Mechanisms of Musical Analgesia

The Pain-Relieving Effects of Self-Selected Music as Mediated by Endogenous μ-Opioids and Expectation

A Pilot Study

Claudia Pazmandi

Submitted as Master's thesis at the Department of Psychology,

University of Oslo

Spring 2021

Acknowledgements

Thank you to Guro Løseth for taking me under your wing. You have been such a wonderful teacher and guide to me. Thank you for knowing me better than I know myself, pulling me out of rabbit holes, and keeping me on the right track. Your support, patience, and steady belief in me has built me up and kept me going. I am so lucky to have such a mentor!

Thank you to Bruno Laeng and Dan-Mikael Ellingsen for your advice along the way and in particular for seeing me through the finish line. Your advice and feedback were invaluable and I am so grateful for your willingness to accommodate 11th hour edits.

Thank you to Christina Thunberg (programming help), Connor Speich (eye tracker help), Martin Trøstheim & Eira Nordeng Jensen (effect size help), and the LAB lab team. Your considerate help taught me the joys of the collaborative nature of research, thank you for sharing your knowledge and time!

Thank you to Cecilia Vezzani, I simply could not have completed data collection without you. Your cheerful and hardworking nature is infectious!

Thank you to my classmates and roommates for making these two years, pandemic and all, engaging and rewarding. In particular I want to thank Nina and Andrea - your daily support and pep talks kept me going through the thesis writing and I am awe-struck and inspired by you ladies every day!

Thank you to my family for supporting me through all my international whims and academic endeavors. Everything I have ever achieved is thanks to your love and support.

Mechanisms of Musical Analgesia

Author: Claudia Pazmandi

Supervised by: Cand. Psychol. Guro Løseth[1]

Co-Supervised by: Prof. Bruno Laeng[12] & Ph.D. Dan-Mikael Ellingsen[3]

Department of Psychology, University of Oslo, Norway[1]

RITMO Centre for Interdisciplinary Studies in Rhythm Time and Motion[2]

Norwegian Center for Mental Disorders Research (NORMENT Center of Excellence), Oslo, Norway[3]

Abstract

Musical analgesia, also commonly referred to as audio-analgesia or music-induced analgesia, is the phenomenon of experiencing pain relief while listening to music. Music therapy employs musical analgesia in clinical settings as an inexpensive, drug-free alternative or adjunct treatment for pain. Experimental studies have explored possible mechanisms of action with the aim to increase the effectiveness and validity of music therapy. However, few studies have attempted to disentangle how music's capacity as a reward and our inherent expectations for music's effects on our subjective state are potential driving forces of the music analgesic effect. Because both reward and expectancy are regulated by endogenous μ-opioids, we ask whether music-induced analgesia is mediated by the endogenous opioid system and whether the expectation of pain relief is a driving force of this effect. To answer this question, a randomized, placebo-controlled study design utilizing full μ-opioid blockade is planned. In a non-pharmacological pilot study, study protocols and procedures were developed to establish the efficacy of our design and conduct an informed sensitivity analysis to determine sample size for the future study. A novel expectation manipulation was developed to determine how expectations for pain relief during music listening impact a musical analgesic effect. Subjective state was captured by self-reported measures of affect, arousal, musical enjoyment and physiological state by pupil size. Thirty-two participants were tested in the lab with the full experimental task and procedures, and 129 participants were surveyed online to validate our expectation manipulation. In line with previous research, preferred music, as compared to noise, was successful in inducing an analgesic effect. Furthermore, reward was a significant moderator of the analgesic effect. Decreased pain and increased musical enjoyment during music listening compared to noise was reflected only by subjective ratings, not pupil size. The expectation manipulation was found to successfully alter expectations in an online validation survey, but we were unable to replicate the success of the manipulation in the smaller pilot. However, participants' expectations for pain relief during music listening were correlated with their pain ratings in the experiment. The methods and results presented in this thesis will be used to inform a future study investigating the role of reward, expectation, and the μ-opioid system as mechanisms of musical analgesia.

Introduction

1. 1 Context for Research Question

Before COVID-19 took siege of lives on a global scale, a different pandemic captured the fear and attention of countries and communities around the world. Specifically, the U.S. opioid epidemic has garnered the public's widespread concern and, unlike COVID-19, this epidemic seems to be on a persistent increasing trend. The number of prescription and illicit opioid overdoses and deaths quadrupled between 2000 and 2016 in the United States (Murthy, 2016). Even more concerning, social distancing and isolation mandates necessitated by the COVID-19 pandemic pose major risk factors for addiction and opioid abuse caused morbidity (Silva & Kelly, 2020). Current clinical prescribing practices are a major player in the escalation of this epidemic, with the increase in the number of opioid related deaths increasing in parallel with the quantity of opioid pain relievers being prescribed (Murthy, 2016). However, simply reducing the number of opioids prescribed is not an effective solution, as refusing opioid access and coverage was measured by only a 0.1-year increase in expected life span. Treating pain safely and effectively remains a critical and significant concern in health care (Silva & Kelly, 2020). It is evident that there is an urgent need for adjuvant, opioid-free, treatments of pain.

1.2 Music Therapy for Pain

1.2.1 Clinical Evidence

Music therapy is one such adjuvant treatment that is used for pain management. One that is safe, noninvasive, and low-cost (Lunde et al., 2018). It has been used to supplement standard care for better treatment results as early as the 1960s and particularly in the last two decades. Hospitalized palliative care patients receiving standard care and cancer patients receiving morphine experienced increased pain relief with music therapy relative to controls (Gutgsell et al., 2013; Krishnaswamy & Nair, 2016). Patients who listened to music while undergoing invasive port catheter placement, bone marrow biopsy, dental procedures, laceration repair, aspiration, or lumbar puncture procedures reported significantly reduced discomfort associated with the procedure than controls who did not listen to music (Gardner et al., 1960; Menegazzi et al., 1991; Nguyen et al., 2010; Shabanloei et al., 2010; Zengin et al., 2013). Music therapy has also been used to treat post-operative pain (Hole et al., 2015). In female breast-cancer patients or patients who underwent total knee arthroplasty and patients after

thoracic surgery, music therapy significantly decreased pain post radical mastectomy or surgery (Allred et al., 2010; Li et al., 2011; Liu & Petrini, 2015). Music therapy interventions have also shown effectiveness as pain treatment in patients where sustained, intensive use of pharmacological intervention is not ideal - successfully decreasing subjective pain ratings in patients with neuropathic and chronic pain (Colwell, 1997; Garza-Villarreal et al., 2014; Guétin et al., 2012; Korhan et al., 2014). Meta-analyses and systematic reviews have also shown that using music for pain control has been implemented broadly in clinical settings and shown success in reducing pain in the patients (Cole & LoBiondo-Wood, 2012; Garza-Villarreal et al., 2017; Kühlmann et al., 2018; Martin-Saavedra et al., 2018). This is a promising result, which garners a need for further exploration of the mechanisms driving these effects. Further exploration could inform and develop the effectiveness of therapeutic music interventions, both as a stand-alone treatment and as an adjuvant care complementing, for instance, pharmaceutical treatments.

1.3 Music-Induced Analgesia

1.3.1 Proposed Mechanisms of Music Analgesia

Several experimental studies on healthy volunteers have been conducted to optimize and capitalize on what it is that makes music therapy effective as a treatment (Antioch et al., 2020; Choi et al., 2018; Gardner et al., 1960; Hsieh et al., 2014; Perlini & Viita, 1996; Mathieu Roy et al., 2008).

Attention

Existing clinical work, particularly in pediatrics, calls music used for pain relief “music distraction”, implicating that a potential driving force of this effect is the diversion of attention (Aitken et al., 2002; Colwell, 1997; Kristjánsdóttir & Kristjánsdóttir, 2011; Kwekkeboom, 2003; Li et al., 2011; Nguyen et al., 2010). However, the term “music distraction” may be a biased term, as attention is typically neither measured nor manipulated in clinical studies. Few studies have designs that discuss varying levels of distraction across music types and none measure this distinction directly. It seems that assuming that musical analgesia is a result of distraction perhaps seems intuitive, but an assumption nonetheless. A study conducted by Mitchell and MacDonald’s research group (e.g., Mitchell & MacDonald, 2006), is one of the few that directly addressed distraction as a potential mechanism of musical analgesia in an experimental setting. They found that preferred music was more effective than humor and a math task distraction in relieving pain and the authors concluded

that preferred music was simply a more effective distractor. Garza Villarreal and colleagues (2012) continued this line of investigation but did so by comparing experimenter-selected pleasant music, nature sounds matched in valence, arousal, and liking to a math task. However, compared to music and sound, the math task was more successful in inducing analgesia to acute heat pain. Mental arithmetic was identified as an active distraction which reduced pain more than music and sounds, identified as the passive distractions. Thus, the authors concluded that the integral component of music-induced analgesia may be at a cognitive level when one attends actively to the music. Furthermore, Garza-Villareal and colleagues (2012), bolstered Mitchell and MacDonald's previous findings, qualifying that familiarity with the music may be crucial to direct the attention to the music via increasing the distraction from the noxious stimulus, thus explaining their contrasting results. However, like the clinical studies before them, none of the above studies measured distraction directly and rather measured affect, pain, and perceived control ratings. Without operationalizing distraction or measuring it directly, it is difficult to conclude that distraction level varied between interventions or make conclusions about distraction's role in a musical analgesic effect. In fact, a later study by (Lu et al., 2019) did directly measure attention by asking participants on a slider how much a pain stimulus attracted their attention and found no significant difference between white noise and preferred participant-selected music. This study had few pain trials and participants, which could have resulted in a Type II error, but it indicates that there may be more to consider when designing studies to investigate attention's role in musical analgesia. Although attention could play a role in mediating the musical analgesic effect, existing experimental work lacks the design to establish a strong foundation for such claims.

Affect

The alternative to an attention-based account of music-induced analgesia, where music is simply posited as a potent stimulus for capturing the patient's attention, is that music elicits pleasure. Music-induced pleasure and its related psychophysiological states are posited to be able to override pain. Undoubtedly, music induces (and expresses) affective, emotional states in a powerful manner (Sloboda & O'Neill, 2011). The valence of the emotion induced by music, referring to pleasantness-unpleasantness on a continuum, has also been suggested as a potential mediator of the strength of musical analgesia. A series of studies explored the role of music-induced emotion on induced acute pain by investigating the effects of pleasant and unpleasant music on pain ratings during heat stimulation (Roy et al., 2012; Roy et al., 2008).

Music was chosen from a pool of experimenter-chosen songs stratified by participant ratings for pleasantness and arousal. Their first study (2008), explored the effects of pleasant and unpleasant music (with high arousal music only) and found that, compared to both unpleasant music and silence, only pleasant music significantly affected pain ratings by decreasing them. Then, in 2012, they compared musical pieces that varied not only in valence but also in arousal. This time not only was there no longer a reduction in pain ratings for pleasant music compared to silence, but also, there was no significant difference in pain between arousal levels for pleasant and unpleasant music types. The authors attributed the failure to replicate their results to a difference in pain application method. Specifying that the electric shock used in this study may have been more surprising during music listening compared to silence leading to overall higher scores during listening. It can be difficult to compare results between different pain induction methods, but also a silent control may not be the most appropriate comparison as it lacks input from the modality being investigated, and could have led to the failure to detect the effect in question. Lu and colleagues (2019), investigated the role of music-induced pleasant emotion in musical analgesia using electric shocks to induce pain. They found that pain ratings were lower during participant-selected preferred music listening, compared to white noise. In addition, ratings of both sound liking and sound pleasantness were rated to be more positive during music compared to the noise control condition and showed a significant association with music-related pain reduction. Participants who enjoyed the music more, showed a stronger reduction in pain. Lu and colleagues interpreted their finding to show that pleasurable music immersed participants in a pleasant emotional state and it is that emotional state which achieved a musical analgesic effect. Taken together with Lu and colleagues findings, perhaps the experimenter chosen music in Roy and colleagues' 2012 study failed to bring about as strong of a pleasant emotional state as did the participant-selected music and thus failed to induce a musical analgesic effect.

Expectation

Uncovering and developing the active ingredient of musical analgesia was instigated by its use on pain patients in clinical settings, and therefore it is relevant to discuss the potential for, and extent of, an expectancy effect. Expectations are thought to play a key role in placebo effects, i.e. symptom improvement following inert treatment. Expectations for pain relief during music listening could be a mechanism of the musical analgesic effect. Two studies have investigated the potential that expectations for pain relief during music listening could be a mechanism of the musical analgesic effect. The first was conducted by Perlini and Viita

(1996) in which participants rated their expectations for pain after each of three pain trials. Pain expectations for pain were lower for those who first heard their preferred music or expected to hear preferred music next, as compared to those who first heard their non-preferred music. In addition, expected pain and pain ratings during the experiment were correlated indicating both that there are expectations for pain relief for preferred music and that they affect subjective pain. However, expectations for pain relief in this study were not manipulated and asking participants for pain expectation directly after receiving some pain, and directly before the proceeding trial may indicate that the findings are likely a result of a demand effect rather than expectation itself. Hsieh and colleagues (2014) continued this line of investigation by increasing expectation for pain relief for either participant-selected preferred music or non-musical sound to investigate whether expectancy would bolster analgesia. They bolstered expectation first through verbal suggestion, followed by conditioning. It was found that in the no-conditioning control group, participants expected much greater pain relief from music than from sound to begin with. In addition, in the conditioned groups, although expectation for pain relief was greater directly after the manipulation, expectations for relief decreased during post-conditioning testing. Prior expectation and an ineffective expectation manipulation could explain why, although music and sound both relieved pain compared to silence, expectancy failed to bolster this result beyond the control group. However, results from Hsieh and colleagues indicate that a prior expectation may be driving analgesia and more studies are needed to investigate this question.

Lack of Clarity

Attention, music-induced pleasant emotion, and expectation are the most indicated factors in the pain-modulatory effect of music. Beyond these, it has also been suggested that anxiety may be a mediator of music induced analgesia. Choi and colleagues (2018) found that experimenter-selected music reduced pain during a cold pressor pain task. However, music was not effective in reducing pain in those with significant general and pain-specific anxiety symptomatology. This, however, is in direct contrast with the clinical literature that often reports a concomitant decrease in anxiety with music-induced analgesia (Allred et al., 2010). Such contradictions are emblematic of the existing body of experimental research, teaching us a mixed bag of lessons with regard to what makes this effect tick. Some report finding that music is most powerful as an analgesic when it is maximally distracting, others when it elicits maximally pleasant emotion, others when expectancy is modulated. Further experimental

work that addresses the methodological weaknesses of previous studies is needed on this topic to identify which, and to what extent, these components are responsible for musical analgesia.

1.3.2 Music as a Reward

Music was listed in the top five categories in a survey where participants were asked what comes to mind when one thinks of pleasure (Dubé & Bel, 2003). This is not surprising, considering how common it is to feel the subjective rewarding effects of listening to music in our day to day lives. Music's rewarding capacity and its value is reflected in society by a willingness to spend money and time on buying albums, music streaming services or attending concerts in order to listen to music. A 2019 (IFPI, 2019) report included results from an online survey of 34,000 international respondents and found that on average people listen to 2.6 hours of music a day with a global increase in the use of on demand music streaming services (+7% from 2018).

Music Reward Responses - Chills and Pupils

Subjective rewarding experiences during music listening are strong, measurable, and are regulated by autonomic, limbic, and neurochemical systems. One particularly intense pleasant emotional and physiological response to music is often referred to as 'chills'. Chills, in the context of music, denote highly positive, pleasant, experiences that take place at specific and predictable moments during a familiar musical piece and may or may not involve bodily reactions (Laeng et al., 2021). A study found that when volunteers listened to self-selected music, they reliably felt chills, compared to experimenter-chosen control songs. The chills were associated with an increase in regional cerebral blood flow patterns of activity in areas known to depend on dopaminergic and opioid neurotransmitters; similar to what is observed in other studies of euphoria and pleasant emotion (Blood & Zatorre, 2001). This result is further reflected by increased pupil size during the experience of music-related pleasure and chills. One study (Alnaes et al., 2014) showed that pupil size was larger in the time window surrounding chills compared to pupil size during passive listening. The pupils dilating response during chills implicates the norepinephrine-locus coeruleus system's engagement which is thought to optimize integration between diverse neurotransmitter systems like systems centered on mesolimbic/mesocortical dopamine circuits and the endogenous opiate system (Laeng et al., 2016). The involvement of the endogenous opioid system is further supported by a psychopharmacological study in which it was found that the endogenous μ-opioid signaling is not necessary for subjective enjoyment of music but an

opioid blockade dampens pupil responses to peak pleasure (chills), consistent with decreased arousal to music (Laeng et al., 2021). However, a powerful component of music pleasure is the violation of expectation in a music piece, thought to be mediated by dopaminergic neurons in the midbrain (Gebauer et al., 2012). In a within-subjects study (Ferreri et al., 2019), the rewarding subjective experience of music with dopamine precursor (levodopa), a dopamine antagonist (risperidone), and a placebo (lactose) were compared and it was found that the levodopa increased subjective reward of music while risperidone decreased the reward, further implicating dopamine as a player in subjective musical reward. Thus, the subjective reward experienced during music, for example reflected by chills or violation of expectation, involves both the dopaminergic and opioidergic systems whose influence can be measured through subjective reports, like ratings, and physiological measurements like pupil dilation.

1.3.3 Preferred Music in Experimental Work

Chosen methods for music selection vary greatly within the experimental work outlined in section 1.3.1. However, musical selection choices matter when producing analgesia in experimental settings. Within a selection of 6 separate experimenter-chosen songs, participants experienced the most pain relief from their personally most preferred song selections (Perlini & Viita, 1996). Most-loved music induced the most effective analgesia compared to a variety of other experimenter-selected music and sound types (Hekmat & Hertel, 1993; Hsieh et al., 2014; Mitchell & MacDonald, 2006). As long as preferred music elicited positive affect, it was functionally equivalent to other preferred music in producing pain relief. The characteristics of the music, such as genre, tempo, and structure did not impact the analgesic effect (Knox et al., 2011). Such studies bring up the question, what is it about personally preferred music that induces greater analgesia?

