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Title: A118G SNP and OPRM1 Gene Opioid-mediated Effects in Humans

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1. Abstract

a. Provide no more than a one page research abstract briefly stating the problem, the research hypothesis, and the importance of the research.

Abuse of opioids is a significant national health problem. Pharmacogenetics, or personalized medicine, uses genotype information to predict phenotypic response (generally medication efficacy or safety). The field of substance abuse is critically lagging behind in the application of pharmacogenetics for identifying individuals at increased risk for developing an opioid abuse disorder, or using personal genetic information to guide treatment. There is growing evidence to suggest a functional polymorphism (A118G) in the OPRM1 gene that codes for the mu opioid receptor (MOR) mediates individual response to opioid medications and has direct relevance for the development of opioid dependence. To date, no controlled human laboratory studies have examined the effect of the A118G SNP or OPRM1 gene on individual response to opioids. The next logical step is to evaluate whether differences in OPRM1 single nucleotide polymorphisms (SNPs) drive individual response to opioid medications, which will help advance the field of substance abuse towards a pharmacogenetics approach to treatment, and establish a precedent for using controlled and well-validated laboratory methodology to investigate the genotype-phenotype interactions of opioids. We are proposing to conduct a laboratory study to evaluate whether the A118G SNP and additional OPRM1 tagging SNPs are associated with a variety of different MOR-mediated functions by evaluating subjective and physiological response to double-blind administration of an opioid medication. We will also evaluate the contribution of OPRM1 on other complex phenotypes related to the MOR activity or opioid dependence (e.g., pain sensitivity, the endogenous opioid-mediated cortisol stress response, and a delay discounting behavioral economic task). This study will be a between-group evaluation of genotype and sex, and a within-subject evaluation of opioid dose-response that will be conducted over 5 days in a residential clinical research unit. Study completers (n=100) will receive double-blind doses of oral hydromorphone or placebo in a randomized, counter-balanced research design. Self-report, physiological, and salivary cortisol measures of drug effects will be collected at several time points following drug administration, and delay discounting will be administered at screening and during peak drug effects. We will also administer different operant pain tasks that provide quantifiable estimates of pain sensitivity, under conditions of placebo or hydromorphone administration. This study will be the most controlled, rigorous, comprehensive examination of the A118G SNP and OPRM1 gene with opioid-mediated effects to date. We expect that genotype will be associated with several opioid-mediated effects, and that the results will advance our understanding of the contribution of OPRM1 to specific behavioral phenotypes. These data will advance the use of pharmacogenetics for substance abuse and use of laboratory testing for genotype-based hypotheses, and will contribute to the development of opioid dependence prevention strategies and interventions to treat comorbid pain and opioid dependence.

2. Objectives (include all primary and secondary objectives).

The primary aim of this study is to examine the associations of specific polymorphisms in the OPRM1 gene with mu opioid receptor (MOR)-mediated effects. We hypothesize the minor A118G SNP allele and OPRM1 tagging SNPs will predict more reinforcing properties of hydromorphone, (e.g., higher self-report ratings of high and drug-liking) and greater opioid-induced physiological changes (e.g., pupil diameter, blood pressure), decreases in cortisol levels as a function of the degree of pleasant effects of hydromorphone, and steeper discounting curves on a delay-discounting task. The secondary aim of this study is to examine the associations of specific polymorphisms in the OPRM1 gene with laboratory measures of pain. We hypothesize the minor A118G SNP allele and OPRM1 tagging

SNPs will predict higher pain threshold on two experimentally-induced laboratory measures of pain, lower analgesic efficacy in response to experimentally-induced pain, and lower cortisol response to painful stimuli. Finally, as an exploratory aim, we will examine the contribution of sex to the primary and secondary aims.

3. Background (briefly describe pre-clinical and clinical data, current experience with procedures, drug or device, and any other relevant information to justify the research)

Abuse of opioids has resulted in a 57% and 717% increase in treatment admissions between 1992-2008 for heroin and prescription opioids, respectively (1). Opioid dependence is a chronic, relapsing disorder that is associated with greater health care services, more emergency room visits, prolonged disability, missed work days, and frequent overdose, resulting in an annual societal cost of more than $8 billion (8 fold greater than nonabusers; (2, 3). Laboratory evaluations of opioids medications reveal robust and consistent between-person differences in self-report and physiological response to opioids (4-8), however no persistent demographic, drug use, and/or treatment characteristics have been identified that reliably predict susceptiblity to develop opioid dependence (9-15). The phenomenon of varied individual response to drugs is not limited to opioid medications and clinically causes reductions in efficacy of medications and increased side effect profiles (16, 17). Pharmacogenetics, one approach to personalized medicine, addresses this phenomenon by using genotype information to predict phenotypic response (generally medication efficacy or safety; (18, 19). Regarding opioids, there is growing evidence to suggest a functional polymorphism in the OPRM1 gene that codes for the mu opioid receptor (MOR) may mediate individual response to opioid medications and have direct relevance for the development of opioid dependence (20-25). The most commonly researched single nucleotide polymorphism (SNP) of the MOR is a nucleotide exchange in exon 1 at the 118 position of the N-terminal domain (26), which results in an amino acid change from aspargine to asparate at position 118 (Asn40Asp or A118G, rs1799971).

We were the first group to show that the A118G SNP of the OPRM1 gene has functional consequences on human behavior (27). Human laboratory studies have reported a reliable and robust, endogenously-mediated cortisol response to the opioid antagonist naloxone (27-32), a greater adrenocorticotropic hormone stress response (29), and decreased pupillary constriction (33-35) in response to opioid administration. Laboratory studies examining alcohol and nicotine have reported functional associations between the A118G SNP of the OPRM1 and behavioral outcomes such as increases in striatal dopamine following nicotine exposure (36), decreased cerebral blood flow following nicotine abstinence (37), increased MOR binding potential (38) and MOR availability (39), finding smoking more reinforcing (40, 41), and greater sensitivity to alcohol. Most recently we have identified that the minor allele predicts lower D2/D3 receptor binding potential in the nucleus accumbens (Wand and co-workers, editorial review in preparation). Altogether, these data are now being used to predict clinical differences in treatment response based on OPRM1 status, such as efficacy of naltrexone for alcohol treatment (42, 43), nicotine replacement products for nicotine (44, 45), and severity of nicotine withdrawal symptoms (46). The field of medicine has embraced the promise of pharmacogenetics more broadly and has published several studies that use genotype to guide treatment for alcohol (47), breast cancer (48), hepatitis C (49), warfarin (see (50), leukemia (51), AIDS (52), P450 enzymes (53), psychotropic medications (54), and pain (55). This approach has great potential because it prevents physicians from prescribing medications that have a lower likelihood of working or are unsafe within different genetic subgroups, ultimately increasing efficiency, reducing time to positive therapeutic response, and minimizing potential side effects of medications. Patients also support the use of genetics to guide prescribing (56).

The field of substance abuse is critically lagging behind in the application of pharmacogenetics for identifying individuals at increased risk for developing an opioid abuse disorder, as well as using personal genetic information for tailoring treatment decisions. The majority of studies that have evaluated the contribution of the OPRM1 gene to human behavior are either Genome Wide Association Studies (GWAS) that provided self-report surveys to large patient samples and looked for associations, or retrospective chart reviews that collected genetic information from clinical patient populations who received opioid medications for pain (21, 57-66). The primary pitfalls of these approaches are that the phenotype (e.g., substance abuse) is either poorly defined or unreliably measured. The field of genetics has begun moving away from these association models; they do not yield consistent results because the measures used to define the phenotype are very heterogeneous across studies and are ultimately poor representations of the target phenotypes. Two independent reviews have recently argued that controlled laboratory examinations of endophenotypes that are evaluated under rigorous experimental conditions better approximate the complex phenotype of drug dependence and produce stronger effect sizes than large-scale association studies that rely on retrospective self-report (67, 68).

