COLORADO MULTIPLE INSTITUTIONAL REVIEW BOARD
CAMPUS BOX F-490 TELEPHONE: 303-724-1055 Fax: 303-724-0990

Protocol #: 21-2886
Project Title: Opioid Modulation and Neural Reward Activation in Healthy Adults
Principal Investigator: Dr. Joshua Gowin
Version Date: 10/28/21

I. **Hypotheses and Specific Aims**:

1. **Evaluate whether mu-opioid receptor blockade via naltrexone alters reward activation in the ventral striatum.** In a study of *10 healthy, moderate drinking* adults, we will use a crossover design to examine brain activation during a functional MRI scan during placebo and mu-opioid blockade.

Hypothesis 1. Relative to the placebo scan, participants will show reduced reward-related activation in the ventral striatum during the active medication scan.

2. **Evaluate whether mu-opioid receptor blockade alters threat activation in the amygdala.** In the same sample and design from Aim 1, we will use the Emotion Regulation Task to assess threat related activation in the amygdala during a functional MRI scan.

Hypothesis 2. Opioid receptor modulation will not affect threat related activation, consistent with naltrexone primarily targeting reward motivation.

3. **Examine whether mu-opioid receptor blockade via naltrexone alters valuation of alcohol.** In the same sample and study design from Aim 1, we will use the Alcohol Purchase Task to assess subjective value of an alcoholic beverage in order to link opioid-modulation directly to alcohol motivation.

Hypothesis 3. Relative to the placebo session, participants will be willing to pay less money for an alcoholic beverage during the active medication session.

This proposal aims to fill a gap in understanding how endogenous opioid function affects reward response in the brain. One of the leading hypotheses is that individuals who drink to maximize reward are more likely to benefit from naltrexone treatment, and the current proposal would provide evidence for this in a healthy sample. Further, this work could lead to a clinical study of reward-related brain activation and response to naltrexone treatment in adults with an Alcohol Use Disorder. This stands to improve precision medicine approaches to tailor treatments for alcohol use disorder.

II. **Background and Significance**:

Reward processing contributes to survival for all animals, including humans, since identifying sources of sustenance is among the most important information learned during life (Berridge and Kringelbach 2008). Dysfunctional reward processing underlies many psychiatric disorders, such as depression (Ng, Alloy, and Smith 2019) and substance use disorders (Hyman, Malenka, and Nestler 2006), thereby reducing quality of life and leading to premature death. A critical brain structure in the reward circuit is the nucleus accumbens. Electrophysiological work in primates shows that nucleus accumbens activation occurs when an animal sees a cue that predicts the delivery of a favorite food (Schultz, Dayan, and Montague 1997) and functional MRI work in humans shows that activation occurs when humans see cues that predict the arrival of money (Knutson et al. 2001). While dopamine neurons that originate in the ventral tegmental area and terminate in the nucleus accumbens account for a major part of reward signaling (Adamantidis et al. 2011), other neurotransmitter systems impinge on these neurons (Heilig et al. 2011). In ongoing functional MRI work, we have shown that we can elicit nucleus accumbens activation using monetary reward in healthy adults. The nucleus accumbens has a dense population of mu-opioid

receptors (Nummenmaa et al. 2018), but their role in human reward processing is poorly understood. Determining how modulation of mu-opioid receptor activity affects reward response in the nucleus accumbens would improve our understanding of reward circuitry and its effects on mental health.

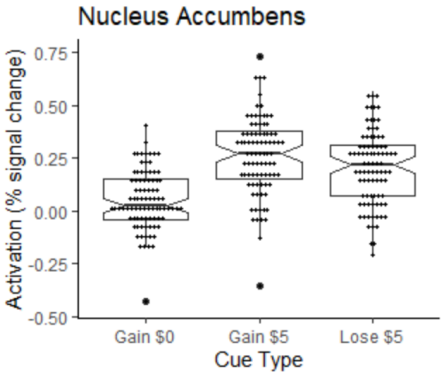
Neurotransmitter systems that interact with dopamine to encode reward are poorly understood. Animal studies show that mu-opioid antagonism blunts dopamine and ventral striatal activity (Walters et al. 2005). However, this has not been studied in humans. Psychopathology, such as substance use and mood disorders, are associated with altered nucleus accumbens activation to reward (Treadway and Zald 2013; Morales et al. 2018) and lower dopamine receptor concentration (Volkow et al. 1993). The treatment of such psychopathology is timely and important given the impact of the coronavirus (COVID-19) on increased incidence of depression (Salari et al. 2020) and substance use problems (Ahmed et al. 2020). Alcohol use remains one of the leading causes of death globally (Rehm et al. 2009), accounting for 88,000 annual deaths in the United States alone (Centers for Disease Control and Prevention 2004). The opioid system is involved in emotion (Nummenmaa and Tuominen 2018) and the mu-opioid receptor is involved in reward processing (Nummenmaa et al. 2018) but little is known about how this relates to psychopathology such as substance and alcohol use disorders.