1.3.4 Expectations of Analgesic Effects of Music

As seen in the experiment by Hsieh and colleagues (2014), where expectation for pain relief was increased for either participant-selected preferred music or non-musical sound to investigate whether expectancy would bolster analgesia, the control group had prior expectations for pain to be reduced during music-listening than during noise. With such results, it is important to consider that people consciously use music as a tool to successfully moderate affect and thus have expectations for music to change their state (Saarikallio, 2010). During an experimental pain task, subjects were actively employing music and noise by frequently changing the volume via a knob, tapping their feet and singing along, perhaps in

an attempt to 'control' their pain (Morosko & Simmons, 1966). By actively engaging with the music in such an uncomfortable strange environment like a lab, it is likely that it was done with the intent to cope with pain. This behavior illustrates that participants had an expectation that immersing themselves in the music would aid in their discomfort. People naturally engage with music in such ways to modulate their state and alleviate their pain.

1.3.5 Reward and Expectation as Potential Mechanisms of Musical Analgesia

The driving forces and neural underpinnings of musical analgesic effect are largely unknown (Lunde et al., 2018). Although attention, affect, and expectation have been identified as potential mediators of music-induced analgesia, there is still uncertainty surrounding the neural mechanisms of action of this phenomenon. As Lunde and colleagues wrote in their 2018 review, this lack of clarity can be attributed to the heterogeneity and inconsistency of methods in experimental studies of musical analgesia. Such inconsistencies include: the delivery method of the music, who chooses the musical stimulus, methods of pain induction, and type of control sounds (nature sounds, news/weather reports, frequency-filtered noise). Crucially, there is a deficit of pharmacological studies when investigating music-induced analgesia. Since the neural underpinnings and driving forces of the analgesic effect are largely unknown, there is a need for more stringent design to disentangle the underlying mechanisms while properly controlling for potential contextual placebo effects. Music's capacity as a reward and our natural engagement and expectations for music's effects on our subjective state, call for further experimental investigation into reward and expectation as mechanisms of musical analgesia. The opioid system is situated at the helm of both reward and expectation effects. Reward induced analgesia is well described in the animal literature, where the endogenous opioid system is clearly implicated. Expectation effects, or rather placebo effects on pain, are well described through experimental studies in humans, and also here, are endogenous opioids implicated. Working towards a pharmacological study that aims to test the hypothesis that music elicits analgesic effects via endogenous opioid signaling, my thesis work has been dedicated to designing an experiment that enables us to test the mechanisms driving music-induced analgesia reliably.

1.4 Reward-Induced Analgesia

Whether it be through the action of serotonergic, noradrenergic, cholinergic, nicotinic, dopaminergic, or opioidergic neurochemical agents, the activation of reward and limbic structures via non-pharmaceutical rewarding stimuli robustly reduces pain.

1.4.1 Reward Analgesia in Animals

From studies in non-human animals, we have learned that anticipating or ingesting a reward induces analgesia (Georgiadis et al., 2012; Kringelbach et al., 2012). Reward's role in producing analgesia in rats began with a study that found that the expectation of receiving a rewarding piece of candy increased pain threshold as measured by paw-lick latency on a hot plate. This was completely blocked by mu-opioid antagonist naloxone (Dum & Herz, 1984). This finding is well-established in rodent-models and is naloxone/naltrexone-reversible (Anseloni et al., 2002; Blass et al., 1987). Chronic ingestion (14 days) of a sucrose solution in rats produced an increase in pain threshold of heat pain as measured by tail withdrawal latencies in a tail-flick test (Irusta et al., 2001; Miyase et al., 2005). This effect was either reduced or completely reversed by a serotonergic, noradrenergic, cholinergic, and nicotinic antagonists (Irusta et al., 2001; Miyase et al., 2005; Rebouças et al., 2005). Heat pain threshold as measured by tail-flick were decreased by administration of a serotoninergic antagonist in the dorsal raphe nucleus after chronic consumption (14 days) of a sucrose solution (Miyase et al., 2005). Foo and Mason (2005), found that reward-analgesia occurs not only with ingestion of sucrose but also in adult rats while feeding in general. Pain thresholds as measured by paw withdrawal were higher during ingestion of sweet foods as well as sugar-free chow as compared to grooming. Reward-induced analgesia is not exclusive to food reward in animals. A naloxone-reversible antinociceptive effects of rewarding sexual behavior is also observable in rats (Forsberg et al., 1987; Szechtman et al., 1980).

1.4.2 Reward Analgesia in Humans

Sweet-solution induced analgesia has been used clinically to induce analgesia in infants, often connected to evolutionary motivation of the sweetness of mother's milk (R. G. Barr et al., 1995; Miller et al., 1994; Pepino & Mennella, 2005). Later, this was extended to adults by Kakeda and colleagues (2008), who found that adults holding sucrose solution in the mouth increased pain threshold relative to distilled water. The same group replicated these results in 2010, and also found decreased arousal, increased valence, and dominance via VAS self-assessment manikins and attenuated activation in the 'pain matrix' for consumption of sweet gelatin vs neutral gelatin during a cold pressor task (Kakeda et al., 2010). Lewkowski and colleagues (2008) found that the increase in pain threshold and mood in adults when holding sucrose solution in their mouth compared to water, was attenuated by naltrexone, implicating opioids as a mediator of reward-analgesia in adults.

Photographs can also be used as a reward in human experimental designs. Pleasant, neutral, and unpleasant pictures were presented to healthy volunteers during an electrical stimulation pain task while measuring N150 and P260 ERPs, meant to be indicative of pain and arousal processes, respectively. Both had greater amplitudes for painful stimulation compared to nonpainful, however they were modulated by the contents of the pictures being viewed. When viewing pictures that participants rated as pleasant, pain intensity was lower and N150 ERP amplitude was reduced (Kenntner-Mabiala & Pauli, 2005). Relative to viewing a photo of a stranger and an active distraction task, viewing photos of a romantic partner activated reward and limbic regions and suppressed activation in pain-processing regions, which was reflected in the behavioral results as a reduction in self-reported pain (J. Younger et al., 2010). Viewing pleasant images that induced positive affect increased pain threshold compared to neutral images, while unpleasant images that induced negative affect (fear) had the opposite effect compared to neutral images (Meagher et al., 2001). While showing participants pleasant images (mainly erotic) that induce positive affective state, the mu-opioid antagonist naloxone also increased subjective pain intensity and unpleasantness but failed to alter subjective pleasure or mood (Kut et al., 2011). To conclude, no matter the modality of the reward, be it gustatory or visual, rewarding stimuli are capable of inducing pain relief in humans.

1.5 Expectation-Induced Analgesia

1.5.1 Placebo in Animals

Placebo effects, which are intervention effects not attributable to the intervention itself and are rather attributable to one's expectations for the intervention, are known to influence intervention's outcomes. Yet, researchers continue to work to find the nuances of what drives it, what its separable categories are, and how to best elicit its effects. Placebo treatments and opiate drugs are thought to have common effects on the opioid system and pain-related brain processes and these effects are additive and dissociable (Atlas et al., 2012; Bingel et al., 2011). The phenomenon is sparingly investigated in animal research as it can be difficult to successfully measure expectation in animals. One method is through conditioning by learning to pair a cue with decreased pain. A conditioned expectation effect paired with placebo successfully induced an increased pain threshold in rats relative to controls, an effect which was reversed with both dopamine and opioid antagonists (Lee et al., 2015; Nolan et al., 2012). When mice were cue conditioned with an opioid agonist (morphine), the cue

conditioned placebo analgesia was reversible via an opioid antagonist (Guo et al., 2010). However, if they were cue conditioned with an NSAID (aspirin), opioid antagonists failed to reverse the effect suggesting that both opioid and non-opioid mechanisms are at play during placebo analgesia, potentially mediated by previous context. Others failed to find placebo effects in rats at all, suggesting that inducing expectation in rats is not reliable (McNabb et al., 2014).

1.5.2 Placebo in Humans

Early on in placebo analgesia research, it was discovered that the opioid antagonist naloxone is capable of blocking placebo analgesia in humans (Grevert et al., 1983; Levine et al., 1978). In humans, placebo analgesia can be induced behaviorally via suggestion, conditioning, and treatment history (Colloca, 2019). Some argue that in humans, placebo analgesia is a learning effect, characterized by prior experience, time lag, and classical conditioning (Colloca & Benedetti, 2006). In both clinical and experimental settings, a positive and rewarding context is capable of inducing analgesia through opioid, cannabinoid, and dopamine systems (Carlino & Benedetti, 2016; Colloca, 2019). When conditioning was performed with nonopioid drugs, nonopioid mechanisms seem to be involved in placebo analgesia (Amanzio & Benedetti, 1999). Expectation effects were reliably reversed with an opioid antagonist, but placebo effects based on conditioning alone did not. Expectation-driven placebo analgesia were shown to be reversible via naloxone (Berna et al., 2018). Pharmacological evidence of the role of mu-opioids is further corroborated by neuroimaging studies of placebo effect that indicate increased activity in brain regions with greater mu-opioid volume (Zubieta, 2005). Although there is still a debate about the mechanistic components of which cognitive and physiological components drive which parts of a placebo analgesic effect, it is clear that endogenous opioids play a crucial role in expectation driven placebo analgesia.

1.6 The Opioid System of the Human Brain

Mu-opioid receptor (MOR) availability can be tracked and measured in vivo via positron emission tomography (PET) studies. Such studies have shown us that MORs are expressed widely throughout the brain, with the highest densities of in brain regions associated with reward and pain processing, including the periaqueductal grey area in the brain stem, and the amygdala, hippocampus, thalamus, hypothalamus, cingulate gyrus, ventral and dorsal striatum (Koepp et al., 2009; Nummenmaa & Tuominen, 2018; Petrovic et al., 2010; Pfeiffer et al., 1982; Sprenger et al., 2005; Tracey, 2010). Furthermore, expectation of

pleasure/reward can indirectly, through increased phasic dopamine signaling in the ventral tegmental area, increase mu-opioid release in the nucleus accumbens (Leknes & Tracey, 2008; Scott et al., 2007). In rats, we see that microinjections of an opioid agonist in the NAcc shell (hedonic hot spot) increased the occurrence of positive hedonic reactions to taste (Berridge & Robinson, 2003; Pecina, 2005; Peciña & Berridge, 2000). In humans, while viewing images of appetizing vs. bland food, mu-opioid receptor (MOR) availability was inversely related to BOLD responses in the reward and emotion circuit (Nummenmaa et al., 2018; Nummenmaa & Tuominen, 2018). These results are in line with MOR's involvement in reward as a promoter of survival and homeostasis motivated behavior in humans (Leknes & Tracey, 2008; Nummenmaa et al., 2018). Music-induced reward does not clearly align with this interpretation of reward and the pleasurable hedonic experiences that accompany them, as music is not clearly necessary for survival or homoeostasis interpretation of rewards. However, reward circuits are implicated in experiences above and beyond basal survival contexts (Berridge & Robinson, 2003). By blocking MORs with naltrexone, an opioid antagonist that preferentially inhibits mu-opioid receptors, there was a dampened physiological response to pleasant music, especially during chills as compared to placebo (Laeng et al., 2021). Naltrexone did not have an effect on subjective reports of musical enjoyment, thus implicating that the relationship between musical reward and accompanying physiological arousal are likely complex. Nevertheless Laeng and colleagues showed that μ-opioids are involved in musical reward.

1.7 The Planned Study

Considerations for Our Study Design

Although the inconsistencies in previous studies make it difficult to draw a cohesive picture, they have allowed for an exploration of which methods will allow future studies the opportunity to capitalize on the most effective parts of musical analgesia. Thus, our study design is informed by existing work to best investigate possible neural mechanisms underlying a non-drug pain management tool. In particular, we are addressing the overarching success of participant-selected preferred music in inducing pain relief. Across studies exploring different questions and using varied methodology, preferred music consistently stands out amongst silence, noise, non-music controls, and unpleasant music controls, as the most potent in producing analgesia. The success of music pleasure in inducing analgesia suggests that music is successful as an analgesic because of its rewarding

quality. Furthermore, inherent expectations for music's effects on our subjective state, further corroborated by Hsieh and colleagues' (2014) results of the control group holding prior beliefs, suggest that expectations for pain relief may drive musical analgesia.

Because both reward and expectancy are regulated by endogenous opioids, we ask whether music-induced analgesia is mediated by the endogenous opioid system and whether the expectation of pain relief is a driving force of this effect. To answer this question our study will utilize a within and between-subject crossover, randomized, placebo-controlled design that is powered to detect small effects, as well as a comprehensive pilot with the objective to establish the efficacy of our design and set up. We expect to find that:

1. Naltrexone blocks or reduces the analgesic effects of music listening compared to placebo
2. Music is perceived as less rewarding with naltrexone vs placebo
3. The effects of the expectation manipulation is less with naltrexone vs placebo

Three-Phase Pilot

A pilot of this study design was conducted with 30 participants completing one session. The data from these participants is necessary to test our study protocols and procedures, assess feasibility of the study, and perform a suitable sensitivity power analysis. Protocols and methods developed and informed by this pilot will be peer-reviewed to obtain an in-principle acceptance of a manuscript including the introduction, protocol, methods, and detailed analysis plan before the start of data collection. This pilot is the content of the following thesis, aiming to improve and develop a subsequent study. The pilot consisted of three stages. The first stage, identified as "Pre-Pilot", was concerned with testing that music indeed appears to have an analgesic effect compared to listening to brown noise. Stage 2, identified as "Video Validation", focused on establishing an expectation manipulation that does indeed affect expectation, and to quantify effects of the manipulation on self-reported expectation of analgesia. At this stage, we tested and tweaked the design of the expectation manipulation to be as engaging, professional, and convincing as possible. We measured the believability of the manipulation directly through an online study to address the lack of measures of prior beliefs of expectancy of music for pain relief and ineffective expectation manipulations in the existing literature. The online study was designed so that we could reasonably detect an estimated small effect size of $f = 0.2$ with 80% power, which based on an a priori power analysis performed in GPower was 120 participants (Faul et al., 2007). Stage 3, identified as

"Pilot", was a full experimental setup including an expectation manipulation. It addressed all pilot predictions that:

1) Listening to self-selected preferred music dampens the painfulness of acute thermal stimulation compared to listening to brown noise.
2) The magnitude of the music-induced analgesic effect is positively correlated with:
    a) Expectations of analgesic effects of music (measured as self-reported prior expectation)
    b) Experience of reward during music listening (measured as self-reported musical enjoyment after listening experience)
3) Manipulating expectations to music holding certain characteristics (such as slow/fast tempo) will affect the analgesic effect of this specific music.
4) Pupil size will be affected so that:
    a) While receiving pain stimulation, pupil size while listening to noise will be larger than pupil size while listening to music
    b) Pupil size is positively correlated with reward (measured as self-reported musical enjoyment after listening experience)
    c) Pupil size is larger for high arousal music, as compared to noise and smaller for low arousal music as compared to noise

The Pilot only differs from the future data collection full setup in number of sessions, and drug condition. Data from this stage was used as a foundation for further power analyses to figure out an ideal sample size for the future study of neurochemical mechanisms involved in music analgesia. Further description and evaluation of the results of these preliminary studies were used to plan and structure the main study.

Methods & Materials

2.1 Participants

A total of 161 participants were recruited. We tested 129 participants for the Video Validation via the survey site Prolific. Participants were informed at the beginning of the survey that all submissions are completely anonymous and that they would be asked to give ratings and watch a video. All reported normal vision, hearing, and English fluency. Thirty-two participants were recruited for the Pre-Pilot and Pilot via acquaintances and locally distributed flyers. Participants were informed in an online recruitment form about exclusion/inclusion criteria and general study procedures, including the use of a thermode to

induce moderate thermal pain. All participants were healthy, reported normal hearing, normal or corrected-to-normal eyesight, and minimal to no musical training. Exclusion criteria were current skin conditions on the arms and current use of pain medication. Participants were reminded the day before the data collection session to not consume any analgesic medication prior to testing, and to make sure that sleep duration, intake of food, caffeine, and nicotine leading up to the experiment, were kept as typical as possible. Participants were compensated with 150 NOK (approx. 25USD) for taking part in the experiment. Written informed consent was obtained before the commencement of study procedures from all participants, and the study was conducted in accordance with the Declaration of Helsinki. Ethical permission for the study was obtained from the internal ethics committee at the Department of Psychology at the University of Oslo, and the handling of personal information was approved by the Norwegian Centre for Research Data (NSD).

2.1.1 Pre-Pilot

We tested 11 participants for the Pre-Pilot. One participant was excluded due to high pain tolerance that resulted in failure to induce moderate heat pain, meaning inability to achieve a calibrated pain rating of 55 with a temperature equal to or less than 50°C. This resulted in a final sample size of 10 participants (6 females, 4 males) aged 22-30 years (mean = 25.4, SD = 2.2) included in analysis for the Pilot 1 set up.

2.1.2 Video Validation Online Survey

A total of 129 participants were recruited, 13 participants who spent less than 5 minutes or more than 25 minutes on the task were excluded. Finally, 116 participants were included in analysis. Due to data loss related to linking excluded participant id numbers to demographic data, the gender and age data is based on 120 participants (50 females, 70 males) aged 18-60 years (mean = 25.23, SD = 7.9). Finally, 116 participants were included in analysis.

2.1.3 Pilot

We tested 21 participants for the Pilot. One participant was excluded due to inability to achieve a calibrated pain rating of 70 with a temperature equal to or less than 50°C. 20 participants (12 females, 9 males) aged 19-38 (mean = 26.35, SD = 5.14) were included in the final analysis, with 9 participants in the group manipulating expectation along low arousal music (4 males, 5 females) and 11 participants in the group manipulating expectation along high arousal music (4 males, 7 females).