To date, no controlled human laboratory studies have examined the effect of the A118G SNP or the larger OPRM1 gene on individual response to opioids. The proposed laboratory study is a necessary first step to evaluate

whether differences in OPRM1 single nucleotide polymorphisms (SNPs) drive individual response to opioid medications, which will help advance the field of substance abuse towards a pharmacogenetics approach to treatment, and establish a precedent for using controlled, reliable, and well-validated laboratory methodology to investigate the genotype-phenotype interactions of opioids. Laboratory studies are ideal for targeting specific behavioral phenotypes because they are rigorously controlled, permit within-subject evaluations of drug effects and double-blind dose administration, identify dose-graded effects, target well-established and specific phenotypes of drug use behavior that can be directly compared to existing literature to guide interpretation of effects, permit cross-laboratory and cross-species comparisons and replication of effects, are robust and reliable predictors of clinical outcomes, and produce larger effect sizes than GWAS studies (67, 68). Our group has specific expertise in conducting laboratory assessments of opioid effects using a model that is considered the standard by the Food and Drug Administration, industry, and national organizations (69). Thus, we are proposing to conduct a laboratory study to evaluate whether the A118G SNP and additional OPRM1 tagging SNPs are associated with a variety of different MOR-mediated functions by evaluating subjective and physiological response to double-blind administration of an opioid medication, pain sensitivity and response to medications for pain (secondary aim, beginning Page 9), the endogenous opioid-mediated cortisol stress response, and a delay discounting behavioral economic task that has been reliably associated with the drug abusing phenotype. This study will advance our understanding of the contribution of genotype to abuse liability, pain, endogenous stress response, and delay discounting. These data can be used to modify analgesic doses and treatments, reduce potential for side effects from opioid medications, predict susceptibility to opioid dependence (70, 71) and develop innovative intervention strategies, and advance the use of controlled laboratory testing to evaluate genotype-based hypotheses. This study will also provide a unique opportunity to identify whether these effects are evident among people that demonstrate increased abuse potential for opioids prior to the development of opioid dependence.

There is also substantial individual variation in the subjective response to nociceptive (e.g., painful) stimuli. Genetic factors have been shown to contribute to that variation (72-75) and growing evidence indicates the functional action of the OPRM1 may influence the associated endogenous opioid response (mediated by beta-endorphin) to pain (26, 76). Several studies report that the minor allele of the A118G may confer a resistance to nociceptive (e.g., painful) stimuli. First, carriers of the minor allele have lower beta-endorphin-mediated serum concentrations of circulating proinflammatory cytokines (77), and a robust beta-endorphin mediated increase in cortisol release following naloxone administration (27-32). Second, controlled human experimental studies report higher pain thresholds in minor allele carriers (78-81), and a longitudinal cohort study reported higher positive affect across 30 days of repeated measurement among A118G minor allele variants with the chronic pain disorder fibromyalgia (82), which is consistent with preclinical studies that reported increased pain sensitivity following direct administration of beta-endorphin (83, 84). The minor allele of the A118G has also been consistently associated with increased medication requirements to achieve analgesia. For example, preclinical and human laboratory studies report that A118G minor allele carriers experience less relief from opioid agonists (85-87), and several retrospective chart reviews report greater postoperative medication requirements (35, 88-96).

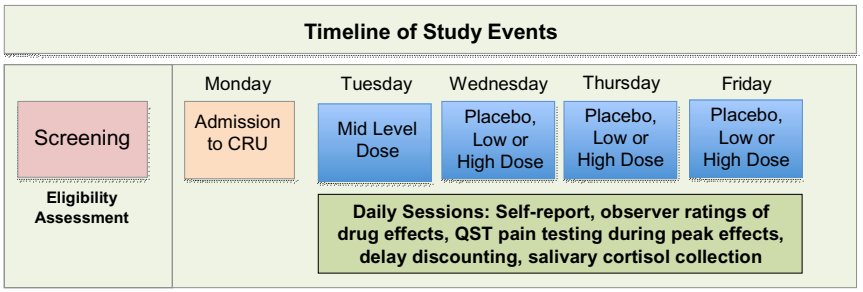
Additional, well-controlled studies are needed to understand and advance our knowledge regarding the effect of the A118G SNP and additional OPRM1 tagging SNP variants on baseline pain tolerance, and pain tolerance under conditions of opioid analgesic treatment. Only two studies have examined the contribution of the A118G on pain tolerance in controlled laboratory settings (78, 81). The first study reported positive associations between A118G and pain on two of the three pain tolerance tests conducted, but did not evaluate tolerance to pain following opioid agonist administration (78, 81). The second study reported a positive association with OPRM1 on one test of pain following oxycodone administration, but did not evaluate additional pain tolerance tests, which is important given the high rate of individual variability among pain testing methods (78, 81). Neither study sampled across the OPRM1 gene and or was designed to prospectively evaluate gender, which is critical because of the robust gender-specific effect that exists on pain and its potential interaction with the OPRM1 gene (41). The current study will extend upon these studies by evaluating the effect of the A118G SNP and OPRM1 gene using a quantitative sensory testing (QST) paradigm, under conditions of double-blind and placebo-controlled hydromorphone administration, and will conduct gender specific analyses. Women will only be enrolled during the first two weeks of their cycle (confirmed via progesterone analysis) to minimize hormonal effects on pain, which will be the most rigorous evaluation of OPRM1 and pain within gender to date. Further, one of our pain tests will activate the diffuse noxious inhibitory control (DNIC) system, which is mediated by the endogenous opioid system, hypothesized to be a model of chronic pain, and believed to contribute to opioid-induced hyperalgesia (97). No prior study has used the DNIC procedure to evaluate OPRM1 effects on pain tolerance, which is critical because of the established functional relationship between the OPRM1 and the endogenous opioid response (26, 98). The results will advance our understanding of the OPRM1 contribution to pain tolerance and response to medication, help elucidate the

mechanism behind opioid-induced hyperalgesia, and may be used clinically to guide development of chronic pain interventions and prescribing guidelines. These results will also be compared to abuse liability outcomes, which- to our knowledge- will be the first within-person comparison of opioid abuse liability and pain effects as a function of genotype.

4. Study Procedures

a. Study design, including the sequence and timing of study procedures (distinguish research procedures from those that are part of routine care).

Study Overview: This study will be a between-group (total completer N=100) evaluation of genotype and sex, and a within-subject evaluation of opioid dose-response. The study will be conducted over 5 days (4 nights) at the Behavioral Pharmacology Research Unit (BPRU) and a residential clinical research unit (CRU) (Figure 1). Day 1 will be an Admission day. Days 2-5 will consist of blinded study drug administration in the morning, 1 pain testing session, and self-report/physiological measures of drug effects for 6 hours post dosing. Participants will receive double-blind doses of oral hydromorphone (2mg, 4mg, 8mg) (99-102) or weight and color-matched placebo in a randomized, counter-balanced research design.



Screening Visit: Participants will complete a brief phone screen to determine initial eligibility and will be invited to complete an in-clinic screen. Participants will sign an informed consent form with a study staff member to begin the Screening visit. To reduce expectancy effects, the consent will inform participants the study drug may consist of one or more of numerous different drug classes (e.g., opioids, stimulants, over-the-counter medications), a common practice in laboratory based studies of drug effects (103, 104). Following consent, a staff member will administer a battery of measures to establish study eligibility (see Study Measures below). A urine sample will be collected and tested for evidence of illicit drugs and to establish medical eligibility. A blood sample of no more than 2 tablespoons will also be collected from eligible participants for medical and genotype analysis. Participants will also be asked to provide a second blood sample (2 additional tablespoons) that will be collected at either Screening or Admission and stored for future genetic analysis. This will be voluntary. Participants who meet eligibility criteria (including results of laboratory blood tests) will be scheduled for an Admission day.