This will be among the first investigations of how mu-opioid antagonism via naltrexone affects human monetary reward response. One previous study examined the effect of naltrexone on monetary reward response (Nestor et al. 2017), but this study used a monetary reward of less than $1. Previous studies have shown that rewards of less than $1 induce low levels of nucleus accumbens activation (Knutson et al. 2001). Further, the previous study administered only a single dose of naltrexone instead of using a steady-state during imaging (Nestor et al. 2017), so medication effects may have been absent for some participants. The study also used multiple study sites and scanners, which can increase variance. We will use a single site, a single scanner, a steady state of naltrexone during scanning, and a larger reward value of $5 that has been shown to induce large activation levels in the nucleus accumbens (Figure 2).

Our goal is to better understand the neurobiology of reward processing by probing other neurotransmitter systems that interact with dopamine to regulate reward. The objective of this application is to establish whether healthy adults show reduced reward-related activation in the nucleus accumbens when ramped up to steady state dosing with naltrexone. Naltrexone is a mu-opioid antagonist and one of three medications approved by the Food and Drug Administration for the treatment of alcohol use disorder. We will also examine threat processing as a control paradigm to establish that opioid modulation primarily affects reward processing. Since diminishing reward signaling may reduce incentive to seek alcohol, we will also examine a measure of subjective alcohol evaluation. We are prepared to conduct this study based on our expertise in psychopharmacology and functional MRI studies of reward and threat processing as well as psychiatric disorders. This qualified group of investigators will make certain that our research adheres to both neuroscientific theory of reward processing as well as clinical application.

III. **Preliminary Studies/Progress Report:**

In a currently ongoing study, we have recruited 45 adults to complete the monetary incentive delay task and we have shown a strong main effect of cue type in nucleus accumbens activation ($F_{2,210} = 58.8$, $p < 0.001$), where activation is greatest during cues to gain $5 (Figure 1).



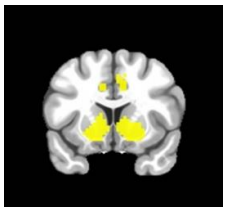


Figure 1. Using the monetary incentive delay task, we found a main effect of cue on nucleus accumbens activation ($F_{2,210} = 58.8$, $p < 0.001$).

IV. Research Methods

A. Outcome Measure(s): There are three outcomes for this study: nucleus accumbens activation to monetary reward, amygdala activation to threat, and subjective alcohol evaluation. For nucleus accumbens activation, we will use percent signal change in response to the cue to earn $5 during the Monetary Incentive Delay task. For the amygdala response to threat, we will use the percent signal change in response to negative images in the Emotion Regulation task. For the valuation of alcohol, we will use the Alcohol Purchase Task to assess the greatest expenditure on drinks (price paid multiplied by number of drinks).

B. Description of Population to be Enrolled:

a. Recruitment, sample description and inclusion/exclusion criteria. Participants will be recruited through advertisements on social media, paper flyering, advertising on local list-servs, and by peer recruitment. Our lab has successfully attracted over 340 participants to complete our screening measures using these methods in the past year. Interested individuals can contact the laboratory via email or phone call to arrange a phone screen, where we will assess likely eligibility for the study.

