

Protocol Title: OPRM1 A118G SNP Effect on Striatal Dopamine Response to an IV Opiate

Abbreviated Title: Genetics, DA Release & Opiate

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Total requested accrual (*separately specify planned accrual for each subject group*)

(0) Patients

(120) Healthy Volunteers

Project Uses Ionizing Radiation:  No  Yes (*attach RSC/RDRC documentation*)

Medically-indicated only

Research-related only

Both

IND/IDE  No  Yes (*attach FDA documentation*)

Drug/Device/# IND 54135 for 11C Raclopride

Sponsor: NIH Clinical Center-PET Imaging Dept

Durable Power of Attorney  No  Yes

Multi-institutional Project  No  Yes

Institution#1 \_\_\_\_\_ FWA # \_\_\_\_\_

Date of IRB approval \_\_\_\_\_ (*attach IRB documentation*)

Institution#2 \_\_\_\_\_ FWA # \_\_\_\_\_

Date of IRB approval \_\_\_\_\_ (*attach IRB documentation*)

*(If NIH is the coordinating site, list each institution; attach documentation of IRB approval for each site)*

*(If NIH is not the coordinating site, list the coordinating site; attach documentation of IRB approval for lead site)*

Data and Safety Monitoring Board  No  Yes

Technology Transfer Agreement  No  Yes

Agreement type and number #30749-10 Expiration Date July 29, 2016 with  
University of Memphis to cover the AutoSense in the opiate PET study

Samples are being stored  No  Yes

Flesch-Kincaid reading level of consent form: 8.6

*(exclude boilerplate in assessing reading level)*

## **Précis:**

### Objectives

Mesolimbic dopamine (DA) release is a key signal for drug reward, and endogenous opioids are thought to exert their effects in part by modulating the activity of this system. A functional  $\mu$ -opioid receptor (OPRM1) A118G single nucleotide polymorphism (SNP) has been associated with increased risk for heroin addiction in some studies. This polymorphism has been shown to confer differential pain sensitivity and to alter the release of DA following an alcohol challenge. The objective of this study is to examine the role of the A118G OPRM1 polymorphism for responses to a challenge of an opiate (morphine) with regard to psycho-physiological variables measured in the laboratory and for brain dopamine release measured by [ $^{11}\text{C}$ ]raclopride PET.

### Study population

Healthy male participants who have had experience with oral prescription analgesics (e.g., Oxycontin, Vicodin, Percocet, oxycodone) will be recruited for the study. These volunteers will be screened to obtain samples of two groups of subjects: 1) persons homozygous for the major 118A allele (118AA genotype); 2) persons carrying one or two copies of the variant 118G allele (118AG or 118GG genotype, hereafter called 118GX). We will recruit up to 120 participants to obtain 40 completers per genotype for the study.

### Design

We will compare the response of these groups to a challenge with morphine given intravenously. Participants will receive a standardized IV challenge of morphine (10.0 mg/70 kg over 1 minute; morphine concentration 2 mg/ml). Pre and post injection measures will be made in two areas: 1) subjective response as measured by standardized questionnaires, and 2) measures of physiological response, including pupil response to light, respiratory rate, oxygen saturation and a pain rating from putting a hand in cold water and blood chemistries. In addition, during this visit, participants will wear the AutoSense mobile physiological monitor; parameters measured by AutoSense include respiration rate, heart rate, heart-rate variability, skin conductance, and activity level. The injection will be repeated in all participants in the PET scanner, once with morphine and once with normal saline. Dopamine release will be assessed by determining the difference between the binding potential for [ $^{11}\text{C}$ ]raclopride, a positron emitter labeled ligand which binds preferentially to D2 receptors during saline administration and its binding potential during morphine administration.

### Outcome measures

We hypothesize that 118GX subjects will have significantly different subjective response to the challenge than 118AA subjects as observed in participants receiving alcohol in a similar study (Ramchandani et al. 2011). However, the genotype effect on the response may be opposite from the effect of genotype on the alcohol response. We also hypothesize that the PET studies with [ $^{11}\text{C}$ ]raclopride will show that 118GX subjects have less dopamine release during morphine administration than 118 AA subjects.

## Table of Contents

1. Introduction/ Scientific Rationale .....	5
2. Study objectives or hypotheses .....	8
3. Subjects .....	9
4. Study Design and Methods .....	11
5. Storage of data and samples:.....	19
6. Additional Considerations .....	20
7. Risks/ discomforts.....	20
8. Subject monitoring.....	23
9. Outcome measures .....	23
10. Statistical Analysis.....	24
11. Human Subjects Protection.....	25
12. Anticipated Benefit .....	28
13. Summary/ classification of risk: .....	28
14. Consent documents and process .....	29
15. Data and Safety monitoring .....	29
16. Quality Assurance .....	30
17. Adverse event reporting .....	31
18. Alternatives to participation or alternative therapies .....	31
19. Confidentiality .....	31
20. Conflict of Interest .....	31
21. Technology Transfer .....	32
22. Research and Travel Compensation.....	32
23. References.....	33
Appendix I - Questionnaires .....	38
Appendix II. Recruiting advertisements .....	49
Appendix III. Screening questionnaires for Patient Recruitment Office .....	50
Appendix IV. Investigator’s Brochure.....	53
Appendix VI. Other – Evaluation to sign consent and expected answers .....	54
Appendix VII. Results from mouse study.....	56
Appendix IX. 1SE Inpatient Nursing Information Sheet.....	57
Appendix X. Plan for clinical monitoring of participants receiving morphine at NIDA .	59
Appendix XI. Plan for clinical monitoring of participants receiving morphine at the NIH Clinical Center in the Course of PET sessions .....	61

## List of Abbreviations

ACTH - Adrenocorticotrophic hormone  
aCPRS – abbreviated Comprehensive Psychopathology Rating Scales  
BPI - Brief Pain Inventory  
DA – dopamine  
DEQ – Drug Effects Questionnaire  
DTI – Diffusion Tensor Imaging  
OPRM1 – gene for  $\mu$  opiate receptor subtype 1  
PSQI - Pittsburgh Sleep Quality Index  
ROI – Region of Interest  
VAS - visual analog scale

# 1. Introduction/ Scientific Rationale

## Background

Dependence on oral prescription analgesics has become an increasing problem for young people in the US. Among adolescents, prescription and over-the-counter medications account for most of the commonly abused illicit drugs by high school seniors (<http://www.drugabuse.gov/publications/topics-in-brief/prescription-drug-abuse>). Prescription opioids interact with  $\mu$ -opioid receptors. All opioids can be highly addictive and altering the route of administration (e.g., snorting or injecting) can intensify the effect. Furthermore some abusers of prescription opioids report moving from prescription opioids to heroin or resorting to using heroin when they cannot get the pills. Of all abused substances, opioid abuse has the highest death rate among users (Callaghan et al. 2012). Abuse or dependence on prescription opioids is estimated to occur in about 1.9 million people in the U.S. Furthermore, men were more likely than women to endorse addictive behaviors related to prescription opioids (early prescription refills, physician refusal to prescribe because of abuse concerns; odds ratio 1.95; (Glass et al. 1993)).

The heritability for opioid dependence has been estimated to be about 50% (Tsuang et al. 1996;Tsuang et al. 1998). Genetic variation at the  $\mu$ -opioid (OPRM-1) gene locus is an important candidate factor for susceptibility to alcoholism, differential alcohol responses, and differential treatment responses to pharmacological treatments and is also thought to be a factor in opiate abuse. The endogenous opioid system is central to the function and modulation of mesolimbic dopaminergic signaling, a physiological system likely involved in the reinforcing properties of all drugs of abuse with the exception of sedative-hypnotics (Kreek et al. 2002;Contet et al. 2004). Quantitative trait locus studies and experiments with OPRM1 knockout mice have demonstrated the critical role played by this receptor in both opiate and alcohol self-administration (Becker et al. 2000;Belknap and Crabbe 1992;LaForge et al. 2000;Matthes et al. 1996;Roberts et al. 2000).

A common functional A118G single-nucleotide polymorphism (SNP) has been identified in the coding region of exon 1 of the OPRM1 gene which has a high-enough frequency (10.5% in a New York City population (Bond et al. 1998), 26% in a European population (Bart et al. 2004), 24% in a European-American population (Zhang et al. 2006), 40% in a study of a Chinese population (Zhang et al. 2007), and up to 60% in some Asian populations (Tan et al. 2003)) to make it a practical candidate for prospective research. In one study the A/G genotype was present in significantly more heroin users than in controls (25.6% vs. 3.8%)(Drakenberg et al. 2006), but another study did not find a greater incidence of the A/G genotype in either heroin or alcohol abusers (Franke et al. 2001).

Human association studies have been inconsistent in demonstrating a relationship between and heroin addiction and the A118G SNP. Szeto et al. (2001) found a significant association with the 118G variant among 200 heroin addicts in Hong Kong compared to 97 healthy controls. The G allele was present in 39.5% of the addict population, vs. 29.4% of controls, with 16.5% of the addicts being GG homozygotes vs. 11.3% in controls ( $p=0.02$ ). Tan et al. (2003) found highly significant differences between ethnic groups among four different Asian populations in allele and genotype frequencies; however, the only significant association between heroin dependence and

genotype distribution ( $p=0.024$ ) and allele frequency ( $p=0.009$ ) was in Indians. Bart et al. (2004) as well as Drakenberg et al. (2006) found an association between G-allele carrier status and heroin dependence, but another study did not find a greater frequency of the G allele in either heroin or alcohol abusers (Franke et al. 2001). Inconsistent findings in association studies are common in psychiatric genetics, in part due to limited power of highly heterogeneous categorical disease phenotypes. Experimentally based intermediate phenotype approaches are therefore an important complement to association studies.