Study Day 1, Admission Visit: Participants will complete a brief history and physical to determine final study eligibility and will be admitted to the CRU for 5 days (Admission day + 4 session days). During the Admission day, participants will be familiarized with the pain testing procedures and the measures used during session days 2-5.

Study Days 2-5, Session Days: The morning of each session, participants will be provided with a standardized breakfast, and cigarette smokers will be permitted 1 cigarette the morning prior to a session (1 additional cigarette will be permitted every 2 hours during the session). Sessions may take place either at the CRU or the BPRU, however all participants will reside overnight in the CRU. Participants will receive a standardized lunch and dinner. Beginning on Day 2, participants will receive a blinded study drug in the morning, will conduct a pain testing session approximately 60-90 minutes post-dose, and will provide self-report, observer-ratings, and physiological measures of drug effects for an additional 6-hours post-dosing. Each element is described in more detail below. Participants will also provide a blood sample (no more than 1 tablespoon) that will be analyzed for metabolic status. This sample can be collected at any point during Study Days 2-5.

Pain Testing: Participants will undergo 1 pain-testing session each day. Sessions will consist of pressure pain threshold, conditioned pain modulation, and temporal summation testing. Participants will be oriented to the study measures and pain testing apparatuses during the Admission Day. During the sessions, study drug will be administered under double-blind conditions in the morning. Salivary cortisol samples will be collected following completion of the pain testing session to evaluate the acute stress response to each pain stimulus, as a function of

drug (placebo vs. hydromorphone). Ratings of numeric rating scales will be used for data analysis, and participants will be asked to identify whether a placebo or active drug had been administered.

Pressure Pain Threshold: The pressure pain threshold is defined as the kilopascals (kPA) value at which a mechanical stimulus is first judged to be painful. An electronic algometer with a $1cm^2$ hard rubber probe will be applied two times to two different muscle groups in randomized order. Pressure will be applied at a constant rate of 30kPA/s until the participant first reports pain. A 30 second interval will be imposed between each stimulus presentation (105). The values in kPA will be averaged across administrations and muscle groups to create an index of pressure pain threshold. Administering the stimulus at multiple anatomic locations increases the reliability of the pressure pain threshold. Possible effects of laterality and muscle group will be evaluated to determine if threshold outcome measures should be separated into subgroups.

Conditioned Pain Modulation: We will also conduct a test of conditioned pain modulation, formerly known as diffuse noxious inhibitory controls. Conditioned pain modulation is the process by which the pain induced by a tonic conditioning stimulus inhibits the pain produced by another noxious stimulus at a distal anatomic location. This procedure initiates a robust activation of the endogenous opioid system that is attenuable by administration of naltrexone (106). Our conditioned pain modulation procedure will measure the change in pressure pain threshold at the trapezius muscle induced by immersion of the contralateral hand in cold water. Two baseline pressure pain threshold readings will be assessed via algometry. Participants will then immerse their hand in a circulating ice water bath maintained at 10 +/- 1ï C and will not be told the duration of time for which their hand will be submerged. After 20 seconds, with their hand still submerged, participants will rate their pain on a 0-100 numeric rating scale (NRS; (107), where 0=No Pain and 100=is Worst Pain Imaginable, and after 45 seconds participants will be asked to remove their hand from the water bath. Simultaneously with hand withdrawal, an algometer will be applied and pressure pain threshold in kPA will be recorded. This procedure will be repeated contralaterally after a 5-minute interval. The order of administration with respect to laterality will be randomized. Conditioned pain modulation will be indexed as the percent change in pressure pain threshold from the baseline administration to the conditioned administration, averaged across the 2 trials (105). Conditioned pain modulation can be conducted with a variety of heterotopic conditioning stimuli, including thermal, ischemic, and electrical stimuli. Cold-water immersion (also known as the cold pressor test) is an attractive choice for the conditioning stimulus because noxious cold stimuli activate cutaneous A∂ and C fibers, resulting in rapid nociceptive neurotransmission (108). This conditioned pain modulation method has been selected for this study because it has been independently validated as a sensitive and reliable measure of descending pain inhibition (109), and it activates the endogenous system that is hypothesized to be altered by the OPRM1 gene.

Mechanical Temporal Summation**.** Temporal summation of pain will be assessed using repetitive mechanical stimuli. The assessment of temporal summation involves rapidly applying a series of identical noxious stimuli and determining the increase in pain across trials; animal studies have suggested that temporal summation occurs centrally in second-order neurons in the spinal cord as a consequence of sustained C-fiber afferent input (149-151). For temporal summation of mechanical pain, pain ratings in response to a single punctate noxious stimulus will be compared to pain ratings in response to a sequence of identical punctate noxious stimuli (152). A weighted pinprick stimulator with a flat contact area will be used to deliver either a single pinprick stimulus or a train of 10 pinprick stimuli repeated at a constant rate. Following the single stimulus and the 10-stimulus train, the subject is asked to give a pain rating. Single pinprick stimuli are alternated with the trains of 10 stimuli. Similar procedures using this type of weighted pinprick stimulator have been used in diabetic neuropathy (153), healthy subjects (154), and to assess temporal summation (155-156).

Acute Effects of Hydromorphone: Participants will ingest medication under staff supervision and complete a battery of self-report, physiological, and cortisol measures will be collected prior to dosing and up to 15 time points post-dose (up to 6 hours). During the final assessment each day, participants will complete a Drug vs. Money Questionnaire modeled from the Multiple Choice Procedure to indicate the dollar value they would place upon the drug received and this measure will be repeated immediately prior to the next day's drug administration. These experimental procedures are consistent with those used in a previous study by our group (110). Participants will be discharged from the study on the final day after completing the final study assessment and a field sobriety test that indicates no persistent opioid effects. We will conduct a follow-up phone call with participants the day following discharge to document any adverse events and to administer the final Drug vs. Money Questionnaire.

General Study Assessments: We have included several measures during the Screening visit to enable us to characterize the population and collect preliminary information that might differentiate between genetic groups or be used to support future research applications, including a questionnaire to evaluate domains the NIDA Genetic

Council recommends be documented in genetic studies. The measures comprise both pharmacologically and qualitatively nonspecific measures (e.g., any drug effect) and qualitatively specific measures (e.g., liking, good effects, bad effects, nausea), which have been recently recommended by a consensus panel and the FDA. OPRM1 has recently been associated with negative side effects of opioid medications so we will specifically assess negative symptoms and side effects following drug administration.

Study Measures to Establish Eligibility and Characterize Sample:

- BPRU Demographic Questionnaire: A questionnaire designed by us to document standardized demographic items (e.g., age, gender, marital status).
- Alcohol/Drug subscale of the Addiction Severity Index (111): A 31-question subscale that assess past 30 day and life-time use of drugs, history of treatment, and interest in treatment. Subscales provide a measure of severity.
- MINI International Neuropsychiatric Instrument: A standardized semi-structured diagnostic interview assesses participants for evidence of psychiatric disorders. The MINI will be used to verify that participants do not meet DSM-IV-TR dependence on alcohol and/or drugs.
- NIDA Genetic Questionnaire: This questionnaire lists several specific questions recommended by the NIDA Genetic Council for any study conducting research on the pharmacogenetics of substance abuse.
- Brief Pain Inventory (BPI; (112)): A 9-item widely-used, standardized, self-report measure to characterize the presence and severity of pain, as well as the interference of pain in every-day activities. The BPI has been modified to be appropriate for self-administration, and for use in an opioid-treatment population.
- Fagerström Test for Nicotine Dependence (FTND; (113)): A 6-item measure to determine presence of smoking and severity of nicotine dependence.
- Profile of Mood States-2 (114): A 65-item questionnaire that is a standardized and normed measure of personality that yields a measures of different personality features (anger-hostility, confusion, depression, fatigue, tension-anxiety, vigor, and friendliness).
- Barratt Impulsiveness Scale (115): A 30-item, widely used measure of impulsivity that will provide a self-report comparison to the delay discounting test of impulsivity.
- The Positive and Negative Effect Scale (PANAS; (116)): A 20-item self-report measure to characterize positive and negative emotional responses to pain.
- Coping Strategies Questionnaire- Short Form (CSQ Short Form; (117)): A 16-item self-report measure to characterize emotional response to pain and perceived efficacy of coping strategies.
- Pain Catastrophizing Scale (PCS; (118)): A 14-item self-report measure to characterize the emotional response to pain, with particular emphasis on anxiety and/or fear of pain.
- McGill Pain Questionnaire-Short Form (MPQ Short Form; (119)): A 15-item widely-used standardized, self-report measure to characterize presence and severity of pain.