i. Inclusion criteria: A) Between 18 and 35 years of age,

ii. Exclusion criteria: A) AUDIT Score less than or equal to 14, B) Non-drinker C) positive result on urine drug screen or breathalyzer at the start of any study visit, D) inability to complete MRI (e.g. presence of ferromagnetic objects in body), E) current use of medications that alter the hemodynamic response, such as insulin, F) history of trauma resulting in loss of consciousness longer than 15 minutes, G) for females, pregnancy, H) current (within the last 3 months) use of opioids to avoid acute precipitated withdrawal due to opioid receptor antagonism I) treatment for a DSM5 disorder other than anxiety or depression through therapy J) treatment for a DSM5 disorder through medications, K) greater than 10 uses of illicit substances in the last year.

b. Feasibility: We have recruited 45 participants to our laboratory's studies in the past year and conducted 45 MRI scans. To recruit ~10 participants in one year, we need to recruit 1 participant per month and conduct 2 scans per month. This is well within the capacity of our laboratory operations.

C. Study Design and Research Methods. After completing an informed consent procedure, participants will sign a consent form. They will then complete a psychiatric interview and receive 5 capsules. Participants will return five days later for visit 2. On visit 2, they will complete a medication side-effect questionnaire. If they report side effects that are more

than mild (i.e. if they report moderate or severe side effects), a medical professional (i.e. nurse or physician) will follow up to confirm that they are ok to complete the MRI and provide care as needed. All participants will complete a breathalyzer and saliva drug test to confirm sobriety. Females will be asked to provide a urine sample for a pregnancy test. Participants will then complete an MRI and the Alcohol Purchase Task. They will be sent home with 5 days' worth of capsules. They will return after 5 days and repeat the same procedures.

a. Dosing. We will administer doses of naltrexone and placebo. Dosing will be double-blind and counter balanced. Participants will receive 5 capsules on their first visit to take during the week. They will receive 5 additional capsules to take each day of the following week. Naltrexone will be over-encapsulated by the Investigational Drug Service at the School of Pharmacy. Matching placebo capsules will contain methylcellulose power. Naltrexone will be taken once daily by mouth and ramped up by the following schedule: Monday-25mg, Tuesday-25mg, Wednesday-50mg, Thursday-50mg, Friday-50mg. A study coordinator blind to the contents of the capsules will deliver the capsules to the participant. The Investigational Drug Service will maintain blinding and will track dosage, to be revealed following study completion. Half of participants will receive placebo during week 1 and half will receive placebo during week 2. Remote video monitoring will also be used to assess adherence. Participants will receive a text message on their mobile phones at 9 am each morning reminding them to take the medication and instructing them to upload a video of themselves doing so to a secure data repository (REDCap). A study-provided mobile phone will be available for participants who do not own a mobile phone or who are not willing to use their phone for this purpose (i.e., due to concerns about mobile data cost). The digital timestamps of these videos will subsequently be extracted, and their contents reviewed, to verify that the participant ingested the medication at the instructed time. Other studies of non-treatment-seeking individuals have reported success with remote video monitoring (DeWorsop et al. 2016).

b. MRI. We will conduct a Magnetic Resonance Scan. This will be completed at the Brain Imaging Center (BIC) at the University of Colorado Anschutz Medical Campus. Scanning will be conducted on a Siemens Skyra 3.0 T MR system that is equipped with high performance gradient coils (45mT/m @ 200 T/m/s), a head volume RF coil, and a 32-channel phased-array RF neurovascular coil. The system has a 70 cm bore opening and a weight capacity of 550 lbs. This will be a head-first, supine scan of the brain. Each scan will last one hour. A fMRI run sensitive to blood-oxygenation level-dependent (BOLD) contrast will be collected using T2*-weighted parameters. For anatomical reference, a high-resolution, magnetization prepared rapid gradient echo (MPRAGE) T1-weighted image will be collected during the same session.

i. **Monetary Incentive Delay task:** The monetary incentive delay task (MID) was developed at the National Institute on Alcohol Abuse and Alcoholism (Knutson et al. 2001). The task has reliably shown that cues signaling a chance to win money recruit activation in the nucleus accumbens, and larger potential gains induce greater activity (Knutson et al. 2001). The task is comprised of three levels of monetary reward (-$5, +$0, +$5). This task reliably recruits nucleus accumbens activation and has a large effect size, with a Cohen's d > 2.0. In the MID task, visual stimuli, such as circles, squares, and triangles, are utilized as incentive cues that code the probabilities and magnitudes of outcomes. The first box shows the cue types presented, with circles indicating the potential to win money (gain cue), triangles indicating the potential to lose money (loss cue),

and a square indicating no money will be won or lost (neutral cue). A cue is presented for 500 ms, followed by a fixation cross (2,000 to 2,500 ms) and then the target square (160 to 260 ms), during which the participant is instructed to press a button as quickly as possible to win or avoid losing money. A feedback screen (1,650 ms), in which the top number indicated the amount of money won or lost during that trial and the bottom number indicates the participant's total amount, is presented at the end of each trial.

