stimuli (duration of one minute and above), in line with classification of experimental pain in animal models (Le Bars et al., 2001).

Antagonist effects on different pain modalities were also investigated, by sorting outcomes according to stimulus modality. The categories were thermal pain (heat or cold stimuli), mechanical pain (pressure, stroke, or pin-prick stimulation), ischemic pain, chemical pain (e.g., capsaicin), electrical pain (shocks), and exertion pain (i.e., exercise-induced global pain from demanding physical activity).

Lastly, outcomes were categorized based on the physical location of pain stimulation on the body, so that potential dissimilarities in antagonist effects in this domain could be uncovered. Categories were extremities (hands, arms, legs, feet), core (back, abdomen), and head. The division was based on rankings of threat value (Sambo et al., 2012) rather than anatomical features such as cutaneous innervation density (Corniani & Saal, 2020).

2.8 Risk of Bias Assessment

All studies were assessed individually according to the Jadad scale (Jadad et al., 1996) in order to evaluate risk of bias. This scale was developed specifically for use in pain research and presents both high validity and reliability in this area (Olivo et al., 2008). The Jadad scale assigns up to five points (possible range: 0-5) to each study based on the presence and method of randomization, presence and propriety of blinding, and whether information about subject withdrawals and dropouts is provided. Higher scores indicate better study quality, and thus lower risk of bias. Assessments were done at study level. A meta-regression was conducted to

determine a potential relationship between quality and study results. Two reviewers independently assessed study quality (the thesis author assessed the quality of most included studies, and a second review team member assessed the quality of a smaller percentage).

Publication bias was assessed by generating funnel plots and conducting an Egger's test to measure funnel plot asymmetry (Egger et al., 1997). This test regresses standardized effects sizes on their respective precisions and is expected to intercept at zero if there is no publication bias. In the presence of reporting bias, funnel plots are expected to be asymmetrical; if none exists, effect sizes plotted against their standard errors are distributed in a manner reminiscent of an inverted funnel (Higgins & Thompson, 2002). Contour-enhanced funnel plots were used to evaluate the significance level at which effects might be unpublished (Peters et al., 2008). Funnel plots were also visually assessed for trends indicative of small-study effects, i.e., the tendency for smaller studies to produce effect estimates that differ from those produced by larger studies (Sterne et al., 2000). Finally, we used the trim-and-fill method to estimate the number of studies that might be missing in the meta-analysis due to publication bias (Duval & Tweedie, 2000).

2.9 Sensitivity Analyses

Since correlation coefficients were imputed for studies with within-subjects design to facilitate calculation of Hedges' *g*, we assessed the robustness of the results by repeating the analyses with lower ($r = 0.25$) and higher ($r = 0.75$) imputed correlations.

A post-hoc analysis was conducted to determine whether the summary effect differed between studies that employed non-drug interventions and studies in which conditioning stimuli were not utilized.

3 Results

3.1 Study Selection

An overview of the study selection process is displayed in the figure below (Figure 3). The database search 2514 records in total, of which 1189 remained after deduplication. Full-text articles were obtained and assessed for 109 records that were identified as potentially eligible. Of these, 88 articles met all inclusion criteria and were included in the review. After requesting data from study authors, 28 articles were missing data items that were necessary for analysis (i.e., treatment means and standard deviations, the number of participants in each condition). Thus, the final sample included in the analyses consisted of 60 studies.

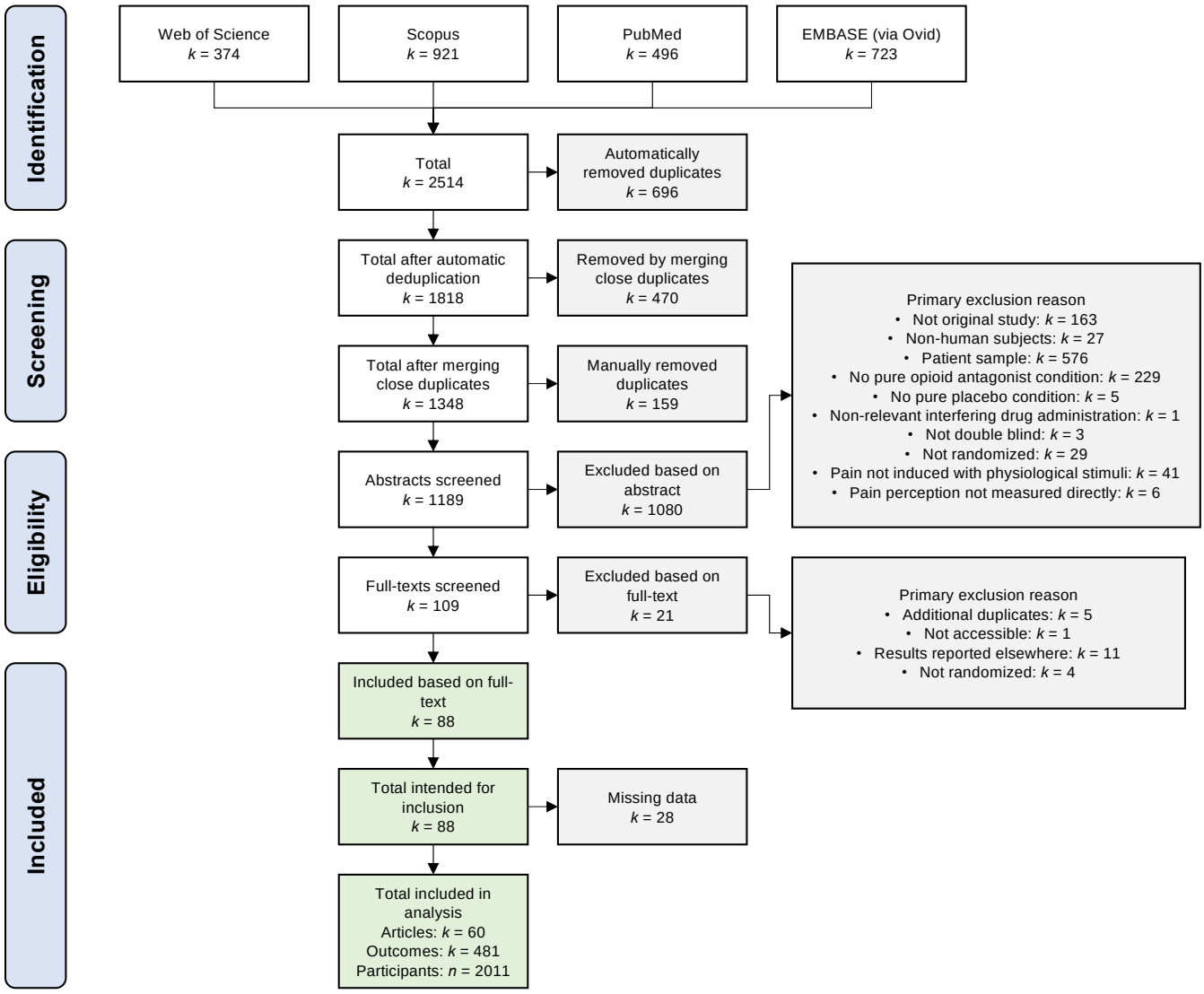

Figure 3. PRISMA flow diagram depicting the study selection process. *k* refers to number of articles unless otherwise specified.

3.2 Study and Sample Characteristics

The characteristics of each included study, as well as the reported range of effects, are presented in Table A1 (Appendix A). In total, 2011 participants were evaluated in the included studies, and the meta-analysis encompassed 481 separate effects.

3.2.1 Sample Characteristics

Table 1 shows participant characteristics based on reported data. Sample sizes ranged from 6 to 151 participants; the average was 24 participants per sample. Samples were predominantly male (67.8%). The number of female participants was not reported in 15 samples (18%), and the mean age of participants was not reported in 32 samples (39%). Participant ages ranged from 19 to 55 years in the samples where age was reported.

Table 1

Characteristics of samples (*k* = 83)	
Age (mean)	29.7 years
Female participants	32.2 %
Nationality (study location)	*k* samples
USA	36
Germany	12
Italy	7
Denmark	6
France	6
Canada	4
Australia	3
Netherlands	2
UK	2
Austria	1
Belgium	1
Israel	1
Sweden	1
Switzerland	1

3.2.2 Study Characteristics

The majority of studies used naloxone in the antagonist condition (48 out of 60 studies). Eleven studies used naltrexone and one study used nalmefene. The study design was within-subjects for 43 studies, and between-subjects for 18 (with one study using both designs). Experimental inter-session intervals in within-subjects studies ranged from 1 day to 8 weeks. An overview of study design and inter-session intervals, as well as antagonist doses and estimated blockade at the time of pain testing, is shown in Table A2 (Appendix A). Most studies (58%) were published between the years 2000 and 2020, whereas the remaining 42% had been published between 1977 and 1999. Pain intensity was the most used measure of pain perception, followed by threshold, unpleasantness, and tolerance. The most prevalent methods of pain induction were forms of thermal stimulation (performed in 25 studies), electrical stimulation (19 studies), and ischemia-inducing tasks (13 studies). Thirty-eight studies employed non-drug interventions in addition to the treatment of interest, including stress-induction tasks, meditation, suggestions of analgesia, placebo treatments, and sensitizing pretreatments.

3.3 Overall Effect

The main analysis included 481 effects across 60 studies. The distribution of effects is displayed in Figure 4. The results showed that experimental pain induction overall generated more pain in the antagonist conditions compared to the control (inactive drug) conditions, although the effect was small (Hedges' g [95% CI] = 0.21 [0.1, 0.3], p = .0003). A clear majority of effects (81.1%) had confidence intervals overlapping zero. Cochran's Q test suggested that the variation in effect sizes was due to systematic variance and not sampling error alone ($Q(480) = 1221.3$, $p < .0001$). There was considerable heterogeneity present (I^2 = 77.2%). The estimated amount of heterogeneity (T^2) was 0.21. The standard deviation of the summary effect was relatively high (T = 0.45), indicating a wide distribution of true effects, with the majority falling in the range of -0.69 to 1.11. A strong correlation was found between effects within studies (intraclass correlation coefficient = 0.78), and most of the global heterogeneity (60.4%) represented between-cluster variance, i.e., variability between groups of study outcomes rather than within-study dispersion. In sum, a high amount of variance could not be explained by random error and reflected differences in effect sizes that varied between studies. We aimed to explore the underlying reasons for the observed heterogeneity in the following subgroup analyses. For primary, secondary, and additional outcomes, analyses were limited to studies that achieved full blockade during pain testing ($k_{articles}$ = 50).