Session Measures:

- Clinical Opiate Withdrawal Scale (120): A widely used, 11-item observer-rating of opioid withdrawal symptoms.
- Vital Signs: We will assess blood pressure, pulse, respiration, and oxygen saturation.
- Pupillary Diameter: Pupil size will be assessed using an electronic Neuroptics Pupilometer
- Visual Analog Rating Scales: Participants will rate the following items on a scale of 0 (none at all) to 100 (strongest possible): good effects, bad effects, high, withdrawal, sick, like how I feel, nausea, sedation, feel sleepy
- Opioid Agonist/Antagonist Rating Scale: This questionnaire assesses positive and negative effects of opioids.
- Drug vs. Money Questionnaire: This questionnaire is a frequently used measure to assess relative monetary value of a drug and is being used to assess relative reinforcing effects of the drug compared to hypothetical monetary values.
- Neurocognitive Assessment: A battery of tasks designed to measure neurocognitive performance that has been developed by our research unit will be adapted for this study. Functions to be tested include psychomotor speed/pattern recognition (e.g. digit symbol substitution) and short-term/working memory and focused attention (e.g. N-back task).
- Hypothetical Purchase Task: This questionnaire is used to measure the abuse liability of drugs by assessing the magnitude and persistence of demand for a given drug under cost constraints. Participants indicate how many drug units (i.e., capsules) they would purchase at increasing price points for a 24-hour period of use.

Delay Discounting (DD):

- We will administer monetary and commodity-based DD tasks via computer to participants during the Screening visit, and each session day during peak drug effects (30 minutes). DD for $100 will be assessed using a computer program developed by Co-investigator Johnson that has been utilized in numerous studies (121-126) and will be quantified using the area-under-the-curve (AUC) method (127). The hypothesized results are that the minor allele of the A118G and other minor alleles of OPRM1 tagging SNPs will be associated with steeper discounting curves (less AUC).

Genetic Analysis: Genotyping will be supervised by Co-Investigator Wand. All blood genetic samples will be collected and transported to the main Hopkins hospital on a weekly basis for analysis. A chain of custody will be kept to ensure proper delivery and receipt of all samples. No participants name will be written on any genetic sample and participants will not be informed of the results of the genetic testing.

For all SNPs across the OPRM1 gene including the A118G and ancestral markers (see Statistical Analysis), genotyping will be performed with GoldenGate Genotyping Assay. The DNA sample used in this assay is activated for binding to paramagnetic particles. This activation step is a highly robust process that requires a minimum input of DNA (250ng at 50ng/µl). Depending upon the multiplex level, this equates to only 160pg of DNA per SNP genotype call. Assay oligonucleotides, hybridization buffer, and paramagnetic particles are then combined with the activated DNA in the hybridization. Three oligonucleotides are designed for each SNP locus. Two oligos are specific to each allele of the SNP site, called the Allele-Specific Oligos (ASOs). A third oligo that hybridizes several bases downstream from the SNP site is the Locus-Specific Oligo (LSO). All three oligonucleotide sequences contain regions of genomic complementarity and universal PCR primer sites; the LSO also contains a unique address sequence that targets a particular bead type. Up to 1,536 SNPs may be interrogated simultaneously in this manner using GoldenGate technology. During the primer hybridization process, the assay oligonucleotides hybridize to the genomic DNA sample bound to paramagnetic particles. Because hybridization occurs prior to any amplification steps, no amplification bias can be introduced into the assay. Following hybridization, several wash steps are performed, reducing noise by removing excess and mis-hybridized oligonucleotides. Extension of the appropriate ASO and ligation of the extended product to the LSO joins information about the genotype present at the SNP site to the address sequence on the LSO. These joined, full-length products provide a template for PCR using universal PCR primers. Universal PCR primers P1 and P2 are Cy3- and Cy5-labeled. After downstream-processing 5 the single-stranded, dye-labeled DNAs are hybridized to their complement bead type through their unique address sequences. Hybridization of the GoldenGate Assay products onto the Array Matrix or BeadChip allows for the separation of the assay products in solution, onto a solid surface for individual SNP genotype readout. After hybridization, the BeadArray Reader is used to analyze fluorescence signal on the Sentrix Array Matrix or BeadChip, which is in turn analyzed using software for automated genotype clustering and calling.

b. Study duration and number of study visits required of research participants.

Participants will complete a Screening visit and will be enrolled in this study for a total of 5 days (Admission Day + 4 session days; Figure 1).

c. Blinding, including justification for blinding or not blinding the trial, if applicable.

Participants will be informed in the consent will that the study drug may consist of one or more of numerous different drug classes (e.g., opioids, stimulants, over-the-counter medications). This is a common practice in laboratory-based studies of drug effects, a practice that we have followed for several years, and one that is recommended in peer-reviewed guidelines for abuse liability testing (103, 104). We will inform participants of the risks associated with hydromorphone (without revealing the drug type) in the consent form so they will be able to make an informed decision about study participation. Participants will receive double-blind oral doses of hydromorphone or placebo during the study to prevent expectancy effects and enable a rigorous evaluation of opioid administration on a variety of clinically-relevant outcome measures. Staff members will also be blinded to what study drug is being provided.

d. Justification of why participants will not receive routine care or will have current therapy stopped.
N/A

e. Justification for inclusion of a placebo or non-treatment group.

A placebo condition has been included as a comparison for opioid-induced effects.

f. Definition of treatment failure or participant removal criteria.

Participants will be removed from study participation if they ask to be removed, if they exhibit an allergic reaction to the study drug, if they fail to comply with the requirements of the study, or if new information becomes available that suggests that continued study participation may put them at increased risk of adverse events.

g. Description of what happens to participants receiving therapy when study ends or if a participant's participation in the study ends prematurely.

Participants are healthy controls. They are not treatment-seeking and do not have any underlying medical condition that would warrant continued treatment upon completing the study. Participants will be informed they can agree to begin the study now and withdraw later without any penalty. Participants who request to discharge from the study prior to completing the study will be assisted with contacting medical professionals upon request from the participant.

5. Inclusion/Exclusion Criteria

We will recruit healthy volunteers who may have consumed prescription opioids in the past either recreationally or for medical purposes, but are not currently dependent (Table 1). Restricting our sample to completely opiate naïve individuals would not be feasible, because verifying a history of no opiate use may not be possible, and because we did not want to exclude participants who received clinically-indicated prescriptions. Non-dependent drug using participants have been strategically chosen to minimize potential confounds with current opioid dependence (e.g., tolerance, presence of pain) and human subject concerns regarding need for treatment. Use of non-dependent participants is a common practice in laboratory studies of drug effects and there is no evidence to suggest this exposure puts individuals at risk for developing drug dependence (see Human Subjects section). Finally, females will be enrolled in the study during the 1st two weeks of their menstrual cycle (confirmed via diary and progesterone) to control for hormonal influence on opioid effects and pain. We will not exclude females taking birth control but may include birth control as a covariate in the statistical analyses.