In that context, a [ $^{11}\text{C}$ ]raclopride PET study showed a robust release of dopamine following an alcohol challenge compared to placebo in social drinkers who carried the G allele (Ramchandani et al. 2011). The effect of the G allele on  $\mu$  opioid receptor binding with PET and [ $^{11}\text{C}$ ]carfentanil has been studied in alcohol-dependent participants and in smokers (Weerts et al. 2012; Ray et al. 2011). There was no effect of alcohol dependence on the binding of [ $^{11}\text{C}$ ]carfentanil, but carriers of the G allele had lower global binding potential ( $\text{BP}_{\text{ND}}$ ) for the radioligand (Weerts et al. 2012). A similar lower binding potential was observed in smokers carrying the G allele, and the lower binding potential in some of the regions assayed was associated with greater subjective reward from smoking (Ray et al. 2011).

The effect of the G allele on pain perception has been studied in various conditions. Fillingim et al. (2005) demonstrated a significant difference in pressure pain threshold in individuals with the 118G variant SNP. Interestingly, this threshold difference was higher among men and lower among women with the 118G SNP as compared to controls. Nonetheless, the effect of fentanyl on perceived labor pain was greater in women carrying the G allele than in women homozygous for A resulting in the need for less analgesic (Landau et al. 2008). However the effect was opposite in a study by Oertel et al. (2008).

Bond et al. (1998) using *Xenopus* oocyte cells, found that receptors encoded by the variant 118G allele showed increased affinity for the endogenous  $\mu$ -opioid ligand  $\beta$ -endorphin *in vitro*, leading to increased activation of G-protein activated inwardly rectifying potassium channels following binding of  $\beta$ -endorphin (Bond et al. 1998). However, Befort et al. (2001) found no difference in binding affinity of  $\beta$ -endorphin between wild type and A118G-coded  $\mu$ -opioid receptors using an *in vitro* model with COS cells. Similarly, Beyer et al. (2001) found no difference in  $\mu$ -opioid binding between wild type and A118G coded receptors using an *in vitro* HEK 293 cell model. These discrepant *in vitro* results reinforce the importance of examining the functional role of the  $\mu$ -opioid receptor in the correct context, both at the cellular level, where coupling, etc. may be determined by cell type, and at the level of the integrated organism.

Using mouse lines that were modified to express either the human A allele or G allele for the OPRM1, Ramchandani et al. (2011), found a 4-fold greater dopamine release from an ethanol infusion in the mice carrying the G allele. However, *in vitro* autoradiography of the  $\mu$ -opioid receptor with [ $^3\text{H}$ ]DAMGO showed no difference between mouse lines. Current studies in collaboration with Drs. Shippenberg and Chefer at the NIDA IRP are testing dopamine release by microdialysis and with Mr. J. Elliot Robinson at the University of North Carolina are testing the effects on intracranial self stimulation (ICSS)

in these mouse lines in response to various opioid agonists. The results of these studies showed that morphine, but not fentanyl, oxycodone or buprenorphine reduced the threshold for ICSS in the mice homozygous for the A allele, but not in those mice homozygous for the G allele. The microdialysis studies with morphine were consistent with the effects on ICSS; morphine induced release of dopamine was lower in the G/G mice than in the A/A mice. These results suggest that the discrepant results observed in genetically engineered cells designed to evaluate the effect of the 118G allele on  $\beta$ -endorphin binding and G-protein mediated intracellular effects (Bond et al. 1998; Beyer et al. 2004) may have been a result of using cells artificially modified to express the A118G-coded receptor and thus may be an artifact of the cell line or genetic engineering model selected.

The mixed results in human population studies of the relationship between the A118G polymorphism and substance dependence may be confounded by two important issues. First is the inherent difficulty of isolating a small factor such as SNP genotype in a large and diverse population. Second, the role played by the A118G SNP in heroin dependence (if any exists) may be more evident in the initiation or development of the drug-taking behavior in younger or more naïve populations than its maintenance in mature or dependent populations. A previous [<sup>11</sup>C]raclopride PET study showed a robust release of dopamine following an alcohol challenge compared to placebo in social drinkers who carried the G allele (Ramchandani et al. 2011). PET studies of dopamine release after an opioid challenge have been inconsistent (Spreckelmeyer et al. 2011; Hagelberg et al. 2002; Daglish et al. 2008). The reasons for these inconsistencies are unknown, but may be due to the genetic makeup of the participants, which was not tested or to the opioid used as the challenge. In the study by Spreckelmeyer et al. (2011), remifentanyl (0.3  $\mu$ g/kg) was used and the authors suggest that this drug is most similar to the endogenous opioid,  $\beta$ -endorphin. No human studies to date have tested the effect of morphine on dopamine release, but it is expected to cause dopamine release as observed with microdialysis in rodents.

As the study outlined in the present protocol aims to examine whether OPRM1 A118G variation moderates the mesolimbic DA response to  $\mu$ -opioid receptor activation, it is important that we have a signal that could be modified. Using non-human primates, it was previously found that the rhesus ortholog of the human OPRM1 A118G SNP only moderated alcohol-induced psychomotor stimulation (a marker of mesolimbic DA activation) in males but not in females (Barr et al. 2007). This difference was presumably due to the negligible overall psychomotor response in the latter group. In agreement with those observations, a subsequent human PET study (Urban et al. 2010; Barr et al. 2007) found only marginal alcohol-induced DA release (measured as % change in [<sup>11</sup>C]raclopride binding potential) in females, while a robust response was present in males. Alcohol activates mesolimbic DA neurons indirectly, through release of endogenous opioids and their actions at  $\mu$ -opioid receptors (Heilig et al. 2011; Urban et al. 2010). Because endogenous opioid release in response to alcohol does not differ between men and women (Mitchell et al. 2012), it must be concluded that the observed sex differences are likely to be postsynaptic. A similar sexual dimorphism is therefore likely to exist for actions of an exogenous  $\mu$ -agonist. Taken together, these observations thus

make it unlikely that the hypothesis of the present study can be addressed in females, providing a compelling scientific rationale for restricting recruitment to males.

We will therefore examine the role of the A118G SNP for subjective and objective responses to a standardized intravenous morphine challenge in opioid experienced, non-dependent males. Subjective psychological and objective psycho-physiological responses, as well as brain dopamine release will be examined in response to the challenge, all using well-established methods as described below. To effectively investigate a relationship between this polymorphism (A118G) and the endophenotype in question (persons likely to abuse/become dependent on opiates), we plan to identify an adequate sample of subjects with and without the genotype in question, but without opiate dependence, and compare their responses to an opiate under controlled conditions. As the frequency of the G allele varies among ethnic populations, we will obtain self-reports of race and ethnicity and obtain ancestry scores from large SNP panels to control for potential differences between carriers of the G allele and A allele homozygotes.

The following tests are known to be sensitive to opioid effects and will be used in this study:

1. McGill Pain – Cold Pressor Test (Posner et al. 1985)
2. Pupillary constriction
3. Drug Effect Questionnaire (DEQ) (Holdstock and de Wit 1999)
4. Respiratory Rate
5. Pulse Oximetry (a measure of oxygen saturation in blood).

Name and description of treatment(s): [<sup>11</sup>C] raclopride will be administered under an IND held by the Clinical Center PET Department.

Morphine (10 mg/70 kg) will be administered as a sterile solution of 2 mg/ml over 1 minute.

Subjects will also receive an MRI (about 5 minutes) for co-registration with the PET. In addition, a resting scan (approximately 5 minutes) to assess connectivity of brain regions at rest and a diffusion tensor imaging (DTI) scan (about 7 minutes) to assess white matter integrity will be acquired. The purpose of these MRI scans is to determine if there is an effect of the OPRM1 genotype on brain connectivity (resting state), and its relationship to genetic-induced alterations in dopamine release. In addition, the structural MRI is required for the PET scan (and functional scan) alignments. These tests will be done only once and not performed while the participant is receiving the opiate challenge. The DTI scans are being performed to examine the relationship of white matter integrity to the above dependent variables.

Statement of GCP: The study will be carried out following standard guidelines for good clinical practice.

## **2. Study objectives or hypotheses**

### **a. Hypotheses:**

Homozygous carriers of the 118A allele of the  $\mu$ -opioid receptor will show greater positive subjective response to the morphine than those carrying one or two copies of the



118G allele (118GX), paralleled by lower binding potential for [<sup>11</sup>C] raclopride following morphine injection because of greater dopamine release than carriers of the G allele.

Objectives:

**a.Primary objectives:** To examine the role of the  $\mu$ -opioid receptor polymorphism OPRM1 A118G on the subjective and objective effects, including dopamine release, of the  $\mu$ -opioid agonist morphine.

**b.Secondary objectives:** A number of exploratory analyses will be carried out with the collected data to generate hypotheses on the relation of patient characteristics (e.g. family history of alcohol/drug use disorders, pain, mood, etc.), physiological opiate responses (e.g., pupil constriction, cardiovascular parameters such as respiratory rate and % oxygen saturation as measured by Pulse Ox), endocrine responses (cortisol, ACTH), pain threshold (measured by cold pressor test), MRI scans, and positive psychological effects (as measured via visual analog scale) with DA release measured by PET.

### 3. Subjects

#### a. Description of study populations

##### *i. Brief description of subject groups*

Subjects will be 21-55 year-old, non-nicotine dependent male volunteers who have taken prescription pain pills without negative effects in the past, but are not currently taking them or dependent on them. All participants will be in good health, non-smokers or light smokers (< 20 cigarettes/week), current non drinkers or social drinkers; participants should not meet DSM IV criteria for alcohol abuse or alcohol dependence. Subjects will be recruited into two groups: 1) subjects homozygous for the major 118A allele (118AA genotype) of the OPRM1 polymorphism; 2) subjects carrying one or two copies of the variant 118G allele (118AG or 118GG genotype, hereafter called 118GX) of the OPRM1 polymorphism.