2.2 Procedures

2.2.1 Pre-Pilot

This Pre-Pilot tested the effectiveness of our study design for testing our first prediction: listening to self-selected, preferred, music dampens the painfulness of acute thermal stimulation compared to listening to brown noise. Before arriving for testing in the lab, volunteers were asked in an online form to provide a list of personally preferred musical pieces that they have enjoyed long term and were between 4:30-5:00 minutes in length. Three of which were fast-paced/exciting songs, and the other three slow-paced/calm. Participants came in for one testing session of approximately 1.5 hours during which the participant and experimenter were wearing masks. After reviewing COVID-19 safety information, participant information, and consent, participants underwent a pain calibration procedure. Following a heat pain calibration designed to achieve a personalized temperature predicted to induce moderate pain [detailed in section 2.2.3], participants completed a brief demo version of the main task (3 minutes) to be familiarized with the format of the study, rating scales, and to set a comfortable volume. After the demo task, a curtain was drawn between the experimenter and the participant so that they could not see their left arm, only their own screen. Participants then proceeded to a brief pupil calibration, which was immediately followed by the main task.

Main Task: Listening and Rating

The main task began with the initiation of a sound clip. Sound clips were presented according to one of four pseudo-random orders. The sound presentation order was counterbalanced between participants, in which no sound type (music/noise) was played more than twice in a row and the sound clip played throughout the entirety of one task block. A block began with a 25 second immersive listening period during which participants were requested to keep their gaze onto the screen and within a gray fixation circle, allowing themselves to immerse in the sound. Then, the fixation circle turned blue 5 seconds before three subjective state VAS sliders were presented. All slider ratings were response-timed. Shortly following each rating (3 seconds), the fixation circle turned red to indicate the first painful stimulus, thus potentiating the fear or startle responses that are known to influence pain. After the presentation of the heat pain stimulus there was one rating of the felt pain on the VAS. Each painful stimulus was followed by a 29 second inter-stimulus interval of listening while keeping the eyes within the gray fixation circle to prevent sensitization/habituation of the arm to heat caused by successive heat trials. There were 3 more pain stimulations in the

experiment (totaling 4), followed by the same three subjective-state ratings as at the beginning of the stimulus (totaling 6) and the sound was allowed to play to the end with a gray fixation circle on the screen. There were a total 9 blocks that repeated the same procedures but with different sound clips playing. Every second task block, the experimenter changed the thermode location in a fixed pseudo-random manner, so that no site on the arm received more than 8 heat stimulations in a row, preventing heat sensitization/habituation. The site order was counterbalanced between participants. Participants were offered a break after the 4th block to prevent drift in participants for whom the prolonged sitting may cause discomfort and affect pain ratings. At the conclusion of the final task block, participants were fully debriefed to collect feedback to inform future task and study development and received a gift card for their participation.

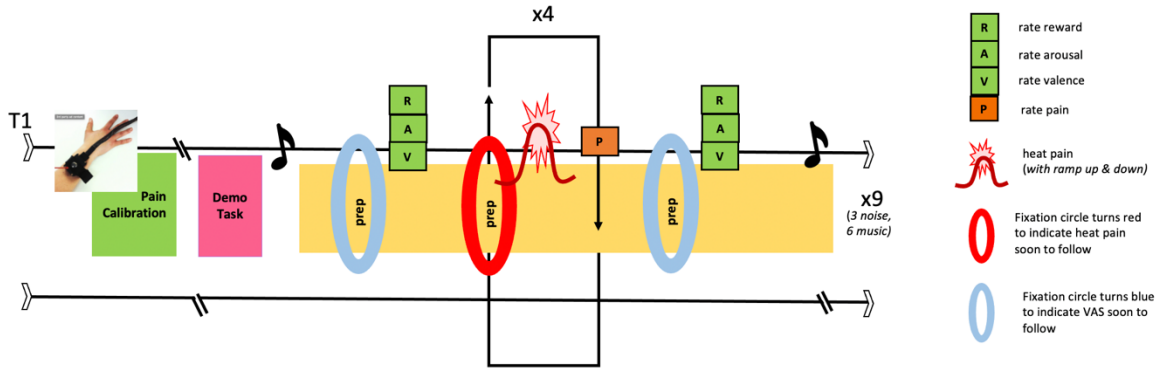

Figure 1. Pre-Pilot study timeline. A session began with a pain calibration involving a sequence of heat stimulations and pain ratings to establish an individually calibrated temperature predicted to elicit moderate pain for each participant. Calibration was followed by a brief demo task that familiarized participants with the main task layout and subjective state ratings. Then, as indicated by the first musical note in the figure, a sound clip was initiated according to a pseudo-random counterbalanced scheme and played throughout the entirety of a block, and a fixation circle appeared on the screen. There was a 25 second immersive listening block, then the fixation circle turned blue to prepare participants for a reward and two state (musical enjoyment, arousal, valence) ratings. There were 4 heat pain trials during one sound clip, preceded by a red fixation circle and followed by pain ratings. Pain trials were separated by 29-second inter-stimulus intervals. After the last pain rating, participants once again rated reward and state, and the block was terminated by the end of the sound clip (max 5 min). The main task block was repeated a total of 9 times to include all musical and noise clips.

2.2.2. Video Validation Online Survey

To validate our videos' effectiveness as an expectation manipulation before implementing them in our design, we distributed them together with a battery of 20 visual sliding scales. Participants were asked to rate how they would expect music to affect their perception of pain and other sensory impressions. They then watched one of the two prepared videos and once again answered the same sliders that they did pre-video. Participants were randomly assigned, via the online survey's built-in randomization scheme, to watch a video that increased expectations of pain for either low or high arousal music. Participants were only requested to make ratings pertaining to the music type that was later manipulated in their assigned video. The presentation of scales was counterbalanced for ratings of each modality to present either negative or positive valence words first. Finally, participants were asked to evaluate the quality of the video by selecting from a checklist of adjectives and to rate their attention to the video to allow us to have feedback on quality to determine whether the video needed to be further developed.

2.2.3 Pilot

This phase of piloting investigated the effectiveness of our study design to test our second and third predictions, regarding how expectations affect the magnitude of analgesia. Same protocols were followed as during the Pre-Pilot, with some additions. Before the demo task, participants completed an adapted version of the video validation survey, where they were asked to rate their current expectations to both high and low arousal music before and after viewing the video described in the previous section. Assignment of manipulating expectations across either low or high arousal and survey question valence order were counterbalanced between participants to adhere to one of the four orders of music presentation later in the study. In line with the video intervention's script intending to manipulate expectation effects pertaining to multisensory perception (tactile, visual), the blue fixation circle was changed to be deliberately ambiguous, an even mix of blue and green. Then an unnumbered VAS slider with a center tick and default start at the center mark, was added before the subjective state ratings asking, "*'How did you perceive the color the circle changed to?"* (anchors ="*green*", *blue*"). At the conclusion of the final task block, participants were fully debriefed about video deception as well as to collect feedback about what is now the complete design involving all planned manipulations (excluding drug and two-session set up).

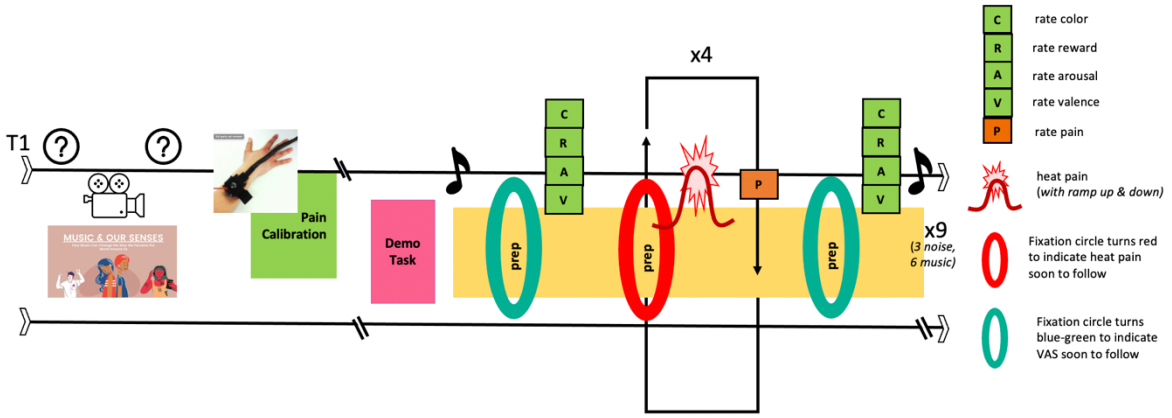

Figure 2. Pilot study timeline. Same procedures were followed as during the Pre-Pilot and also, before pain calibration, participants viewed a validated video designed to manipulate expectations about one type of music. Expectations for pain were manipulated for either high arousal or low arousal music, to expect higher pain during that music type, aiming to eliminate inherent expectations for pain relief for one music type. The videos presented the study as investigating music's effects on multi-sensory experiences. Thus, to prevent participants from guessing the musical analgesic nature of the study, the blue fixation circle prepping for ratings was made to be evenly blue-green and a color perception rating was collected after the fixation circle changed to the blue-green color.

2.2.4 Acute Experimental Pain

Heat Apparatus

Thermal stimuli were produced by a 3x3 cm contact thermode (Medoc Ltd. Advanced Medical System, Israel) comfortably strapped on to the anterior surface of the forearm. Baseline temperature of the thermode was 32°C, with a ramp up rate of 4°C/second, and an 8 second duration at the destination temperature. After a brief demo of the Medoc thermode at 40°C, a calibrated temperature specific to the participant was established via the pain calibration procedure detailed in the next section. The individual goal temperature was used as the painful stimulus during the experiment session and was kept constant throughout the rest of the experiment. To avoid habituation, the thermode was placed on one of three different skin locations on the forearm after every two sound clips in a pseudo-random manner counter-balanced with sound type, with a total of 9 sound clips and 4 instances of pain stimulation per clip. Experimenters were trained to manually deliver the heat at the onset of the red fixation circle, the beginning of temperature ramp up.

Pain Calibration

Pain limits and thresholds for each participant were established at the beginning of each session in order to control for individual differences in pain perception and day-to-day fluctuations. During calibration, the participants' left arm was behind a curtain to allow them to focus on the sensation and to block temperature information visible on the experimenter screen. Participants were informed that they would be told whenever heat was starting and to wait until the experimenter's prompt to rate the pain between 0 ("*no pain*") and 100 ("*unbearably painful*"). Experimenters always asked, "*Was that painful?*" before asking for a rating value to prevent participants rating heat intensity rather than pain. Participants were informed that they could stop thermal stimulation at any point if stimulation was intolerable by asking the experimenter to stop. Similar to the well-established heat pain calibration from (Price et al., 1999), set temperatures were applied to establish a stimulus-response curve, and previous ratings were input into a linear regression to predict a temperature that will produce a desired pain rating. Calibration involved 20 stimulations and ratings, the first 10 were identical for all participants. Specifically, 10 pseudo-random temperatures were chosen between 44 and 49C via a random number generator, with half predicted to be painful and half non-painful. In our design however, the next 10 stimulations were temperatures generated by a linear regression to evoke pain ratings between 40 and 65. Then these temperatures and ratings were used to calculate a temperature that was predicted to elicit a moderate pain rating of 55 in the Pre-Pilot. In the Pilot, due to low average ratings during the main task in the Pre-Pilot, the targeted pain rating for moderate heat was changed to 70. No site was stimulated more than twice in a row during calibration, and if there was a pain rating over 50 and the next stimulation was for the same site, there was a 29 second rest in between. These procedures were developed to establish a temperature that is generally regarded as moderately painful for the participant while keeping the session as concise as possible and avoiding carry-over habituation or sensitization into the main experiment.

2.3 Outcome Measures

2.3.1 Subjective State Measures

In order to capture the hedonic experiences of music listening, we recorded, with visual sliding scales, subjective ratings of arousal ("*How are you feeling?*", anchors = "*Calm*", "*Excited*"), and valence ("*How are you feeling?*", anchors = "*Bad*", "*Good*"). These mood-state ratings all had a default start at the center marker, labeled with a tick, and anchor

descriptors were selected to capture the affective experience of the participant and typical measures in similar studies (Bradley & Lang, 1994). Subjective mood and state were response-timed and measured at 6 time points during a block (see timeline, figs. 1 & 2) using the same VAS sliders with each repetition.

2.3.2 Reward Measure

The rewarding quality of the music is a central question in our design and was captured by ratings of musical enjoyment. In order to measure participant's enjoyment of the music we recorded, with a visual sliding scale, subjective ratings of reward ("*How has your listening experience been so far?*", anchors = "*Very unpleasant*", "*Very enjoyable*").

2.3.3 Pain Measures

Pain intensity was also rated on a visual sliding scale. After a ramp down of heat, pain was rated on a slider ("*How painful was the heat?"* anchors = "*Not painful*", "*Unbearably painful*") without a center marker and a default start on the far left. Pain ratings were response-timed and measured at 4 time points during a sound clip (see timeline, figs. 1 & 2) using the same VAS sliders with each repetition.

2.3.4 Expectation Measures

In the Video Validation and Pilot, expectation was measured by participants reading a conditional statement, "*How do you think listening to (fast-paced, exciting music/slow-paced calm music) would make you perceive the following things?*" and were then asked to "*please indicate by dragging the slider with your first impression*". Then 10 visual sliders were presented with a sensation above it. Participants indicated what they would expect to perceive by sliding on a scale with anchors "*not intense at all*", "*as usual*", and "*extremely intense*". The center anchor "*as usual*" was included to indicate perceiving the sensation as without music. The sliders were 0-100, and a box below the slider indicated the value that the participant selected. The visual sliding scales accompanying the video validation online survey, and Pilot were the same. They only varied in what types of music they probed. The items served to keep participants engaged in the 'story' of the manipulation that music affects many senses simultaneously. This was designed to avoid participants guessing the true nature of the study as a musical analgesia study, which would prevent any attempt to reduce expectations for pain relief. See table 1 for an overview of the included items. Table 1 presents the items in the correct order, however about half of the participants were randomized to an order that inverts the order within-modality. The same questions and items were always presented both before and after the video.

1.Color	2.Brightness	3.Pain	4.Pleasure	5.Unpleasant smell
6.Pleasant smell	7.Unpleasant taste	8.Pleasant taste	9.Unpleasant heat	10.Pleasant heat

Table 1. Items were listed as above with a visual slider directly beneath them.

2.3.5 Pupil Measurement

To explore the use of pupil dilation as a physiological measure of different aspects of participants' sensory experience - pain, reward, and arousal, pupil size was measured. Pupillometry, is the study of changes in the diameter of the pupil as a function of cognitive processing and has been used in clinical settings, for example, as an objective measure of pain in pediatrics where patients may have difficulty appraising pain in subjective pain ratings (Connelly et al., 2014; Sirois & Brisson, 2014)**.** Pain appraisal, measured in our study as subjective pain ratings, has been shown to mediate autonomic nervous system's responses to painful stimulation (Mischkowski et al., 2019). Pupil size is also impacted by rewarding stimuli and arousal and therefore pupil recordings during immersive listening periods allowed for an exploration of the relationship between pupil and reward (as measured by reward ratings) and also to pupils and arousal (Alnaes et al., 2014; Blood & Zatorre, 2001; Laeng et al., 2021). Binocular pupil diameter responses were measured using infrared light (SMI, iMotions) with a 250 Hz sampling rate. Before initiating the main experiment task, a 5-point pupil calibration was performed. After the calibration, pupil diameter was measured continuously until the end of the last block. All stimuli presented on the screen were luminance matched and the lighting in the test room was exclusively from overhead lighting and was therefore stable at all times of day and for all participants.

2.4 Materials

2.4.1 Auditory Stimuli

Individual music choices were determined according to a list of criteria designed to specify a controlled set of participants' personally preferred music. Prior to coming to the first visit, participants were asked to provide a set of 6 songs or musical pieces that are no less than 4 minutes 35 seconds and no more than 5 minutes long, that they have enjoyed long-term, and contain no other sounds other than the song itself. In addition, 3 of the songs should be

slow-paced, calm and the other 3 fast-paced and exciting. Participants were also informed that it was okay to choose multiple songs from the same artist and songs in any language and of any genre. Upon receiving the music submission and ensuring that the criteria were met, the music was peak amplitude matched to one another and to the non-musical noise control to ensure there were no jarring volume intensity shifts. The non-musical auditory control stimuli were alternating, frequency-filtered noise clips, a choice based on studies showing that filtered brown noise is less distressing to participants than white noise (Hongisto et al., 2015; Vassie & Richardson, 2017). Three noise clips were presented to each participant, edited to match the length of 3 of the participants' 6 self-chosen musical pieces. All auditory stimuli were listened to through headphones to maintain as high quality as possible, as well as to promote immersion. Auditory stimuli were presented in a pseudo-random order where neither music nor noise were presented more than twice in a row. This was counter-balanced and order was assigned to participants sequentially to ensure even distribution of participants in each group.

2.4.2 Video

Two videos were developed specially for this study as a persuasive tool to manipulate expectations across low or high arousal music. A script was established that described real studies that measured outcomes of music's effects on taste, smell, sight, and touch as well as information on music therapy and pain reduction in clinical settings. Two versions of the script were made involving minimal deception where the studies were described as involving the assigned level of arousal and there was a concluding slide that explicitly stated that

> *"In particular,* ***fast-paced/slow-paced*** *music is powerful in heightening our senses, increasing the intensity of our perceptions and making us take in the finer details of things. While this can make for instance colours look more intense and vibrant, it can also enhance unpleasant sensations and make pain worse."*

The videos showed text and images accompanied by a voice over. The videos were created and animated in Canva and sound was recorded and processed by a professional sound engineer to produce a believable professional-quality informational video. (Videos are available for viewing on the Open Science Framework available online as supplemental digital content link found in Appendix A.).

2.5 Analysis

2.5.1 Exploratory Analyses

As this was a pilot study with the main aim being to establish whether the varying elements of our study design are effective, exploratory analyses were conducted to allow me to inform my decisions when establishing an analysis plan.

Video Validation Online Survey

In order to determine whether our expectation manipulation videos indeed affected expectation in the Video Validation online survey, and to quantify the effects of the manipulation on self-reported expectation of analgesia, a linear mixed model (LMM) was built using the mixed effect model command MIXED in SPSS (version 27, IBM). The LMM had the fixed main effect *rating time*, denoting the time of expected pain ratings relative to video watching. As this was a survey designed to evaluate the quality and effectiveness of our expectation manipulation, and there were two videos shown between-subjects, I also wanted to check for any potential differences between-conditions. Therefore, the fixed effect *condition* was added to the LMM, denoting whether the participant's expectations were manipulated for high arousal or low arousal music. To account for dependencies in the data due to the music type (high or low arousal) being manipulated, the random effect structure included a per-participants and per-music type crossed random intercept.