Table 1. Study Eligibility Criteria

Inclusion	Exclusion
1. Provide a urine sample that tests negative for opioids, methadone, buprenorphine, oxycodone, amphetamine, cocaine, and benzodiazepines	1. Answer "yes" to question 1 of the Brief Pain Inventory (89) to assess the presence of chronic pain.
2. Negative ethanol breath test (0.000)	2. Current use of opioids or other medications for pain
3. Aged 21-50	3. Meet DSM-5 criteria for current or lifetime alcohol or drug use disorder (excluding nicotine)
4. Deemed medically eligible to take hydromorphone	4. Self-report any illicit drug use in the past 7 days
	5. Self-report opioid use $\geq$5 days in the past 30
	6. Evidence of opioid physical dependence at screening or following 1st night in CRU (following confirmed opioid abstinence)
	7. Allergy to hydromorphone or other opioid agonists
	8. Experience an adverse event that warrants opioid antagonist treatment following 1st hydromorphone dose.
	9. If female, not be pregnant or breastfeeding
	10. Presence of any clinically significant medical (e.g., chronic renal insufficiency, history of myocardial infarction, seizure disorder) and/or psychiatric illness (e.g., schizophrenia, bipolar disorder) that may interfere with study participation.
	11. BMI >30 (obese category)

6. Drugs/ Substances/ Devices

a. The rationale for choosing the drug and dose or for choosing the device to be used.

We will administer oral hydromorphone in this study. We have selected hydromorphone because it is a prototypic, selective, potent, full MOR agonist that is frequently used in abuse liability studies as a surrogate for heroin, it is not subject to P450 metabolism, it has good oral bioavailability, there are no apparent sex effects of metabolism, it is used clinically for the treatment of pain, there is a wealth of literature available to which our research findings can be compared and interpreted, and we have several years experience administering it under double-blind, laboratory conditions (4, 7, 8, 128-135). The doses of hydromorphone chosen for this study are supported by a study conducted by Walsh et al., (2008), in which oral hydromorphone was administered to non-dependent individuals in doses up to 25mg (136). The authors of that study had no instances of respiratory depression or reductions in Sp02 at any of the doses administered. That study compared the reinforcing effects of oral hydromorphone with the two prominently abused opioid agonists hydrocodone and oxycodone within healthy volunteers who had a history of recreational opioid use but were not opioid dependent. Prior to beginning the study, the researchers conducted a pilot study wherein 5 doses of oral hydromorphone [2.5 mg – 35mg] were administered to enable identification of several safe, tolerable and detectable doses for study administration. The researchers reported the 2 lowest doses [2 and 5mg] were inactive within a population of recreational but non-dependent individuals, and ultimately selected three mid-range doses [10mg, 17.5mg, 25mg] based upon evidence of safety and tolerability.

We have chosen to deliver doses lower than those administered by that study so we may evaluate whether there are individual differences in the ability to detect the low dose of hydromorphone. Participants in our study will receive three active hydromorphone doses. The first dose participants will receive is the mid-level dose, which will be administered on study Day 2. Any participant who has a negative reaction to this dose will be discharged from the study. The participants will receive placebo, low, and high doses on Days 3-5 of the study; the order of doses will be randomized and counter-balanced. These doses were deemed safe in the same participant population in the Walsh study, they produced a documented detectable range of subjective effects, and they represent supratherapeutic doses consistent with those abused recreationally. Ultimately, we feel confident these dose values do not impose any unique risk above and beyond that presented by the administration of hydromorphone.

Generic powdered hydromorphone will be encapsulated using size 0, opaque, hard gelatin capsules; placebo will be size and weight matched and will contain microcrystalline cellulose filler. A pharmacy member with no participant interaction will conduct the dose randomization. All medication administration will be supervised and a medical staff member will be available to address any adverse events or to break the medication blind.

b. Justification and safety information if FDA approved drugs will be administered for non-FDA approved indications or if doses or routes of administration or participant populations are changed.

Hydromorphone hydrochloride is a pure mu opioid agonist that can be administered in oral, rectal, intramuscular (IM), or intravenous (IV) formulations for the indication of acute and/or chronic pain. We have chosen to administer hydromorphone orally in this study to reduce the potential for side effects and adverse events associated with the more direct parenteral administration, and to increase the clinical relevance of the study by administering the most commonly-abused formulation of hydromorphone. This will also extend the time course of hydromorphone effects, which will help us to achieve our study aims. When administered orally, hydromorphone is rapidly absorbed through the gastrointestinal tract and subjected to extensive first-pass metabolism. Exposure to hydromorphone is proportional to the dose range, with *in vivo* bioavailability of an 8mg tablet being approximately 24%. Following oral administration, peak plasma level of hydromorphone is generally attained in 30 – 60 minutes, and approximately 95% of hydromorphone is metabolized to hydromorphone-3-glucuronide via glucuronidation in the liver. There is no apparent effect of sex on the pharmacokinetics of hydromorphone.

c. Justification and safety information if non-FDA approved drugs without an IND will be administered.

N/A

7. Study Statistics

a. Primary outcome variables. The primary outcome variables are self-reported ratings of drug high and liking, changes in pupil diameter and blood pressure, pain threshold on 2 laboratory measures of pain, and analgesic efficacy in response to laboratory-induced pain. We expect that individuals with the minor allele of the A118G SNP and other

tagging SNPS across the OPMR1 gene will find hydromorphone more reinforcing (e.g., higher self-report ratings of high and drug-liking) and exhibit greater opioid-induced physiological changes (e.g., pupil diameter, blood pressure). Research also suggests the OPRM1 gene may also confer greater sensitivity to the negative effects of opioids, which may be observed as measuring greater ratings of nausea. We also hypothesize the minor allele and other tagging SNPs across the OPRM1 gene will produce higher pain threshold on 2 laboratory measures of pain, and lower analgesic efficacy in response to laboratory induced pain.

b. Secondary outcome variables. Decreases in cortisol levels as a function of the degree of pleasant effects of hydromorphone and painful stimuli, delay-discounting curves, and the effect of sex on all outcome measures. We hypothesize that individuals with the minor alleles will have greater decreases in cortisol levels as a function of the degree of pleasant effects of hydromorphone, lower cortisol in response to painful stimuli, and steeper discounting curves on a delay-discounting task. Finally, although we expect that the A118G will be associated with these effects, we hypothesize that several of the additional OPRM1 tagging SNPs will contribute to effect magnitude.

c. Statistical plan including sample size justification and interim data analysis: The aims of the proposed study will explore the impact of genetic variation across the OPRM1 gene on hydromorphone response. There are specific steps we describe below to assure that we interrogate all known functional variation across the OPRM1 gene, adequately cover all other variation using appropriately selected tagging SNPs, prevent spurious associations due to population stratification by accounting for local ancestry, and properly control the Type I error rate.

Association Model: Our primary goal is to demonstrate the impact of SNP variation across OPRM1 on variability in response to hydromorphone across time and dose. Association analyses will be performed by modeling genotype at each SNP in a general linear model. In its simplest form this test, commonly termed the measured genotype analysis (137), models the influence of genotypic variation at a given locus on variation in the quantitative trait, essentially a one-way Analysis of Variance (ANOVA). This approach provides a distinct advantage in that it can easily be extended to a two-way ANOVA to model genotype effects across 3 different doses of drug. In addition, the flexibility of the GLM allows incorporation of ancestry covariates and environmental moderators or mediators. We will examine the impact of OPRM1 at each time-point in separate analyses and account for the multiple testing using LD-adjusted Bonferroni correction (see below).