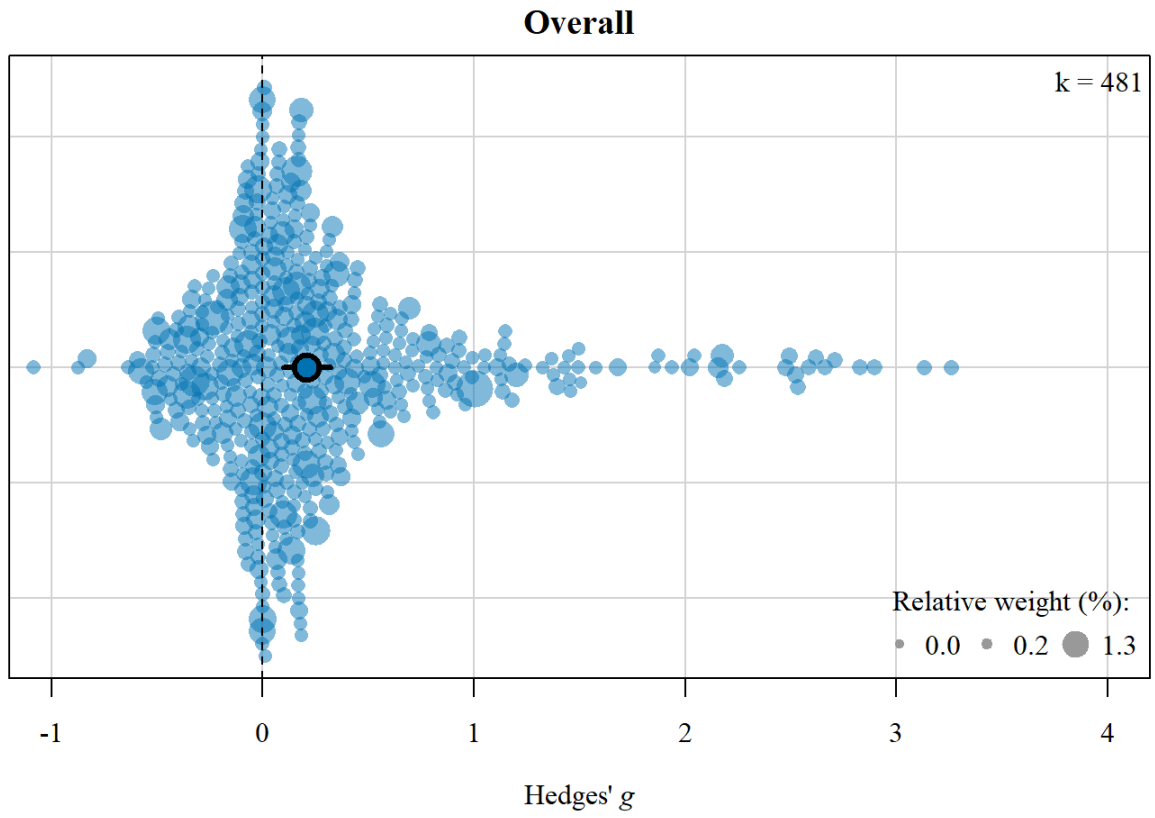

Figure 4. Orchard plot (Nakagawa et al., 2021) illustrating the overall distribution of effects. Blue transparent circles depict individual effect sizes. The relative weight of each effect is illustrated by the size of the circle. Minimum, mean, and maximum weight is indicated by the gray dots. The summary effect (with 95% CIs) is marked by the solid black circle with horizontal bars. Dashed vertical line indicates no difference between treatment conditions. Dashed vertical line indicates no difference between treatment conditions.

3.4 Blockade

Grouping of studies and effects according to the level of μ-opioid receptor occupancy during pain induction revealed that full blockade was largely achieved in the included studies. Most studies ($k_{articles}$ = 50) assessed pain during a window of full μ-opioid blockade ($k_{effects}$ = 326). Twelve studies induced and measured pain during medium blockade ($k_{effects}$ = 90), while only a small subset ($k_{articles}$ = 8) performed pain testing with minimal blockade ($k_{effects}$ = 49). There was some overlap between studies, as a few included multiple dosage conditions (see Table A1). Classification of excessive blockade presented as a challenge based on available imaging evidence, and thus this category was incorporated into the full blockade group. None of the included studies administered ultra-low antagonist doses.

3.4.1 Full Blockade

Pain was significantly higher in the antagonist conditions compared to pharmacologically inactive treatments when testing was performed during full blockade (i.e., >90% μ-opioid receptor occupancy; Hedges' *g* [95% CI] = 0.23 [0.10, 0.36], $p < .0001$).

3.4.2 Medium Blockade

When experimental pain testing was performed during a window of medium blockade, a similar effect was found, with significantly more pain following antagonist administration compared to control conditions (Hedges' *g* [95% CI] = 0.25 [0.06, 0.43], $p < .0001$).

3.4.3 Minimal Blockade

No statistically significant effect of opioid antagonism on pain was found in studies assessing pain at a time of minimal blockade, and pain levels were similar in antagonist and control conditions (Hedges' *g* [95% CI] = 0.07 [-0.06, 0.43], $p = .11$).

3.4.4 Investigation of Dose-Response Relationship

A meta-regression analysis demonstrated a statistically significant positive relationship between level of blockade and magnitude of the summary effect (*B (SE)* = 0.10 (0.03), $z = -1.06$, $p = .002$). Higher levels of blockade were associated with more pain. The summary effect was in fact marginally higher for medium blockade than full blockade; however, the estimated effect of minimal blockade was close to zero, indicating that pain might indeed increase as a function of antagonist dosage (i.e., the achieved level of μ-opioid blockade).

3.5 Primary Outcomes

The results of the primary and secondary analyses are depicted in Figure 5.

3.5.1 Pain Intensity

The majority of the included studies ($k_{articles} = 38$) reported data on pain intensity, and this primary analysis included 222 effect estimates. Overall, pain intensity was significantly higher following antagonist administration compared to pharmacologically inactive treatments (Hedges' *g* [95% CI] = 0.26 [0.08, 0.43], $p < .0001$). The mean effect was, however, again small. As for the overall effect, effect sizes for pain intensity were relatively spread out around the mean ($T = 0.54$).

3.5.2 Pain Tolerance

Pain tolerance outcomes were reported in seven studies ($k_{effects} = 11$). Of these, two studies used the cold pressor test as the method of pain induction and two used the submaximal effort tourniquet test. Tolerance to thermal or electrical stimuli was assessed in three studies. The analysis results showed that pain tolerance was significantly lower with antagonist drugs compared to pharmacologically inactive treatments, but the effect was small (Hedges' *g* [95% CI] = 0.18 [0.06, 0.30], $p = .003$).

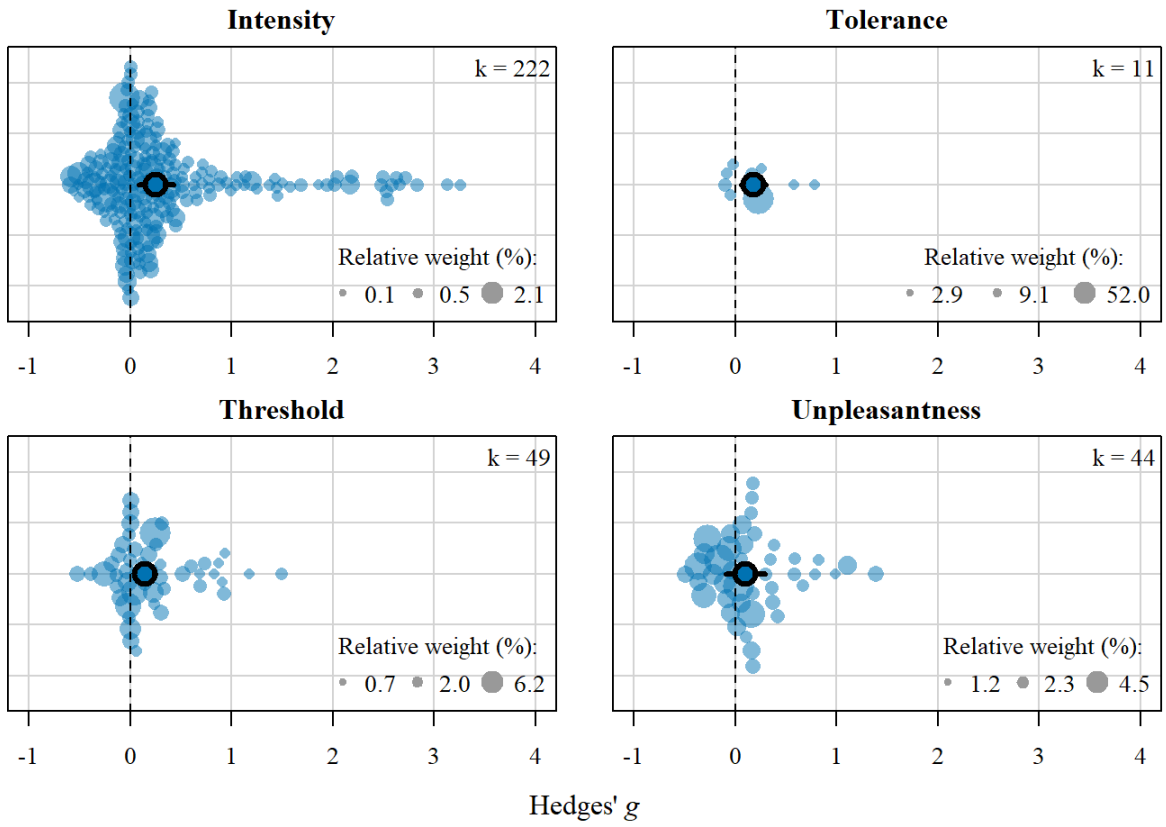

Figure 5. Orchard plot illustrating the distribution of effects for measures of pain intensity, pain tolerance, pain threshold, and pain unpleasantness. Blue transparent circles depict individual effect sizes. The relative weight of each effect is illustrated by the size of the circle. Minimum, mean, and maximum weight is indicated by the gray dots. The summary effect (with 95% CIs) is marked by the solid black circle with horizontal bars. Dashed vertical line indicates no difference between treatment conditions.

3.6 Secondary Outcomes

3.6.1 Pain Unpleasantness

Twelve studies reported pain unpleasantness data, generating 44 poolable effect sizes. We found no significant evidence of higher pain unpleasantness in the antagonist conditions compared to control treatments (Hedges' g [95% CI] = 0.11 [-0.09, 0.30]), p = .290).

3.6.2 Pain Threshold

Pain thresholds were assessed in sixteen studies ($k_{effects}$ = 49). There was a small effect of opioid antagonism on pain, with an overall decrease in pain thresholds in the antagonist drug conditions relative to control groups (Hedges' g [95% CI] = 0.15 [0.04, 0.25], p = .007).

3.7 Subgroup Analyses

An overview of the summary effects for all subgroup analyses is shown in Figure 6.