Video Effectiveness in the Pilot

For the Pilot, I wanted to ascertain not only whether the video effectively manipulated expectations in a lab setting, but also whether those expectations influenced pain during the experiment. To evaluate whether the video effectively manipulated expectations, a repeated measures analysis of variance (ANOVA) was run using jamovi software (The jamovi project, 2021) with repeated measures *rating time* denoting the timing of expected pain ratings, *music type* denoting whether participants were asking about high arousal or low arousal music, and between-subjects factor *condition* denoting which *music type* was manipulated in the video. Then, due to the small sample size of the Pilot, a Bayesian model of the repeated measures ANOVA described above was run to establish the strength of the evidence in the data so that my interpretation of the ANOVA could be tempered appropriately. Bayes factors were computed as compared to a null model and interpreted based upon recommendations by Lee and Wagenmakers (2013). Beyond this, I investigated the associations between manipulated and unmanipulated expectation and actual pain ratings during the in-lab experiment. Exploring this association was enabled by the experiment design in which participants rated their expectations for both types of music that they would hear during pain administration and thus allowed an investigation of the effectiveness of the expectation manipulation to

influence acute pain experiences. For this, I inspected correlations between expected pain ratings and experimental pain ratings by generating a correlation matrix inspecting Pearson product moment correlation coefficients (r), p-values in jamovi (The jamovi project, 2021).

Prior Expectation

To evaluate the potential for prior expectations for pain relief to differ between high and low arousal music, Bayesian and frequentist Independent Samples Student's t-tests were run in jamovi (The jamovi project, 2021) for both the Video Validation online survey and the Pilot.

2.5.2 Behavioral Analysis

To improve statistical power for the analysis of reward and pain ratings, data was compiled from the Pre-Pilot and the Pilot, which had identical procedures for the main experimental task (rating of pain and reward). Data from a total of 30 participants was included in the behavioral analysis. The two experiments differed in that the Pilot included an expectation manipulation, but exploratory analyses revealed that the expectation manipulations did not significantly affect‑pain ratings. Besides an increased target pain (55/70) during calibration and the added question about the fixation circle included in the Pilot in line with the video intervention's script pertaining to multisensory perception (tactile, visual), procedures were identical for the two experiments.

Linear Mixed Model for Pain Ratings

Linear mixed models were chosen as the preferred method for analysis because of their ability to handle missing data, account for changes in response due to time or order effect, and as a more flexible approach compared to ANOVAs for analysis of within-subjects repeated measures data (Baayen et al., 2008; Barr, 2013; Gueorguieva & Krystal, 2004). Analyses were conducted using the mixed effect model command GENLINMIXED in SPSS (version 27, IBM). A LMM was established to evaluate effects on pain as measured by pain ratings, total 1077 pain ratings across 30 participants, during the main task. The model had the fixed effects: *sound type, block number,* and *pain trial number*. *Sound type*, reflecting whether high arousal music, low arousal music, or noise was playing during the block, is the fixed effect included to test whether musical analgesia was induced during the study. *Block number* was included as a fixed effect to account for order effects caused by the 9 consecutive blocks of the main task. *Pain trial number* reflects pain stimulation per block (1-4), included to account for habituation or sensitization effects caused by the study design of repeated heat trials. A random effects structure was established to account for

dependencies in the data. Since individuals may vary in how repeated pain trials and blocks affect their pain ratings via habituation or sensitization, the random effects structure included per-participant random intercepts for the trial and order variables *block number,* and *pain trial number,* respectively.

Effect of Reward on Pain Ratings

Discrete or continuous covariates for which a linear mixed model needs to be adjusted for can be included to a mixed model as a fixed factor (Hintze, 2007). Therefore, to test how experience of reward during music listening affected the magnitude of the music-induced analgesic effect, the pain rating LMM as described above was run with *reward* added as a fixed effect covariate. Reward was measured as self-reported musical enjoyment after listening experience of each sound type. During each block of sound listening reward was recorded twice, once after the first 25 seconds of listening, then again at the end of all the pain trials. The second reward rating asks, retroactively, how much the participant had enjoyed the sound clip since the pain trials had begun. Because this rating captures the reward experience in the time period during which pain was administered, only the second reward rating was included in the analysis.

2.5.3 Pupil Analysis

Pre-Processing

Data from 24 participants from the Pre-Pilot and Pilot were included in pre-processing and analysis. Reasons for excluding data were: one participant's data was a faulty recording resulting in identical values for the entire session, one participant's data failed to convert to a readable data format, and 4 participants' recordings failed to save and were lost. Raw eye tracker output was converted to a standard format containing raw pupil size time series for the left and right eyes, signal segmentation, and sound type information in R (R Core Team, 2020) using functions from *dplyr* and *tidyr* in the *tidyverse* library (Wickham et al., 2019). Pupil size was recorded as diameter in millimeters. Signal segmentation contained information about what event was occurring during the session, necessary for splitting the recording into only the relevant segments. The four pain trials occurring during each block including the 8 second ramp up time, *pain*, were chosen as the indicators of pain intensity. The study design included a 25 second immersive listening period in the beginning of each task block, during which participants were instructed to rest their eyes on a screen with a gray fixation circle and allow themselves to be immersed in the sound they are hearing. This listening period of each block, *listening*, was selected as the segment in which pupil

diameter's association with sound enjoyment and arousal could be evaluated. All pre-processing of the standard format pupil data was also conducted in R with the *pupillometryR* library and its associated functions (Forbes, 2020). First, a linear regression was conducted of the left pupil against the right pupil and then the reverse. The data was regressed over each participant, trial, and time. Then left and right pupil diameters (mm) were averaged, and the mean pupil sizes were used for the rest of the pre-processing. The mean pupil data was filtered with a median absolute deviation as a robust, and outlier resilient, data dispersion metric (Kret & Sjak-Shie, 2019). To smooth across blinks, a linear interpolation was performed. After filtering and smoothing, data was downsampled from 250 Hz to 60 Hz to reduce the autocorrelation in the residuals (van Rij et al., 2019).

Pupils During Pain

As subjective pain experienced can be captured by pupil size, pupil diameter was measured during pain stimulation as an objective measure of pain. Potential fear and startle pupil responses to pain were attenuated via the fixation circle turning red as a warning of heat ramp up (Mischkowski et al., 2019). During one session, there were 12 total events of pain stimulation for each sound type. In order to compare pupil size for pain stimulation during music listening compared to noise listening, I computed grand means during event *pain* by all sound conditions: high arousal music, low arousal music, and noise listening. Pupil recordings from these events were averaged for each participant across each condition. A repeated measures ANOVA was conducted in jamovi (The jamovi project, 2021) using the participant's grand means.

Pupils During Listening

The primary motive to include pupillometry was as an objective outcome measure of pain. However, as the subjective reward experienced during music listening can be captured by pupil size (as described in section 1.3.2) pupil diameter was measured during the experiment as an objective measure of reward. During one session, there were 9 total *listening* events. Grand means were computed for all *listening* events by all sound conditions: high arousal music, low arousal music, and noise listening. The grand means for *listening* events were used for all following analyses. I inspected correlations between pupil size and reward ratings by generating a correlation matrix inspecting Pearson product moment correlation coefficient (r) and p-value in jamovi software (The jamovi project, 2021). Then, in order to compare pupil size during music of varying arousal compared to noise listening, I computed grand means by all sound conditions: high arousal music, low arousal music, and noise listening.

Pupil recordings from these events were averaged for each participant across each condition. A repeated measures ANOVA was conducted in jamovi (The jamovi project, 2021) using the participant's grand means.

2.5.4 Power Analysis

One practical aim of this pilot study was to estimate effect sizes of our main effects to perform a sensitivity analysis for the main trial's sample size calculation. A weighted average effect size of naltrexone on pain was established via existing literature (Bruehl et al., 1996; Cook et al., 2000; France et al., 2007; Frew & Drummond, 2007, 2008; King et al., 2013; Koltyn et al., 2014; Pontén et al., 2020; Taneja et al., 2020; Tarr et al., 2017; J. W. Younger et al., 2009). Effect size for music and expectation manipulation's effects on pain were calculated using the G*Power f-test MANOVA for repeated-measures within factors, using the variance of pain ratings during music and noise listening (Faul et al., 2007). To estimate the effect size using variance information for the power analysis, high arousal and low arousal music were combined under one category of "music". Data from the Pre-Pilot and Pilot were used to establish variance for music's effect on pain, and data from the Video Validation online survey was used to establish variance for expectation's effects on pain. Standard error and number of observations were used to calculate variance, which was used to establish the effect sizes. Pairwise contrasts (Table 3), standard errors for error variance, and variance attributable to music and expectation were retrieved from the residual effects were generated by the LMMs described in the previous sections "Video Validation Online Survey" and "Linear Mixed Model for Pain Ratings". The sensitivity analysis was informed by the calculated effect sizes and conducted in G*Power (Faul et al., 2007).

Results

3.1 Exploratory Results

Descriptive Analysis

Descriptive statistics of the responses to the video validation survey were inspected, ensuring that the data fulfilled the assumptions of the further analyses, generated in jamovi (*jamovi*, version 1.6). During the Video Validation online study, participants were asked to evaluate the quality of the expectation manipulation videos by selecting from a checklist of adjectives describing the quality of the video. Because our expectation manipulation was novel in

format and content for musical analgesic effect, it was important for me to establish how it was received by participants. Not only in its effectiveness in manipulating expectations for pain, but also in its perceived quality and believability as a source of information. Participants in the Video Validation online survey's feedback for the quality of the video revealed that it was regarded positively, as convincing, clear, and informative (Fig. 1).

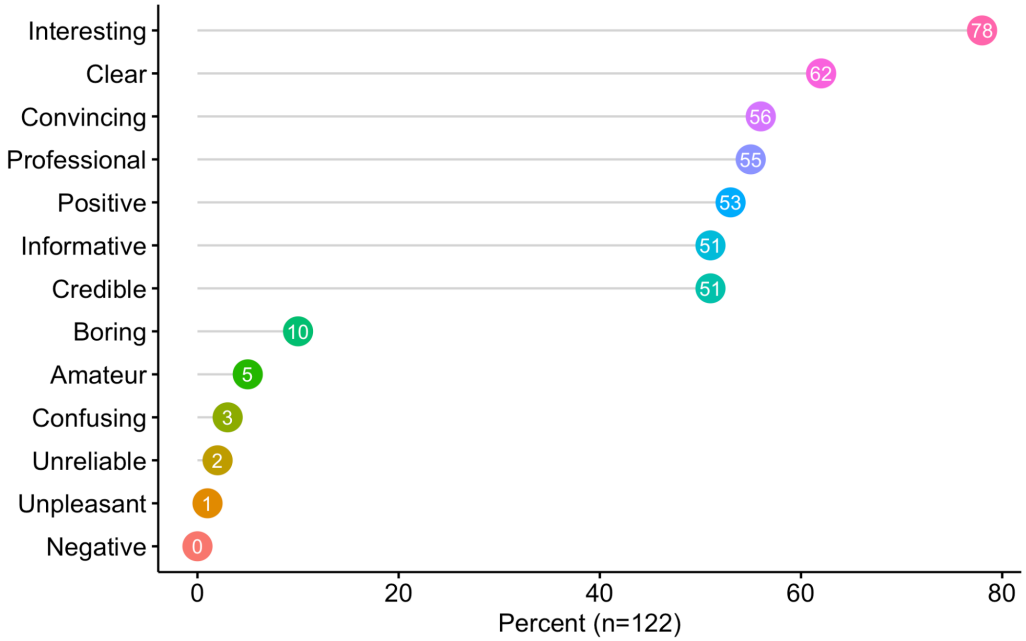

Fig. 1 Percentages (N = 122) of responses to a checklist of adjectives describing the video manipulations, responses for both videos combined. There was no limit to the number of adjectives participants could select during rating. Visualized in R using the *ggpubr* package (Kassambara, 2020).

During the Pilot study, participants were asked about their expectations of pain for both high arousal and low arousal music before watching a video. Then they watched a video intended to raise expectations of pain for the music type. At first glance of the pain expectation means (Fig. 2), there seem to be greater prior expectations for pain relief while listening to high arousal as compared to low arousal music. Excluding expectations for low arousal music in the high arousal manipulation condition, expectations for pain were increased after video watching for both manipulation conditions. These descriptive indicators are investigated in greater depth in the proceeding exploratory statistical analyses and results.

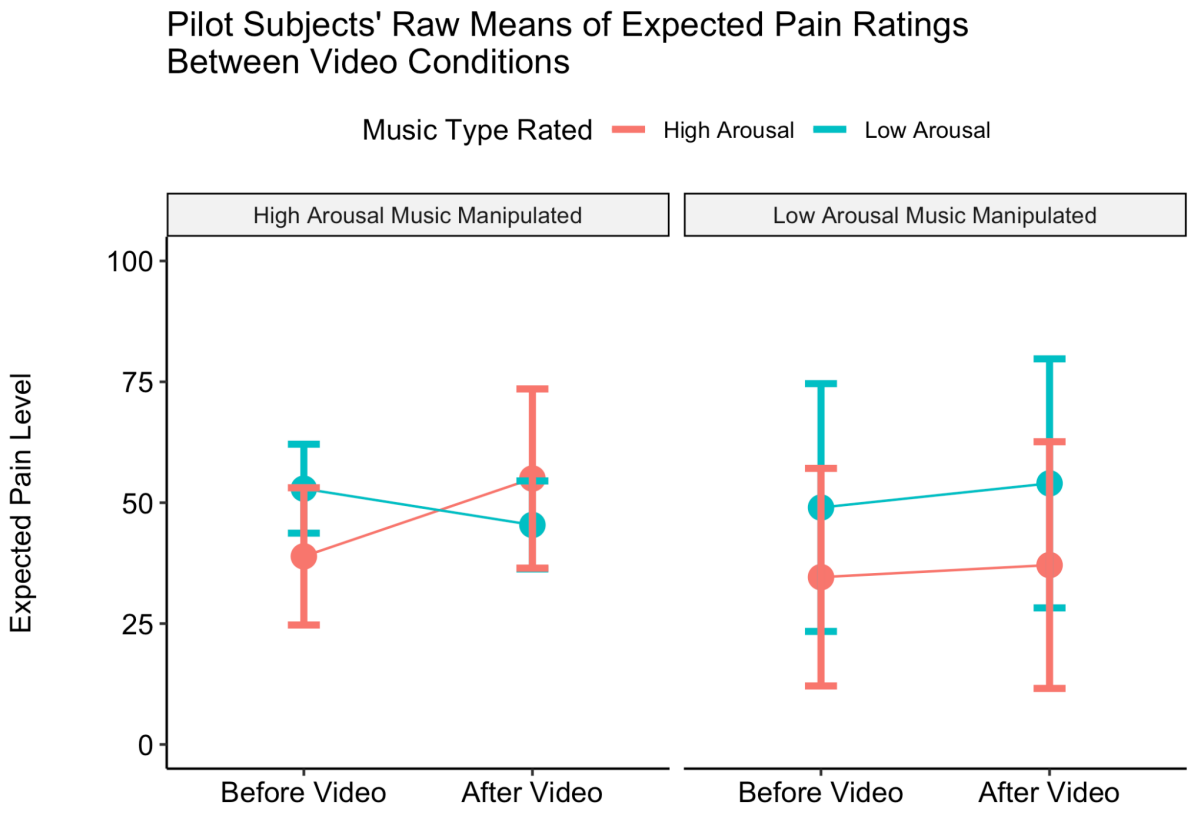

Fig. 2 Descriptive statistics for expected pain ratings for Pilot participants, error bars indicate 95% CI. All participants (N = 20) rated pain expectations for high and low arousal music. Music type rated indicates which music type was manipulated to expect higher pain for (High arousal music: (N = 11),Low arousal music: (N = 9)). Visualized in R using the *ggplot2* package (Wickham, 2016).

Video Effectiveness in Video Validation Online Survey

In line with the aim of the expectation manipulation, the videos were successful in increasing the expectation of pain. There was a significant increase in expected pain ratings after video watching, as compared to ratings before video watching ($F_{1,115}$ = 11.276, p = 0.011) and no significant difference for expectation between conditions ($F_{1,114}$ = 0.899, p = 0.345). As seen in Fig. 3, this difference was reflected as a mean increase in expected pain (*estimated means (SEM)* pre: μ = 40.261 (2.351), post: μ = 48.787 (2.351)).