Power and Correction for Multiple Testing: Power (1-Type II error) and correction for multiple testing (control of Type I error) are important considerations in any genetic association study. Typically investigators attempt to control the Type I error rate of a given set of hypothesis tests by controlling the family wise error rate through manipulation of the alpha level of the test. This is not problematic when a handful of tests are performed at an alpha level (false positive level) of 5%, where the usual approach is the Bonferroni correction. We anticipate performing tests across multiple time-points and must adjust our target alpha level. Importantly, our 71 selected SNPs across the OPRM1 gene (see below) do not represent 71 independent tests due to correlation between SNPs. We will use an approach, termed SNPSpD (138) which uses spectral decomposition to estimate the number of independent tests across the region. Using available HapMap data, we estimate 44 independent tests across the region. We calculate power for the GLM approach modeling the effect of genotype on phenotype. If we assume a constant effect size difference between genotype groups (at each dose other than placebo), at an alpha level conservatively adjusted for 50 tests to .001, using the entire completer sample (N= 100) or the sex subsamples (N=50), we have >80% power for modest effect sizes of ~.45 and .67 respectively. In our calculations, effect size (Cohen's d) is the mean difference between genotype groups in standard deviation units. Importantly, this effect size is well within the range seen by other studies examining drug response difference in OPRM1 (e.g., Ray et al., 2004: effect size = 1.2).

SNP selection (for association testing and control of Local Ancestry): SNPs will be genotyped across the OPRM1 region to allow us to test associations across the gene and account for genetic ancestry (ethnicity). Typing all available SNP markers across the candidate gene set is inefficient and not necessary since many will provide redundant information due to linkage disequilibrium (LD). A more cost-effective strategy is to select a subset of representative SNPs (tagSNPs) that are in strong LD (measured through pairwise r^2) with other SNPs that are therefore not required for genotyping in order to capture the haplotypic diversity across the gene. We followed this approach across OPRM1 with the Tagger algorithm at an r^2 value of 0.8 and a minor allele threshold of 0.05 (139). We force included all predicted functional variation with allele frequency > .03 to allow for secondary hypothesis testing across OPRM1 functional SNPs. We used the latest Data release 21 (CEU Sample) of HapMap (http://www.hapmap.org) in conjunction with the NCBI build 35/hg17 of gene and EST data at UCSC Genome Browser (http://genome.ucsc.edu/cgi-bin/hgGateway;). Using this approach, we selected 71 SNPs across the OPRM1 region for association testing and control of local ancestry. In our approach, we will re-estimate local ancestry for

each SNP association, dropping the tested SNP from ancestry estimation. Our approach to accounting for population stratification is detailed below.

SNP Quality Control: Maximizing genotype accuracy is a key step in maximizing our ability to detect true genotype-phenotype relationships. We will perform the usual checks to ensure data precision. We will first test Hardy-Weinberg equilibrium (HWE) and eliminate markers that exhibit large deviations from expected genotype distribution. The rationale for this step is to eliminate markers that may exhibit systematic genotype error (e.g., excess failure of a particular allele due to allele loss). We do realize that minor deviations from HWE will be observed by chance when testing such a large number of markers. Thus, we will use a stringent criterion for dropping markers exhibiting Hardy-Weinberg disequilibrium ($p < 0.001$). Additionally we will eliminate markers that exhibit a high level (>5%) of missing data. A high level of missing data is generally interpreted as an indicator of a poor quality marker. We will eliminate individuals with a high level (>5%) of missing genotypes as this is likely due to poor DNA quality.

Population Stratification: In the context of genetic association testing, population stratification refers to genetic differences between subpopulations. This is especially problematic in instances where the investigators are blind to the presence of stratification and/or the population substrata also differ phenotypically. In these cases, association analyses will be prone to generate spurious genotype-phenotype relationships. Previously, several methods, including genomic control (140) and STRUCTURE (141) were proposed to deal with this issue by either correcting the test statistic for the average level of stratification or a priori grouping of population subsets. At the onset of the GWAS era, the wealth of genome-wide data gave rise to additional approaches which rely on the correlation structure of genetic information to identify cryptic population structure. EIGENSTRAT (142) is an example of an approach that uses genomewide data to infer principal components of population membership. Recently, several investigators (143, 144) have noted that differences in global (genomewide) versus local (at a gene or LD block) ancestry exist, especially in admixed populations (e.g., African-American). This is particularly germane to our setting given: 1) the genetic ancestry of the ECA sample is a mix of European-Americans and African-Americans, 2) the focus on a relatively small number of genes, and 3) the lack of genome-wide SNP data. We will use an approach (WinPOP/LAMP; (144, 145) to estimate proportions of local ancestry at each tested gene and account for stratification based on those mixing proportions in subsequent GLM tests. In order to accomplish this we will densely tag OPRM1.

Analysis of pain testing sessions: We hypothesize that OPRM1 SNP variants will exhibit greater pressure pain thresholds, greater CPM efficiency, lower hydromorphone-related analgesia, and lower cortisol stress response than participants with the major allele, and that results will be positively correlated with response to self-report and physiologically-mediated hydromorphone drug effects collected during days 3-5. Pressure pain threshold will be estimated by averaging the results of the 2 administrations to radiobrachialis and quadriceps muscle groups. We will generate a CPM index for each participant [(mean pressure pain threshold during cold pressor)/(mean pressure pain threshold prior to cold pressor)] x 100 (90). These values will provide a quantitative measure of percent change in the test stimulus (i.e., pressure point threshold). Mixed effects regression models with a random intercept will be used to compare dependent variables (pressure pain threshold, DNIC threshold, DNIC index) as a function of within-subject drug effects (placebo vs. hydromorphone), and between-subject effects of OPRM1 SNP analysis will be conducted as specified in Aim 1. Gender, age, and race have been associated with response to pain and the CPM test (146-148). Gender will be evaluated using independent subgroup analyses and age and race will be included as covariates. Finally, subscale scores of self-report measures of pain and coping strategies will be compared between the two genotype groups using independent samples t-tests, and drug identification data will be compared using a chi-square for each drug condition. We expect that both experimental manipulations will induce pain in the minor and major allele groups, and that participants with the minor allele will demonstrate lower sensitivity to pain (e.g., greater pressure pain magnitudes), lower NRS ratings of pain, lower cortisol response to painful stimuli, and lower analgesic relief from hydromorphone compared to the major allele participants.

d. Early stopping rules. The safety of participants is a priority of BPRU. If a participant experiences a serious side effect that seems to be related to the study drug, s/he will be discontinued from the study. If clinically indicated the participant will be referred to the Johns Hopkins Bayview Medical Center (JHBMC) Emergency Department for further treatment. BPRU and the CRU have policies in place to ensure safety of all participants and staff, and some policies may lead to administrative discharge of a participant, and therefore, discontinuation from the study. Participants who do not abide by the BPRU policies and procedures, or who demonstrate behaviors abusive of staff or other participants may be discontinued from the study. Intercurrent illness or condition changing a participant risk may result in medical discharge from the study.

Stopping criteria for the study: In addition, an independent panel of consultants will be contracted for review of the risks-benefits ratio of the study. At mid-point during enrollment this panel will be provided with a report of adverse events and participant outcome data (subjective response to hydromorphone) by unlabeled sex groups (A vs. B). This information will be used to assess whether the risks reported are considered to be of unusual magnitude or frequency across any specific group, and to determine whether the study should be ended prematurely due to participant safety concerns or because a result has been unequivocally demonstrated. It is unlikely that such a small study would be interrupted because of a large rate of adverse events, particularly given the noninvasive administration method of the medication (i.e., oral) and our experience at administering this drug, however we will ensure that a qualified panel of reviewers makes this decision.

8. Risks

a. Medical risks, listing all procedures, their major and minor risks and expected frequency.