ii. **Emotion Regulation task:** In the emotion regulation task (Waugh et al. 2016), we will examine subjects' ability to change how they are feeling by changing the way they think. Participants will be shown a series of pictures from the International Affective Picture System that have been validated and grouped by rating (e.g. typically induces negative affect) (Lang, Bradley, and Cuthbert 1997). Some pictures are designed to be neutral, such as pictures of nature scenes, and some are designed to evoke strong negative feelings, such as disgust or horror. Prior to viewing each picture, the participant will be instructed to either "Look" or "Decrease". When instructed to "Look", participants are told to keep their eyes on the picture and feel any feelings naturally without trying to change them. This instruction occurs prior to all 15 neutral pictures and prior to 15 of the negative pictures. When instructed to "Decrease" prior to the remaining 15 negative pictures, participants are told to keep their eyes on the picture and find an aspect of it that is not as bad as it seems at first. After viewing each image, participants will be asked how negative they feel. They will be shown a five-point Likert scale from one (not at all) to five (extremely negative), and they will record their response by pressing one of five buttons on a button box attached to their hand. Two sets of pictures will be available so that each participant will see a new set of images on each scan. Order of the sets will be counterbalanced across participants.

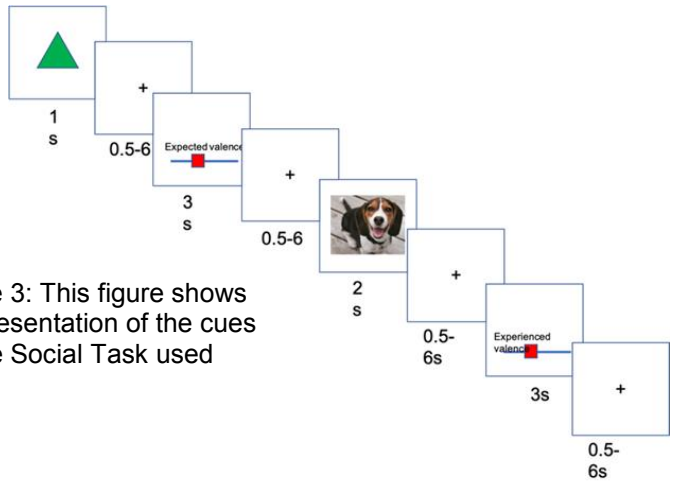


Figure 3: This figure shows the presentation of the cues for the Social Task used

iii. **Social Task:** The task, adapted from Jepma et al and Willroth et al, requires participants to rate their expected and experienced valence of varying types of images (Fig. 3). Three distinct geometric shapes acted as cues that predicted the category of image that the individual would subsequently view (erotic, non-erotic pleasant, or neutral). On presentation of the cue, the subject is asked to rate the expected valence of the image using a slider device on a scale of unpleasant to pleasant (coded as 0 to 100). After rating their expectation, they

are presented with the image and are required to rate the experienced valence of that image using the same slider device. Subjects rate expected and experienced valence of 90 unique images: 30 erotic images, 30 non-erotic pleasant images (e.g., scenes of nature, cute animals), and 30 neutral images (e.g., forks, furniture). The same images are presented to each participant, but participants are randomly assigned to one of four random orders. The task takes place over 2 runs of approximately 8 minutes each. Prior to starting the task, participants are given instructions that included 3 practice cue/image pairs from each category to introduce them to the task and the relationship between each cue and image category. All images were publicly available and/or from affective picture sets currently used in research (Lang et al. 2005)(Marchewka et al. 2014)(Kurdi et al. 2017).