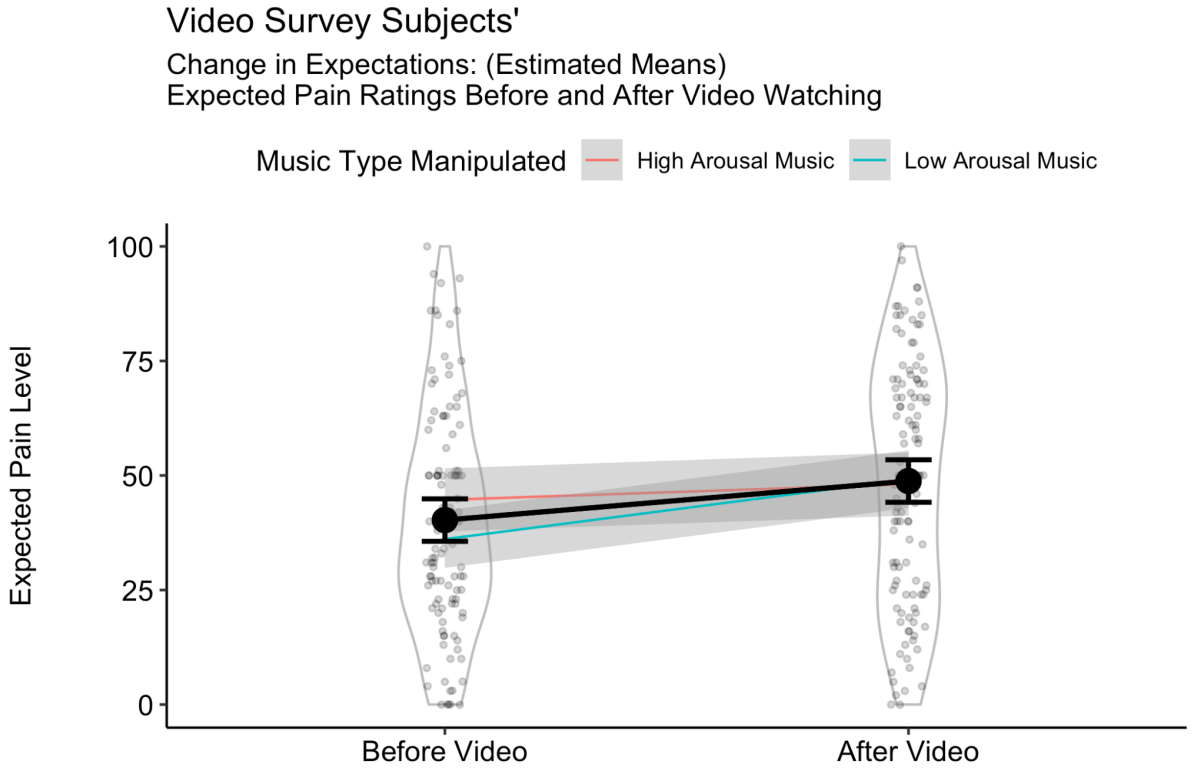

Fig. 3 Estimated means of expected pain ratings (in black) for before and after video-watching in the Video Validation online survey, error bars showing 95% confidence interval. No significant difference in expected pain ratings between video conditions manipulating either high arousal music or low arousal music. 95% CI per condition shown by gray ribbons. Individual data points (N=116) jittered for each rating time. Visualized in R using the *ggplot2* package (Wickham, 2016).

Video Effectiveness in the Pilot

Indicating a failure of the expectation manipulation to achieve the intended outcome, in the Pilot, there was no statistically significant difference between pre and post expectation for pain ($F_{1,18} = 1.608$, $p = 0.221$). Although there was no significant difference between pre and post expectations, there was also no evidence for the null ($B_{10}=0.1292$) between pre and post expectation ratings. This indicates that we cannot draw reliable conclusions about the effectiveness of the expectation manipulation video during the Pilot. However, I also explored associations between manipulated and unmanipulated expectation and actual pain ratings during the Pilot experiment (N = 20). The correlations paint a picture (Fig. 4) more aligned with the results of the online study, where the video did influence expectations. The strength of the correlations are interpreted per the recommendations by Schober and

colleagues (2018). Indicative of a successful expectation manipulation, pain ratings during the manipulated sound condition had a moderate strength correlation with expected pain rating after the video (r = 0.410, p = <0.0001) and were not correlated with prior expectation (r = 0.005, p = 0.939). The opposite stood true, where pain ratings during the unmanipulated sound condition had a moderate-strength correlation with prior expectancy ratings (r = 0.316, p = <0.0001) and only a weak correlation with post-video expectancy rating (r = 0.15, p = 0.02).

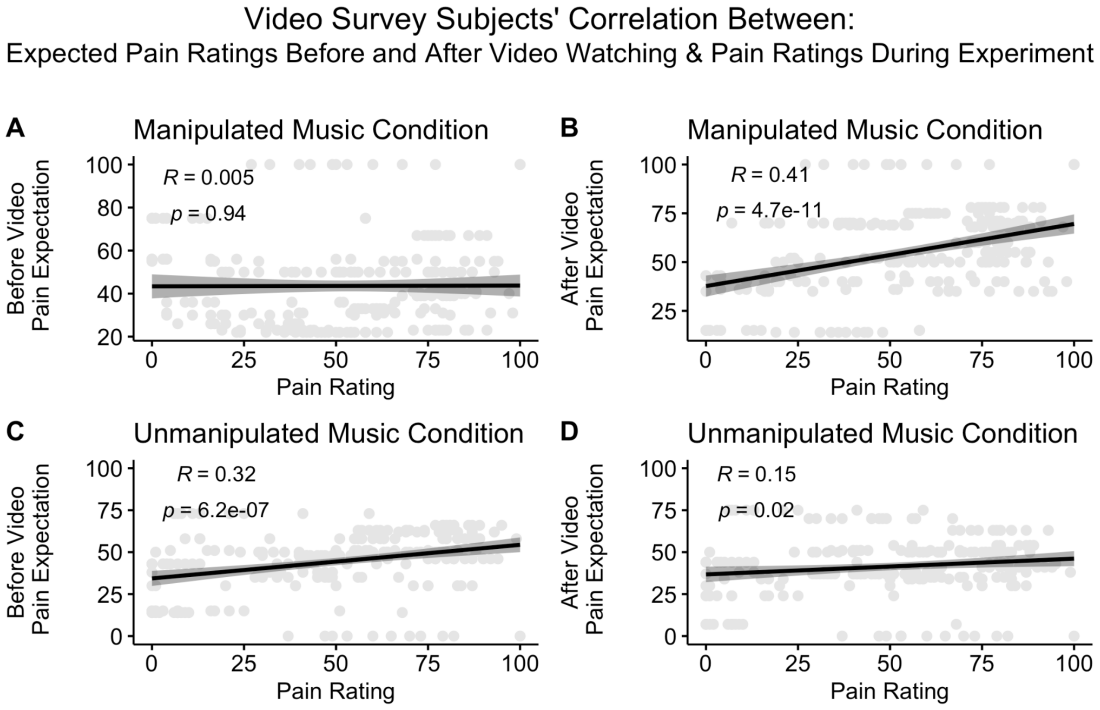

Fig. 4 Correlations of expectations with gray ribbons indicating 95% CI, for pain and actual pain ratings during the experiment for manipulated and unmanipulated music. Pain ratings during the manipulated music condition only correlate with expected pain after watching the expectation manipulation videos. Pain ratings during the unmanipulated music condition correlate with expected pain before and after watching videos manipulating the other music condition. Visualized in R using the *ggpubr* package (Kassambara, 2020).

Prior Expectation

In both the Video Validation online survey, and the Pilot, participants had prior expectations for pain relief that were significantly different between high arousal music and low arousal music. Interestingly, in the Video validation survey prior expectations for pain relief were stronger for low arousal music compared to high-arousal music (t = 1.93, df = 114, p = 0.057,

BF_{10} = 1.04). In contrast, during the Pilot, prior expectations for pain relief were stronger for low arousal music (t = -2.49, df = 38, p = 0.017, BF_{10} = 3.3). Importantly, there was little to no evidence in the Pilot, based on the Bayes factors, that there was truly a significant difference between prior expectations for pain relief between music types.

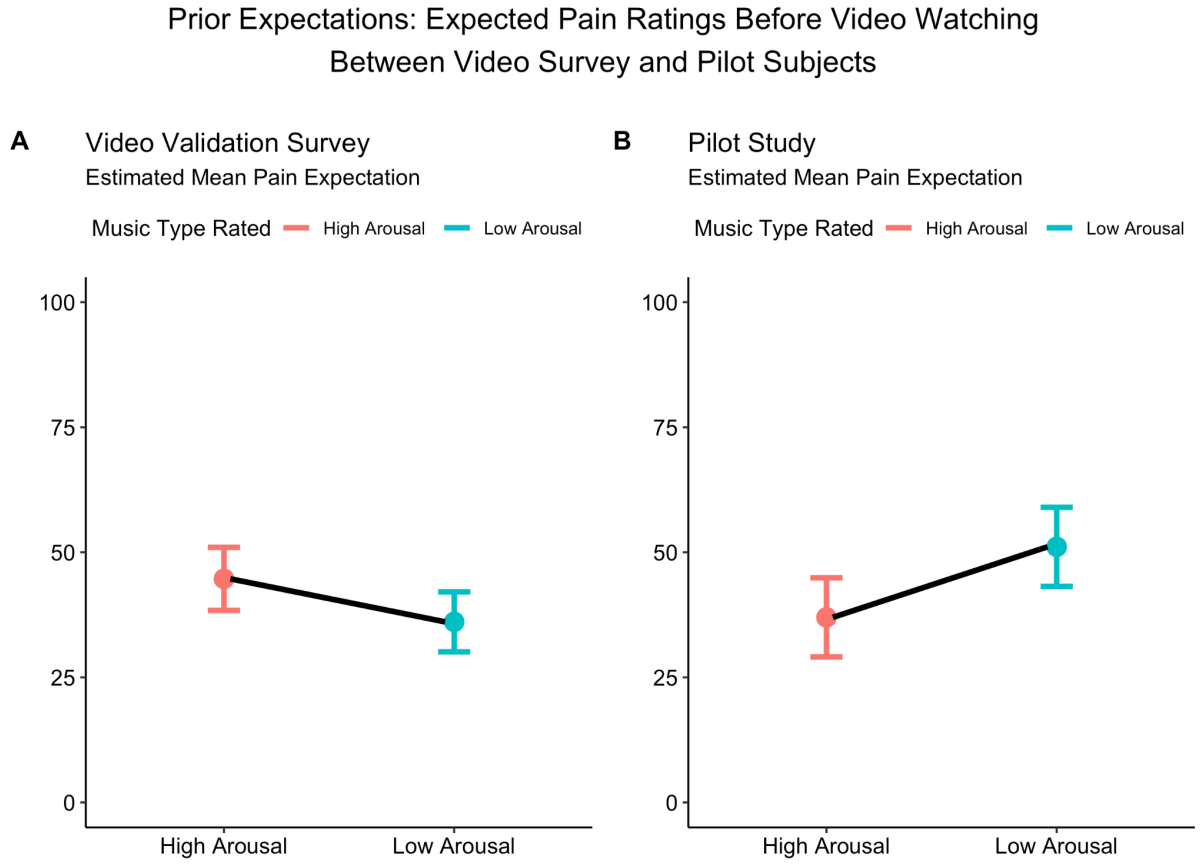

Fig. 5 Mean expectations for pain prior to watching the expectation manipulation video for (A) Video Validation online survey (B) Pilot. Error bars show 95% CI. Visualized in R using *ggplot2* and arranged with the *ggpubr* packages (Wickham, 2016; Kassambara, 2020).

Study	Sound Type	N	Mean	SD	Std. Error
Video Survey	High Arousal Music	55	44.7	24.0	3.24
Video Survey	Low Arousal Music	61	36.1	23.9	3.06
Pilot	High Arousal Music	20	37.0	18.0	4.02
Pilot	Low Arousal Music	20	51.1	18.0	4.03

Table 1 Descriptive statistics corresponding to Fig. 5 for expected pain ratings before watching the expectation manipulation video in the Video Validation Survey and Pilot studies. Visualized in R using the *ggpubr* package (Kassambara, 2020).

3.2 Behavioral Results

In line with a musical analgesic effect, there was a statistically significant main effect of *sound type* (*Table 2, $F_{2, 1063}$* = 8.331, p = 0.000), with pain ratings being significantly lower for both music types compared to noise (*mean* (*SEM*): high arousal music: 41.201 (4.427), low arousal music: 43.168 (4.434), noise: 49.660 (4.465), high arousal music < noise (β = -8.458, p = 0.000), low arousal music < noise (β = -6.492, p = 0.003), see Fig. 6A). Block number ($F_{8,1063}$ = 2.087, p = 0.034) and pain trial ($F_{3,1063}$ = 35.005, p = 0.000) also significantly affected pain ratings, reflecting a general habituation effect within each block (Fig. 6B).

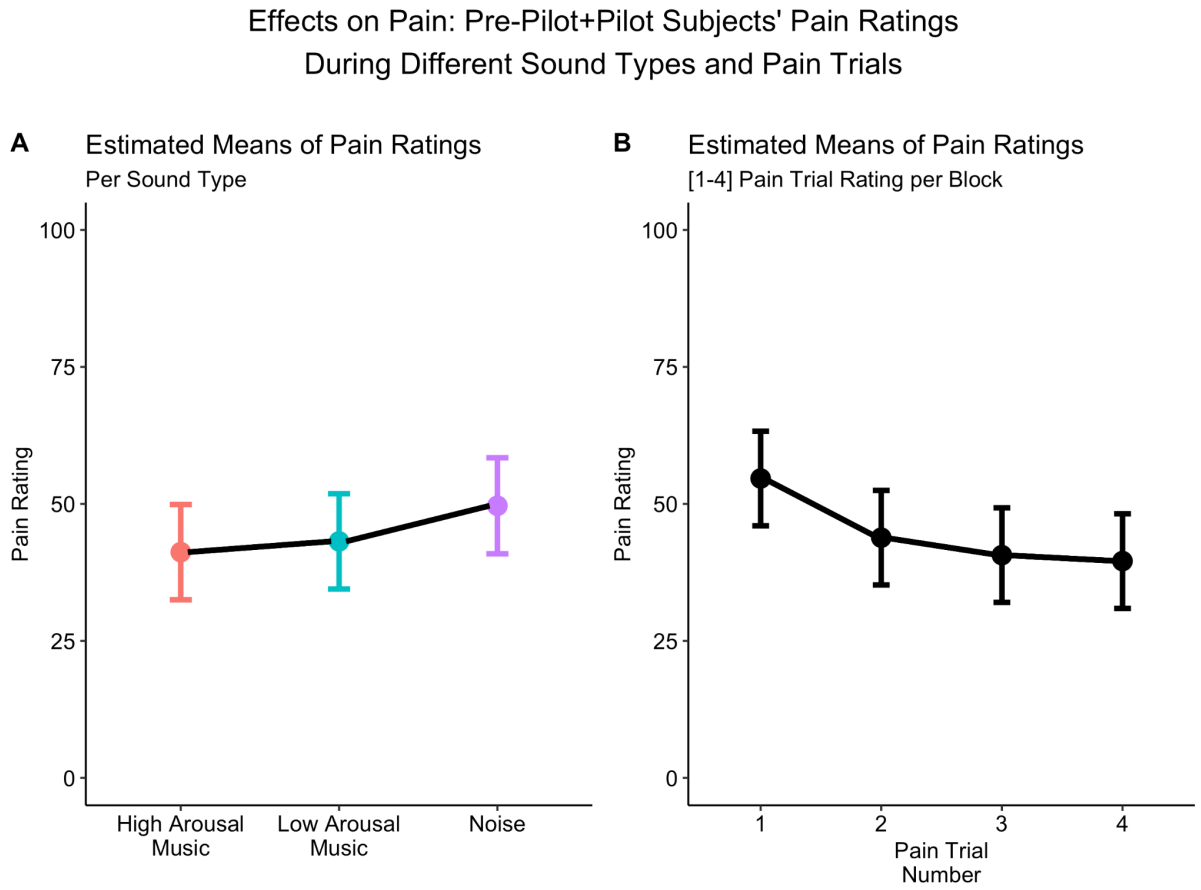

Fig. 6 Estimated means for pain ratings for Pre-Pilot and Pilot participants (N = 30) (A) between sound types (B) within one block of music or noise listening, error bars indicate 95% CI. Visualized in R using the *ggplot2* package (Wickham, 2016).

Sound Type	Contrast Estimate	Std. Error	t	df	Adj. Sig.
High Arousal Music - Low Arousal Music	-1.967	1.899	-1.036	1063	3.01e-01
High Arousal Music - Noise	-8.458	2.108	-4.012	1063	6.45e-05
Low Arousal Music - Noise	-6.492	2.151	-3.018	1063	3.00e-03

Table 2 Pairwise contrast parameters comparing pain ratings during music to noise for Pre-Pilot and Pilot participants (N = 30). Showing no significant difference between the two music parameters. Visualized in R using the *ggpubr* package (Kassambara, 2020).

Reward significantly affected pain ratings ($F_{1, 1062} = 7.131$, p = 0.008), with a small decrease in pain ratings (β = -.156). Notably, there was no longer a significant difference in pain ratings between music and sound once reward was added to the model as a covariate ($F_{2, 1062} = 0.453$, p = 0.636), reflecting that reward is a significant moderator of the musical analgesia effect. This moderation of the analgesic effect is further reflected in shifted estimated means for pain ratings (*mean* (*SEM*): HA = 43.476 (4.389), LA = 45.165 (4.377), N = 45.380 (4.630)), seen in Fig.7, where reward was fixed at its mean value (69.43) for the estimated means calculations. Block number ($F_{8,1062} = 2.033$, p = 0.040) and pain trial ($F_{3,1062} = 35.003$, p = 0.000) remained significant factors. Furthermore, reward ratings of musical enjoyment had a small correlation with pain ratings (r = -0.275, p = <0.001, see Fig. 7B) so that greater reward was associated with lower pain ratings (Schober et al., 2018).

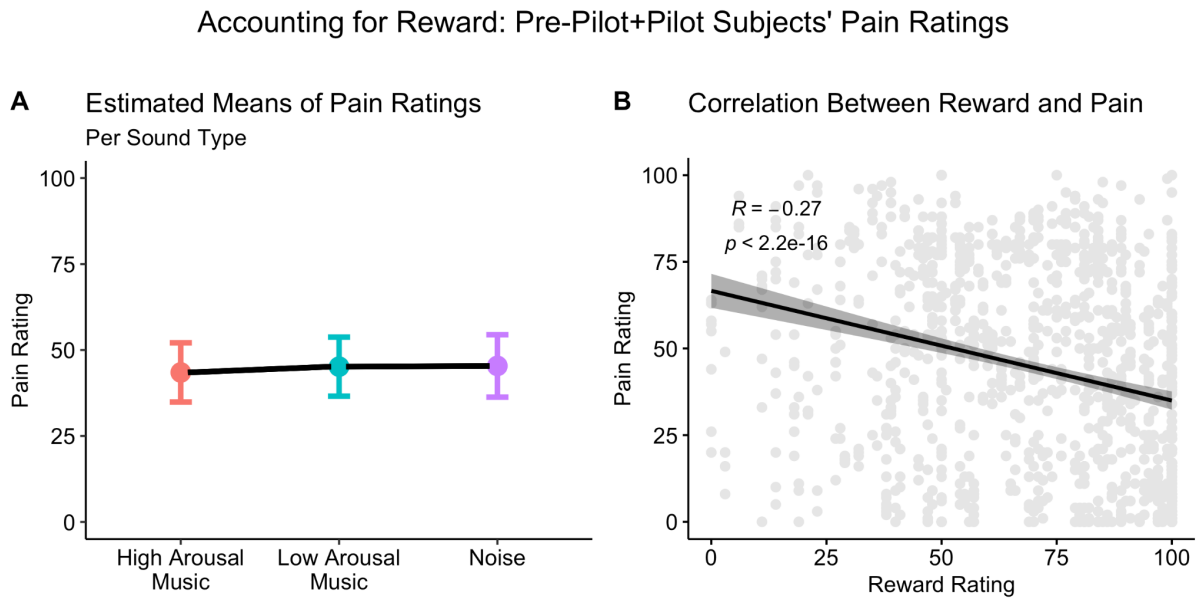

Fig. 7 (A) Diminished musical analgesia (see Fig. 6) after adjusting for reward reflected by estimated means for Pre-Pilot and Pilot participant's pain ratings between sound types. Error bars represent 95% CI. (B) Correlation scatterplot with gray ribbon indicating 95% CI, showing association between decreased pain ratings with higher reward, measured by musical enjoyment, ratings. Visualized in R using *ggplot2* and arranged with the *ggpubr* packages (Wickham, 2016; Kassambara, 2020).