Hydromorphone: Our research team has extensive experience administering hydromorphone under controlled laboratory conditions. Administration of any drug involves some risk because it is not always possible to predict individual response to drugs. The most likely risk in this study is that subjects will experience side-effects of the drugs that may be unpleasant. Common side effects of hydromorphone are light-headedness, dizziness, sedation, nausea, vomiting, sweating, flushing, dysphoria, euphoria, dry mouth, and pruritus. Less frequently observed side effects are weakness, headache, agitation, tremor, uncoordinated muscle movements, alterations of mood (nervousness, apprehension, depression, floating feelings, dreams), muscle rigidity, paresthesia, muscle tremor, blurred vision, nystagmus, diplopia and miosis, transient hallucinations and disorientation, visual disturbances, insomnia, increased intracranial pressure, flushing of the face, chills, tachycardia, bradycardia, palpitation, faintness, syncope, hypotension, hypertension, bronchospasm and laryngospasm, constipation, biliary tract spasm, ileus, anorexia, diarrhea, cramps, taste alteration, urinary retention or hesitancy, antidiuretic effects, urticaria, other skin rashes and diaphoresis. Side effects of hydromorphone are generally temporary, dissipate within several hours, and are dose-dependent. Thus, participants may only experience side-effects occasionally during the research study. Serious potential adverse effects of hydromorphone administration that are possible but extremely unlikely to be encountered in this study are respiratory depression and loss of consciousness. The most serious potential adverse event following hydromorphone administration is opioid agonist overdose, which is characterized by respiratory depression, somnolence progressing to stupor or coma, skeletal muscle flaccidity, cold and clammy skin, constricted pupils, and sometimes bradycardia and hypotension. In serious overdosage, particularly following intravenous injection, apnea, circulatory collapse, cardiac arrest and death may occur. We believe the risk of respiratory depression leading to overdose in this study to be low for the following reasons: we have extensive experience in the administration of hydromorphone, we have chosen doses that have been demonstrated directly by us in several previous laboratory studies (99-102) to be safe and tolerable in an identical participant population, we will exclude any individual who has a medical condition that may increase the risk for respiratory depression and/or other adverse events, all medication doses will be administered by a trained research nurse, and participants will be monitored 24-hours by trained staff who will have access to the opioid antagonist naloxone to reverse the opioid agonist effects.

Chance That Participants May Begin Abusing Opioids Following Exposure to Study Hydromorphone: There is a small but potential risk that exposing non-drug abusing individuals to doses of hydromorphone designed to elicit reinforcing effects may precipitate subsequent drug-use behavior. Enrolling non-drug users into controlled laboratory studies is a conventional strategy that has been used extensively to evaluate the abuse liability potential of numerous drugs of abuse both within our laboratory and by other laboratories. We feel confident this strategy imposes minimal risk on the participants, and have provided justification for this assertion in the Protection Against Risks section.

Risks Associated with Genetic Analysis: We have established several precautions to prevent breach of confidentiality regarding genotype status that are discussed in the Protection Against Risks section. There is a low risk for breach of confidentiality regarding genotype status and that a participant's genetic information can be linked to his or her questionnaire assessments.

Discomfort from Pain Testing: Participants will likely find the pain testing procedures uncomfortable for a brief period of time. This effect is expected to be transient and to produce only mild levels of discomfort.

Breach of Confidentiality: Although staff members are highly trained to maintain participant confidentiality, there is always a risk that some of the confidential information collected could be revealed to people who are not involved in

the research study. This could be embarrassing to the participant if the participant preferred to keep his or her study participation secret, or if sensitive information became known to an individual outside the study. We have an extensive history of conducting research among substance abusers and have instituted several practices to prevent a breach from confidentiality from occurring (see below); thus, we believe this risk to be minimal.

b. Steps taken to minimize the risks.

Prior to study enrollment, a medical staff member carefully screens participants to exclude those with an increased risk of adverse events. Trained nursing staff member conducts all medication administrations, and a code to break the drug blinds is always available if needed. Specific protection against risks are outlined below.

Protection Against Hydromorphone Risks: We also have extensive experience in the administration of hydromorphone and other opioid agonists in controlled laboratory settings and therefore anticipate few problems. Any individual who may be prone to the risks associated with hydromorphone will be excluded from participating. The FDA has identified the following conditions as increasing the risk for respiratory depression or other serious side effects following hydromorphone administration: patients with status asthmaticus; chronic obstructive pulmonary disease; reduced respiratory function; high blood pressure; impairment of hepatic, pulmonary or renal functions; myxedema or hyperthyroidism; adrenocortical insufficiency; gall bladder disease; acute alcoholism; history of convulsive disorders; history of head injury; currently taking sedatives, hypnotics or phenothiazines; and sulfite allergy. Mean exposure to hydromorphone is also increased 4-fold among patients with hepatic impairment and 3-fold among patients with renal impairment. Therefore, to further reduce the risk serious side effects, we will exclude any potential participant who exhibits one or more of these conditions. Second, although research staff and participants will be blinded to the exact medication provided, both groups will be informed of the potential side effects and risks associated with the study drug administration. Third, participants will be free to discontinue study participation at any time without consequence. Great care has been taken in selecting the appropriate drug doses to ensure hydromorphone is well-tolerated and safe for our participants. Fourth, standard concomitant medications (e.g., anti-nausea medications) will also be available as needed to treat any unpleasant side effects that may occur as a result of hydromorphone administration and participants will also be able to discontinue study participation at any time. Finally, the most serious risk associated with hydromorphone administration is respiratory depression. We believe this risk is minimal in this study for several reasons. First, we have selected a range of equipotent doses that we have previously demonstrated to be safe and well-tolerated in the same participant population (non opioid dependent individuals) (99-102) and based upon a previous study that piloted and ultimately administered identical doses to an identical research sample (136). Second, we have developed several standard criteria that are followed by nursing staff and research personnel to monitor participants who have been provided with a study medication. All nursing and research staff are informed of these standards, and a list of these standards is posted in each testing room. The standards are as follows: If respiratory rate drops below 8 breaths/minute and is accompanied by sedation, participants are prompted verbally to breath. In our experience, verbal and physical stimulation is often sufficient to prompt breathing and restore a normal respiratory rate. If respiratory rate drops further and/or if oxygen saturation rates fall below 90% saturation, successive dosing is terminated. Patients are monitored carefully with a "watch and wait" approach; during this period they are accompanied continuously by a medical and/or nursing staff member, evaluated by a staff physician, and are given supplemental oxygen at 2L/min via a nasal cannula (available on site in the exam testing room). If clinical evaluation determines that a participant's sedation level is increasing, the opioid antagonist naloxone can be promptly administered via intramuscular route to produce an immediate reversal of opioid effects. There have been very few incidents throughout our >15 year experience administering opioid agonists to human participants in controlled laboratory conditions that have necessitated actual intervention (oxygen and/or naloxone), however our equipment and medical/nursing staff is always prepared for this possibility. We feel these procedures will sufficiently protect participants from possible adverse and serious adverse events. Finally, we will submit an annual review of study-related adverse and serious adverse events to the Johns Hopkins IRB and an independent group of consultants who will serve as our data safety monitoring board for evaluation.