The requirement that there is experience with prescription pain pills is in place because we do not wish to administer an opiate to naïve volunteers. Prior experience with opioid pain medications enhances safety and aids in the exclusion of potential participants who have had negative experiences (e.g., allergic response, excessive nausea and/or vomiting, itching) as these side effects could make obtaining the PET scan difficult. The experience of an individual who has taken even one or two prescription pain pills should provide enough information regarding negative side effects to eliminate people with allergies or negative experiences. In addition, participants will be excluded if they have used pills chronically (> 6 weeks) or recently (within the previous 3 months). The purpose of these criteria is to remove possible residual effects on the receptor.

We have chosen to restrict the study population to ages 21-55 years to reduce age-related variability in the study. Since the ventral striatum shrinks an average of 3.6 to 3.7% per decade (Gunning-Dixon et al. 1998), a wider age range introduces significant and avoidable variance in the study group.

We have chosen to restrict the study to men only based on the lack of an effect in women on DA release during the administration of alcohol as the effect of alcohol on DA release

is presumed to occur through the  $\mu$ -opioid receptor (Barr et al. 2007; Mitchell et al. 2012; Urban et al. 2010).

- ii. *Target number of completers*: 80 completers
- iii. Withdrawals or dropouts will be replaced

**b. Inclusion criteria**

1. Male participants between 21-55 years of age.
2. Good health as determined by medical history, physical exam, EKG and lab tests.
3. Current non-smokers or light smokers or e-cigarette users (<20 cig/week) who can easily abstain from smoking or using e-cigarettes for 1-2 days/week.
4. Current non-drinkers or social drinkers who do not meet past or current DSM IV criteria for alcohol abuse or alcohol dependence.
5. An equal number of final participants will be of OPRM1 118 A/A vs. 118A/G or 118G/G genotype. This means that after the first group (n40) is complete then only participants with the required genotype for the other group will be included.
6. Prior opiate use, at least one experience with one of the opiates in the list in Appendix 1 (p. 34).
7. Comprehension/fluency with English Language.

**c. Exclusion criteria**

1. Current or prior history of any significant disease, including cardiovascular, respiratory, gastrointestinal, hepatic, renal, endocrine, or reproductive disorders, or a positive hepatitis or HIV test at screening, disorders that could make administration of an opiate more risky (e.g., asthma, COPD, sleep apnea, or other breathing disorders; liver or kidney disease; thyroid disorder; trouble swallowing, or a blockage in the digestive tract (stomach or intestines); neurologic disorders (e.g., a history of head injury or brain tumor, epilepsy or other seizure disorder, CVA, migraine in treatment, etc.); low blood pressure; hypertension; neuromuscular disorder; gallbladder disease; Addison's disease or other adrenal gland disorders; enlarged prostate, urination problems)
2. Current Axis-I psychiatric illness as determined by the Structured Clinical Interview for DSM IV disorders (SCID).
3. Current or prior history of any alcohol or drug dependence as determined by the Structured Clinical Interview for DSM IV disorders (SCID).
4. Positive result on urine screen for illicit drugs.
5. Medication use:
  - a) Current chronic prescription or over the counter medications or use of prescription or OTC medications known to interact with dopamine receptors within 2 weeks of the study.
  - b) Drugs known to inhibit or induce enzymes that metabolize opiates should not be used for 4 weeks prior to the study. These include chlorzoxazone, isoniazid, metronidazole and disulfiram.
  - c) Cough-and-cold preparations that contain anti-histamines or opiate pain medicines will be withheld for at least 72 hours prior to each study session.

- d) Drugs that may interfere with the BOLD MRI signal within 2 weeks of the study. These include, but may not be limited to: muscle relaxants or respiratory, cardiovascular or anticonvulsant medications.
6. Morbid obesity (BMI >40 kg/m<sup>2</sup>)
  7. Previous negative effects of opioid administration
  8. Presence of certain implanted devices (cardiac pacemaker or neurostimulator, some artificial joints, metal pins, surgical clips or other implanted metal parts), body morphology, or claustrophobia. *Justification:* Implanted devices may increase the risk of MRI scanning and/or adversely affect the quality of the data; body morphology may prevent optimal positioning in the scanner and thus affect the quality of the data; participants with claustrophobia may find the MRI scan too unpleasant and may exhibit excess movement that will adversely affect the quality of the data. *Assessment tool(s):* Prospective participants will fill out an MRI screening questionnaire and undergo an interview with an MR technologist. Questions concerning suitability for scanning will be referred to the Medical Advisory Investigator. Prospective participants will be questioned about symptoms of claustrophobia and placed in the mock scanner during their first visit to assess for possible difficulty tolerating the confinement of the scanner and for ability to fit into the scanner.
  9. Conditions restricting participant's ability to lie flat for up to two hours (such as coagulopathies, superficial or deep vein thrombosis, or musculoskeletal abnormalities). *Justification:* PET scanning sessions require participants to lie flat on their backs and remain perfectly still for approximately two hours. Therefore, conditions that would make that difficult (e.g. chronic back pain, significant scoliosis) or dangerous (e.g. familial hypercoagulability syndrome, history of thrombosis) will be exclusionary. *Assessment tool(s):* History and physical examination by a qualified IRP clinician, supplemented with a trial of lying in the mock scanner to assess comfort.
  10. Head trauma leading to loss of consciousness for more than 5 min or hospitalization
  11. Exposure to ionizing radiation from research studies that, in combination with the study tracer, would result in cumulative exposure of >5 rem within the previous 12 month period.
  12. Self-reported and/or observed signs, symptoms, or diagnosis of Raynaud's or Buerger's disease (e.g., pain in hands or feet at times of rest, during/following cold exposure or stress, any significant color changes in hands or toes). Additionally, medical staff will be present to watch for these symptoms during the actual cold pressor test.

## 4. Study Design and Methods

### a. Study overview –

The study will require at least 4 visits by the participants. Each visit will last approximately 5-8 hours. Visits 1 (consent visit) and 2 (toleration visit) will be at the BRC in Baltimore, the other two visits (Visits 3 and 4) will be conducted in the NIAAA Outpatient clinic and the PET scanning facility of the Clinical Center in Bethesda. The

first two visits will be outpatient visits. Participation in 10-DA-N457 will be encouraged, but not required. Protocol 10-DA-N457 provides for more generalized genetic testing in conjunction with other neuropsychological tasks. Each participant will be scheduled for structural, resting state and diffusion tensor imaging MRI scans on one of the study days or on a separate day if that is not possible in Baltimore. The participants will spend the night before each of the PET studies in the NIAAA inpatient ward (1SE Unit) at the NIH Clinical Center.

During the screening visit performed under the screening protocol (06-DA-N415), MMG will screen healthy participants from a population who have had experience with oral prescription analgesics to obtain samples of two groups of subjects: 1) subjects homozygous for the major 118A allele (118AA genotype) of the OPRM1 polymorphism; 2) subjects carrying one or two copies of the variant 118G allele (118AG or 118GG genotype, hereafter called 118GX) of the OPRM1 polymorphism. At the time of consent to 13-AA-0061 the entire study will be explained and the participant given the opportunity to enroll in the study. During the consent visit (hereafter called Visit 1 of the protocol) or the next visit, participants will be given a questionnaire to evaluate his previous response to oral prescription analgesics (See Appendix I). During Visit 1 or another visit, the participant will undergo the MRI portion of the study.

During Visit 2, participants, who successfully pass the initial screening and enroll in the study after the consent interview, will receive a placebo injection and subsequently a standardized opiate challenge. The opiate challenge will be a standardized IV injection of morphine, (10.0 mg/70 kg; 2 mg/ml) administered over 1 minute.

Post-placebo) and post-morphine injection measures will be made in two areas: 1) subjective response as measured by a standardized questionnaire (DEQ), and 2) objective measures response. We will determine their response to a cold pressor pain test, and a measurement of their pupillary constriction as well as several other tests of their response to the morphine, including continuous Pulse Ox monitoring and intermittent monitoring of their respiratory rate, heart rate, EKG and blood pressure. Blood will be collected to measure plasma ACTH and cortisol levels as they may be suppressed by activation of the  $\mu$ -opioid receptor. Retrospectively, participants will be asked to evaluate their peak experience to the morphine on a Likert-like visual analog scale (VAS), which will include sedation, euphoria, dizziness, fatigue, depression, tremors, anxiousness, upset stomach/nausea, dry mouth, and headache, which are common side effects of opiate administration (Appendix I). We hypothesize that 118AA subjects will have significantly higher subjective response to the morphine challenge than 118GX subjects.

On two more separate visits, at least a week apart (Visits 3 and 4), participants will report to the Clinical Center in Bethesda for an overnight stay before each PET scan. We will repeat the injection procedure in all participants twice in the PET scanner, once injecting morphine and once injecting saline, and assess the binding potential of [ $^{11}\text{C}$ ]raclopride, a positron emitter labeled ligand which binds preferentially to D2 dopamine receptors. The difference between the binding potential following the saline injection and that during the morphine injection will provide a measure of dopamine release. We hypothesize that 118AA subjects will have a lower binding potential with [ $^{11}\text{C}$ ]raclopride from the morphine injection relative to placebo, indicating greater amounts of dopamine release than those subjects carrying one or two copies of the G allele.

## **b. Recruitment**

This study employs an experiment of nature, i.e. genetic variability, as a basis for group assignment. To obtain the respective groups, this protocol will be conducted in the following phases:

1. Recruitment of potential participants through approved advertising media (see draft ad in Appendix II).
2. Thorough screening of selected participants according to 06-DA-N415 (“Screening Protocol for the Evaluation of Potential Research Subjects”).
3. Genotyping, which will allocate subjects into two groups: A) 118AA homozygotes, and B) 118AG heterozygotes, and 118GG homozygotes
4. An invitation to members of each group to join the study with an effort to balance the groups demographically, most likely by trying to match group B.