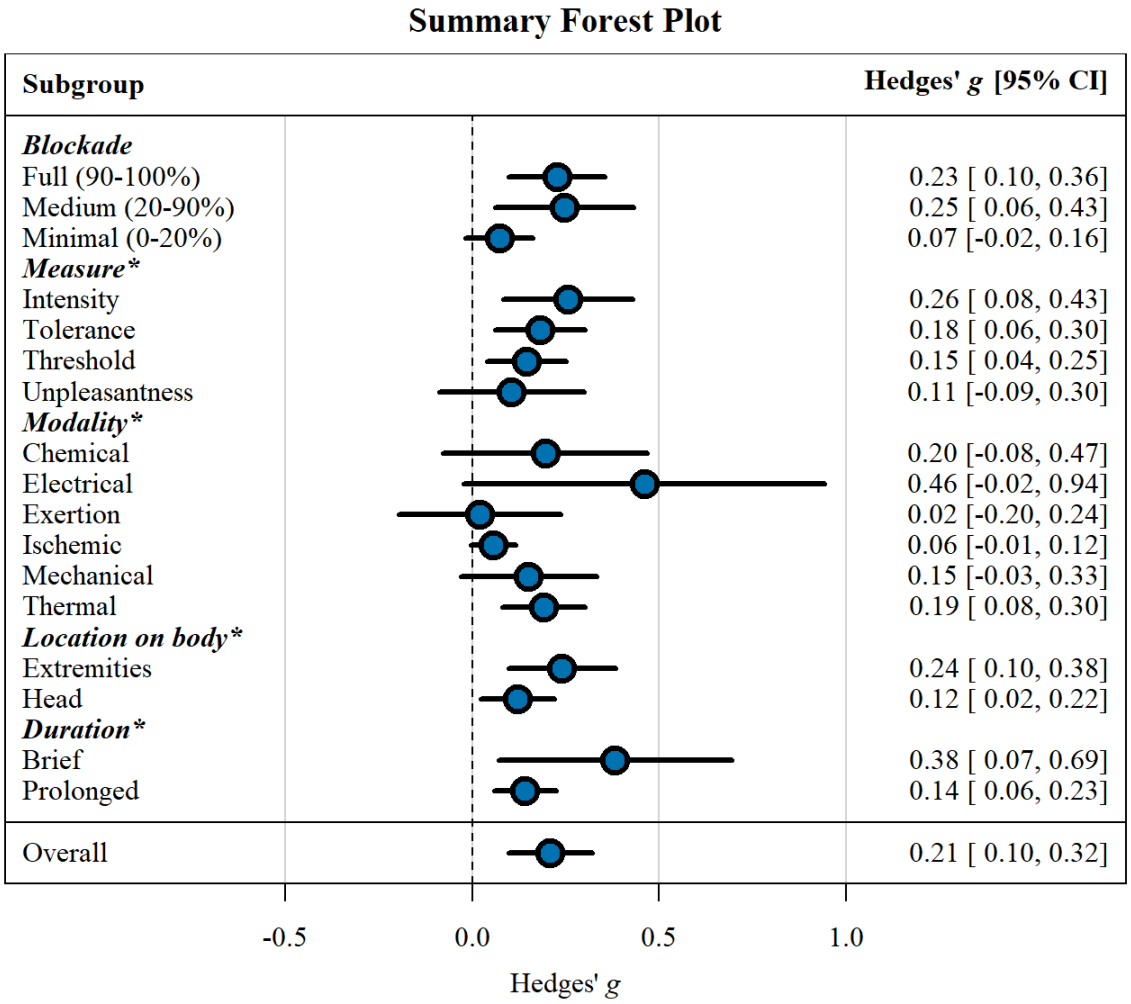

Figure 6. Forest plot illustrating the summary effects for all subgroup analyses. * indicates that the analysis was restricted to full blockade studies. Summary effects (with 95% CIs) are displayed by the solid black circles with horizontal bars. Dashed vertical line indicates no difference between treatment conditions.

3.7.1 Location of Noxious Stimulation on the Body

Pain was induced on the arms, legs, hands, or feet in most of the included studies ($k_{articles}$ = 46, $k_{effects}$ = 301). In three studies, pain induction was performed on the head ($k_{effects}$ = 20). None of the included studies induced pain on the core (torso). When testing was performed on the extremities, opioid antagonism had a small but significant effect in the direction of more pain compared to the control conditions (Hedges' *g* [95% CI] = 0.24 [0.10, 0.34], *p* = .001). A small significant difference in pain was also found for antagonist drugs versus pharmacologically inactive treatments when noxious stimuli were applied to the head (Hedges' *g* [95% CI] = 0.12 [0.02, 0.22], *p* = .017).

3.7.2 Stimulus Modality

Opioid blockade did not lead to significantly more pain in comparison with the control conditions for most of the assessed stimulus modalities (see Figure 7). The exception was thermal stimulation ($k_{effects}$ = 110), which generated more pain overall in the antagonist conditions compared to controls (Hedges' *g* [95% CI] = 0.19 [0.08, 0.30], *p* = .001). For electrical stimuli ($k_{effects}$ = 74), there was a moderate but nonsignificant effect of opioid blockade compared to inactive treatments (Hedges' *g* [95% CI] = 0.46 [-0.02, 0.94], *p* = .062). Similarly, there was a small effect of opioid antagonism on pain for mechanical stimuli ($k_{effects}$ = 49), but again this was not statistically significant (Hedges' *g* [95% CI] = 0.15, *p* = .104 [-0.03, 0.33]). The standardized mean difference indicated similar levels of exercise-induced pain with antagonist drugs and inert treatments ($k_{effects}$ = 8; Hedges' *g* [95% CI] = 0.02 [-0.20, 0.24], *p* = .857). Furthermore, there was no significant mean difference between the antagonist conditions and control conditions for ischemic pain ($k_{effects}$ = 68; Hedges' *g* [95% CI] = 0.06 [-0.01, 0.12], *p* = .074). The summary effect for chemical stimuli ($k_{effects}$ = 17) indicated that opioid antagonism overall did not significantly increase pain as compared with inert substances (Hedges' *g* [95% CI] = 0.20 [-0.08, 0.47], *p* = .163).

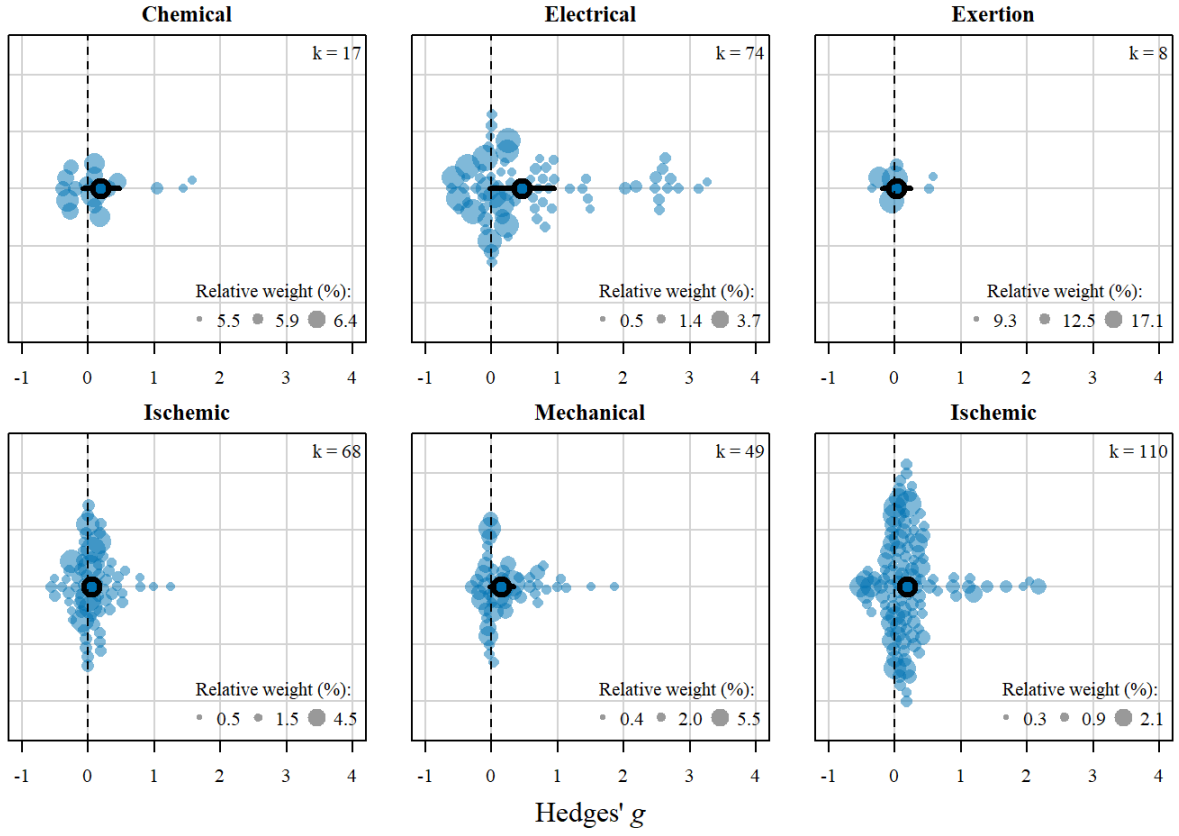

Figure 7. Orchard plot illustrating the distribution of effects for all stimulus modalities. Blue transparent circles depict individual effect sizes. The relative weight of each effect is illustrated by the size of the circle. Minimum,

mean, and maximum weight is indicated by the gray dots. The summary effect (with 95% CIs) is marked by the solid black circle with horizontal bars. Dashed vertical line indicates no difference between treatment conditions.

3.7.3 Duration of Noxious Stimulation

Subgroup analysis of stimulus duration displayed overall significant effects of antagonist drug administration on pain for both brief and prolonged stimuli. There was a medium effect for brief stimuli, with significantly higher pain in the antagonist conditions compared to control conditions (Hedges' *g* [95% CI] = 0.39 [0.07, 0.70], p = .016). For prolonged stimuli, the summary effect was small, but also indicated an overall tendency towards more pain with opioid blockade as compared with inactive treatments (Hedges' *g* [95% CI] = 0.14 [0.06, 0.23], *p* = .001).

3.8 Risk of Bias Assessment

The risk of bias assessment was based on all included studies (i.e., the total included in the main analysis).

3.8.1 Quality Assessment

In general, the included studies were of moderate to high quality. Due to the inclusion criteria, the lowest possible score was 2 on the Jadad scale. Most studies (57 out of 60) were given a third point for describing an appropriate method of double blinding. Twenty-two studies received an additional point for including descriptions of dropouts. Five studies also described the method to generate the sequence of randomization. The vast majority of studies thus received a score indicative of moderate study quality (*M* = 3.4, *SD* = 0.59). Meta-regression was performed to investigate study quality as one possible source of heterogeneity. The analysis found a negative correlation between the size of the summary effect and study quality, but this relationship was not statistically significant, *B (SE)* = -0.10 (0.10), *z* = -1.03, *p* = .305. However, high study quality was associated with smaller effect sizes, and there was less variability across effects in the most rigorously reported studies (see Figure 8). Thus, although the quality assessment indicated generally satisfactory quality of the included evidence, a tendency towards inflated effect estimates in lower quality studies might contribute to an overestimation of the overall effect.