3.3 Pupil Size

Pupils During Pain

In line with results from Mischkowski and colleagues (2019) we predicted that the musical analgesic effect will be reflected as larger pupil size, indicating greater pain, during noise listening compared to during music listening. Although sound had a significant effect on pupil diameter, $F(2, 46) = 5.89$, $p = .005$, $\eta^2_p = 0.204$, pupils were not larger during noise listening compared to music – contrary to what we had expected. On average pupil diameter (mm) during pain trials was biggest during listening of high arousal music ($\mu = 4.84$, $SD = 1.000$) and smallest during listening of low arousal music ($\mu = 4.70$, $SD = 0.976$) as compared to noise ($\mu = 4.76$, $SD = 0.943$).

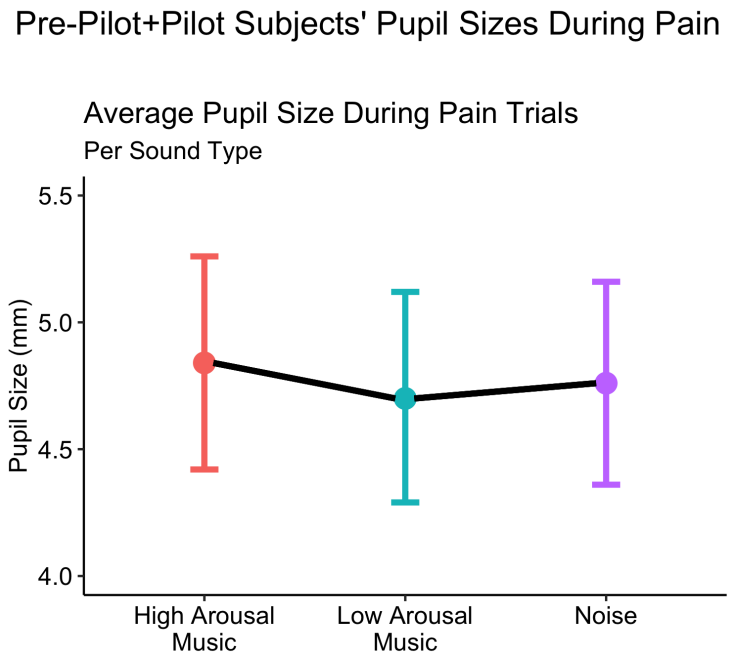

Fig. 8 Estimated marginal means of pupil size (diameter in mm) for Pre-Pilot and Pilot participants (N = 24) during pain stimulations between sound types. Error bars indicate 95% CI. Visualized in R using the *ggplot2* package (Wickham, 2016).

Pupils During Listening

To test the association between the rewarding experience of listening to one's preferred music and pupil diameter, a correlation matrix was generated inspecting Pearson product moment correlation coefficients (r) and p-values in jamovi software (The jamovi project, 2021). We predicted that pupil size would be positively correlated with reward (measured as self-reported musical enjoyment after listening experience). Contrary to our prediction, there was no correlation (r = 0.058, p = 0.630, see Fig. 9A) between pupil size and musical enjoyment ratings (Schober et al., 2018). Beyond reward, as an indicator of arousal, we predicted that pupil size would be larger for high arousal music, as compared to noise and smaller for low arousal music as compared to noise. A repeated measures ANOVA showed that sound had a significant effect on pupil diameter, $F(2, 46) = 5.35$, $p = .008$, $\eta^2_p = 0.189$, and pupil size was in line with our predictions. On average pupil diameter (mm) was biggest during listening of high arousal music ($\mu = 4.97$, $SD = 1.025$) and smallest during listening of low arousal music ($\mu = 4.80$, $SD = 0.909$) as compared to noise ($\mu = 4.87$, $SD = 0.909$).

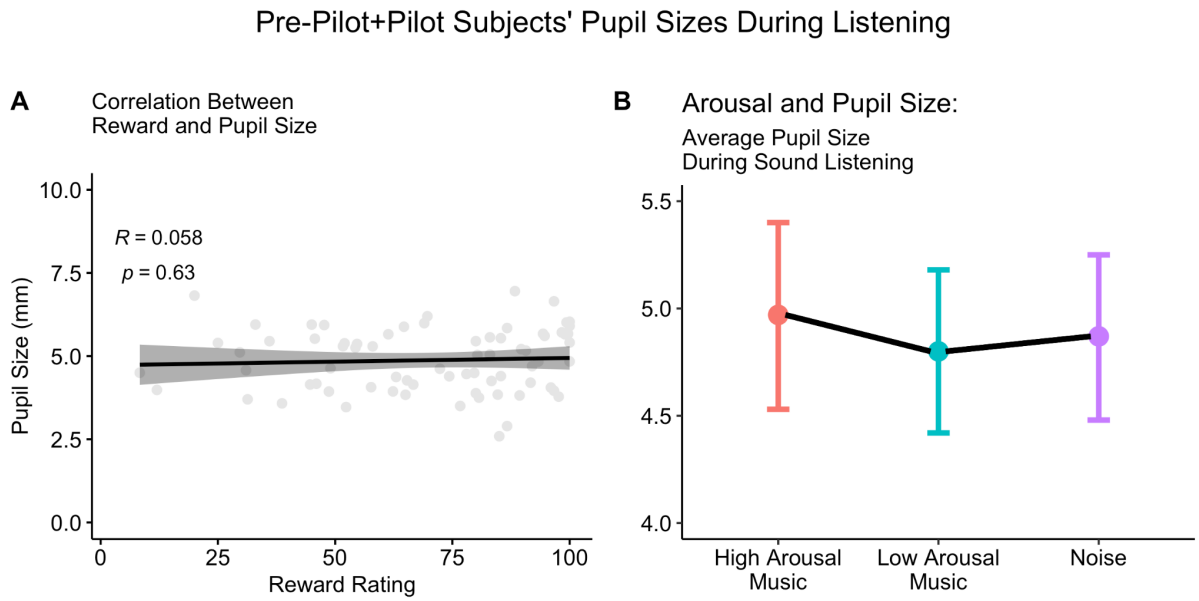

Fig. 9 (A) Correlation between reward, as musical enjoyment ratings, and pupil diameter (mm) does not show a meaningful association. Grand means of pupil size across sound type, per-participant and corresponding reward ratings also averaged across sound type, per-participant are jittered in gray. (B) Estimated marginal means of pupil diameter (mm) of Pre-Pilot and Pilot participants (N = 24), during the 25 second listening period at the beginning of each block, across sound types. Truncated axis to show small differences inherent to average pupil size changes more clearly. Visualized in R using *ggplot2* and arranged with the *ggpubr* packages (Wickham, 2016; Kassambara, 2020).

3.4 Power Analysis

The standard errors used to calculate variance, and in turn, effect size, are listed in Table 3. Effect size interpretation is based on Cohen's (1988) recommendations. The effect size of musical analgesia based on the combined data from Pre-Pilot and Pilot participants (N = 30) was partial η^2 = 0.02, effect size f = 0.14 indicating a small effect. The effect of the expectation manipulation, based on expected pain ratings in Video Validation online survey (N = 116), was a very small effect with a partial η^2 = 0.002, effect size f = 0.04. The effect size for naltrexone on pain was established based on existing literature, is Hedges' g = 0.15, a small effect. With our within-subjects repeated measures design, in order to detect our main effects on pain with 80% power sample sizes required would be: musical analgesia = 11, expectation manipulation = 137, naltrexone = 11. However, our main research question is not investigating these main effects but rather interaction effects, which require larger samples as

their effects are typically smaller and need more power to be detected (Lakens, 2021). Using G*Power's sensitivity analysis, for a repeated within-subjects design such as ours, an effect as small as f = 0.06 would be able to be reasonably detected with 80% power and α = 0.05, with 60 participants.

A

Sound Type	Contrast Estimate	Std. Error	t	df	Adj. Sig.
Music - Noise	-7.524	1.906	-3.948	1064	8.398e-05

B

Rating Time	Contrast Estimate	Std. Error	t	df	Adj. Sig.
Before Video - After Video	-8.526	2.539	-3.358	230	0.001

Table 3 (A) Pairwise contrast parameters (N = 30, # total pain trials = 1077) from the Pre-Pilot and Pilot from which variance information (SEM) was used to inform the power analysis for musical analgesia effect size. (B) Pairwise contrast parameters (N = 116, # total ratings = 232) from the Video Validation online survey from which variance information (SEM) was used to inform the power analysis for expectation manipulation effect size.

Discussion

The main aims of the present pilot study were to determine whether music has an analgesic effect as compared to noise, assess musical pleasure's role in music-induced analgesia, and to manipulate and measure expectations' effect on pain relief during music-listening. Practical aims included improving and developing a subsequent pharmacological study by testing study protocols and procedures, assessing feasibility of the study, performing a sensitivity power analysis, and providing preliminary evidence of efficacy potential. Accounting for inconsistencies in existing musical analgesic literature, a study design and protocol were developed, and data from 161 healthy participants were collected and analysed to establish whether our design successfully addressed our main aims. Based on an evaluation of the outcome of these aims, a future large-scale pharmacological study (N = 60) is planned, to assess potential changes in musical analgesia associated with μ-opioid blockade.

4.1 The Main Findings

Pain ratings during the Pre-Pilot and Pilot were lower while listening to both high and low arousal music. Subjective pain ratings indicated that listening to self-selected preferred music dampened the painfulness of acute thermal stimulation compared to listening to brown noise. Thus confirming the effectiveness of our design to elicit a musical analgesic effect. There appeared to be a habituation effect resulting in lower pain ratings over time within a block, and across a session. However, as sound types were counterbalanced and pseudo-randomized, the habituation effect is unlikely to have affected comparisons between sound types.

The magnitude of musical analgesic effect was significantly affected by the rewarding capacity of music, as measured by self-reported musical enjoyment. Furthermore, higher subjective ratings of musical enjoyment had a small association with lower pain ratings.

Preliminary success of the expectation manipulation videos was also assessed. Analysis of the online validation survey found that the videos were received as believable, and effectively manipulated expectations for pain during music listening. There was a significant increase in expected pain ratings after video watching, as compared to ratings before video watching. Despite the success of videos in manipulating pain expectations in a large survey, the videos failed to increase expected pain in the Pilot with 20 participants. There was no significant difference between expected pain ratings during music listening from before watching the manipulation video and after the video. However, pain ratings during the manipulated music type were only correlated with expected pain ratings after video-watching, not before. Higher expected pain ratings after video-watching were associated with higher pain ratings while listening to the manipulated music type. This indicates a successful manipulation of expectation for reduced pain relief which in turn increased subjective pain. Investigating prior expectations of pain relief during music listening had inconclusive results as the video validation survey and the Pilot showed opposing differences in prior expectations for pain relief between high and low arousal. Regardless, self-reported prior expectation of pain relief during music listening was correlated with actual ratings of pain during music listening, reflecting that the magnitude of musical analgesia increases with increased prior expectations for pain relief.

We predicted that, as a reflection of greater musical enjoyment during listening periods, pupil diameter would be larger during music listening blocks as compared to noise blocks. Furthermore, we predicted that increased pain would be reflected by larger pupil diameter during pain events in noise blocks compared to music blocks. Neither reward nor

pain were reflected as larger pupil diameter during listening or pain events as expected. This negative finding may have been the result of a few potential causes. One possibility is that subjective ratings are more sensitive than pupil diameter. Therefore, the pupil analysis may require more statistical power which would only be captured by the larger planned main study. Additionally, it is possible that the current pupil analyses were not fine-grained enough. Event flags were used to mark the listening and pain events, and there is a possibility that pupil data was included that did not correspond to the timing of the pupillary pain response, therefore diluting the data and dampening the effects in the analysis. Furthermore, a potential artifact could have arisen if participants did not observe the request to keep their gaze rested on the fixation circle. A future, more sensitive, fine-tuned analysis that includes gaze artifact rejection and accounts for dependencies as in the subjective rating analyses could reveal why pupil diameter did not have the expected result.

The hypotheses of the planned study all investigate the interaction of naltrexone with pain, reward, and expectation. Data from all stages of the pilot allowed us to calculate the effects on pain for two of our main effects; musical analgesia and expectation. The effect of naltrexone on pain was determined via existing literature. All the main effects were found to be small. Therefore, it had to be taken into account that the sample sizes needed to detect our main effects would not be suitable for the main study as an interaction effect would have to be quite large to be detected by a sample size suited for a single main effect (Lakens, 2020). Furthermore, effect sizes drawn from pilots face the same imprecision as any study with a small sample size (Leon et al., 2011). Therefore, the sensitivity analysis was deliberately performed accounting for that likelihood that the calculated effect sizes had the potential for over-estimation, and that the interaction effect would be smaller than the main effects. As a result, the planned study is planned to reasonably detect a very small effect size of Cohen's f = 0.06.

4.2 Consistency with Previous Studies

In line with previous musical analgesic research outlined in section 1.3, music was successful in inducing an analgesic effect compared to noise. The present pilot went further to quantify the musical enjoyment experienced while listening to preferred music and found it to be a significant moderator of the analgesic effect. Showing that once enjoyment is controlled for, the musical analgesic effect is diminished. Previous work supports our reward findings, any kind of music will not work with the same kind of efficacy as well-loved music(Hekmat &

Hertel, 1993; Hsieh et al., 2014; Knox et al., 2011; Mitchell & MacDonald, 2006; Perlini & Viita, 1996). Taken together with the results of our pilot study, it is clear that highly enjoyable, favorite music is very effective in inducing a musical analgesic effect which should be taken into account when making decisions in clinical pain treatment settings. Given our findings that pain was lower during music than noise, our pupil size outcomes during pain were not consistent with previous studies that found higher pupil size during greater subjective pain (Mischkowski et al., 2019). There also was no correlation between reward and pupil size, which is not in line with previous findings in which pupil size reflects the rewarding capacity of music (Alnaes et al., 2014). However, pupil size was larger during high arousal music and lower during low arousal music as compared to noise. Thus indicating that we were successful in capturing arousal through pupil diameter, a result which is well supported in previous work studying pupillary responses to music (Bowling et al., 2019; Gingras et al., 2015).

4.3 Expectation

One main aim of the present pilot study was to establish the efficacy of the expectation manipulation. In some respects our expectation manipulation affected our outcome measures as predicted. In the Video Validation Survey, expected pain was greater after watching the expectation manipulation as compared to before watching the video. In the Pilot, expected pain ratings after manipulation correlated with pain ratings during the manipulated song type in the study itself. However, the associations between the non-manipulated sound type were weaker than expected. Specifically, the expected pain ratings after video-watching were less correlated with pain ratings than expected pain before watching the video. This indicates a spill-over effect of the video manipulation not only affecting the manipulated music type, but also the non-manipulated music type. In our design, intact expectation for pain relief is represented by pain ratings in the unmanipulated music condition. The effects of reduced expectation for pain relief on musical analgesia are represented by pain ratings in the manipulated music condition. Therefore, the presence of a spill-over effect would indicate that the unmanipulated music condition is no longer a reliable comparison to the manipulated music. It would be difficult to make comparisons between the effects of intact expectation and the effects of reduced expectation for pain relief, if the unmanipulated music condition does not represent intact expectation after all. At present, only Hsieh and colleagues (2014) have also directly investigated expectation's role in musical analgesia in an experimental

setting. Hsieh and colleagues found that, although their conditioning increased expectation for pain relief during music listening, the significant changes in expectancy did not link to pain relief. In fact, they found that music was the most pain relieving irrespective of conditioning and increasing expectations for pain relief added no further analgesia. This aligns with our results, in which there was no significant difference in pain ratings between manipulated and unmanipulated music types and music in general was more pain-relieving than noise, irrespective of manipulated music condition. Perhaps unspecific contextual treatment factors increase the magnitude of musical analgesia in clinical settings, but it seems that in an experimental context on healthy participants, it may be difficult to capture.

4.4 Limitations

Due to practical constraints the present pilot study happened in only one session. The planned, complete, study will be a double-blind placebo-controlled experiment with a within-subjects design, which requires testing at two separate sessions. A two-session pilot would have allowed for a calculation of a potential session effect and an evaluation of the suitability for a two-session design for future studies of music analgesia.

Another limitation of the current pilot is the failure to capture musical enjoyment or pain experiences in pupil diameter. Typically, strongly rewarding music and acute pain experiences are robustly reflected by pupil diameter (Laeng et al., 2021; Mischkowski et al., 2019). However, arousal also affects pupil diameter (Bowling et al., 2019; Gingras et al., 2015) . Our choice to have participants select music of two arousal levels was chosen as a believable “story” that would be realistic for participants to achieve. However, the varying arousal levels seem to have affected our pupils and obscured reward and pain effects, particularly due to the design of having participants actively chose music of varying arousal. Considering participants could easily introspect as to what is arousing physiologically, it follows that pupils should have been larger while listening to high arousal music.