Ads will be placed in approved media seeking healthy volunteers between ages 21-55 years. The ads will specify that volunteers must have had experience with prescription analgesics (e.g., Oxycontin, Vicodin, oxycodone), but are no longer needing these medications. They must be willing to undergo genotyping to determine whether they are homozygous, heterozygous, or negative for the 118G SNP (as per methodology of Skarke et al. (2004)).

## **c. Screening**

An initial screen will be accomplished by phone using the standard MMG phone screen. If the participants appear to qualify based on the inclusion and exclusion criteria, they will be invited for an in-person screening visit, consisting of informed consent procedures, blood draws for genotyping and basic medical evaluation for inclusion/exclusion criteria. The screening evaluation will include:

1. medical history
2. drinking and drug use history using the Drug Use Survey
3. Structured Clinical Interview for DSM-IV (SCID) psychiatric diagnoses including alcohol or drug dependence as currently administered by MMG.
4. physical examination, including EKG
5. fasting blood samples for routine blood chemistry, complete blood count (CBC), liver function, hepatitis B and hepatitis C, a serum HIV screen, and TSH
6. urine sample for illicit drug screen
7. MRI screen.
8. The determination of a lack of nicotine dependence will be based on the potential participant’s stated ability to regularly abstain from smoking for 1-2 days/week.
9. Tuberculin skin testing

The study will be open to participants of all races. All participants will be recruited through approved advertising media. Participants go through a consent interview during which the study will be fully explained. If they chose to enroll, they will sign the informed consent form prior to enrollment in the study.

## **d. Study Procedures**

Study Overview:

Visit 1 – This visit will involve reviewing the consent and if the person agrees to participate, may involve additional questionnaires and the MRI scans. It may be necessary to schedule these procedures on a separate day.

Visits 2-4 -Subjects will receive four injections on three separate days, two morphine injections and two placebo injections. During Visit 2, the first injection will be placebo (normal saline) and the second will be morphine to measure its subjective and physiological effects prior to the PET sessions; the other two injections, morphine or placebo, will be given while the participant is undergoing a PET scans with [<sup>11</sup>C]raclopride (Visits 3 and 4). The injections during Visit 2 will be conducted at the BRC. The injections during Visits 3 and 4 will be conducted during PET in the Nuclear Medicine department at the NIH Clinical Center.

During the study visits that do not involve the PET scans, participants will arrive at the BRC by 8:00AM on the morning of testing, following an overnight fast, and after abstaining from alcohol and all medications for 72 hours. To minimize variance in caffeine consumption, patients will be asked to report to the clinic having had no caffeinated coffee, tea, or soda in the morning.

On days when participants could receive morphine (Visits 2, 3 or 4), they will be brought to the BRC or Clinical Center in a taxi paid for by NIH. Participants will receive a light breakfast (~300 cal). A breathalyzer test will be performed to ensure a zero breath alcohol concentration and a CO test for recent smoking or exposure to second hand smoke (a CO over 15 ppm indicates likely recent smoking and participant will be disqualified). For subjects reporting use of e-cigarettes, recent use will be assessed by performing a cotinine salivary test such as NicAlert (a cotinine value over 1 indicates likely recent e-cigarette use and participant will be disqualified).

During Visit 1 after giving informed consent or in a subsequent separate visit (herein termed Visit 1a), participants will complete assessments of baseline pain, sleep, and depression/anxiety as these may impact subjective assessment of drug effects - Brief Pain Inventory (BPI), abbreviated Comprehensive Psychopathology Rating Scales (aCPRS), WHO QOL Bref, Sleep Disorders Questionnaire, and Pittsburgh Sleep Quality Index (PSQI). The MRI scans, which will be completed before Visits 3 and 4, may occur during Visit 1 or 1a.

During Visit 2, physiological responses (RR, HR, EKG and oxygen saturation) will be monitored using standard instrumentation (EKG, pulse oximetry) continuously for 60 min after injections or until they return to normal and values for these parameters as well as for BP will be recorded approximately every 5 min for the duration of the drug effect. In addition, physiological responses will be collected continuously on the AutoSense, a wearable suite of sensors for physiological monitoring in the daily environment. AutoSense was developed by a group of computer scientists at the University of Memphis, led by Dr. Santosh Kumar. Parameters measured by AutoSense include respiration rate, heart rate, heart-rate variability, skin conductance, and activity level. The sensors are hosted on a tiny computing platform called a wireless sensor mote (with dimensions similar to those of the memory cards used in digital cameras). Sensors are attached to an elastic belt that is worn around the chest. Two electrodes, similar to those used in ECGs, are placed on the chest and connected to the sensors in the belt. Data are

sent wirelessly to a pre-programmed smart phone for temporary storage. These data collected during administration of morphine under controlled laboratory conditions will be used to develop an algorithm to detect opioid administration in future field studies.

**MRI scans:**

All MRI scans will be performed in the Trio at the BRC. The participants will not receive any drug before these scans. Under this protocol or 10-DA-N457, a structural MRI of the brain for co-registration with the PET scan as well as resting state and DTI MRI scans of the brain will typically be obtained on the day of Visit 1 or 1a at the BRC. As many participants enroll in multiple studies, the participant may have already enrolled in 10-DA-N457 and the scans acquired under that protocol may have already been performed and will be used for this protocol.

Repeating the MRI scans - Because excessive head movement is an obstacle to obtaining quality fMRI data, participants who generate unusable data (or if there is an equipment failure) during their scanning session may be asked to repeat the session. Repeating the session will maximize the risk/benefit ratio of the MRI scanning as it will allow completion of all aspects of the study.

Participants will be given a meal once collection of dependent measures is complete. After the meal, orthostatic vitals will be obtained and if there is no evidence of symptomatic orthostasis the participant will be discharged.

Visit 2 Morphine injection with subjective and physiological measures (Toleration Study):

The purpose of this visit is two-fold. The first is to reduce the likelihood that side effects like vomiting will occur in the PET scanner. The second is to obtain data on the participant’s responses (pain response and pupil response) to morphine at a time when the participant is not in the scanner when such measurements would be more difficult to acquire. Following a prestudy nursing assessment, which includes urine toxicology, breathalyzer, CO monitoring, HR, BP, RR, and weight, two indwelling intravenous catheters will be inserted, one each into the antecubital vein of the arm using sterile technique. The following table summarizes the prestudy nursing assessments obtained:

<b>Measurement</b>	<b>Parameter(s) to proceed</b>
Breathalyzer	Negative
CO test	≤ 15 ppm
Urine toxicology	Negative
Heart rate*	≥50 bpm and ≤105 bpm
Systolic blood pressure*	≥95 mmHg and ≤135 mmHg
Diastolic blood pressure*	≥60 mmHg and ≤90 mmHg
Respiratory rate*	≥8 and ≤24 breaths per minute
Oxygen saturation*	≥94%

One catheter will be used for the placebo and morphine injections and the other will be used for blood sampling. A nurse will monitor HR, BP, RR, ECG and blood oxygen saturation throughout the study. Measurements will be recorded at 5 min intervals after either the placebo or morphine injection. The measurements will be recorded for at least

60 min or until values have returned to the normal range. A nurse will place the AutoSense ECG electrodes and respiration band on the participant's chest for continuous physiological monitoring throughout the session. Monitoring by the AutoSense will be in addition to monitoring by traditional methods.

Baseline measurements (as described below) will then be obtained. After this, the placebo injection (normal saline in a volume equal to that used for the morphine injection) will be given (at approximately 9:45AM).

At baseline as well as during and after the injection, the following measures will be obtained, following the schedule outlined in the study-session flow-sheet (Table 1): Subjective questionnaires: a visual analog scale of high and intoxication See Appendix I. for Drug Effects Questionnaire (DEQ) (Holdstock and de Wit 1999). The DEQ consists of four visual analog scales used to describe current drug effects (feel drug effects, none (0) to a lot (100)]; like the effects they feel, dislike (0) to neutral (50) to like extremely (100); feel high, not at all (0) to a lot (100); and (4) would like more not at all (0) to a lot (100).

Objective Measurements: 1) Pupil constriction(Grace et al. 2010), cold pressor test (Posner 1991), and 2) Blood will be drawn at baseline and at 10 min after the injection for analysis of blood chemistries potentially responsive to an opiate challenge (including, but not limited to ACTH and cortisol) (Jaffe 1985).

Cold Pressor Test: The cold water pressor test evaluates discomfort or pain when the nondominant hand is first placed in a warm water bath (37° C) for 2 min, then transferred to an ice water bath (0° C). The participant is instructed to immerse his/her hand in the ice water for up to 3 min, but could withdraw the hand at any time if the pain becomes too great. Participants are instructed to report verbally when they first sensed pain. This time measures pain threshold. Time to withdrawal measures pain endurance (Posner 1991). See scoring sheet in Appendix I.

Pupil constriction test: Participants will be instructed to sit quietly, blinking as little as possible while the pupillograph is positioned over the right eye by the operator. While the participant focuses on a fixed point. The resting pupil diameter will be measured for 2 s every 20 s for a total of one minute. This test is a modification of a previously reported method (Grace et al. 2010) to remain within the time of the expected drug effect and will commence four minutes after the participant puts his hand in the cold water.

The participant will be returned to the lounge for a relaxation period and a snack or lunch. In early afternoon, the participant will return to the study room for the morphine injection, which will be administered by an NIH Credentialed physician with ACLS certification. Participants will receive an injection of 10 mg/70 kg morphine (2 mg/ml) administered by an NIH Credentialed physician over 1 minute. The tests described above will be repeated. The participant will be monitored by an NIH Credentialed physician for the next 60 min or until HR, RR, BP, EKG and oxygen saturation have returned to normal ranges. Values will be recorded approximately every 5 min (+/- 1) for the next 60 min after the injection or until they have returned to normal ranges.