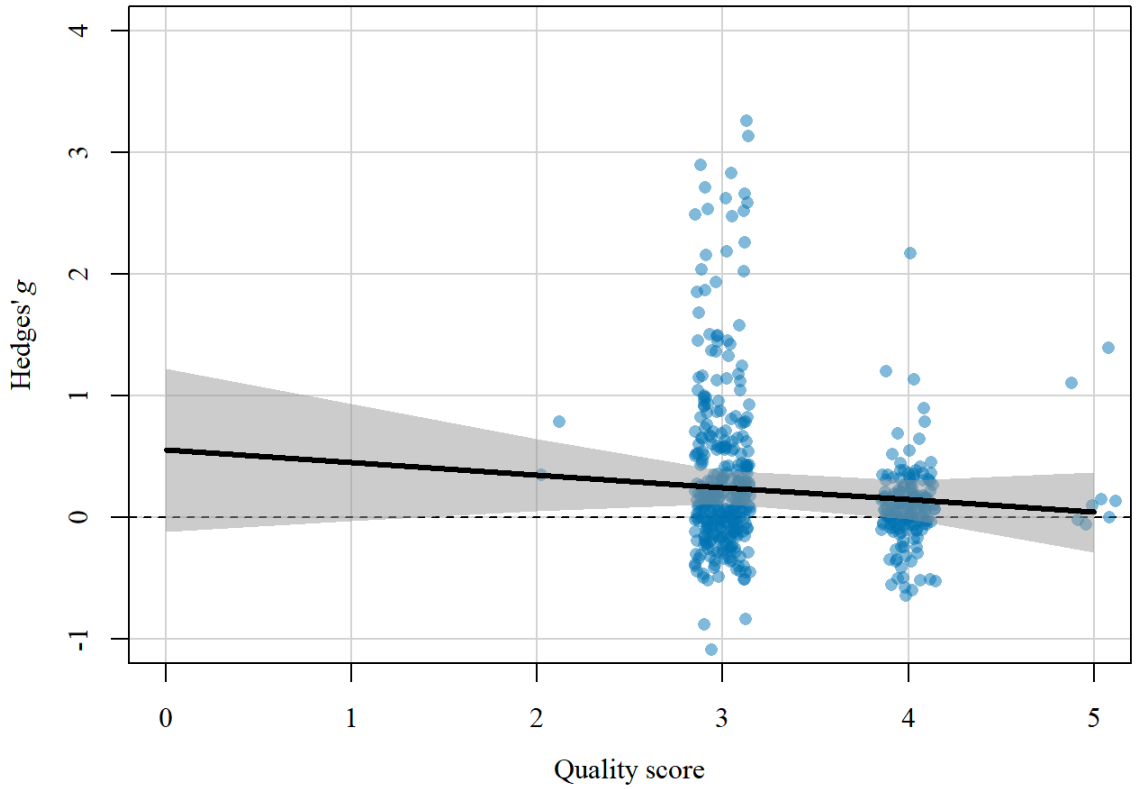

Figure 8. Scatter plot illustrating the relationship between total quality score and the observed effect for all included studies. The black line is the regression line, and the gray bands display 95% CIs. Jitter has been added on the x-axis to visualize individual effects more easily. The nearest integer thus reflects the quality score of each data point. Dashed vertical line indicates no difference between treatment conditions.

3.8.2 Publication Bias and Small-Study Effects

Some evidence of reporting bias was found for the overall effect (see Figure 9). Visual inspection of the funnel plot revealed asymmetry in observed effects, with less precise studies finding greater effects of opioid blockade. The Egger's test was statistically significant, *B (SE)* = 6.01 (0.42), z = -14.40, $p < .0001$, indicating that smaller studies tended to show more pronounced effects of opioid blockade (in the direction of more pain) than larger studies. The contour-enhanced funnel plot suggested that published reports of significant effects showing more pain in the control condition with a significance level of $p < .01$ might be missing, as there was a high prevalence of significant effects at this level pointing towards more pain in the antagonist condition. There appeared to be a small tendency towards biased reporting of significant antagonist effects at $p < .05$ and $p < .1$ as well. A post-hoc analysis showed that when symmetry was restored by removing these large effects (Hedges' $g > 1$, as well as Hedges' g <-1), the summary effect was substantially reduced, but remained significant (Hedges' g [95% CI] = 0.09 [0.03, 0.14], $p = .003$). The same was true when extreme values

were removed for pain intensity (Hedges' *g* [95% CI] = 0.07 [0.01, 0.14], *p* = .045). Overall, however, funnel plot assessment showed that effects were predominantly clustered around the mean. The trim-and-fill method estimated that no effects were missing on the left side of the plot, albeit with a standard error of 12.40. In sum, these plots indicated that a combination of reporting bias and exaggerated results from smaller studies might have skewed the results slightly in favor of increased pain with antagonist drugs. The summary effect appeared to be influenced by this tendency to some extent.

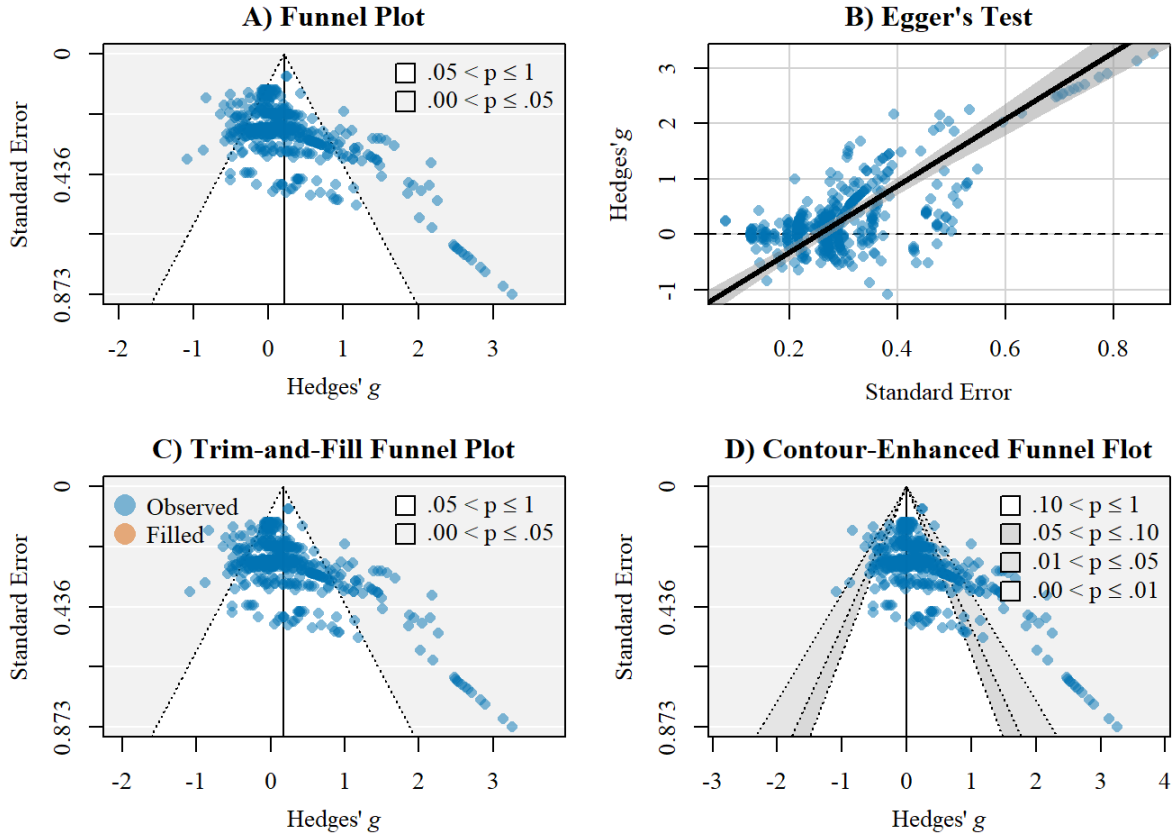

Figure 9. Funnel plots illustrating the risk of publication bias across all included studies. Blue circles depict observed effect sizes and orange circles depict missing effect sizes estimated by the trim-and-fill method. The shaded regions correspond to different significance levels for the effects. For the Egger's test, the black line is the regression line, the gray bands display 95% Cis, and the dashed vertical line indicates no difference between treatment conditions.

3.9 Sensitivity Analyses

Two sensitivity analyses were performed with within-subjects correlation coefficients of 0.25 and 0.75, respectively. These analyses showed that using different imputations left the results largely unchanged. The overall effect was similar with a within-subjects correlation of 0.25 (Hedges' *g* [95% CI] = 0.19 [0.09, 0.29], p = .0001) and 0.75 (Hedges' *g* [95% CI] = 0.22 [0.10, 0.22], p = .0002). This was also true for the main outcomes; summary effects for

pain intensity and pain tolerance were virtually identical with all attempted values. When a correlation coefficient of 0.75 was used, an increase in the summary effect for the minimal blockade subgroup was seen (Hedges' *g* [95% CI] = 0.22 [-0.24, 0.69], p = .350); however, confidence intervals were wide and still overlapped with zero. Overall, then, these analyses demonstrated the robustness of our initial findings.

An additional sensitivity analysis was conducted post-hoc to determine whether the use of non-drug interventions significantly altered the summary effect in full blockade studies. Non-drug interventions were used in 31 articles ($k_{effects}$ = 154), whereas pain testing was performed without any conditioning stimuli in 34 articles ($k_{effects}$ = 172). The results showed that the use of non-drug interventions was associated with a slightly greater summary effect (Hedges' *g* [95% CI] = 0.29 [0.11, 0.46], p = .001) compared to when pure pain testing was performed (Hedges' *g* [95% CI] = 0.15 [0.01, 0.28], p = .040). This suggests that the use of conditioning stimuli may explain some of the observed variability across effects.

4 Discussion

4.1 Summary of Evidence

The results of the main analyses showed that in sum, μ-opioid receptor blockade produced a slight increase in pain compared to pharmacologically inert treatments, consistent with the hypothesis that endogenous μ-opioid activity can downregulate pain in humans. The magnitude of the response depended on the achieved degree of opioid antagonism. Medium blockade effects were overall slightly higher than full blockade effects compared to controls, while minimal blockade did not generate more pain in the antagonist conditions. Specifically, pain intensity increased, pain tolerance was reduced, and pain thresholds were significantly lowered in the antagonist condition. The perceived unpleasantness of pain, however, was not significantly altered by opioid antagonism compared to placebo. Moreover, all the meta-analytically derived effects were small, ranging from 0.15 to 0.38. There was also substantial heterogeneity among studies, and we found indications of reporting bias in the included evidence.

The findings in this meta-analysis are in alignment with prior research indicating that endogenous μ-opioids can instigate pain relief when humans are exposed to painful stimuli (Bencherif et al., 2002; Zubieta et al., 2001). Overall, full blockade of μ-opioid receptors led to an increase in pain intensity in treated versus untreated participants. In contrast, a previous systematic review concluded that μ-opioid antagonism produced either no effect or ambiguous effects on pain (Werner et al., 2015). Consistent with this more cautious interpretation of the literature, the small size of the observed summary effects in the present meta-analysis should be noted. Most of the observed effects also had confidence intervals which spanned zero. Thus, while we observed an effect in the expected direction, the impact of opioid blockade on experimental pain measures in the published human literature was modest. Early research generated the suggestion, based on observed bidirectional effects of naloxone on pain in humans, that endogenous μ-opioids may have a "modulatory rather than strictly analgesic role" (Buchsbaum et al., 1977). The significant – but modest – effects observed in the present meta-analysis can be interpreted as evidence of this, and may place these results more in line with opioid fine-tuning of pain relief (Eikemo et al., 2021).

Some theorists hold that opioid analgesics can reduce pain unpleasantness without interfering with the sensory component of pain, leading to the phenomenon known as pain asymbolia (Gerrans, 2020). Interestingly, the results showed that the affective component of pain, indexed by unpleasantness ratings, was not significantly worsened by opioid blockade.