c. The Structured Clinical Interview for the Diagnostic (SCID-5) and Statistical Manual for Mental Health Disorders 5, research version (First, Williams et al. 2015) will be administered to all participants by a psychiatrist or mental health professional (e.g. post-doc with clinical background). Diagnoses will be determined via a consensus meeting with the study psychiatrist, Dr. Penner. SCID-5 is a semi-structured interview guide for making diagnoses according to the diagnostic criteria published in the American Psychiatric Association's Diagnostic and Statistical Manual for Mental Disorders (DSM). With the release of the fifth edition (DSM-5), the SCID for DSM-5 (SCID-5) was published in 2013 and is the latest version available.

d. We will administer questionnaires using Research Electronic Data Capture (REDCap), as the University of Colorado is part of the REDCap consortium (Patridge and Bardyn 2018). These metrics will establish history of substance use, psychopathology such as anxiety and depression levels, and trait levels of cognitive reappraisal.

i. **Adult Self Report:** (ASR) is used to obtain measures of internalizing and externalizing pathology (Rescorla and Achenbach 2004).

ii. **Alcohol Use Disorders Identification Test** (Saunders et al. 1993): (AUDIT) is a 10-item screening tool developed by the World Health Organization (WHO) to assess alcohol consumption, drinking behaviors, and alcohol- related problems. This study uses a self-report version of the AUDIT. Patients should be encouraged to answer the AUDIT questions in terms of standard drinks. A chart illustrating the approximate number of standard drinks in different alcohol beverages is on the questionnaire for reference. A score of 8 or more is considered to indicate hazardous or harmful alcohol use. The AUDIT has been validated across genders and in a wide range of racial/ethnic groups and is well suited for use in primary care settings.

iii. **Substance Use Risk Profile Scale**: (SURPS) (Woicik, Stewart et al. 2009), measures hopelessness, anxiety sensitivity, impulsivity, and sensation-seeking, and has been shown to reliably predict alcohol use.

iv. **Beck Depression Inventory**: (BDI) is a 21-item, self-report rating inventory that measures characteristic attitudes and symptoms of depression (Beck, et al., 1961). The BDI has been developed in different forms, including the 13-item short form and the more recent BDI-II by Beck, Steer & Brown, 1996.Internal consistency for the BDI ranges from .73 to .92 with a mean of .86. (Beck, Steer, & Garbin, 1988). Similar reliabilities have been found for the 13-item short form (Groth-Marnat, 1990). The BDI demonstrates high internal consistency, with alpha

coefficients of .86 and .81 for psychiatric and non-psychiatric populations respectively (Beck et al., 1988).

v. **The Barratt Impulsiveness Scale:** BIS (Patton et al., 1995) is a questionnaire designed to assess the personality/behavioral construct of impulsiveness. It is the most widely cited instrument for the assessment of impulsiveness and has been used to advance our understanding of this construct and its relationship to other clinical phenomena for 50 years (for review see Stanford et al., 2009).

vi. **Customary Drinking and Drug Use Record** (Brown et al. 1998)**:** CDDR provides current (past 3 months) and lifetime measures of four alcohol- and other drug-related domains: level of involvement, withdrawal characteristics, psychological/behavioral dependence symptoms, and negative consequences.

vii. **COGA Family History Assessment Module** (Vogel-Sprott, Chipperfield, and Hart 1985)**:** (COGA FHAM) is semi-structured diagnostic instrument intended for clinicians and non-clinicians to assess major DSM-III-R psychiatric disorders among relatives of the participant. The following psychiatric disorders are ascertained: alcoholism, drug abuse/ dependence, depression, mania, schizophrenia, antisocial personality and unspecified psychiatric disorder.

viii. **Difficulties in Emotion Regulation Scale (DERS)** (Gratz and Roemer 2004)**:** A measure of subjective emotion ability, as defined by a prominent clinically derived model of emotion regulation. The model upon which it is based proposes four broad facets of emotion regulation: (a) awareness and understanding of emotions; (b) acceptance of emotions; (c) the ability to control impulses and behave in accordance with goals in the presence of negative affect; and (d) access to emotion regulation strategies that are perceived to be effective for feeling better.