Additionally, the expectation manipulation failed to significantly affect pain in the Pilot. It is likely that the failure to capture this effect is a result of a small sample size that did not have the power to detect the effect. As can be seen from the properly powered Video Validation Survey, the expectation manipulation did have a significant effect on expected pain. However, the size of that effect was very small ($f = 0.04$). This means that it would not be feasible to conduct a study the size needed to detect that effect, particularly to assess naltrexone’s impact on the expectation effect.

Furthermore, in both the online survey and pilot, participants were only asked about their expectations for pain relief for high and low arousal music. A weakness of this design has been not asking participants for their prior expectations for noise as well which would have allowed for a more complete assessment of prior expectations of pain relief during music listening, as compared to noise rather than a different type of music.

4.5 Future Directions

Due to limitations surrounding the expectation manipulation, it could not be feasibly incorporated into our study design. Practical constraints do not allow for the sample size needed to detect the effect of the expectation manipulation as it is likely to be smaller than the f = 0.04 of the main effect. However, non-specific contextual effects are likely to be a mechanism of musical analgesia (Hsieh et al., 2014; Lunde et al., 2018; Perlini & Viita, 1996) and therefore are worthwhile to investigate. Until now, no studies have directly quantified prior expectations separable from a manipulation. Therefore, it would be advisable in the planned study to do an assessment of prior expectation for pain relief during music listening as compared to a control sound. This assessment would take place at time of recruitment at least three days before the first session to reduce chances of a demand effect. Such an assessment would quantify prior expectation to see if it is present, or large enough to have not only a statistically significant, but also a clinically relevant effect on pain relief during music listening. Collecting such information from the study participants would also allow for exploratory post-hoc analyses of expectations' effects on participant's pain ratings during the experiment.

By altering the design of the study to exclude the expectation manipulation, there is also no longer a need for the participant-selected music to be of low and high arousal, which were chosen to align with the expectation manipulation. Because the varying arousal concealed the objective pupil diameter measures of reward and pain, music selection criteria should be altered for the main study. Participants should select three, not six, pieces of well-loved music that is of a nature that is "not particularly calming or exciting". In addition to these preferred musical selections, three experimenter-selected widely unknown musical pieces can be added to the design besides the 3 instances of brown noise. Thus resulting in a design of equal length and instances of music vs. noise blocks as the present pilot, while allowing for a design that allows an assessment of reward more completely. This would allow the rewarding quality of music to be manipulated by having a non-rewarding music selection

as a comparison, and noise as a non-musical control.

Conclusion

In conclusion, the objective of the pilot study to provide preliminary evidence of efficacy of music in reducing pain has been met and the study design and protocol were shown to be well-equipped to study musical analgesia. The information gathered in the present comprehensive pilot study confirms that by making modifications to the assessment of expectation and musical stimuli, the planned study will be ideally equipped to answer questions about mu-opioids and reward's involvement in musical analgesia. Our planned study will have the power to detect the effect of mu-opioid blockade on music induced analgesia and music enjoyment and allow us to learn more about the mechanisms underlying the musical analgesic effect.

References

Aitken, J. C., Wilson, S., Coury, D., & Moursi, A. M. (2002). The effect of music distraction on pain, anxiety and behavior in pediatric dental patients. *Pediatric Dentistry*, 5.

Alboukadel Kassambara (2020). ggpubr: 'ggplot2' Based Publication Ready Plots. R package version 0.4.0. https://CRAN.R-project.org/package=ggpubr

Allred, K. D., Byers, J. F., & Sole, M. L. (2010). The Effect of Music on Postoperative Pain and Anxiety. *Pain Management Nursing*, *11*(1), 15–25. https://doi.org/10.1016/j.pmn.2008.12.002

Alnaes, D., Sneve, M. H., Espeseth, T., Endestad, T., van de Pavert, S. H. P., & Laeng, B. (2014). Pupil size signals mental effort deployed during multiple object tracking and predicts brain activity in the dorsal attention network and the locus coeruleus. *Journal of Vision*, *14*(4), 1–1. https://doi.org/10.1167/14.4.1

Amanzio, M., & Benedetti, F. (1999). Neuropharmacological Dissection of Placebo Analgesia: Expectation-Activated Opioid Systems versus Conditioning-Activated Specific Subsystems. *The Journal of Neuroscience*, *19*(1), 484–494. https://doi.org/10.1523/JNEUROSCI.19-01-00484.1999

Anseloni, V. C. Z., Weng, H.-R., Terayama, R., Letizia, D., Davis, B. J., Ren, K., Dubner, R., & Ennis, M. (2002). Age-dependency of analgesia elicited by intraoral sucrose in acute and persistent pain models. *Pain*, *97*(1), 93–103. https://doi.org/10.1016/S0304-3959(02)00010-6

Antioch, I., Furuta, T., Uchikawa, R., Okumura, M., Otogoto, J., Kondo, E., Sogawa, N., Ciobica, A., & Tomida, M. (2020). Favorite Music Mediates Pain-related Responses in the Anterior Cingulate Cortex and Skin Pain Thresholds. *Journal of Pain Research*, *Volume 13*, 2729–2737. https://doi.org/10.2147/JPR.S276274

Atlas, L. Y., Whittington, R. A., Lindquist, M. A., Wielgosz, J., Sonty, N., & Wager, T. D. (2012). Dissociable Influences of Opiates and Expectations on Pain. *Journal of Neuroscience*, *32*(23), 8053–8064. https://doi.org/10.1523/JNEUROSCI.0383-12.2012

Baayen, R. H., Davidson, D. J., & Bates, D. M. (2008). Mixed-effects modeling with crossed random effects for subjects and items. *Journal of Memory and Language*, *59*(4), 390–412. https://doi.org/10.1016/j.jml.2007.12.005

Barr, D. J. (2013). Random effects structure for testing interactions in linear mixed-effects models. *Frontiers in Psychology*, *4*. https://doi.org/10.3389/fpsyg.2013.00328

Barr, R. G., Young, S. N., Wright, J. H., Cassidy, K.-L., & Hendricks, L. (1995). "Sucrose Analgesia" and Diphtheria-Tetanus-Petussis Immunizations at 2 and 4 months. *Developmental and Behavioral Pediatrics*, *16*(4), 220–225.

Berna, C., Leknes, S., Ahmad, A. H., Mhuircheartaigh, R. N., Goodwin, G. M., & Tracey, I. (2018). Opioid-Independent and Opioid-Mediated Modes of Pain Modulation. *The Journal of Neuroscience*, *38*(42), 9047–9058. https://doi.org/10.1523/JNEUROSCI.0854-18.2018

Berridge, K. C., & Robinson, T. E. (2003). Parsing reward. *Trends in Neurosciences*, *26*(9), 507–513. https://doi.org/10.1016/S0166-2236(03)00233-9

Bingel, U., Wanigasekera, V., Wiech, K., Ni Mhuircheartaigh, R., Lee, M. C., Ploner, M., & Tracey, I. (2011). The Effect of Treatment Expectation on Drug Efficacy: Imaging the Analgesic Benefit of the Opioid Remifentanil. *Science Translational Medicine*, *3*(70), 70ra14-70ra14. https://doi.org/10.1126/scitranslmed.3001244

Blass, E., Fitzgerald, E., & Kehoe, P. (1987). Interactions between sucrose, pain and isolation distress. *Pharmacology Biochemistry and Behavior*, *26*(3), 483–489. https://doi.org/10.1016/0091-3057(87)90153-5

Blood, A. J., & Zatorre, R. J. (2001). Intensely pleasurable responses to music correlate with activity in brain regions implicated in reward and emotion. *Proceedings of the National Academy of Sciences*, *98*(20), 11818–11823. https://doi.org/10.1073/pnas.191355898

Bowling, D. L., Graf Ancochea, P., Hove, M. J., & Fitch, W. T. (2019). Pupillometry of Groove: Evidence for Noradrenergic Arousal in the Link Between Music and Movement. *Frontiers in Neuroscience*, *12*, 1039. https://doi.org/10.3389/fnins.2018.01039

Bradley, M. M., & Lang, P. J. (1994). Measuring emotion: The self-assessment manikin and the semantic differential. *Journal of Behavior Therapy and Experimental Psychiatry*, *25*(1), 49–59. https://doi.org/10.1016/0005-7916(94)90063-9

Bruehl, S., Carlson, C. R., Wilson, J. F., Norton, J. A., Colclough, G., Brady, M. J., Sherman, J. J., & McCubbin, J. A. (1996). Psychological coping with acute pain: An examination of the role of endogenous opioid mechanisms. *Journal of Behavioral Medicine*, *19*(2), 129–142. https://doi.org/10.1007/BF01857603

Carlino, E., & Benedetti, F. (2016). Different contexts, different pains, different experiences. *Neuroscience*, *338*, 19–26. https://doi.org/10.1016/j.neuroscience.2016.01.053

Choi, S., Park, S.-G., & Lee, H.-H. (2018). The analgesic effect of music on cold pressor pain

responses: The influence of anxiety and attitude toward pain. *PLOS ONE*, *13*(8), e0201897. https://doi.org/10.1371/journal.pone.0201897

Cohen, J. (1988). *Statistical power analysis for the behavioral sciences* (2nd ed., pp. XXI, 567). Laurence Erlbaum.

Cole, L. C., & LoBiondo-Wood, G. (2012). *Music as an Adjuvant Therapy in Control of Pain and Symptoms in Hospitalized Adults: A Systematic Review*. 20.

Colloca, L. (2019). The Placebo Effect in Pain Therapies. *Annual Review of Pharmacology and Toxicology*, *59*(1), 191–211. https://doi.org/10.1146/annurev-pharmtox-010818-021542

Colloca, L., & Benedetti, F. (2006). How prior experience shapes placebo analgesia. *Pain*, *124*(1), 126–133. https://doi.org/10.1016/j.pain.2006.04.005

Colwell, C. M. (1997). Music as Distraction and Relaxation to Reduce Chronic Pain and Narcotic Ingestion: A Case Study. *Music Therapy Perspectives*, *15*(1), 24–31. https://doi.org/10.1093/mtp/15.1.24

Connelly, M. A., Brown, J. T., Kearns, G. L., Anderson, R. A., St Peter, S. D., & Neville, K. A. (2014). Pupillometry: A non-invasive technique for pain assessment in paediatric patients. *Archives of Disease in Childhood*, *99*(12), 1125–1131. https://doi.org/10.1136/archdischild-2014-306286

Cook, D. B., O'Connor, P. J., & Ray, C. A. (2000). Muscle pain perception and sympathetic nerve activity to exercise during opioid modulation. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *279*(5), R1565–R1573. https://doi.org/10.1152/ajpregu.2000.279.5.R1565

Dubé, L., & Bel, J. L. (2003). *The content and structure of laypeople's concept of pleasure*. 35.

Dum, J., & Herz, A. (1984). Endorphinergic modulation of neural reward systems indicated by behavioral changes. *Pharmacology Biochemistry and Behavior*, *21*(2), 259–266. https://doi.org/10.1016/0091-3057(84)90224-7

Faul, F., Erdfelder, E., Lang, A.-G., & Buchner, A. (2007). *G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences*. 17.

Ferreri, L., Mas-Herrero, E., Zatorre, R. J., Ripollés, P., Gomez-Andres, A., Alicart, H., Olivé, G., Marco-Pallarés, J., Antonijoan, R. M., Valle, M., Riba, J., & Rodriguez-Fornells, A. (2019). Dopamine modulates the reward experiences elicited by music. *Proceedings of the National Academy of Sciences*, *116*(9), 3793–3798.

https://doi.org/10.1073/pnas.1811878116

Forsberg, G., Wiesenfeld-Hallin, Z., Eneroth, P., & Södersten, P. (1987). Sexual behavior induces naloxone-reversible hypoalgesia in male rats. *Neuroscience Letters*, *81*(1–2), 151–154. https://doi.org/10.1016/0304-3940(87)90356-9

France, C. R., al'Absi, M., Ring, C., France, J. L., Harju, A., & Wittmers, L. E. (2007). Nociceptive flexion reflex and pain rating responses during endogenous opiate blockade with naltrexone in healthy young adults. *Biological Psychology*, *75*(1), 95–100. https://doi.org/10.1016/j.biopsycho.2006.12.005

Frew, A. K., & Drummond, P. D. (2007). Negative affect, pain and sex: The role of endogenous opioids. *Pain*, *132*(Supplement 1), S77–S85. https://doi.org/10.1016/j.pain.2007.04.010

Frew, A. K., & Drummond, P. D. (2008). Stress-evoked opioid release inhibits pain in major depressive disorder. *Pain*, *139*(2), 284–292. https://doi.org/10.1016/j.pain.2008.04.022

Gardner, W. J., Licklider, J. C. R., & Weisz, A. Z. (1960). Suppression of Pain by Sound. *Science*, *132*(3418), 32–33.

Garza Villarreal, E. A., Brattico, E., Vase, L., Østergaard, L., & Vuust, P. (2012). Superior Analgesic Effect of an Active Distraction versus Pleasant Unfamiliar Sounds and Music: The Influence of Emotion and Cognitive Style. *PLoS ONE*, *7*(1), e29397. https://doi.org/10.1371/journal.pone.0029397

Garza-Villarreal, E. A., Pando, V., Vuust, P., & Parsons, C. (2017). Music-Induced Analgesia in Chronic Pain Conditions: A Systematic Review and Meta- Analysis. *Pain Physician*, 14.

Garza-Villarreal, E. A., Wilson, A. D., Vase, L., Brattico, E., Barrios, F. A., Jensen, T. S., Romero-Romo, J. I., & Vuust, P. (2014). Music reduces pain and increases functional mobility in fibromyalgia. *Frontiers in Psychology*, *5*. https://doi.org/10.3389/fpsyg.2014.00090

Gebauer, L., Kringelbach, M. L., & Vuust, P. (2012). Ever-changing cycles of musical pleasure: The role of dopamine and anticipation. *Psychomusicology: Music, Mind, and Brain*, *22*(2), 152–167. https://doi.org/10.1037/a0031126

Georgiadis, J. R., Kringelbach, M. L., & Pfaus, J. G. (2012). Sex for fun: A synthesis of human and animal neurobiology. *Nature Reviews Urology*, *9*(9), 486–498. https://doi.org/10.1038/nrurol.2012.151

Gingras, B., Marin, M. M., Puig-Waldmüller, E., & Fitch, W. T. (2015). The Eye is Listening:

Music-Induced Arousal and Individual Differences Predict Pupillary Responses. *Frontiers in Human Neuroscience*, *9*. https://doi.org/10.3389/fnhum.2015.00619

Grevert, P., Albert, L. H., & Goldstein, A. (1983). Partial antagonism of placebo analgesia by naloxone: *Pain*, *16*(2), 129–143. https://doi.org/10.1016/0304-3959(83)90203-8

Gueorguieva, R., & Krystal, J. H. (2004). Move Over ANOVA: Progress in Analyzing Repeated-Measures Data andIts Reflection in Papers Published in the *Archives of General Psychiatry*. *Archives of General Psychiatry*, *61*(3), 310. https://doi.org/10.1001/archpsyc.61.3.310

Guétin, S., Giniès, P., Siou, D. K. A., Picot, M.-C., Pommié, C., Guldner, E., Gosp, A.-M., Ostyn, K., Coudeyre, E., & Touchon, J. (2012). The Effects of Music Intervention in the Management of Chronic Pain: A Single-Blind, Randomized, Controlled Trial. *The Clinical Journal oF Pain*, *28*(4), 329–337. https://doi.org/10.1097/AJP.0b013e31822be973

Guo, J.-Y., Wang, J.-Y., & Luo, F. (2010). Dissection of placebo analgesia in mice: The conditions for activation of opioid and non-opioid systems. *Journal of Psychopharmacology*, *24*(10), 1561–1567. https://doi.org/10.1177/0269881109104848

Gutgsell, K. J., Schluchter, M., Margevicius, S., DeGolia, P. A., McLaughlin, B., Harris, M., Mecklenburg, J., & Wiencek, C. (2013). Music Therapy Reduces Pain in Palliative Care Patients: A Randomized Controlled Trial. *Journal of Pain and Symptom Management*, *45*(5), 822–831. https://doi.org/10.1016/j.jpainsymman.2012.05.008

Hekmat, H. M., & Hertel, J. B. (1993). Pain Attenuating Effects of Preferred Versus Non-preferred Music Interventions. *Psychology of Music*, *21*(2), 163–173. https://doi.org/10.1177/030573569302100205

Hintze, J. L. (2007). Mixed Models—General. In *NCSS Statistical System* (p. 82). NCSS, LCC.

Hole, J., Hirsch, M., Ball, E., & Meads, C. (2015). *Music as an aid for postoperative recovery in adults: A systematic review and meta-analysis*. *386*, 13.

Hongisto, V., Oliva, D., & Rekola, L. (2015). Subjective and objective rating of spectrally different pseudorandom noises—Implications for speech masking design. *The Journal of the Acoustical Society of America*, *137*(3), 1344–1355. https://doi.org/10.1121/1.4913273

Hsieh, C., Kong, J., Kirsch, I., Edwards, R. R., Jensen, K. B., Kaptchuk, T. J., & Gollub, R. L. (2014). Well-Loved Music Robustly Relieves Pain: A Randomized, Controlled

Trial. *PLoS ONE*, *9*(9), e107390. https://doi.org/10.1371/journal.pone.0107390

IFPI. (2019). *Music Listening 2019*. IFPI.

Irusta, A. E. C., Savoldi, M., Kishi, R., Resende, G. C. C., Freitas, R. L., Carvalho, A. D., & Coimbra, N. C. (2001). Psychopharmacological evidences for the involvement of muscarinic and nicotinic cholinergic receptors on sweet substance-induced analgesia in Rattus norvegicus. *Neuroscience Letters*, *305*(2), 115–118. https://doi.org/10.1016/S0304-3940(01)01824-9

Kakeda, T., Ogino, Y., Moriya, F., & Saito, S. (2010). Sweet taste-induced analgesia: An fMRI study. *NeuroReport*, *21*(6), 427–431. https://doi.org/10.1097/WNR.0b013e3283383df5

Kenntner-Mabiala, R., & Pauli, P. (2005). Affective modulation of brain potentials to painful and nonpainful stimuli: Affective modulation of somatosensory-evoked potentials. *Psychophysiology*, *42*(5), 559–567. https://doi.org/10.1111/j.1469-8986.2005.00310.x

King, C. D., Goodin, B., Kindler, L. L., Caudle, R. M., Edwards, R. R., Gravenstein, N., Riley, J. L., & Fillingim, R. B. (2013). Reduction of conditioned pain modulation in humans by naltrexone: An exploratory study of the effects of pain catastrophizing. *Journal of Behavioral Medicine*, *36*(3), 315–327. https://doi.org/10.1007/s10865-012-9424-2

Knox, D., Beveridge, S., Mitchell, L. A., & MacDonald, R. A. R. (2011). *Acoustic analysis and mood classification of pain-relieving music*. 11.