Accordingly, we did not find support for the hypothesis that (endogenous) opioids particularly mediate the affective aspect of pain perception.

4.1.1 Experimental Versus Clinical Pain

This meta-analysis aimed to elucidate the role of the endogenous opioid system in healthy humans during exposure to painful stimuli. Experimental pain models are largely used to provide insight into the mechanisms underlying various clinical pain phenomena (Staahl & Drewes, 2004). Thus, an important question concerns how well findings translate to (non-experimental) acute and chronic pain states. Animal models are also commonly used in this endeavor, and much of the current knowledge about pain physiology and pathophysiology stems from experimental pain testing in animals (Berge, 2013). Yet there are some concerns about the validity of experimental pain models in animals and humans.

First, animal models use observable behaviors, such as motor withdrawal, startle reactions, avoidance, vocalizations and more complex reactions (e.g., conditioned responses), to infer the experience of pain (Le Bars et al., 2001). Functional imaging, electrophysiology, biomarkers and genetics can also be used, sometimes in conjunction (Berge, 2013). Human studies, on the other hand, primarily rely on self-report. The use of indirect pain assessments in animals means that important aspects of the human pain experience may not be captured (Corder et al., 2018).

Second, context and beliefs about the meaning of pain are powerful determinants of the pain experience (Pasternak & Pan, 2013). Thus, there may be important differences between experimental and clinical pain that prevent direct comparison between the two. Compared to clinical pain, pain induced in an experimental setting is finite and predictable, assuredly not tissue-damaging, can be stopped at any moment, and does not have similar emotional significance and lifestyle implications (Edens & Gil, 1995). Clinical pain, on the other hand, is often fluctuating, indicative of tissue injury, of unknown duration, and can be both emotionally and functionally impairing. As a result, while experimental pain models can provide valuable information and insight, they may have limited applicability in describing clinical situations (Pasternak & Pan, 2013). Thus, although we found that complete opioid blockade slightly increased pain in healthy humans in pain experiments, this observation may not generalize to clinical situations. For instance, chronic low back pain is not worsened by opioid antagonism (Bruehl et al., 2002).

4.1.2 The Role of Endogenous Opioids in Clinical Pain

The view that endogenous opioids cause hypoalgesia largely stems from work in animals. For instance, tissue injury has been shown to induce prolonged basal activity of μ-opioid receptors in mice, which promoted naltrexone-reversible analgesia (Corder et al., 2013). Microinjections of μ-opioid agonists into brain areas known to be involved in analgesia and descending pain inhibition (e.g., the insular cortex, amygdala, hypothalamus, PAG, DLPT, and RVM, all of which contain high concentrations of μ-opioid receptors) decrease responses to pain in animals (Fields, 2004). Similarly, electrical stimulation of the PAG in humans produces analgesia, which is reversed by opioid blockade (Hosobuchi et al., 1977). Recently it was discovered that naloxone reverses analgesia in humans that lack of the sodium channel $Na_v1.7$ (leading to congenital insensitivity to pain), and this effect was also observed in $Na_v1.7$ knockout mice (MacDonald et al., 2021). But extrapolating findings from animals to humans is potentially problematic. It has been noted previously that animal models in pharmacological research suffer from a lack of predictive validity, and that many animal findings do not directly translate to humans (Venniro et al., 2020). Rodents are used in almost all animal pain models (Le Bars et al., 2001). However, there may be fundamental differences in how pain relief occurs in rodents compared with humans (Eikemo et al., 2021). Many forms of analgesia can be fully reversed by opioid antagonism in rodents (Fields, 2004). In contrast, we observed just a small hyperalgesic effect following μ-opioid antagonism in humans. Thus, endogenous opioids may not carry the same indispensable function in humans in terms of pain relief, and may instead exert a modulatory role in pain perception (Eikemo et al., 2021).

Early research in humans showed that postoperative pain was worsened by naloxone injections (Levine et al., 1978). On the other hand, exogenous μ-opioid agonists reliably produce analgesia when given in the short term to pain patients (Fields, 2011). Both findings seem to suggest that μ-opioid agonism normally lessens clinical pain. Altered opioid signaling has been proposed as a reason for chronic pain states, as these may result from changes in descending inhibitory pathways which are largely mediated by endogenous opioids (Bruehl & Chung, 2004). In the brain, reduction in μ-opioid receptors availability have been observed in chronic pain patients compared to healthy controls in multiple PET studies, indicating diminished efficacy of endogenous opioids (Harris et al., 2007; Jones et al., 1999, 2004; Klega et al., 2010; Maarrawi et al., 2007; Sprenger et al., 2006; Willoch et al., 2004). However, these studies cannot determine whether the observed differences in the endogenous opioid system are a result of chronic pain, if they preceded the condition or are medication-

induced (Thompson et al., 2001). Our findings suggest that μ-opioid receptor activation does not substantially mitigate non-clinical pain in healthy people. Interestingly, multiple studies support a less essential role of endogenous opioids in clinical pain as well. Opioid blockade has not been found to increase pain in many clinical conditions, including chronic low back pain, tension headache, and neuralgia (Bruehl et al., 2002; Hosobuchi et al., 1977; Langemark, 1989; Lindblom & Tegnér, 1979). Consequently, it has been suggested that "the endorphin system does not offer protection of any importance in chronic pain" (Lindblom & Tegnér, 1979, p. 65). Thus, one may argue that endogenous opioids do not offer substantial protection against acute pain either.

Recently, other neurotransmitter systems have been proposed to act in conjunction with endogenous opioids to reduce pain. Some forms of analgesia previously thought to be mediated by endogenous opioids alone, such as exercise-induced hypoalgesia, are now believed to primarily involve endocannabinoids (Siebers et al., 2021). Endocannabinoids have also been shown to relieve both acute and chronic pain in animal models, and have in some human clinical trials been reported to be as effective as morphine in the treatment of neuropathic pain (Zogopoulos et al., 2013). Norepinephrine and serotonin pathways also appear to be involved in antinociception (Bruehl et al., 1999; Singewald & Philippu, 1998). To determine the role of endogenous μ-opioids in the relief of clinical pain, future studies should systematically assess effects reported in the opioid antagonist literature (extending the current review) and consider the how multiple signaling pathways may be interrelated.

One important distinction between clinical and experimental pain, which may influence the respective importance of endogenous opioids, is the duration. Clinical pain is usually longer lasting than pain induced in experiments, unless real injury is provoked (e.g., Springborg et al., 2020). A possible scenario is that a certain duration of pain is necessary for endogenous opioids to impact on pain perception. Certain experimental pain tasks included in this review, such as the submaximum effort tourniquet test (e.g., Grevert & Goldstein, 1977) produce prolonged pain which may be comparable to the duration of some acute pain states. However, we did not find substantial differences in pain between brief and prolonged stimuli when μ-opioid receptors were blocked (effects largely overlapped). This adds credence to the hypothesis that endogenous opioids may not significantly mitigate clinical pain. To further investigate this, we will assess the relationship between stimulus duration and experienced pain in a meta-regression analysis in future work.

4.1.3 The Use of Antagonist Drugs to Infer Endogenous μ-Opioid Functions

In order to gain a greater understanding of the importance of endogenous opioids in pain, we assessed studies which attempted to block μ-opioid agonist responses to observe the impact on pain perception. However, the antagonist drugs also block other opioid receptors. The drugs used in the included studies were naloxone, naltrexone, and nalmefene. All of these are non-selective opioid antagonists that block multiple receptor subtypes (Márki et al., 1999). For instance, PET data has demonstrated that the most commonly used naltrexone dose (50 mg) blocks approximately 95% of μ-opioid binding by the μ-selective agonist carfentanil, and 20-25% of δ-opioid binding by a specific δ-opioid agonist (Weerts et al., 2008). To attribute observed effects to inhibition specifically of μ-opioid signaling, selective antagonists are needed. Examples of such drugs are cyprodime and GSK1521498, which are both highly selective for μ-opioid receptors (Giuliano et al., 2015; Schmidhammer et al., 1989). In this systematic review, we did not locate any eligible studies using selective antagonists. To increase certainty regarding which opioid receptor class is driving potential antagonist effects, future research should implement the use of selective antagonists.

Moreover, it has been suggested that naltrexone, naloxone, and nalmefene exhibit some partial agonist activity, and that these compounds do not produce complete inhibition of agonist responses at μ-opioid receptors (Kelly et al., 2015). However, the method on which this suggestion is based has been criticized (Wang & Sadee, 2015). Naloxone has also been reported to have a dose-dependent biphasic effect on clinical and experimental pain in animals and humans, with low doses producing analgesia (Buchsbaum et al., 1977; Levine et al., 1978; Ueda et al., 1986; Woolf, 1980). Furthermore, it has been proposed that continuous infusion of naloxone, which was used in several of the included studies, might potentiate the activity of opioid receptors through up-regulation (Gan et al., 1997). Up-regulation of beta-adrenergic receptors have been observed within 30 minutes following cardiopulmonary bypass (Schwinn et al., 1991), suggesting that such physiological changes may take place inside the timeframe of experimental pain testing. If so, agonist binding may no longer be inhibited, which counteracts the aim of deactivating μ-opioid receptors to deduce their function in pain.

Finally, some full agonists are capable of eliciting a maximal biological response through binding to less than 1% of the total receptor pool. This has been demonstrated for both acetylcholine and noradrenaline (Rang et al., 2016; Stephenson, 1956). If endogenous μ-opioids possess such characteristics, this would interfere with the proposed usefulness of

antagonist drugs, unless complete μ-opioid receptor occupancy is achieved and maintained during experiments.

In sum, the above findings suggest that some receptor activation may occur despite the use of the most common opioid antagonist drugs, and that multiple opioid receptor classes may contribute to the observed response. Thus, these compounds are somewhat limited in what they can reveal about the function of endogenous μ-opioid agonists. This underscores the need for selective, pure antagonists as the new standard in research. Meanwhile, these drawbacks must be considered when interpreting the results of antagonist studies.

4.2 Limitations and Directions for Future Research

Several limitations were present in this review and should be acknowledged.

4.2.1 Sample Size, Missing Data and Reporting Bias

There was a high prevalence of small samples in the included studies. Small samples are more likely to be biased, thus providing less statistical power (due to higher levels of variability; Gravetter & Wallnau, 2014). This can be problematic particularly if the treatment effect is small. Larger samples are generally more accurate – however, if the sample size is too large, there is a risk of a study being overpowered, which means that it may obtain statistically significant differences even if the actual effect size is very small (Sakai, 2016). Only a few large samples were included in this review; most studies had a sample size of less than 30, indicating that underpowering was a more prevailing issue. Power to test the summary effect is higher in a meta-analysis because samples are combined (Borenstein, 2009). However, small samples tend to produce results that are more extreme than larger samples (Turner et al., 2013); thus, there is a risk of bias in many studies included in this review which carries over to the meta-analytic output (Higgins & Green, 2011). The pooled variability may also be higher due to increased risk of sampling error in individual studies.

At the review level, an important limitation concerned incomplete retrieval of data. Twenty-eight eligible studies could not be included due to missing necessary data, which was not obtained in time for the analysis. Data which is received subsequently will be included in future analyses. A total of 481 effects and more than 2000 individual participants were included in the meta-analysis; however, incomplete reporting and inaccessible statistics did lead to some loss of data, which may have affected the overall outcome. There seemed to be a tendency towards less detailed reporting of non-significant findings. Thus, the data overall may be slightly biased due to selective outcome reporting.