ix. **Emotional Regulation Questionnaire:** The Emotion Regulation Questionnaire (Gross and John 2003) is a 10-question survey that assesses trait levels of suppression and reappraisal. Each question has a 7-point Likert-rated response (1 strongly disagree to 7 strongly agree). Emotion regulation assessed via questionnaire has high internal consistency (Cronbach's alpha = 0.80) (Gross and John 2003). The reappraisal scale consists of 6 items and the suppression scale consists of 4 items. An example of reappraisal is, "When I want to feel more positive emotion, I change the way I'm thinking about the situation." An example of suppression is, "I control my emotions by not expressing them."

x. **Friendship, Loneliness, Life Satisfaction, Perceived Rejection from the NIH Toolbox Emotion Battery** (Salsman et al. 2013): F, L, LS, & PR evaluate perceptions that one is alone, lonely, or socially isolated from others, perceptions of the availability of friends or companions with whom to interact or affiliate, One's cognitive evaluation of life experiences and whether one likes his/her life or not, and Perceptions of being ignored or others not listening or responding to requests for help.

xi. **State-Trait Anxiety Inventory**: (STAI) is a commonly used measure of trait and state anxiety (Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983). It can be used in clinical settings to diagnose anxiety and to distinguish it from depressive syndromes. It also is often used in research as an indicator of caregiver distress (e.g., Greene et al., 2017, Ugalde et al., 2014). Form Y, its most popular version, has 20 items for assessing

trait anxiety and 20 for state anxiety. State anxiety items include: “I am tense; I am worried” and “I feel calm; I feel secure.” Trait anxiety items include: “I worry too much over something that really doesn’t matter” and “I am content; I am a steady person.” All items are rated on a 4-point scale (e.g., from “Almost Never” to “Almost Always”). Higher scores indicate greater anxiety. The STAI is appropriate for those who have at least a sixth-grade reading level. Internal consistency coefficients for the scale have ranged from.86 to .95; test-retest reliability coefficients have ranged from .65 to .75 over a 2-month interval (Spielberger et al., 1983). Test-retest coefficients for this measure in the present study ranged from .69 to .89. Considerable evidence attests to the construct and concurrent validity of the scale (Spielberger, 1989).

xii. **Childhood Trauma Questionnaire**: (CTQ) contains 5 subscales, emotional abuse, physical abuse, sexual abuse, emotional neglect and physical neglect (Bernstein et al. 1998).

xiii. **Marlowe–Crowne Social Desirability Scale:** (MC–SDS) is a 33-item self-report questionnaire that assesses whether or not respondents are concerned with social approval. The scale was created by Douglas P. Crowne and David Marlowe in 1960 in an effort to measure social desirability bias, which is considered one of the most common biases affecting survey research (Marlow and Crowne 1961).

e. We will collect behavioral measures using a laptop during the study visit.

i. **Alcohol Purchase task (APT):** (Kaplan et al. 2018): After the scan, we will administer this task to determine the amount individuals are willing to pay for alcohol. In the alcohol purchase task, participants first read the following vignette: “Imagine that you and your friends are at a bar from 9 p.m. to 2 a.m. to see a band. The following questions ask how many drinks you would purchase at various prices. The available drinks are standard size beer (12 oz.), wine (5 oz.), shots of hard liquor (1.5 oz.), or mixed drinks with one shot of liquor. Assume that you did not drink alcohol before you went to the bar and will not go out after.” Participants will then be asked “How many drinks would you consume if they were ____ each?” 14 costs will be listed: $0 (free), $0.25, $0.50, $1.00, $1.50, $2.00, $2.50, $3.00, $4.00, $5.00, $6.00, $7.00, $8.00, and $9.00.

ii. **Iowa gambling task:** (IGT) is a psychological task thought to simulate real-life decision making. It was introduced by Antoine Bechara, Antonio Damasio, Hanna Damasio and Steven Anderson, then researchers at the University of Iowa (Bechara et al. 1994). We will use the often computer based version of the task. The task was originally developed to detect problems patients with damage to the ventromedial prefrontal cortex. This part of the brain is, among other things, involved in processing risk, fear, emotion, and decision making.

iii. **Delay-Discounting Task**: (DD) is a measure of temporal discounting, the tendency for people to prefer smaller, immediate monetary rewards over larger, delayed rewards. Participants complete a series of 5 questions that each require choosing between a smaller, immediate reward (e.g., $500 today) versus a larger, later reward (e.g., $1000 in 25 days). The 5 items are divided into three groups according to the size of the larger amount (small, medium, or large) (Koffarnus and Bickel 2014). Modeling techniques are used to fit the function that relates time to discounting. The main dependent measure of interest is the steepness of the discounting

curve such that a more steeply declining curve represents a tendency to devalue rewards as they become more temporally remote.