Table for Visit 2

Minutes	BL	0	5	10	15	20	25	30	35	40	45	50	55	60 (plus additional records q. 10 min if not within normal range)
Morphine or placebo administration		x												
HR, BP, RR, O2 Sat, EKG recorded *	x		x	x	x	x	x	x	x	x	x	x	x	x
Cold Pressor Test			x											
DEQ	x			x		x		x		x		x		x
Pupil constriction	x			x		x		x						
Blood draw for ACTH/cortisol*	x		x					x						
VAS														x

Please note the timing for these parameters will be as close as possible to the stated times, but may not be at exactly these times. Data from cold pressor test, DEQ and pupillary constriction will be collected in sequence, not at the same time.

\*Please note monitoring of these parameters will be continuous until they have returned to normal ranges and recording of these parameters will continue at 10 min intervals if they have not returned to normal ranges. It is expected that following the placebo injection, these values will be normal throughout the study, but they will be monitored for at least 60 min.

\*\* First blood draw for ACTH/cortisol will be after cold pressor test and DEQ, but before pupil constriction test.

The AutoSense electrodes and respiration belt will be removed at the end of the session.

Visits 3 and 4: PET studies of [<sup>11</sup>C]raclopride binding with and without morphine:

Participant will be brought to the Clinical Center via taxi during the afternoon or early evening of each day before the scheduled PET studies.

Outline of PET session procedures:

- 1) Two IV lines will be started, one for injection of [<sup>11</sup>C]raclopride, the other for the morphine or placebo (normal saline in a volume equal to that used for the morphine injection) injection.
- 2) Subjects will be placed in the PET scanner and administered a bolus of [<sup>11</sup>C]raclopride.
- 3) Subjects will be administered either a morphine or a placebo (normal saline) injection using the procedure identical to that used in session 0.
- 4) The subjective questionnaires will be administered at baseline and during the morphine or placebo (normal saline) challenge, identical to the procedures used in Visit 2. Blood samples for blood chemistries will be drawn as described for Visit 2.

*Detailed description of PET procedure:*

Scans will be performed with the GE Advance tomograph that acquires 35 simultaneous slices with 4.25 mm inter-slice distance. Scans will be acquired in 3D mode with septa removed producing a reconstructed resolution of 6 mm in all directions. The subject will be placed in the PET scanner, with the brain in the field of view. A thermoplastic mask will be made for each subject to gently immobilize the head while in the scanner. Each individual's mask will be used for each PET scan in that individual, to ensure similar head positioning. First, a transmission scan lasting approximately 8-min will be acquired with two rotating rod sources for the purpose of attenuation correction. The tracer will be prepared and tested for purity just prior to use.

At 5 min following the morphine, which will be administered by an NIH Credentialed physician with ACLS certification, or placebo injection, up to 12 mCi [<sup>11</sup>C]raclopride will be administered as a bolus over one minute into the IV line in the opposite arm. Monitoring of HR, RR, BP, EKG and oxygen saturation will begin immediately by an NIH Credentialed physician. Values will be recorded every 5 min for the duration of the PET scan by the nurse or nurse practitioner monitoring the study. The participant will be asked how he feels in addition to the administration of the DEQ in the event he feels nauseous or needs to vomit. He will also be encouraged to tell the study staff at any time if he feels nauseous or needs to vomit. In the event that the participant experiences either of these side effects, he will be removed from the scanner until the effects subside or if they do not, the study will be discontinued. As the participant cannot easily view the DEQ in paper form or on a computer while he is in the scanner, he will be asked to provide a number between 0 and 100 in response to the four DEQ questions. An NIH Credentialed physician with ACLS certification, will continue to monitor these values for 60 min or until the values return to normal ranges. Dynamic PET scans will begin with the [<sup>11</sup>C]raclopride injection and continue approximately 90 minutes to obtain optimal signal.

The PET data from the placebo day will be used as a measure of baseline raclopride binding potential, using the Simplified Reference Tissue Model outlined by Lammertsma and Hume (1996). PET measurements taken following the morphine injection will be compared to that derived from placebo injection to measure the reduction in specific binding due to competition with dopamine endogenously released by the morphine challenge. The percent change from baseline has been shown to be proportional to the magnitude of dopamine release (Watabe et al. 2000).

Table for Visits 3 and 4

Minutes	BL	0	5	10	15	20	25	30	35	40	45	50	55	60	70	80	90	95
Morphine or placebo administration		x																
[ <sup>11</sup> C]Raclopride injection, start scanning			x															
HR, BP, RR, O2 Sat, EKG recorded*	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
DEQ	x			x		x		x		x		x		x				
End scanning																		x
VAS																		x

Please note timing for these parameters will be as close as possible to the stated times, but may not be at exactly these times.

\*Please note monitoring of these parameters will be continuous throughout the scan and recording of these parameters will continue at 10 min intervals after the scan if they have not returned to normal ranges. It is expected that during the visit in which the participant receives placebo, these values will be normal throughout the study, but they will be monitored for the same duration and at the same frequency as that for the morphine session.

After the study is completed, subjects will return to the NIAAA inpatient area (1 SE) where they undergo a post scan assessment. If any medical complaints or concerns are voiced, appropriate medical attention will be provided. Subjects will be taken home by taxi paid for by NIH after receiving medical clearance from study investigator.

### **End of Participation**

Participants will undergo a post-study assessment, typically on the last study session (Visit 4). The evaluation will include vital signs, EKG, CBC and blood chemistry. This evaluation will occur after the participant has been cleared for discharge.

Visit 5 (by phone) The Lead Investigator or her designate will contact participants by phone the approximately one week after the study to ensure that there have been no unexpected adverse events. No other need for re-contact is anticipated. However, should the need arise for any reason, the names and contact information will remain on file with the NIH office of volunteer recruitment. No information on the results of the genetics test or the results of the PET scans will be shared with the participants.

## **5. Storage of data and samples:**

Plasma samples for cortisol and ACTH collected at the BRC will be stored temporarily in a freezer located in the Freezer room. Plasma samples for cortisol and ACTH collected at the Clinical Center will be stored in a controlled access freezer room at the LCTS inside the NIH Clinical Center. Samples collected at the BRC will be transferred to the Clinical Center by the NIDA courier at convenient intervals (once every month to two months depending on the number of participants). The samples will be kept until they are analyzed, which is expected to be when all participants have completed the protocol. All sample and data analyses are expected to be completed within 6 months of the last PET-scan.

Collection of blood for OPMR1 genotyping will be processed under the approved NIDA screening protocol (06-DA-N415). Samples will be used to identify carriers of the G allele and those individuals who are homozygous for the A allele. The DNA will also be collected during Visit 1. It will be used to determine ancestry scores from large SNP panels. Biological specimens obtained at the Clinical Center and those sent from the BRC will be stored in coded form (serial numeric codes) in freezers located in the access controlled Building 10 Hatfield CRC 1SE laboratory area of NIAAA/LCTS.

Blood collected for genetic testing will be kept indefinitely for future analysis of relevant gene polymorphisms about which new data may emerge in the future literature. Blood obtained as part of this protocol will be stored in coded form (serial numeric codes) in a

freezer in the NIDA Freezer Room until they are shipped to NIAAA for analysis. Once they are at NIAAA, they will be stored in freezers located in the access controlled Building 10 Hatfield CRC 1SE laboratory area of NIAAA/LCTS. They will be analyzed for various biomarkers (at the NIAAA Lab of Clinical and Translational Studies).

De-identified data from the AutoSense and age, gender, and drug use history will be sent to Dr. Kumar at the University of Memphis (Material Transfer Agreement #30749-10; expiration date 7/29/2016) and to Dr. Yixin Chen at Washington University (Data Transfer Agreement in process) for analysis.

## 6. Additional Considerations

### *a. Research with investigational drugs or devices*

#### *i. Medications/devices requiring IND/IDE:*

The radiotracer,  $^{11}\text{C}$  raclopride, will be administered under IND #54135 held by NIH Clinical Center-PET Imaging Department. The radiation exposure is for research purposes only.

#### *ii. Source of materials/storage/handling*

The radiotracers will be made by the radiochemists in the PET department.

#### *iii. Attach investigator's brochure*

No investigator's brochure is available.

### *b. Gene Therapy*

#### *i. Identify if gene therapy is used (if so, attach RAC documentation)*

No gene therapy will be done.

## 7. Risks/ discomforts

### Known risks and discomforts associated with study procedures and medications:

#### *Morphine injection:*

Side effects from opiates can include sedation, euphoria, dizziness, fatigue, depression, tremors, sleeplessness, anxiousness, flu-like symptoms, upset stomach, vomiting, dry mouth, pupil constriction, itching, transient rash, hallucination, delirium, sweating, muscle and bone pain, confusion, extreme irritability, constipation and muscle spasms. Severe side effects can include severe respiratory depression, confusion or stupor, coma, clammy skin, circulatory collapse and cardiac arrest. Sometimes, even in the case of individuals who later become addicted, initial injections may produce unpleasant sensations such as headache, dizziness, and nausea. Participants will be encouraged to take extra fluids to avoid constipation.

With the exception of allergic reactions, adverse effects of morphine are largely dose-dependent, and the level of risk is therefore associated with the dose chosen. The FDA approved label recommends i.v. morphine doses between 4 – 10 mg/70 kg that can be repeated every 4 hours. In the present study, subjects will receive no more than a single morphine dose of 10 mg/70 kg on any given day. A literature search shows that when this dose was previously used in research volunteers, these experienced several central morphine effects, such as for instance feeling sleepy, clumsy, dizzy, high or intoxicated (Zacny et al. 1994a; Zacny et al. 1994b; Zacny et al. 1997a; Zacny et al. 1997b; Stoops et

al. 2012). It is therefore to be expected that some or all of these effects will be experienced by our participants. These effects are reversible, may cause discomfort, but do not pose a significant medical safety concern.