We used the Egger's test and various funnel plots to estimate the risk of reporting bias in this review. The results indicated that some publication bias might indeed be present. Future work will further investigate the risk of publication bias using z-curve and p-curve (Bartoš & Schimmack, 2020; Simonsohn et al., 2014), which was not done in the present review due to time restraints.

Taken together, the combination of many small samples and a likelihood of reporting bias (favoring increased pain in antagonist conditions) suggest that the summary effect may be slightly inflated, due to compounding of exaggerated treatment effects from small studies. Removal of result indicative of publication bias resulted in a smaller, yet still significant summary effect; thus, this tendency may not greatly alter the interpretation of the findings. However, as a result of these limitations, our ability to reach solid conclusions in this review is somewhat reduced.

4.2.2 Variability

This meta-analysis aimed to determine the conditions under which pain modulation is opioid-dependent. The results showed that a substantial amount of variability was not explained by the analyses conducted thus far. Small samples leading to imprecise effect size estimates may be one reason for the considerable observed heterogeneity. Contrary to evidence cited in the introduction, endogenous opioid modulation of pain perception did not seem to vary according to differences in nociceptor activation, as stimulus modality generally did not have a significant effect on pain following opioid antagonism. However, two tenable reasons for the observed variance were not explored. First, a wide array of experimental pain interventions was used in the included studies. Pain produced by different methods may be influenced endogenous opioids to various degrees, which may have caused some variability in the results. Second, approximately half of the included studies employed non-drug interventions in addition to the treatment of interest. A previous systematic review concluded that endogenous opioids seem to regulate specific kinds of analgesic responses, i.e., stress-induced analgesia and analgesia induced by rTMS (Werner et al., 2015). Thus, various conditioning stimuli can possibly explain some variability in the observed effects. The impact of opioid blockade on pain modulation by non-drug interventions will be investigated in future work.

4.2.3 Residual Drug Effects

Insufficient time between experimental sessions presented a notable limitation in some included studies. The activity of naltrexone in the brain is long-lasting, with 30% of receptors

still occupied by the antagonist a full week after administration of a single 50 mg dose (Lee et al., 1988). Two days following administration, μ-opioid receptor blockade was at 91%. As shown in Table 3, the intersession intervals in the within-subjects naltrexone studies lasted up to one week. This allows for some carry-over effects from the previous session within participants who received naltrexone in the first study session. In fact, four studies repeated testing within two days. In these studies, there is reason to believe that residual drug effects were present, interfering with the validity of the control condition. Similarly, a single dose of 1 mg nalmefene resulted in high blockade that remained at 53,3% at the 24 hour time point after administration (Kim et al., 1997). The one included study that used nalmefene was within-subjects, and retested pain after only one day. Thus, μ-opioid receptor occupancy might still have been at a moderate level during the second session. Future repeated-measures studies should ensure that residual antagonist effects are adequately controlled for. Residual drug effects were not investigated in the current meta-analysis, but we plan to quantify the impact in this dataset.

4.2.4 Estimated Level of Blockade

As the level of blockade was estimated from an extrapolation of multiple study results, there is some uncertainty regarding the calculations. Few PET studies detail the pharmacokinetics of all the most common opioid antagonists, including a precise time-course for onset of effects and time to elimination from the brain. Importantly, the doses required to achieve 100% μ-opioid receptor occupancy need to be established. Only two studies (Ingman et al., 2005; Villemagne et al., 1994) have reported this level of blockade for nalmefene and naloxone, but these studies did not report the exact time to maximum effect or its duration. Naltrexone is more consistently demonstrated to produce blockade above 90% with a single dose of the standard 50 mg dose (Lee et al., 1988; Rabiner et al., 2011). However, it is uncertain how quickly full blockade occurs. Most included naltrexone studies allowed one hour for absorption, as peak plasma levels are reached within one hour after oral administration (Kranzler et al., 1997). Central activity is thus inferred from plasma levels, which may be erroneous; Weerts et al. (2008) found no clear correlation between plasma levels and receptor binding potential. Assuming that a one-hour waiting time is long enough, however, 50 mg (which was the minimum dose used in all included studies in this meta-analysis) is sufficient to induce significant to complete μ-opioid receptor blockade according to the available information.

Furthermore, the relevant molecular imaging studies were either single-dose or conducted following a multiple-day dosage regimen. In contrast, many studies in the meta-analysis

employed an extended dosage regimen, where a bolus injection of naloxone was followed by continuous infusion of the antagonist drug. While we expect that continuous infusion maintains receptor occupancy levels throughout dosing, the effect of continuous antagonist infusion on μ-opioid receptor occupancy and availability over time has not been demonstrated.

In sum, future PET studies are needed to determine the necessary doses of each antagonist to induce absolute μ-opioid blockade in humans, as well as a comprehensive timeline for receptor blockade.

4.2.5 Dose Categories

Pain was overall higher during moderate blockade compared to full blockade, suggesting that dose categories may have been too wide. It is possible that a maximal response was induced at a lower level of μ-opioid receptor occupancy, and that the cut-off should have been set below 90%. Several effects may also stem from testing performed at receptor occupancies only slightly below this percentage, producing almost identical responses. It is also possible that opioid antagonism induces a stronger hyperalgesic effect at moderate doses. Alternatively, the small difference may be spurious. The medium blockade category contained fewer studies and effects relative to the full blockade category, which means that the summary effect was more susceptible to the limitations (risk of bias) already discussed.

We originally intended to include two additional categories in the analyses, one for ultra-low dose antagonism (defined as uptake on the microgram level for naloxone, and <1 mg dose for per oral naltrexone) and one for excessively high blockade (i.e., antagonist doses at 50% or above the drug amount necessary to achieve full blockade). Due to challenges in estimating the level and duration of blockade, which we were not able to resolve in time for the analyses, these were combined with the remaining categories. Based on the respective summary effect sizes in the medium and full blockade groups, it appears that excessive blockade did not substantially alter pain perception compared to moderate and high doses. Thus, this category could be omitted in future analyses. Instead, the existing categories may be modified to determine the cut-off for maximal antagonist effects. A possible division is 0-20%, 20-40%, 40-60%, 60-80% and >80% μ-opioid receptor occupancy, which may enable the creation of a more detailed dose-response curve.

4.3 Conclusion

Overall, the meta-analysis showed that experimental pain responses were significantly higher in conditions where moderate or full μ-opioid blockade was achieved via opioid antagonist administration, compared to conditions where participants received a pharmacologically inert substance. The summary effect was consistently small across subgroups and analyses. In a sensitivity analysis where we removed extreme values possibly suggestive of biased reporting, the overall estimate was diminished further. Importantly, we found evidence of a dose-response response relationship, which may explain some of the observed heterogeneity. Notably, the confidence intervals of 81.1% of effects included in the main analysis overlapped zero. This means that a substantial majority of the overall effects in the literature might be null findings, indicative of a minimal role of endogenous opioid pain relief in these studies. We found the largest effect estimate for electrical pain, but there was high variability and again zero was included in the confidence interval. Exertion pain presented with a null effect, though with wide confidence intervals and little data. In sum, we observed great variability in effect sizes of opioid antagonist effects on experimental pain in healthy humans, even when analysis was restricted to pain testing during estimated blockade of at least 90% of μ-opioid receptors. Subgroup analyses revealed some patterns of variability. For instance, effect sizes were on average somewhat larger for measures of pain intensity than for pain unpleasantness. Similarly, the summary effects indicated that brief stimuli might have a greater effect on pain than prolonged stimuli, but confidence intervals showed complete overlap between the two. There was no statistically significant difference between effects on intensity versus unpleasantness ratings, and similarly no credible difference between studies of pain stimuli shorter than one minute compared with prolonged stimuli. Nevertheless, the available data clearly indicates a lack of support for the notion than endogenous opioids selectively regulate the affective dimension of pain, and that endogenous opioids are engaged in regulation preferentially of longer-lasting pain.

Our ability to draw further conclusions is hampered by some limitations in the included evidence at both the study level and review level, including evidence of publication bias, considerable heterogeneity among studies, and small-study effects. In sum, the results highlight the necessity of future high-quality research. As discussed, some methodological limitations, notably the use of non-selective antagonist drugs, prevent strong conclusions about whether observed effects can be attributed to complete μ-opioid blockade. Our findings demonstrated a (non-significant) inverse relationship between the magnitude of the summary effect and study quality, but also showed that insufficient dosage might explain some null

effects. The high prevalence of low precision estimates across analyses especially renders the interpretation of effects uncertain. On the whole, the results illustrate that a lot of underlying variability has yet to be explained. One compelling direction of investigation is assessing the influence of conditioning stimuli on the observed effects. This was beyond the scope of this thesis and will be examined in future work.

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7 Appendix A

Table A1

Author (year)	N	Females (%)	Age M (SD)	Antagonist Drug	Method of Pain Induction	Modality	Stimulus Duration	Pain Measurement Category	Location on Body	Quality Score	Hedges' *g* (range)
Frew & Drummond (2008)	31	54.8%	36.3 (13.2)	Naltrexone	Electrical shock Cold Pressor Test	Electrical Thermal	Brief Prolonged	Intensity Tolerance Unpleasantness	Extremities	4	(-0.05 - 0.32)
Amanzio & Benedetti (1999)	31	41.9%	47.7 (9.3)	Naloxone	Sphygmomanometer cuff	Ischemic	Prolonged	Tolerance	Extremities	2	(0.35 - 0.79)
Benedetti et al. (1999)	173	48.0%	38.1 (8.6)	Naloxone	Capsaicin injection Electrical shock	Chemical Electrical	Prolonged	Intensity Threshold	Extremities	3	(-0.39 - 1.58)
Berna et al. (2018)	20	55%	27 (5.4)	Naloxone	Thermal heat	Thermal	Brief	Intensity Unpleasantness	Extremities	3	(0,08 - 0,59)
Bruehl et al. (2011)	85	60%	33.8 (9.6)	Naloxone	Forgione-Barber finger pressure pain stimulator Ischemic pain task	Mechanical Ischemic	Prolonged	Unpleasantness Intensity	Extremities	3	(-0.08 - 0.17)
Bruehl et al. (1996)	53	0%	NR	Naltrexone	Forgione-Barber finger pressure pain stimulator	Mechanical	Prolonged	Intensity	Extremities	3	(-0.19 - 0.26)
Buchsbaum et al. (1983)	19	47.4%	NR	Naloxone	Electrical shock	Electrical	NR	Unpleasantness	Extremities	3	(-0.36 - (-0.27))
Burton et al. (2017)	39	51.3%	25.7 (5.3)	Naloxone	Capsaicin cream with heating element	Thermal	Prolonged	Intensity	Extremities	3	(-0.45 - (-0.04))

Table A1 (Continued)