Koepp, M., Hammers, A., Lawrence, A., Asselin, M., Grasby, P., & Bench, C. (2009). Evidence for endogenous opioid release in the amygdala during positive emotion. *NeuroImage*, *44*(1), 252–256. https://doi.org/10.1016/j.neuroimage.2008.08.032

Koltyn, K. F., Brellenthin, A. G., Cook, D. B., Sehgal, N., & Hillard, C. (2014). Mechanisms of Exercise-Induced Hypoalgesia. *JOURNAL OF PAIN*.

Korhan, E. A., Uyar, M., Eyigör, C., Hakverdioğlu Yönt, G., Çelik, S., & Khorshıd, L. (2014). The Effects of Music Therapy on Pain in Patients with Neuropathic Pain. *Pain Management Nursing*, *15*(1), 306–314. https://doi.org/10.1016/j.pmn.2012.10.006

Kringelbach, M. L., Stein, A., & van Hartevelt, T. J. (2012). *The functional human neuroanatomy of food pleasure cycles*. 10.

Krishnaswamy, P., & Nair, S. (2016). Effect of Music Therapy on Pain and Anxiety Levels of Cancer Patients: A Pilot Study. *Indian J Palliat Care*, *22(3)*, 307–311. https://doi.org/10.4103/0973-1075.185042

Kristjánsdóttir, Ó., & Kristjánsdóttir, G. (2011). Randomized clinical trial of musical

distraction with and without headphones for adolescents' immunization pain: Trial of musical distraction with(out) headphones. *Scandinavian Journal of Caring Sciences*, *25*(1), 19–26. https://doi.org/10.1111/j.1471-6712.2010.00784.x

Kühlmann, A. Y. R., de Rooij, A., Kroese, L. F., van Dijk, M., Hunink, M. G. M., & Jeekel, J. (2018). Meta-analysis evaluating music interventions for anxiety and pain in surgery: Evidence-based music in surgery. *British Journal of Surgery*, *105*(7), 773–783. https://doi.org/10.1002/bjs.10853

Kut, E., Candia, V., von Overbeck, J., Pok, J., Fink, D., & Folkers, G. (2011). Pleasure-Related Analgesia Activates Opioid-Insensitive Circuits. *Journal of Neuroscience*, *31*(11), 4148–4153. https://doi.org/10.1523/JNEUROSCI.3736-10.2011

Kwekkeboom, K. L. (2003). Music Versus Distraction for Procedural Pain and Anxiety in Patients With Cancer. *Oncology Nursing Forum*, *30*(3), 433–440. https://doi.org/10.1188/03.ONF.433-440

Laeng, B., Eidet, L. M., Sulutvedt, U., & Panksepp, J. (2016). Music chills: The eye pupil as a mirror to music's soul. *Consciousness and Cognition*, 18.

Laeng, B., Garvija, L., Løseth, G., Eikemo, M., Ernst, G., & Leknes, S. (2021). 'Defrosting' music chills with naltrexone: The role of endogenous opioids for the intensity of musical pleasure. *Consciousness and Cognition*, *90*, 103105. https://doi.org/10.1016/j.concog.2021.103105

Lakens, D. (2020). *Effect Sizes and Power for Interactions in ANOVA Designs*. http://daniellakens.blogspot.com/2020/03/effect-sizes-and-power-for-interactions.html

Lakens, D. (2021). *Sample Size Justification* [Preprint]. PsyArXiv. https://doi.org/10.31234/osf.io/9d3yf

Lee, M., & Wagenmakers, E. (2014). *Bayesian Cognitive Modeling: A Practical Course*. Cambridge: Cambridge University Press. doi:10.1017/CBO9781139087759

Lee, I.-S., Lee, B., Park, H.-J., Olausson, H., Enck, P., & Chae, Y. (2015). A new animal model of placebo analgesia: Involvement of the dopaminergic system in reward learning. *Scientific Reports*, *5*(1), 17140. https://doi.org/10.1038/srep17140

Leknes, S., & Tracey, I. (2008). A common neurobiology for pain and pleasure. *Nature Reviews Neuroscience*, *9*(4), 314–320. https://doi.org/10.1038/nrn2333

Leon, A. C., Davis, L. L., & Kraemer, H. C. (2011). The role and interpretation of pilot studies in clinical research. *Journal of Psychiatric Research*, *45*(5), 626–629. https://doi.org/10.1016/j.jpsychires.2010.10.008

Levine, J. D., Gordon, N. C., & Fields, H. L. (1978). The Mechanism of Placebo Analgesia. *Lancet*, *2*, 654–657.

Li, X.-M., Yan, H., Zhou, K.-N., Dang, S.-N., Wang, D.-L., & Zhang, Y.-P. (2011). Effects of music therapy on pain among female breast cancer patients after radical mastectomy: Results from a randomized controlled trial. *Breast Cancer Research and Treatment*, *128*(2), 411–419. https://doi.org/10.1007/s10549-011-1533-z

Liu, Y., & Petrini, M. A. (2015). Effects of music therapy on pain, anxiety, and vital signs in patients after thoracic surgery. *Complementary Therapies in Medicine*, *23*(5), 714–718. https://doi.org/10.1016/j.ctim.2015.08.002

Lu, X., Thompson, W. F., Zhang, L., & Hu, L. (2019). Music Reduces Pain Unpleasantness: Evidence from an EEG Study. *Journal of Pain Research*, *Volume 12*, 3331–3342. https://doi.org/10.2147/JPR.S212080

Lunde, S. J., Vuust, P., Garza-Villarreal, E. A., & Vase, L. (2018). Music-induced analgesia: How does music relieve pain? *PAIN*, *160*(5), 989–993. https://doi.org/10.1097/j.pain.0000000000001452

Martin-Saavedra, J. S., Vergara-Mendez, L. D., & Talero-Gutiérrez, C. (2018). Music is an effective intervention for the management of pain: An umbrella review. *Complementary Therapies in Clinical Practice*, *32*, 103–114. https://doi.org/10.1016/j.ctcp.2018.06.003

McNabb, C. T., White, M. M., Harris, A. L., & Fuchs, P. N. (2014). The elusive rat model of conditioned placebo analgesia. *Pain*, *155*(10), 2022–2032. https://doi.org/10.1016/j.pain.2014.07.004

Meagher, M. W., Arnau, R. C., & Rhudy, J. L. (2001). Pain and Emotion: Effects of Affective Picture Modulation: *Psychosomatic Medicine*, *63*(1), 79–90. https://doi.org/10.1097/00006842-200101000-00010

Menegazzi, J. J., Paris, P. M., Kersteen, C. H., Flynn, B., & Trautman, D. E. (1991). A randomized, controlled trial of the use of music during laceration repair. *Annals of Emergency Medicine*, *20*(4), 348–350. https://doi.org/10.1016/S0196-0644(05)81652-X

Miller, A., Barr, R. G., & Young, S. N. (1994). The cold pressor test in children: Methodological aspects and the analgesic effect of intraoral sucrose. *Pain*, *56*(2), 175–183. https://doi.org/10.1016/0304-3959(94)90092-2

Mischkowski, D., Palacios-Barrios, E. E., Banker, L., Dildine, T. C., & Atlas, L. Y. (2019). Pain or nociception? Subjective experience mediates the effects of acute noxious heat

on autonomic responses - corrected and republished: *PAIN*, *160*(6), 1469–1481. https://doi.org/10.1097/j.pain.0000000000001573

Mitchell, L. A., & MacDonald, R. A. R. (2006). An Experimental Investigation of the Effects of Preferred and Relaxing Music Listening on Pain Perception. *Journal of Music Therapy*, *4*, 22.

Miyase, C. I., Kishi, R., de Freitas, R. L., Paz, D. A., & Coimbra, N. C. (2005). Involvement of pre- and post-synaptic serotonergic receptors of dorsal raphe nucleus neural network in the control of the sweet-substance-induced analgesia in adult Rattus norvegicus (Rodentia, Muridae). *Neuroscience Letters*, *379*(3), 169–173. https://doi.org/10.1016/j.neulet.2004.12.058

Morosko, T. E., & Simmons, F. F. (1966). The Effect of Audio-Analgesia on Pain Threshold and Pain Tolerance. *Journal of Dental Research*, *45*(6), 1608–1617. https://doi.org/10.1177/00220345660450060701

Murthy, V. H. (2016). Ending the Opioid Epidemic—A Call to Action. *New England Journal of Medicine*, *375*(25), 2413–2415. https://doi.org/10.1056/NEJMp1612578

Nguyen, T. N., Nilsson, S., Hellström, A.-L., & Bengtson, A. (2010). Music Therapy to Reduce Pain and Anxiety in Children With Cancer Undergoing Lumbar Puncture: A Randomized Clinical Trial. *Journal of Pediatric Oncology Nursing*, *27*(3), 146–155. https://doi.org/10.1177/1043454209355983

Nolan, T. A., Price, D. D., Caudle, R. M., Murphy, N. P., & Neubert, J. K. (2012). Placebo-induced analgesia in an operant pain model in rats. *Pain*, *153*(10), 2009–2016. https://doi.org/10.1016/j.pain.2012.04.026

Nummenmaa, L., Saanijoki, T., Tuominen, L., Hirvonen, J., Tuulari, J. J., Nuutila, P., & Kalliokoski, K. (2018). μ-opioid receptor system mediates reward processing in humans. *Nature Communications*, *9*(1), 1500. https://doi.org/10.1038/s41467-018-03848-y

Nummenmaa, L., & Tuominen, L. (2018). Opioid system and human emotions. *British Journal of Pharmacology*, *175*(14), 2737–2749. https://doi.org/10.1111/bph.13812

Pecina, S. (2005). Hedonic Hot Spot in Nucleus Accumbens Shell: Where Do -Opioids Cause Increased Hedonic Impact of Sweetness? *Journal of Neuroscience*, *25*(50), 11777–11786. https://doi.org/10.1523/JNEUROSCI.2329-05.2005

Peciña, S., & Berridge, K. C. (2000). Opioid site in nucleus accumbens shell mediates eating and hedonic 'liking' for food: Map based on microinjection Fos plumes. *Brain Research*, *863*(1–2), 71–86. https://doi.org/10.1016/S0006-8993(00)02102-8

Pepino, Y. M., & Mennella, J. A. (2005). Sucrose-induced analgesia is related to sweet preferences in children but not adults. *Pain*, *119*(1–3), 210–218. https://doi.org/10.1016/j.pain.2005.09.029

Perlini, A. H., & Viita, K. A. (1996). Audioanalgesia in the control of experimental pain. *Canadian Journal of Behavioural Science / Revue Canadienne Des Sciences Du Comportement*, *28*(4), 292–301. https://doi.org/10.1037/0008-400X.28.4.292

Petrovic, P., Kalso, E., Petersson, K. M., Andersson, J., Fransson, P., & Ingvar, M. (2010). A prefrontal non-opioid mechanism in placebo analgesia. *Pain*, *150*(1), 59–65. https://doi.org/10.1016/j.pain.2010.03.011

Pfeiffer, A., Pasi, A., Mehraein, P., & Herz, A. (1982). Opiate receptor binding sites in human brain. *Brain Research*, *248*(1), 87–96. https://doi.org/10.1016/0006-8993(82)91150-7

Pontén, M., Fust, J., Kosek, E., Guterstam, J., & Jensen, K. (2020). Naltrexone during pain conditioning: A double-blind placebo-controlled experimental trial. *Molecular Pain*, *16*, 174480692092762. https://doi.org/10.1177/1744806920927625

Price, D. D., Milling, L. S., Kirsch, I., Duff, A., Montgomery, G. H., & Nicholls, S. S. (1999). An analysis of factors that contribute to the magnitude of placebo analgesia in an experimental paradigm: *Pain*, *83*(2), 147–156. https://doi.org/10.1016/S0304-3959(99)00081-0

R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rebouças, E. C. C., Segato, E. N., Kishi, R., Freitas, R. L., Savoldi, M., Morato, S., & Coimbra, N. C. (2005). Effect of the blockade of ?1-opioid and 5HT2A-serotonergic/?1-noradrenergic receptors on sweet-substance-induced analgesia. *Psychopharmacology*, *179*(2), 349–355. https://doi.org/10.1007/s00213-004-2045-x

Roy, M., Lebuis, A., Hugueville, L., Peretz, I., & Rainville, P. (2012). Spinal modulation of nociception by music: Spinal modulation of nociception by music. *European Journal of Pain*, *16*(6), 870–877. https://doi.org/10.1002/j.1532-2149.2011.00030.x

Roy, Mathieu, Peretz, I., & Rainville, P. (2008). Emotional valence contributes to music-induced analgesia: *Pain*, *134*(1), 140–147. https://doi.org/10.1016/j.pain.2007.04.003

Saarikallio, S. (2010). Music as emotional self-regulation throughout adulthood. *Psychology of Music*, 21.

Schober, P., Boer, C., & Schwarte, L. A. (2018). Correlation Coefficients: Appropriate Use

and Interpretation. *Anesthesia & Analgesia*, *126*(5), 1763–1768. https://doi.org/10.1213/ANE.0000000000002864

Scott, D. J., Stohler, C. S., Egnatuk, C. M., Wang, H., Koeppe, R. A., & Zubieta, J.-K. (2007). Individual Differences in Reward Responding Explain Placebo-Induced Expectations and Effects. *Neuron*, *55*(2), 325–336. https://doi.org/10.1016/j.neuron.2007.06.028

Shabanloei, R., Golchin, M., Esfahani, A., Dolatkhah, R., & Rasoulian, M. (2010). Effects of Music Therapy on Pain and Anxiety in Patients Undergoing Bone Marrow Biopsy and Aspiration. *AORN Journal*, *91*(6), 746–751. https://doi.org/10.1016/j.aorn.2010.04.001

Silva, M. J., & Kelly, Z. (2020). The Escalation of the Opioid Epidemic Due to COVID-19 and Resulting Lessons About Treatment Alternatives. *The American Journal of Managed Care*, *26*(7), e202–e204. https://doi.org/10.37765/ajmc.2020.43386

Sirois, S., & Brisson, J. (2014). Pupillometry: Pupillometry. *Wiley Interdisciplinary Reviews: Cognitive Science*, *5*(6), 679–692. https://doi.org/10.1002/wcs.1323

Sloboda, J., & O'Neill, S. (2011). Emotion in everyday listening to music. *Oxford University Press*, 415–430.

Sprenger, T., Berthele, A., Platzer, S., Boecker, H., & Tölle, T. R. (2005). What to learn from in vivo opioidergic brain imaging? *European Journal of Pain*, *9*(2), 117–121. https://doi.org/10.1016/j.ejpain.2004.07.010

Szechtman, H., Hershkowitz, M., & Simantov, R. (1980). *SEXUAL BEHAVIOR DECREASES PAIN SENSITIVITY AND STIMULATES ENDOGENOUS OPIOIDS IN MALE RATS*. 7.

Taneja, P., Olausson, H., Trulsson, M., Vase, L., Svensson, P., & Baad‐Hansen, L. (2020). Modulation of experimental facial pain via somatosensory stimuli targeting sensations of different valence. *Journal of Oral Rehabilitation*, *47*(6), 720–730. https://doi.org/10.1111/joor.12963

Tarr, B., Launay, J., Benson, C., & Dunbar, R. I. M. (2017). 'Naltrexone Blocks Endorphins Released when Dancing in Synchrony.' *Adaptive Human Behavior and Physiology*, *3*(3), 241–254. https://doi.org/10.1007/s40750-017-0067-y

The jamovi project (2021). *jamovi* (Version 1.6) [Computer Software]. Retrieved from https://www.jamovi.org

Tracey, I. (2010). Getting the pain you expect: Mechanisms of placebo, nocebo and reappraisal effects in humans. *Nature Medicine*, *16*(11), 1277–1283. https://doi.org/10.1038/nm.2229

Vassie, K., & Richardson, M. (2017). Effect of self-adjustable masking noise on open-plan office worker's concentration, task performance and attitudes. *Applied Acoustics*, *119*, 119–127. https://doi.org/10.1016/j.apacoust.2016.12.011

Wickham et al., (2019). Welcome to the tidyverse. Journal of Open Source Software, 4(43), 1686, https://doi.org/10.21105/joss.01686

Younger, J., Aron, A., Parke, S., Chatterjee, N., & Mackey, S. (2010). Viewing Pictures of a Romantic Partner Reduces Experimental Pain: Involvement of Neural Reward Systems. *PLoS ONE*, *5*(10), e13309. https://doi.org/10.1371/journal.pone.0013309

Younger, J. W., Zautra, A. J., & Cummins, E. T. (2009). Effects of Naltrexone on Pain Sensitivity and Mood in Fibromyalgia: No Evidence for Endogenous Opioid Pathophysiology. *PLoS ONE*, *4*(4), e5180. https://doi.org/10.1371/journal.pone.0005180

Zengin, S., Kabul, S., Al, B., Sarcan, E., Doğan, M., & Yildirim, C. (2013). Effects of music therapy on pain and anxiety in patients undergoing port catheter placement procedure. *Complementary Therapies in Medicine*, *21*(6), 689–696. https://doi.org/10.1016/j.ctim.2013.08.017

Zubieta, J.-K. (2005). Placebo Effects Mediated by Endogenous Opioid Activity on -Opioid Receptors. *Journal of Neuroscience*, *25*(34), 7754–7762. https://doi.org/10.1523/JNEUROSCI.0439-05.2005

Appendix A. Supplemental digital content

Supplemental digital content associated with this article can be found online at https://osf.io/dwyub/?view_only=15d72518292f460197d3d67f20258ca3.