The most important adverse effect of morphine, which does pose a significant medical risk, is that the drug can depress or stop breathing. Respiratory depression is dose dependent. Unless properly monitored and treated, it may lead to death. The five studies cited above together exposed 65 subjects to 10 mg morphine /70 kg (Zacny et al. 1994a;Zacny et al. 1994b;Zacny et al. 1997a;Zacny et al. 1997b;Stoops et al. 2012). One of the studies (Stoops et al. 2012) also included a 20 mg/70 kg dose, and demonstrated an orderly and linear dose-response relationship for end-tidal CO<sub>2</sub>, such that that 10 mg dose was intermediate between the 5 and 20 mg doses. The latter study also exposed subjects to multiple doses. No serious adverse events were observed after the 10 mg/70 kg morphine dose in any of the 65 published cases, and no serious adverse events were encountered in the paper using a 20 mg dose. In people receiving morphine after surgery, the incidence of respiratory depression has been estimated to less than one in a hundred subjects (Dahan et al. 2010).

i. *Steps taken to minimize risk*

Participants will be screened for a history of negative effects of opiate administration, and excluded if such a history is present. As all participants will have had experience with pain pills, it is expected that they will come to the study with knowledge of their previous experiences. Participants will be excluded if they have used pills chronically (> 6 weeks) or recently (within the previous 3 months). The purpose of these criteria is to remove the possibility of residual effects on the receptor.

The potential adverse consequences of the morphine injection primarily involve respiratory depression, skeletal muscle rigidity, hypotension, bradycardia, nausea, dizziness or other impairment. Following the morphine injection, oxygen saturation, respiratory rate, blood pressure, and heart rate will be monitored continuously by an NIH Credentialed physician with ACLS certification for 60 min or until these values have returned to normal ranges. Values will be recorded approximately every 5 min until they have returned to normal ranges. Subjects who experience significant nausea, breathing difficulties, hypotension, or bradycardia will carefully monitored and supplemental oxygen, intravenous fluids, atropine, and naloxone will be available if needed. Finally, risks of impairment are minimized by monitoring subjects until the effects of the opiate on motor performance or judgment have passed, and sending subjects home in a taxi.

The study is designed to provide the first opiate challenge in the outpatient clinic, so that persons who react unfavorably to the challenge will be identified and excluded before becoming eligible for the PET scan procedure. Participants will be expected to be intoxicated at the conclusion of the opiate challenge. They will be supervised on site until any signs of drug effect have dissipated (e.g., vital signs in normal range and no signs of impairment) and the participant reports that they are not experiencing any drug effects at which time they should be able to safely to leave the NIH premises and will be taken home in a taxi arranged and paid for by the NIH.

*PET scan:*

Potential risk of this procedure is primarily related to radiation exposure. The effective dose from the emission and transmission scans will be 0.62 rem, which is below RSC guidelines of 5.0 rem per year in adults. This risk has been assessed by separate Radiation Safety review, which assures that exposure is minimized, and falls within a widely accepted range. In summary, no harmful effect to humans has been observed from the levels of radiation used here. Additional measures taken to ensure subject safety will be to obtain a careful history of exposure to radiation from other sources or medical procedures to ensure that total exposure remains within an acceptable range.

Some participants have reported a sense of claustrophobia or panic in reaction to placement in an MRI or PET scanner. Participants will be instructed to report such feelings so they can be removed from the apparatus and will not be required to continue with the session if they are uncomfortable with the procedure. If they cannot continue, this will require withdrawal from the study, and they will be paid for their participation up to that point.

In summary, the risk / discomfort of the morphine injection procedure represents more than a minor increase over minimal, but is well justified by the scientific gains.

*MRI:*

MRI scans are routinely carried out both for clinical and research purposes. Individuals will complete an MRI safety checklist. Subjects with metal objects in their body may be negatively affected, and are therefore excluded from this study. The enclosed space of the scanner can provoke anxiety in some individuals. If so, the scan is discontinued, where upon the anxiety resolves. Finally, MRI is a noisy process, so we require participants to wear foam earplugs while inside the scanner. The risk / discomfort of this procedure is judged to be minimal.

*Genetic testing:*

No adverse events are anticipated as a result of the genotyping. However, to protect confidentiality no genotyping data will be entered into the clinical record of participants in the study and no genotype data will be released to participants under any circumstance. No CLIA [Clinical Laboratory Improvement Act] certified genotyping acceptable for a clinical diagnostic or insurance purpose will be performed. In the study, there will be no identification of genetic diseases, and subsequently, and consequently, no relevant information to report to the participants. The participants will be told that the medical relevance of the genotyping in this study is not established, while other information (e.g. a positive family history of drug problems) is a known indicator of susceptibility.

Thus, this is considered a minimal risk / discomfort procedure.

*Pupillary constriction tests:*

This test is a widely used psychophysiological measure with minimal risk and discomfort.

*Cold pressor test:*

Participants may remove their hands when the pain becomes too much to continue. There is a 3 min cut-off, which eliminates any potential damage to the hand. The participant is instructed to immerse his hand in the ice water for up to 3 min, but could withdraw the hand at any time if the pain becomes too great. Participants are instructed

to report verbally when they first sensed pain. This time measures pain threshold. Time to withdrawal measures pain endurance.

*IV placement and Blood sampling:*

Minor discomfort is expected to result from blood draws and placement of IV lines. These procedures will be performed by qualified and credentialed NIH physicians, nurses, or technicians. Pressure will be applied to IV sites after lines are withdrawn to prevent subcutaneous bleeding. This follows standard clinical routine, and is considered to be a minimal risk/discomfort procedure. Less than 450 ml of blood will be drawn as part of this research study.

*Autosense:*

Wearing the AutoSense belt may be inconvenient. Participants may also experience slight discomfort and/or minor skin irritation while wearing the belt and electrodes.

## **8. Subject monitoring**

Participants will be monitored by the medical, nursing and research staff for any untoward effect and the study session will be terminated for the participant if any major or unexpected adverse events occur. Oxygen saturation and heart rate will be monitored continuously throughout the study session. Respiratory rate and blood pressure will be monitored every 5 minutes. The MAI or another NIH Credentialed physician with ACLS certification will perform the morphine administration.

Participants will be assessed at the end of the day of Visits 2, 3 and 4 and will not be released until staff can document normal vital signs, an alert and oriented demeanor as well as a normal pupil response to light and have received clearance from attending study investigator (MD). Any impairment in locomotor coordination or judgment will be monitored by the nursing and research staff. The participant will be sent home in a cab paid for by the NIH.

To evaluate for the possibility of adverse events, a safety assessment evaluation will be conducted at the completion of the last of the PET scan / injection study sessions. This assessment will include vital sign determination, EKG, CBC, and blood chemistry. The Lead Investigator or her designate will contact the participant by phone, approximately one week after the completion of the study to evaluate for the possibility of any unexpected adverse events.

Participants will be withdrawn from the study if they do not follow the instructions outlined in the protocol and informed consent form. Participants will also be withdrawn if they cannot tolerate the morphine injection and demonstrate any signs of untoward effects. Participants may also, at any time, choose to discontinue their participation in the study.

## **9. Outcome measures**

- a. The two primary outcome measures are:
  - 1) [<sup>11</sup>C]raclopride binding potential in striatal areas of the brain
  - 2) Subjective perceptions of morphine intoxication and opiate effects.
- b. Secondary measures are:

- 1) levels of neuroendocrine measures (ACTH, cortisol)
- 2) pupillary constriction, respiratory rate, pain response

## 10. Statistical Analysis

### a. Analysis of data/ study outcomes

For the primary analyses, dependent variables (subjective and objective responses in the laboratory, post-morphine injection raclopride binding potential) will be analyzed using analysis of variance, with genotype as the independent factor. The role of drug and alcohol drinking history and family history of drug abuse/alcoholism as well as self-reports of ancestry and genetically derived ancestry scores will be evaluated as covariates in the analysis. The level of significance will be set at 0.05.

PET Analysis will be based on a Region of Interest (ROI) methodology. MRI scans will be performed on each participant, and co-registered with the participant's PET scans. ROIs will be drawn in the nucleus accumbens, dorsal and ventral caudate and putamen, these being the main areas of post-synaptic dopamine release. An ROI will also be placed on the cerebellum as this region is thought to be devoid of dopamine receptors and is used as the reference region because it provides a measure of nonspecific binding (Lammertsma and Hume 1996). Dopamine release will be estimated by comparing [<sup>11</sup>C]raclopride binding potential derived using a bolus injection and the simplified reference tissue model (SRTM) under two different conditions (baseline vs. morphine) during separate scans conducted in randomized order.

Baseline binding potential, changes in binding potential following morphine challenge, and subjective effects of the challenge as measured by changes in stimulation and intoxication scores on subjective perception scales between dose conditions (opiate vs. placebo) will be correlated.

### a. Power analysis

#### Sample size:

In a study that evaluated the effect of remifentanyl administration on D2 dopamine receptor availability using a different PET radioligand in detoxified alcohol dependent participants (N=11) and controls (N=11) found a significantly reduced dopamine D2/3 receptor availability after administration compared with baseline in the ventral striatum (9.5%) and the dorsal putamen (8.3%) in both groups (Spreckelmeyer et al. 2011). We can expect similar reductions due to DA release after morphine in one or both research groups (118A homozygotes; 118GX). A study that evaluated the effect of the variants of the OPRM1 genotype in social drinkers compared during placebo and alcohol infusions found a significantly greater DA release as measured by  $\Delta$ binding potential in those participants who carried the G allele (N=12) compared to the 118 homozygotes (N=16) (Ramchandani et al. 2011). However, results of the studies in the humanized mouse strains of these two genotypes yielded opposite effects of genotype on DA release from morphine.

If the results of this study yield similar results, 40 completers per group should provide adequate power to detect effects of OPRM1 genotype. We estimate that to have this number of completers, we must screen and genotype at least 120 prospective participants.