Author (year)	N	Females (%)	Age M (SD)	Antagonist Drug	Method of Pain Induction	Modality	Stimulus Duration	Pain Measurement Category	Location on Body	Quality Score	Hedges' *g* (range)
Sprenger et al. (2011)	20	0%	25.8 (0.11)	Naloxone	Heat stimulation Cold Pressor Test	Thermal	Brief Prolonged	Intensity	Extremities	4	(0.02 - 0.35)
King et al. (2013)	33	48.5%	23.5 (3.9)	Naltrexone	Focal heat	Thermal	Brief	Intensity	Extremities	3	1.00
Cook et al. (2000)	12	0%	24 (4)	Naltrexone	Dynamic handgrip exercise	Exertion	Prolonged	Intensity Threshold	Extremities	3	(-0.05 - 0)
de Andrade et al. (2011)	36	33.3%	29.1 (6.0)	Naloxone	Thermal stimulation (cold pain)	Thermal	Brief Prolonged	Intensity Threshold	Extremities	3	(-0.46 - 1,87)
Eippert et al. (2008)	30	0%	NR	Naloxone	Thermal stimulation	Thermal	Brief	Intensity	Extremities	4	0.17
Eippert et al. (2009)	40	0%	25.8 (1.0)	Naloxone	Thermal stimulation	Thermal	Brief	Intensity	Extremities	4	(-0.36 - 2.17)
Esch et al. (2017)	31	77.4%	26.7 (7.7)	Naloxone	Tourniquet test	Ischemic	Prolonged	Tolerance	Extremities	4	(0.11 - 0.13)
Benedetti (1996)	127	NR	NR	Naloxone	Tourniquet test	Ischemic	Prolonged	Intensity	Extremities	3	(-1.09 - 1.24)

Table A1 (Continued)

Author (year)	N	Females (%)	Age M (SD)	Antagonist Drug	Method of Pain Induction	Modality	Stimulus Duration	Pain Measurement Category	Location on Body	Quality Score	Hedges' *g* (range)
France et al. (2007)	151	45.0%	19.4 (0.1)	Naltrexone	Electrocutaneous pain	Electrical	Brief	Tolerance Threshold	Extremities	3	(0.24 - 0.25)
Frew & Drummond (2007)	43	51.2%	20.6 (4.1)	Naltrexone	Cold pressor test	Thermal	Prolonged	Intensity Tolerance Unpleasantness	Extremities	3	(-0.30 - 0.23)
Frid et al. (1981)	52	51.9%	31 (NR)	Naloxone	Tourniquet test	Ischemic	Prolonged	Tolerance	Extremities	3	(-0.84 - 0.43)
Stacher et al. (1988)	24	50%	NR	Naloxone	Radiant heat Electrical stimulation	Thermal Electrical	Brief Prolonged	Tolerance Threshold	Extremities Head	4	(-0.64 - 0.22)
Surbey et al. (1984)	13	0%	22.1 (3.6)	Naloxone	Maximal capacity treadmill run Submaximal endurance treadmill run	Exertion	Prolonged	Intensity	NA	3	(-0.34 - 0.59)
Gordon et al. (1989)	11	0%	26 (3)	Naloxone	Maximal cycle ergometer test	Exertion	Brief	Intensity	Extremities	3	-0.24
Graven-Nielsen et al. (2002)	15	0%	24.4 (NR)	Naloxone	Capsaicin injection	Chemical	Prolonged	Intensity	Extremities	3	(0.09 - 0.45)
Grevert et al. (1983)	30	40%	31.3 (10.4)	Naloxone	Submaximum effort tourniquet technique	Ischemic	Prolonged	Intensity	Extremities	4	(-0.29 - 0.79)

Table A1 (Continued)

Author (year)	N	Females (%)	Age M (SD)	Antagonist Drug	Method of Pain Induction	Modality	Stimulus Duration	Pain Measurement Category	Location on Body	Quality Score	Hedges' *g* (range)
Grevert et al. (1983)	12	0%	24.8 (3.1)	Naloxone	Submaximum effort tourniquet technique	Ischemic	Prolonged	Intensity	Extremities	4	(-0.55 - 0.45)
Hermans et al. (2018)	20	100%	NR	Naloxone	Fisher manual algometry Occlusion cuff	Ischemic Mechanical	Prolonged	Threshold Unpleasantness	Extremities	4	(-0.17 - 0.33)
Hughes et al. (1991)	13	0%	31.7 (8.8)	Naloxone	Cold pressor test	Thermal	Prolonged	Intensity	Extremities	3	(-0.40 - 0.37)
Posner & Burke (1985)	12	NR	NR	Naloxone	Tourniquet task	Ischemic	Prolonged	Intensity	Extremities	3	(-0.09 - 0.31)
Willer & Ernst (1986)	8	50%	NR	Naloxone	Electric shock	Electrical	Brief	Intensity	Extremities	3	(2.49 - 2.89)
Taylor et al. (2013)	14	NR	NR	Naloxone	Cutenous heat stimuli on capsaicin-treated skin	Thermal	Brief	Intensity	Extremities	4	(0.21 - 1.20)
Taylor et al. (2012)	24	50%	24.8 (3.0)	Naloxone	Heat stimulation Cutenous heat stimulation on capsaicin-treated skin	Thermal	Brief	Intensity	Extremities	3	(-0.20 - 2.26)
Julien & Marchand (2006)	20	50%	30.1 (7.7)	Naloxone	Cold water bath	Thermal	Prolonged	Intensity	Extremities	3	(-0.43 - 0.29)

Table A1 (Continued)

Author (year)	N	Females (%)	Age M (SD)	Antagonist Drug	Method of Pain Induction	Modality	Stimulus Duration	Pain Measurement Category	Location on Body	Quality Score	Hedges' *g* (range)
Jungkunz et al. (1983)	29	NR	NR	Naloxone	Electrical stimulation	Electrical	Prolonged	Threshold	Extremities	3	(0.11 - 1.18)
Kern et al. (2008)	12	50%	NR	Naloxone	Thermal grill (cold) Thermal grill (warm) Heat pain Cold pain Paradoxical pain	Thermal	Brief Prolonged	Intensity Threshold	Extremities	4	(-0.14 - 0.18)
Koltyn et al. (2014)	58	50%	21 (3)	Naltrexone	Isometric exercise Pressure pain	Exertion Mechanical	Prolonged	Intensity Threshold	Extremities	4	(-0.08 - 0.10)
Koppert et al. (2005)	15	20%	29.3 (5.9)	Naloxone	Cotton wool tip Electrical stimulation von Frey filament	Electrical Mechanical	Prolonged	Intensity Unpleasantness	Extremities	3	(-0.88 - 1.86)
Lautenbacher et al. (1990)	11	100%	23.1 (3)	Naloxone	Phasic heat Tonic heat	Thermal	Brief Prolonged	Threshold	Extremities	3	(-0.52 - 0)
Leonard et al. (2010)	24	45.8%	24.9 (5.7)	Naloxone	Tonic heat (thermode)	Thermal	Prolonged	Intensity	Extremities	3	(0.14 - 1.68)
Fechir et al. (2012)	14	28.6%	24.3 (0.9)	Naloxone	Electrical stimulation	Electrical	Brief	Intensity	Extremities	3	(-0.32 - 2.15)

Table A1 (Continued)

Author (year)	N	Females (%)	Age M (SD)	Antagonist Drug	Method of Pain Induction	Modality	Stimulus Duration	Pain Measurement Category	Location on Body	Quality Score	Hedges' *g* (range)
May et al. (2018)	32	43.8%	52.5 (4.3)	Naloxone	Electrical stimulation	Electrical	Brief	Intensity Unpleasantness	Extremities	4	(-0.60 - 0.01)
Moret et al. (1991)	8	0%	34.1 (NR)	Naloxone	Cold pressor test	Thermal	Prolonged	Intensity	Extremities	3	(0.25 - 0.40)
Grevert & Goldstein (1977)	12	50%	NR	Naloxone	Submaximum effort tourniquet technique	Ischemic	Prolonged	Intensity	Extremities	3	(-0.29 - 0.27)
Pontén et al. (2020)	27	0%	40.3 (8.3)	Naltrexone	Pressure pain stimulator	Mechanical	Brief	Threshold	Extremities	4	(-0.02 - 0.70)
Zachariae et al. (1998)	20	65%	29.7 (8.2)	Naloxone	Electrical stimulation	Electrical	Brief	Intensity	Extremities	4	(-0.58 - (-0.10))
Wells et al. (2020)	59	49.2%	27.2 (1.8)	Naloxone	Thermal probe	Thermal	Brief	Intensity Unpleasantness	Extremities	5	(-0.05 - 1.39)
Chapman et al. (1983)	14	42.9%	NR	Naloxone	Stimulating electrode	Electrical	Brief	Threshold	Head	3	(0.06 - 0.24)
Roelofs et al. (2000)	60	0%	NR	Naloxone	Electrical stimulation	Electrical	Brief	Intensity	Extremities	4	(-0.51 - 0.16)
Bruehl et al. (2012)	39	39.3%	30.9 (8.3)	Naloxone	Finger pressure pain stimulator Ischemic pain task	Ischemic Mechanical	Prolonged	Intensity Threshold Unpleasantness	Extremities	3	(-0.25 - 0.04)

Table A1 (Continued)

Author (year)	N	Females (%)	Age M (SD)	Antagonist Drug	Method of Pain Induction	Modality	Stimulus Duration	Pain Measurement Category	Location on Body	Quality Score	Hedges' *g* (range)
Lautenbacher et al. (1994)	10	NR	NR	Naloxone	Thermode	Thermal	Prolonged	Threshold	Extremities	3	(0 - 0)
Sharon et al. (2016)	14	NR	NR	Naloxone	Ice water immmersion	Thermal	Brief	Intensity Unpleasantness	Extremities	4	(0.13 - 0.25)
Simmons & Oleson (1993)	20	NR	NR	Naloxone	Electrical pulp stimulation	Electrical	Prolonged	Threshold	Head	3	(-0.16 - 0.56)
Springborg et al. (2020)	38	0%	23.6 (NR)	Naloxone	Pin-prick by punctuate stimulator	Mechanical	Prolonged	Threshold	Extremities	5	(-0.02 - 0.10)
Gal & DiFazio (1986)	6	0%	NR	Nalmefene	Submaximal tourniquet ischemia test	Ischemic	Prolonged	Tolerance	Extremities	3	(-0.48 - (-0.16))
Taneja et al. (2020)	38	100%	NR	Naltrexone	Hypertonic saline infusion (i.m.) Thermal stimulation 0,5 ℃ Thermal stimulation 24,2 ℃ Thermal stimulation 39,6 ℃ Thermal stimulation 45,9 ℃ Pinprick stimulation 256 mN Pinprick stimulation 512 mN Capsaicin cream	Chemical Mechanical Thermal	Brief Prolonged	Intensity Unpleasantness	Head	4	(-0.32 - 0.36)

Table A1 (Continued)