D. Description, Risks and Justification of Procedures and Data Collection Tools:

a. Justification for Exclusion of Vulnerable Populations:

Vulnerable populations are routinely excluded from experimental studies with more than minimal risk and without an established therapeutic benefit.

b. Risks/Discomforts and Benefits Ratio

Benefits: Participation in this study will not provide any known direct benefit. Participants will receive psychiatric assessment and will be notified if anything pertinent to their health is discovered. The primary benefit of this study will be to others. The knowledge gained from this study could help develop treatments and provide information about who those treatments will work best on.
Risks and risk management: Known risks and discomforts associated with study procedures and steps to mitigate or minimize the risks are described below.

MRI: There are no known long-term risks of MRI scans. However, people are at risk for injury from the MRI magnet if they have certain metal objects in their bodies. These include implanted electrical devices such as pacemakers, cochlear implants, delivery pumps or brain stimulators. People with implanted metal such as aneurysm clips (metal clips on the wall of a large artery), permanent eyeliner, shrapnel fragments or some dental implants and prostheses (including metal pins and rods, heart valves) are also at risk. Welders and metal workers are also at risk for injury because of possible small metal fragments in the eye. Individuals with back problems may have back pain or discomfort from lying in the scanner. Individuals with fear of confined spaces may become anxious during an MRI. The noise of the MRI machine can be loud enough to damage one's hearing, especially in people who already have hearing loss.

1. To minimize risk from metal objects, participants will be screened for metal in their body using the MRI safety screening questionnaire, and individuals who report metal in their bodies will be excluded from the study.
2. Volunteers who are pregnant or planning to become pregnant during the study cannot participate. For female participants, a urine pregnancy test will be given no more than 24 hours before each MRI session. If a pregnancy test is positive or uncertain, she may not be in the study.
3. Participants will be able to communicate with MRI technologist during the scan and can ask to be removed from the scanner at any time if they become anxious or feel uncomfortable during the scan.
4. The participant will be fitted with earplugs and headphones to muffle the sound.

c. Confidentiality: Medical records will be handled to prevent breach of privacy at a level considered sufficient for sensitive health care data. Confidentiality and information technology standards will be placed to protect electronic repositories of patient data as well as other clinical patient related materials. Research data not managed electronically will be stored using codes. No personal identifiers will be used. Data will be kept in password-protected computers, either located in access-controlled areas (servers), or, if transferred onto individual workstations, on encrypted media that protect privacy even if storage media are subject to theft. Samples will be kept in locked storage in access-controlled areas. Full access to data will be provided, under conditions of confidentiality, to individuals or groups

conducting quality assurance and appropriate regulatory agencies. With these exceptions, only study investigators and their staff will have access to the samples and data. When reporting the results of this research in medical journals or at scientific meetings, the people who take part will not be named and identified. We will not release any information about subject research involvement without the subject's written permission. The Federal Privacy Act protects the confidentiality of medical records. However, the Act allows release of some information from a subject's medical record without the subject's permission; for example, if it is required by the FDA, members of Congress, law enforcement officials, or other authorized people.

d. Naltrexone:

i. Common and Serious Side Effects of Naltrexone

Nausea, sleepiness, headache, dizziness, vomiting, decreased appetite, painful joints, muscle cramps, cold symptoms, trouble sleeping, toothache.

ii. Serious side effects of naltrexone may include:

Risk of opioid overdose. Accidental overdose can happen in two ways. Naltrexone blocks the effects of opioids, such as heroin or opioid pain medicines. Patients who try to overcome this blocking effect by taking large amounts of opioids may experience serious injury, coma, or death. After receiving a dose of naltrexone, the blocking effect slowly decreases and completely goes away over time. Patients who are taking naltrexone for an OUD can become more sensitive to the effects of opioids at the dose used before, or even lower amounts. Using opioids while on naltrexone can lead to overdose and death.
Liver damage or hepatitis is possible. Patients should tell their practitioner about any of the following symptoms during treatment: stomach area pain lasting more than a few days, dark urine, yellowing of the whites of your eyes, tiredness, Practitioners may need to stop treatment using naltrexone if patients develop signs or symptoms of a serious liver problem, Depressed mood, Pneumonia, Serious allergic reactions, Skin rash, swelling of face, eyes, mouth, or tongue, trouble breathing or wheezing, chest pain, feeling dizzy or faint.