#### Accrual number request, taking into account screening/ dropouts

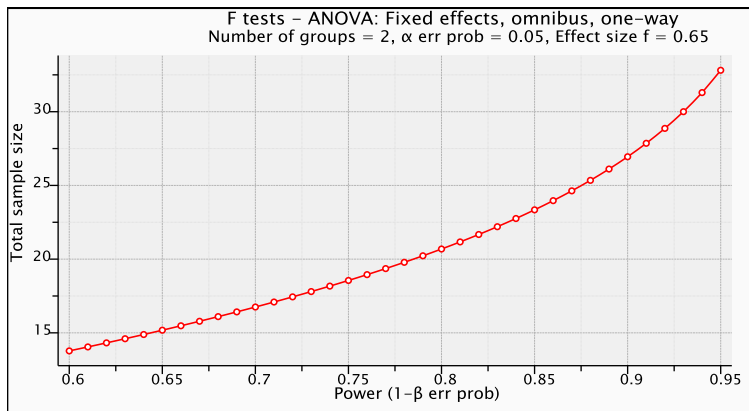


We are requesting an accrual limit of 120 to obtain 80 subjects to complete the study.

### Power Analysis:

<b>Analysis:</b>	A priori: Compute required sample size	
<b>Input:</b>	Effect size $f$	= 0.65
	$\alpha$ err prob	= 0.05
	Power ( $1-\beta$ err prob)	= 0.80
	Number of groups	= 2
<b>Output:</b>	Noncentrality parameter $\lambda$	= 9.2950000
	Critical F	= 4.3512435
	Numerator df	= 1
	Denominator df	= 20
	Total sample size	= 22
	Actual power	= 0.8262998

An effect size of Cohen's  $F = .65$  was observed by Ramchandani et al. (2011). To detect an effect size of Cohen's  $F=0.65$  with a 90% probability at an  $\alpha=0.05$ , a total sample size of 24 would be required. To ensure adequate power to reliably detect somewhat smaller effect sizes, we will recruit a total sample size of 40 males per genotype.



This result is based on the effect size in data acquired by Ramchandani et al. (2011) in a similar study in which the challenge was alcohol.

## 11. Human Subjects Protection

### a. Subject selection

This study will be conducted in healthy 21-55 year-old male participants. The specific inclusion and exclusion criteria are listed in a previous section.

Females will not be recruited. As this study aims to examine whether OPRM1 A118G variation moderates the mesolimbic DA response to  $\mu$ -opioid receptor activation, it is important that we have a signal that could be modified. Using non-human primates, it was previously found that the rhesus ortholog of the human OPRM1 A118G SNP only moderated alcohol-induced psychomotor stimulation

(a marker of mesolimbic DA activation) in males but not in females (Barr et al. 2007). This difference was presumably due to the negligible overall psychomotor response in the latter group. In agreement with those observations, a subsequent human PET study (Urban et al. 2010) found only marginal alcohol-induced DA release, (measured as % change in [<sup>11</sup>C]raclopride binding potential) in females while a robust response was present in males. Alcohol activates mesolimbic DA neurons indirectly, through release of endogenous opioids and their actions at  $\mu$ -opioid receptors (Heilig et al. 2011;Urban et al. 2010). Because endogenous opioid release in response to alcohol does not differ between men and women (Mitchell et al. 2012), it must be concluded that the observed sex differences are likely to be postsynaptic. A similar sexual dimorphism is therefore likely to exist for actions of an exogenous  $\mu$ -opioid agonist. Taken together, these observations thus make it unlikely that the hypothesis of the present study can be addressed in females, providing a compelling scientific rationale for restricting recruitment to males.

Participants will be recruited through advertisement in local media (electronic and print) at NIH and in the greater Baltimore and Washington DC areas. The study will be open to all racial and ethnic groups, without targeting any specific group.

APPROVED STUDY POPULATION						
	FEMALE ENROLLMENT	MALE ENROLLMENT	TOTAL ENROLLMENT	FEMALE COMPLETERS	MALE COMPLETERS	TOTAL COMPLETERS
<b>APPROVED CEILING</b>	0	120	120	0	80	80

NIH TARGETED/PLANNED ENROLLMENT			
ETHNIC CATEGORY	Sex/Gender		
	Females	Males	Total
Hispanic or Latino	0	9	9**
Not Hispanic or Latino	0	111	111
<b>Ethnic Category: Total of All Subjects*</b>	0	120	120*
<b>RACIAL CATEGORIES</b>	0		
American Indian/Alaska Native	0	2	2
Asian	0	13	13
Native Hawaiian or Other Pacific Islander	0	1	1
Black or African American	0	14	14
White	0	90	90
<b>Racial Categories: Total of All Subjects*</b>	0	120	120*

\*The "Ethnic Category: Total of All Subjects" must be equal to the "Racial Categories: total of All Subjects."

**b. Justification for exclusion of children**

Studies involving radiation exposure are counter indicated in children under the age of 18 years. Studies involving administration of opioids are counter indicated in children under the age of 21 years. Therefore, only adult participants over age 21 will be enrolled.

**c. Justification for exclusion of other vulnerable subjects**

Vulnerable participants will not be enrolled.

**d. Justification of sensitive procedures**

To avoid an expectation effect the study will be run single blind. The participant will be told that the injection on any given day may be active drug or placebo. At the end of the study, the participant will be told when he received the active drug.

**e. Safeguards for vulnerable populations**

Women will not be recruited.

**f. Qualifications of investigators**

**Vijay A. Ramchandani, Ph.D.** is the Chief of the Section on Human Psychopharmacology in the Laboratory of Clinical and Translational Studies. He is a clinical pharmacologist with several years of experience conducting human laboratory studies, particularly using the alcohol infusion method, which he co-developed. He participated in the study design and protocol development, and will oversee the study conduct and analysis. Dr. Ramchandani will obtain consent for this study.

**Peter Herscovitch, MD**, is Chief of the PET department in the Clinical Center of NIH. He has several years of experience in conducting imaging studies, including PET studies with radiotracers such as raclopride. He participated in study design and protocol preparation.

**Kenzie L. Preston, Ph.D.**, is Chief of the Treatment Section and Chief of the Clinical Pharmacology and Therapeutics Research Branch, NIDA IRP. Her undergraduate degree is a B.S. in pharmacy, and she is a registered pharmacist. The Archway clinic is part of the Clinical Trials Section and under the leadership of Dr. Preston. She has an established reputation in the field of opioid abuse research and is currently conducting a series of clinical trials of combination treatment including contingency management and buprenorphine maintenance. She participated in study design and protocol preparation and will participate in manuscript preparation.

**Karran Phillips, M.D., M.Sc.**, is a Staff Clinician in the Treatment Section, NIDA IRP. She is Board Certified in Internal Medicine, fellowship-trained in General Internal Medicine, certified in Addiction Medicine, and completed a graduate degree in public health. She has clinical and research experience working with patients with substance dependence and HIV. She will be the Medical Advisory Investigator (MAI), will supervise the medical aspects of the study, and will coordinate the medical care of patients when appropriate. She will administer the morphine dose at the BRC. She will be in the room with the participant for at least 30 minutes following the morphine administration or until vital signs have returned to the normal range. She has current ACLS certification. She participated in study design and protocol preparation and will also participate in data analysis and manuscript preparation and has been designated by the PI to obtain consent.

**Elliot A. Stein, Ph.D.**, is a behavioral neuroscientist and Chief of the Neuroimaging Research Branch, NIDA-IRP. He has more than 20 years of experience in the neurophysiology and neuropharmacology of drugs of abuse. Dr. Stein will assume

responsibility for the supervision and performance of all MRI data analysis and interpretation. He will participate in data analysis and manuscript preparation.

**Primavera A. Spagnolo, M.D.** is a post-doctoral fellow in the Section of Clinical Assessment and Treatment Evaluation, Laboratory of Clinical and Translational Studies, NIAAA/NIH. She is Board Certified in Addiction Medicine and Medical Toxicology. She has clinical and research experience working with patient with alcohol and substance dependence, especially in capacities involving direct patient care of opiate addicts. She will participate in overseeing the administration of morphine during the PET scans and assist with data analysis.

**Reza Momenan, PhD**, is a staff scientist with the Section on Brain Electrophysiology and Imaging (SBEI), Laboratory of Clinical and Translational Studies, and currently its acting Chief. He has over 20 years of experience in conducting research in various medical imaging modalities. In the present study, he is working on experimental paradigm design and implementation, medical imaging, image analysis, and other data analysis. He will not be obtaining consent.

**Nancy Diazgranados, M.D., M.S.** Dr. Diazgranados received her Doctoral Degree in Medicine and Surgery from the Pontificia Universidad Javeriana in Bogota Colombia in 2001. She completed her Psychiatry residency at Albert Einstein Medical Center and a Master in Science Degree in Pharmacology at Thomas Jefferson University in 2007. In 2008 she became a diplomate of the American Board of Psychiatry and Neurology. She continued her training as a Post-Doctoral Clinical-Research Fellow at the intramural program at NIMH; there she worked at the Experimental Therapeutics and Pathophysiology Branch in the Mood and Anxiety Disorders Program. In 2010 she joined the University of Texas Health Science Center at San Antonio as a tenure track assistant professor within the Division of Mood and Anxiety Disorders. In 2012 she joined a private practice and relocated to Maryland. Finally in September 2013 she joined NIAAA as a staff clinician. She will administer the morphine dose for the PET studies at the NIH CC and supervise the procedure until the participant is medically cleared. She is an NIH credentialed physician with current ACLS certification.

**Michelle Leff, M.D., M.B.A.** Dr. Leff is the Chief of Staff at the NIDA IRP. She is board-certified in General Psychiatry and Child/Adolescent Psychiatry. She has served as the MAI on various IRP protocols, including treatment studies for adolescent smokers, and served as the Deputy Clinical Director from 2003-2011. She is NIH-credentialed and JHBMC-credentialed, and is ACLS-certified. She will provide ad hoc coverage for the MAI.