Author (year)	N	Females (%)	Age M (SD)	Antagonist Drug	Method of Pain Induction	Modality	Stimulus Duration	Pain Measurement Category	Location on Body	Quality Score	Hedges' *g* (range)
Tarr et al. (2017)	99	NR	NR	Naltrexone	Blood pressure cuff	Ischemic	Prolonged	Threshold	Extremities	4	(-0.07 - 0.52)
van der Kolk et al. (1989)	8	0%	39.8	Naloxone	Thermode	Thermal	Brief	Intensity Unpleasantness	Extremities	3	(-0.16 - 0.36)
Koppert et al. (2005)	13	0%	31.2 (5.3)	Naloxone	Cotton-wool tip Electrode Intradermal steel nedle and skin surface electrode von Frey filament (pinprick)	Electrical Mechanical	Brief Prolonged	Intensity Unpleasantness	Extremities	3	(-0.31 - 1.45)
Anderson et al. (2002)	9	44.4%	29 (5)	Naloxone	Capsaicin cream with peltier-device heating element	Thermal	Prolonged	Intensity	Extremities	4	(-0.52 - 1.14)
Willer & Ernst (1986)	8	50%	NR	Naloxone	Electrical stimulation	Electrical	Brief	Intensity	Extremities	3	(2.02 - 3.26)
Younger et al. (2009)	10	100%	55 (7.7)	Naltrexone	Fischer dolorimeter (pressure) Thermode (cold)	Mechanical Thermal	Prolonged	Tolerance Threshold	Extremities	4	(-0.09 - 0.31)

Table A2

Author	Antagonist Drug (Route of Administration)	Dose	Blockade	Design	Intersession interval
Frew & Drummond (2008)	Naltrexone (p.o.)	50 mg	Full	Between-subjects	NA
Amanzio & Benedetti (1999)	Naloxone (i.v.)	0.14 mg/kg	Minimal Medium Full	Between-subjects	NA
Benedetti et al. (1999)	Naloxone (i.v.)	0.14 mg/kg	Full	Between-subjects	NA
Berna et al. (2018)	Naloxone (i.v.)	0.15 mg/kg bolus, then 0.2 mg/kg/h infusion	Full	Within-subjects	1 day
Bruehl et al. (2011)	Naloxone (i.v.)	8 mg	Full	Within-subjects	1 week
Bruehl et al. (1996)	Naltrexone (p.o.)	50 mg	Full	Within-subjects	1 week
Buchsbaum et al. (1983)	Naloxone (i.v.)	8 mg	Full	Within-subjects	1 day
Burton et al. (2017)	Naloxone (i.v.)	0.1 mg/kg	Full	Within-subjects	1 week
Sprenger et al. (2011)	Naloxone (i.v.)	0.15 mg/kg bolus, then 0.2 mg/kg/h infusion	Full	Within-subjects	1 week
King et al. (2013)	Naltrexone (p.o.)	50 mg	Full	Within-subjects	2 days

Table A2 (Continued)

Author	Antagonist Drug (Route of Administration)	Dose	Blockade	Design	Intersession interval
Cook et al. (2000)	Naltrexone (p.o.)	50 mg	Full	Within-subjects	1 day
de Andrade et al. (2011)	Naloxone (i.v.)	0.1 mg/kg bolus, then 0.1 mg/kg/hour infusion	Medium	Within-subjects	2 weeks
Eippert et al. (2008)	Naloxone (i.v.)	0.15 mg/kg bolus, then 0.075 mg/kg/hour infusion	Full	Between-subjects	NA
Eippert et al. (2009)	Naloxone (i.v.)	0.15 mg/kg bolus, then 0.2 mg/kg/hour infusion	Full	Between-subjects	NA
Esch et al. (2017)	Naloxone (i.v.)	0.14 mg/kg	Minimal	Within-subjects	1 day
Benedetti (1996)	Naloxone (i.v.)	10 mg	Minimal Full	Between-subjects	NA
France et al. (2007)	Naltrexone (p.o.)	50 mg	Full	Within-subjects	5 days
Frew & Drummond (2007)	Naltrexone (p.o.)	50 mg	Full	Between-subjects	NA
Frid et al. (1981)	Naloxone (i.v.)	2 mg	Medium	Within-subjects	1 week
Stacher-Janotta et al. (1988)	Naloxone (i.v.)	5 mg 20 mg	Medium Full	Within-subjects	2 days

Table A2 (Continued)

Author	Antagonist Drug (Route of Administration)	Dose	Blockade	Design	Intersession interval
Surbey et al. (1984)	Naloxone (i.v.)	0.15 mg/kg	Full	Within-subjects	3 days
Gordon et al. (1989)	Naloxone (i.v.)	4 mg	Full	Within-subjects	1 day
Graven-Nielsen et al. (2002)	Naloxone (i.v.)	1.3 mg	Full	Within-subjects	2 weeks
Grevert et al. (1983)	Naloxone (i.v.)	10 mg	Full	Between-subjectsWithin-subjects	1 week
Grevert et al. (1983)	Naloxone (i.v.)	2 mg bolus, then 0.2 mg/min for 8 hours (11.6 mg). 10 mg bolus, then 0.1 mg/min for 8 hours (58 mg).	Minimal Full	Within-subjects	1 week
Hermans et al. (2018)	Naloxone (s.c.)	0.4 mg	*	Within-subjects	1 week
Hughes et al. (1991)	Naloxone (i.v.)	10 mg	Medium	Within-subjects	1 day
Posner et al. (1985)	Naloxone (i.v.)	1 mg bolus, then 0.1 mg/min continuous infusion	Full	Within-subjects	7 days
Willer & Ernst (1986)	Naloxone (i.v.)	0.06-0.07 mg/kg	Minimal Full	Within-subjects	4 days

Table A2 (Continued)

Author	Antagonist Drug (Route of Administration)	Dose	Blockade	Design	Intersession interval
Taylor et al. (2013)	Naloxone (i.v.)	0.1 mg/kg	Full	Within-subjects	1 week
Taylor et al. (2012)	Naloxone (i.v.)	0.1 mg/kg	Medium Full	Within-subjects	1 week
Julien & Marchand (2006)	Naloxone (i.v.)	0.14 mg/kg bolus (x 2)	Full	Within-subjects	1 day
Jungkunz et al. (1983)	Naloxone (i.v.)	0.8 mg	Medium Full	Between-subjects	NA
Kern et al. (2008)	Naloxone (i.v.)	0.1 mg/kg bolus, then 0.1 mg/kg/h infusion	Full	Within-subjects	1 week
Koltyn et al. (2014)	Naltrexone (p.o.)	50 mg	Full	Within-subjects	2 days
Koppert et al. (2005)	Naloxone (i.v.)	0.05 µg/kg bolus, then 0.4 µg/kg infusion for 20 min, then 0.2 µg/kg/h for 8 min. 0.5 µg/kg bolus, then 4 µg/kg/h infusion for 20 min, then 2 µg/kg/h infusion for 8 min. 5 µg/kg bolus, then 40 µg/kg/h infusion for 20 min, then 20 µg/kg/h infusion for 8 min.	Minimal Medium Full	Within-subjects	1 week

Table A2 (Continued)

Author	Antagonist Drug (Route of Administration)	Dose	Blockade	Design	Intersession interval
Lautenbacher et al. (1990)	Naloxone (i.v.)	5 mg	Full	Within-subjects	1 day
Leonard et al. (2010)	Naloxone (i.v.)	0.02 mg/kg bolus (x 2) 0.14 mg/kg bolus (x 2)	Full	Between-subjects	1 week
Fechir et al. (2012)	Naloxone (i.v.)	0.01 mg/kg	Medium	Within-subjects	1 week
May et al. (2018)	Naloxone (i.v.)	0.15 mg/kg bolus, then 0.2 mg/kg/hour infusion	Full	Within-subjects	1 day
Moret et al. (1991)	Naloxone (i.v.)	0.04 mg/kg	Full	Within-subjects	1 day
Grevert & Goldstein (1977)	Naloxone (i.v.)	2 mg 10 mg	Full	Within-subjects	1 day
Ponten et al. (2020)	Naltrexone (p.o.)	50 mg	Full	Between-subjects	NA
Zachariae et al. (1998)	Naloxone (i.v.)	1 mg	Full	Within-subjects	1 day
Wells et al. (2020)	Naloxone (i.v.)	0.15 mg/kg bolus, then 0.1 mg/kg/hour infusion	Full	Within-subjects	3 days
Chapman et al. (1983)	Naloxone (i.v.)	1.2 mg	Full	Between-subjects	NA

Table A2 (Continued)

Author	Antagonist Drug (Route of Administration)	Dose	Blockade	Design	Intersession interval
Roelofs et al. (2000)	Naloxone (i.v.)	10 mg	Full	Between-subjects	NA
Bruehl et al. (2012)	Naloxone (i.v.)	8 mg	Full	Within-subjects	1 week
Lautenbacher et al. (1994)	Naloxone (i.v.)	5 mg	Full	Within-subjects	3 days
Sharon et al. (2016)	Naloxone (i.v.)	0.1 mg/kg	Full	Within-subjects	1 week
Simmons & Oleson (1993)	Naloxone (i.v.)	0.8 mg	Medium	Between-subjects	NA
Springborg et al. (2020)	Naloxone (i.v.)	3.25 mg/kg	Full	Between-subjects	8 weeks
Gal & DiFazio (1986)	Nalmefene (i.v.)	0.5 mg 1 mg 2 mg	*	Within-subjects	1 day
Taneja et al. (2020)	Naltrexone (p.o.)	50 mg	Full	Within-subjects	1 week
Tarr et al. (2017)	Naltrexone (p.o.)	50 mg 100 mg	Full	Between-subjects	NA
van der Kolk et al. (1989)	Naloxone (i.v.)	2 mg loading dose, then 1 mg booster dose x 2	Full	Between-subjects	2 weeks

Table A2 (Continued)

Author	Antagonist Drug (Route of Administration)	Dose	Blockade	Design	Intersession interval
Koppert et al. (2003)	Naloxone (i.v.)	0.01 mg/kg	Minimal Medium Full	Within-subjects	1 week
Anderson et al. (2002)	Naloxone (i.v.)	0.1 mg/kg	Minimal Medium Full	Between-subjects	2 days
Willer & Ernst (1986)	Naloxone (i.v.)	0.08 mg/kg	Full	Within-subjects	10 days
Younger et al. (2009)	Naltrexone (p.o.)	50 mg	Full	Within-subjects	1 day

* Signifies that we were unable to calculate blockade

8 Appendix B

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	p. ii
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	p.ii
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	pp. 1, 17-18
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	pp. 1, 16-17
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	pp. 20-21, 24-26
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	p. 21
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	p. 21
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	p. 22
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	p. 22
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	pp. 23-24
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	pp. 22-23
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	pp. 26-27
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	p. 24
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	pp. 20-21
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	pp. 23-24
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	p. 24
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	pp. 24-25
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	pp. 25-27

Section and Topic	Item #	Checklist item	Location where item is reported
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	p. 27
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	p. 27
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	p. 25
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	p. 28
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	p. 28
Study characteristics	17	Cite each included study and present its characteristics.	p. 29, I-VIII
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	pp. I-VIII
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	pp. I-VIII
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	pp. 30, 36
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	pp. 30-36
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	pp. 31-39
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	pp. 38-39
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	pp. 37-38
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	pp. 30-36
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	pp. 40-42
	23b	Discuss any limitations of the evidence included in the review.	pp. 45-46
	23c	Discuss any limitations of the review processes used.	pp. 45-46
	23d	Discuss implications of the results for practice, policy, and future research.	pp. 43-50
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	p. 20
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	p. 20
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	p. 48
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	p. 51
Competing interests	26	Declare any competing interests of review authors.	p. 51

Section and Topic	Item #	Checklist item	Location where item is reported
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	NA