Protection Against Chance That Participants May Begin Abusing Opioids Following Exposure to Study Hydromorphone: Another concern is the possibility that exposure of participants with no histories of drug abuse to drugs in our research setting might in some way increase the likelihood of these individuals to begin abusing illicit drugs when they return to the community. The Johns Hopkins IRB closely monitors this issue, and has repeatedly concluded that administering drugs that have reinforcing effects to individuals who do not abuse drugs is not associated with an increased propensity to begin abusing drugs. Administering drugs that may have reinforcing

effects to non-dependent users has substantial precedent in laboratory examinations of drug effects, and we have a rich and extensive history of utilizing this practice. Several research studies that have directly examined the association between study-related drug administration and subsequent drug use behavior have failed to demonstrate that controlled, laboratory drug exposure increases the risk for developing future dependence. For example, authors of a recent study that administered methamphetamine to a sample of non treatment-seeking drug abusers reported no difference between-group differences in drug use behaviors, assessed via the Addiction Severity Index, at a 6-month follow-up assessment (166). Second, a systematic follow-up study reported that alcohol-dependent volunteers randomly assigned to laboratory studies either involving or not involving experimental alcohol consumption have not differed in their follow-up outcomes (167). Third, a recent study concluded that investigational administration of intravenous cocaine to intravenous inexperienced cocaine users did not increase the risk of recreational intravenous use (168). Fourth, a study conducted by our research team administered cocaine and/or opioids to participants with histories of drug abuse and observed no significant changes in number of days of reported drug use, dollar amounts reported spent for various drug classes, or any increases in Addiction Severity Index domain scores at a one month follow-up (169). Finally, the College on the Problems of Drug Dependence, a prestigious international association of drug dependence researchers, supports the practice of enrolling non treatment-seeking individuals into drug abuse liability evaluation studies. Specifically, the College on Problems of Drug Dependence reported that exposure of drug abusers to abused drugs in a controlled research setting does not enhance the desire of an individual to use drugs, worsen addiction, or make addiction more difficult to treat (170). Overall, given the substantial data available in the literature and our own laboratory experience, we feel confident that administration of small quantities of opioids to individuals with recreational histories of drug abuse will not be associated with future drug use behavior. Ultimately, we feel the benefits of using this well-validated and heavily researched design to evaluate the association between OPRM1 status and abuse liability of hydromorphone outweighs the minimal potential risk that exposure to hydromorphone in a controlled research setting could precipitate subsequent drug use behavior. Second, the risk that drug administration may precipitate drug use behavior increases when the route of administration leads to a quicker onset of effects, as is the case with intravenous, intramuscular or intranasal administration. We have therefore chosen to administer hydromorphone in an oral formulation, which has the slowest onset of effects, in part to help mitigate this risk. Finally, both participants and research staff will be informed the study drug may consist of one or more numerous different drug classes (e.g., opioids, benzodiazepines, stimulants, over-the-counter medications); this is a widely used practice that helps reduce drug expectancy effects (97-98). This practice will also help protect against subsequent abuse because participants will not know exactly what compound produced the reinforcing effects they may have experienced.

Protection Against Risks Associated with Genetic Analysis: We believe the risks associated with breach of confidentiality regarding genetic status to be minimal because the proposed study does not fall into any of the categories identified by the NIDA Genetics Workgroup as being high risk for participants involved with genetic studies: (1) pedigree studies that aim to discover the pattern of inheritance of a disease and to catalog the range of symptoms involve; (2) positional cloning studies that aim to localize and identify specific genes; (3) DNA diagnostic studies that aim to develop techniques for determining the presence of specific DNA mutations; and (4) gene therapy research that aims to develop treatments for genetic disease at the DNA level. In addition, we can identify no *a priori* defined ethical imperative that would necessitate disclosure of the genetic testing results. For instance, given the relatively specific eligibility criteria, it is unlikely we will be enrolling members of families. Second, there is not an established association between OPRM1 status and the development of any disease- including substance abuse. If new information becomes available that unequivocally indicates a direct link between a genetic polymorphism in the OPRM1 and the development of disease, we will consult with the Johns Hopkins IRB regarding any ethical imperative that participants be informed of their OPRM1 status.

Discomfort from Pain Testing: It is likely that participants will experience some acute and transient discomfort from the pain testing session, however we will work to mitigate that risk as much as possible. First, participants will be informed of the pain testing procedure during the informed consent and will be able to make an informed decision regarding their study participation. Second, we chose pain tests (pressure pain and conditioned pain modulation) that will produce short-lived effects, and are unlikely to produce any residual pain. Third, we will stop pain administration as soon as participants report the first incidence of "pain" (operationalized as 50% on a scale of 0% (no pain) to 100% (extreme pain)). Time to first pain will serve as our dependent variable in the analyses. Fourth, we will enforce an upper limit on both pain measures to prevent any tissue damage from occurring (e.g., hand cannot be in cold pressor task for >150 seconds). Fifth, participants will be informed that they can revoke their consent to participate in the pain testing at any time without penalty. Finally, the participants will be monitored 24 hours by

study staff and will have access to medical care and concomitant medications to treat residual pain if necessary. We will also document and submit to our DSMB all adverse events reported from the pain testing session and will follow any recommendations they may have regarding the cessation of pain testing.

Protection Against Risks Associated with Confidentiality: To protect confidentiality, all research participants will be assigned unique participant identification codes that will be used on all study–related forms and online websites. Documents that include the participants' full names (e.g., signed informed consent forms) will be stored in an independent binder, consistent with FDA Good Clinical Practice Guidelines, and will be kept in a locked room. Confidential information will never be shared with anyone outside of the research program without the explicit written permission of the research participant. Only selected designated staff members will be approved to share confidential information after explicit written permission is obtained from the participant and the participant will be able to revoke written permission at any time. In accordance with IRB requirements, all research staff will be formally trained in these procedures. No identifying participant information will be used in written reports, manuscripts and/or conference presentations.

c. Plan for reporting unanticipated problems or study deviations.:

All adverse events will be reported to the IRB and other relevant agencies (e.g., FDA, NIDA) as required and as described in the DSMP. The Principal Investigator and Co-investigators are responsible for reporting such events.

d. Legal risks such as the risks that would be associated with breach of confidentiality.:

Although there is always a small risk that confidentiality will be breached, we believe this risk to be very minimal with the current study.

e. Financial risks to the participants. None.

9. Benefits

Description of the probable benefits for the participant and for society: The long-term goal of this research is to investigate individual differences in response to opioids, and identify genetic differences that contribute to variations in the susceptibility of opioid dependence. The objective of this application is to determine whether the OPRM1 gene predict one of several opioid-mediated effects using a rigorous, controlled laboratory study among healthy individuals with no history of drug dependence and whether this effect differs as a function of gender. This study will constitute the most controlled, rigorous, comprehensive examination of the OPRM1 gene association with opioid-mediated effects to date. We expect that genotype will be associated with several opioid-mediated effects. These results are expected to have a positive impact by advancing our understanding of the association between the OPRM1 gene and opioid-mediated effects, including susceptibility for opioid dependence. These data will provide positive contributions to the field of pharmacogenomic medication prescribing, the development of opioid dependence prevention strategies and interventions to treat comorbid pain and opioid dependence, and will have implications for other MOR-mediated effects, including alcohol dependence.

10. Payment and Remuneration

a. Detail compensation for participants including possible total compensation, proposed bonus, and any proposed reductions or penalties for not completing the protocol.

Participants will be paid $30 for completing the Screening visit. Enrolled participants will earn $50 for each session day and will incur a completion bonus that will escalate in value with each subsequent session. Bonuses will be earned for completion of the study only and any participant who ends the study prematurely will receive their session payments but will forfeit the completion bonus. Participants can earn up to $750 in payments and bonuses for study participation.

Study Day	Session Payment	Completion Bonus	
Admission	$50.00	$50.00	
Day 2	$50.00	$75.00	
Day 3	$50.00	$100.00	
Day 4	$50.00	$125.00	
Day 5	$50.00	$150.00	
Total:	$250.00	$500.00	$750.00

11. Costs

a. Detail costs of study procedure(s) or drug (s) or substance(s) to participants and identify who will pay for them.

All study procedures and study drug will be supported by a grant from the National Institute on Drug Abuse and will be provided to participants free of charge.

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