iii. Minimizing risk

Remote video monitoring will also be used to assess adherence. Dosing size will be set before participants receiving the naltrexone and participants will start on smaller doses (25mg) for two days before being raised to the final dosing to allow for adjustment to naltrexone and in case of adverse reaction to naltrexone. We will use a questionnaire to check for adverse reactions and will provide care as needed for moderate or severe side effects, such as nausea.

e. Psychological Tests

i. During the Standardized Clinical Interview for the DSM-V (SCID) participants may discuss negative or traumatic events they have experienced. The SCID will be administered by a trained psychiatrist or by a member of the team trained by the team psychiatrist. Some of the questionnaires may cause emotional distress by asking about traumatic or unpleasant events from a person's life. While most individuals will tolerate this discomfort without problems, it is possible that the memories may trigger unhappiness. To mitigate the risk, study staff will allow participants to take breaks as needed. If serious distress arises, participants will be referred to a psychiatrist from the study team. This protocol may identify mental health problems, such as depression, suicidality, or substance use disorders. Problems with acute danger, such as suicidality will receive immediate attention and intervention. Individuals with diagnoses such as depression or alcohol use disorder will be given referrals for treatment.

E. Potential Scientific Problems: One challenge of our study is that we will not directly observe naltrexone binding. Directly observing naltrexone's activity would require PET imaging to observe displacement of a tracer that binds to opioid receptors, but we will not do that in this study due to cost and time. Thus, even if we observe behavioral or neural effects, we will not know if naltrexone is acting at that site. However, since we are using a randomized, placebo-controlled design, we can still draw conclusions about naltrexone causing the changes even if the changes may be downstream from naltrexone's direct site of action.

F. Data Analysis Plan: Outcomes will be examined using paired t-tests for two times (active medication, placebo). Functional MRI analysis will be conducted with AFNI software (Cox 1996). Functional images will be time-slice corrected and co-registered with anatomical images. Anatomical images will be non-linearly warped to standard space and an identical transformation will be applied to the functional image. Functional images will be blurred using a 4mm Gaussian kernel and values will be scaled to have a mean intensity of 100. A regression algorithm will be applied including six regressors to account for motion. For the monetary incentive delay task, regressors of interest include the gain, loss, and neutral cue. For the emotion regulation task, regressors of interest include neutral, look, and decrease conditions. For the monetary incentive delay task, activation will be extracted from the nucleus accumbens for each of the three cues. For the emotion regulation task, activation will be extracted from the amygdala for each of the three conditions. As an exploratory analysis, we will conduct whole-brain, voxel-wise analysis.

Sample Size and Power Considerations. In terms of evaluating differences in reward activation between placebo and mu-opioid antagonism conditions, a study of mice estimated a large effect size of mu-opioid antagonism on reward response in the nucleus accumbens, with Cohen's d > 1.0 (Walters et al. 2005). A study of kappa-opioid receptor modulation in human reward response measured with functional MRI found a medium to large effect size of Cohen's d = 0.6 (Krystal et al. 2020). We specified significance level alpha=.05, two-tailed, and power of 0.8. A sample of 9 adults would provide 80% power to detect an effect size of 1.0 or greater as significant.

G. Summarize Knowledge to be Gained: We hope to show that reward response is diminished when opioid receptors are blocked in humans. We hope to show that opioid antagonism has specificity for ventral striatal (e.g. nucleus accumbens) activation for rewards. Following this pilot, our goal is to test the effect of naltrexone in adults with an alcohol use disorder. A leading theory is that naltrexone will be more effective in patients who seek alcohol's rewarding effects compared to patients who seek relief of negative symptoms such as anxiety or withdrawal. This would help develop a precision medicine approach to treatment of alcohol use disorder, where clinicians could use reward response as a marker to determine the likelihood of naltrexone's efficacy. We would use this pilot data as part of an R01 application to fund the next study.

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