## **12. Anticipated Benefit**

This study does not offer direct medical or health benefit to participants but is likely to yield generalizable knowledge about the effects of opioids and their potential for abuse by determining the effect of an opiate on dopamine release in the brain, and the role of the opioid receptor (OPRM) polymorphism on this response in humans.

## **13. Summary/ classification of risk:**

This study is classified as “more than minimal risk”.

The overall risk of participation in this study is low, and every precaution and protection to minimize any risks will be taken as described in a previous section. The study is expected to provide important information about the pharmacological effects of opiates, and the role of the opioid receptor (OPRM1) polymorphism, in humans. We believe the benefit:risk ratio of the proposed study is favorable.

## **14. Consent documents and process**

### **a. Designation of those obtaining consent**

Study investigators designated as able to obtain consent in section 11f above will obtain informed consent.

### **b. Consent procedures**

All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions regarding this study prior to signing.

### **c. Consent documents**

The consent form contains all required elements. We will use an “Evaluation to Sign Consent” which has been tailored to this protocol in lieu of a consent quiz as the method to ensure participant understanding of the study.

## **15. Data and Safety monitoring**

### **a. Data and Safety Monitor**

This is a small, single-site Phase 2a / physiologic study of short duration. It is carried out in healthy research volunteers, a population that is neither vulnerable, nor at elevated risk for death or other serious outcomes. The study involves administration of morphine, an FDA approved medication, at a dose that is at the high end, but within the range of what is recommended on the FDA approved label. Accordingly, previous studies administering this dose to healthy research volunteers have not reported any serious adverse events. Although the study therefore does not meet any of the FDA criteria (FDA 2013) for the need for a Data Safety and Monitoring Board (DSMB), we will utilize the standard DSMB for protocols reviewed by the Addictions IRB. The Principal Investigator and Medical Advisory Investigator will also monitor the study.

### **b. Data and safety monitoring plan**

Data and safety monitoring will be done by the PI, the MAI, and the DSMB for Addictions IRB, which will have access to unblinded data.

#### *Data to be monitored*

- 1) Participant accrual
- 2) Safety data
- 3) Adverse events; serious, nonserious, expected, and unexpected
- 4) Study dropout, including the reason(s) for dropout (adverse event, treatment failure, etc.)

#### *Materials submitted to the DSMB*

In addition to the materials identified above that will be sent to the monitor for regularly scheduled reviews, we will report protocol-related serious, unexpected adverse events to the monitor at the same time that we report them to the IRB, the Clinical Director, and other NIH officials. Additionally, we will notify the monitor promptly of any protocol amendments.

#### *Interim Analyses*

An interim analysis of the PET data is planned after the first 5 participants in each group. The purpose of this analysis is to ensure that dopamine release as evidenced by a reduction in binding potential for [<sup>11</sup>C]raclopride by the morphine challenge is observed, at least in those participants who are homozygous for the A allele. If no participant (regardless of genotype) exhibits dopamine release, the study will be stopped. If an alternative approach to the study is deemed more likely to allow the measurement dopamine release, such as the use of a different opiate drug or different radioligand, a revised protocol may be submitted.

#### *DSMB Reports*

The DSMB will make findings and interpret the data, then report to the PI, IRB, and Clinical Director about continuation, modification, suspension, or termination of the protocol based on observed beneficial or adverse effects of the experimental treatment under study. The DSMB will act promptly on any findings indicating the need for an amendment to the protocol or affecting the continuation of the protocol.

#### *Review Schedule*

The DSMB will examine the safety data after the first 10 participants have completed the study. Additional reviews will occur each time the number of study completers (or dropouts) has increased by 20. Additional reviews will be conducted if warranted by the occurrence of serious adverse events or otherwise deemed necessary by the DSMB.

#### **c. Criteria for stopping the study or suspending enrollment or procedures**

The study will be stopped after we complete data collection from our target number (80) of participants who complete the study. The study will be stopped prior to that point if an interim analysis does not show any effect of the opiate administration on the release of dopamine. If there are any serious adverse events following the administration of the opiate that are found to be protocol related, the protocol will be suspended until the safety of continuing the protocol can be established or stopped if the safety of continuing the protocol cannot be established.

## **16. Quality Assurance**

### **a. Quality assurance monitor**

The Principal Investigator, Lead Investigator and the MAI will be monitoring data collection and the study on an ongoing basis. Quality assurance (QA) will be performed by NIDA QA and NIAAA QA staff as indicated in the NIDA and NIAAA Quality Assurance Monitoring Planas described below.

### **b. Quality assurance plan**

Quality assurance, including monitoring of Compliance with Good Clinical Practice

standards, will be carried out by NIDA and NIAAA QA staff. Monitors will review individual Clinical Research Files and establish their agreement with relevant source documents, will review the study Regulatory Binder for completeness and relevant updates, and will confirm compliance with all regulatory requirements including appropriate reporting of AE's. Monitors will bring any deficiencies encountered to the attention of the PI, and will follow up on these through resolution.

## **17. Adverse event reporting**

Serious or unexpected adverse events and other unanticipated problems will be reported orally as soon as possible and in writing within 7 days if life-threatening and within 15 days otherwise. Expected or non-serious adverse events will be reported at the time of continuing review.

## **18. Alternatives to participation or alternative therapies**

Subjects do not receive any treatment in this study or forego any treatment in order to participate in this study. The alternative, therefore, is not to participate.

## **19. Confidentiality**

This protocol is covered by a Certificate of Confidentiality issued by the US Department of Health and Human Services. Strict subject confidentiality will be maintained throughout the study. Confidentiality and information technology standards are in place at the NIAAA/LCTS and NIDA intramural programs to protect electronic repositories of patient data as well as other clinical patient related material. It is reasonably expected that these safeguards will protect participants' medical and personal health information, ensuring their privacy.

Information obtained in the course of being screened for or participating in this protocol will become part of the patient's NIH medical record. This includes potentially sensitive information, such as urine tests positive for illicit drugs. Access to this information may be requested by third parties. Such access will not be granted without the explicit, written consent of the subjects. However, failure on the part of the subject to provide access to the information may in itself be to the disadvantage of the subject, e.g. in the case of a potential employer or insurer requesting the information. This situation will be made clear in the consent.

Samples and data will be stored using codes that we assign. Data will be kept in password protected computers. Samples will be kept in locked storage. Only study personnel will have access to the samples and data.

## **20. Conflict of Interest**

Statement of conflict of interest of investigators:

NIH guidelines on conflict of interest have been distributed to all investigators. There are no conflicts-of-interest to report. There is no commercial sponsor or company for this study.

## 21. Technology Transfer

List tech transfer agreement/s and confidential disclosure agreements:

Agreement type and number       #30749-10       Expiration Date   July 29, 2016    
with University of Memphis to cover the AutoSense in the opiate PET study

Statement of the role of the drug company:

These data collected during administration of morphine under controlled laboratory conditions will be used to develop an algorithm to detect opioid administration in future field studies. De-identified data from the AutoSense and age, gender, and drug use history will be sent to Dr. Kumar at the University of Memphis (Material Transfer Agreement #30749-10; expiration date 7/29/2016) and to Dr. Yixin Chen at Washington University for analysis..

## 22. Research and Travel Compensation

Participants will receive compensation according to the NIH guidelines for payment to normal volunteers. Participants will receive \$1362.50 for completion of the three injection sessions of the study, as described below. In addition, participants will receive compensation for the screening evaluation under the screening protocol. The following table gives the breakdown of procedures (duration and inconvenience units) used in computation of the compensation. If participants are unable to complete the study, they will be paid on a pro-rated (meaning partial payment) basis. Travel to and from the clinic and meals will be covered during their visits to the clinic.

Procedure	Amount
Visit 1 – Study consent interview, questionnaires	\$112.50
MRI	\$75*
Visit 2: IV catheter insertion, morphine injection, blood draws, testing, physiological monitoring with AutoSense	\$300
Visit 3: overnight stay, multiple IV catheter insertion, C-11 raclopride and morphine/placebo injection, blood draws, testing	\$350
Visit 4: overnight stay, multiple IV catheter insertion, C-11 raclopride and morphine/placebo injection, blood draws, testing, safety assessment including physical exam, EKG, CBC and blood chemistry.	\$400
Completion incentive	\$125
<b>TOTAL</b>	<b>\$1362.50</b>

\*If the MRI is not acquired under protocol 10-DA-N457



## 23. References

- Apfelbaum JL, Gan TJ, Zhao S, Hanna DB, Chen C (2004) Reliability and validity of the perioperative opioid-related symptom distress scale. *Anesth Analg* 99:699-709, table.
- Barr CS, Schwandt M, Lindell SG, Chen SA, Goldman D, Suomi SJ, Higley JD, Heilig M (2007) Association of a functional polymorphism in the  $\mu$ -opioid receptor gene with alcohol response and consumption in male rhesus macaques. *Arch Gen Psychiatry* 64:369-376.
- Bart G, Heilig M, LaForge KS, Pollak L, Leal SM, Ott J, Kreek MJ (2004) Substantial attributable risk related to a functional mu-opioid receptor gene polymorphism in association with heroin addiction in central Sweden. *Mol Psychiatry* 9:547-549.
- Becker A, Grecksch G, Brodemann R, Kraus J, Peters B, Schroeder H, Thiemann W, Loh HH, Hollt V (2000) Morphine self-administration in mu-opioid receptor-deficient mice. *Naunyn Schmiedebergs Arch Pharmacol* 361:584-589.
- Befort K, Filliol D, Decaillet FM, Gaveriaux-Ruff C, Hoehe MR, Kieffer BL (2001) A single nucleotide polymorphic mutation in the human mu-opioid receptor severely impairs receptor signaling. *J Biol Chem* 276:3130-3137.
- Belknap JK, Crabbe JC (1992) Chromosome mapping of gene loci affecting morphine and amphetamine responses in BXD recombinant inbred mice. *Ann N Y Acad Sci* 654:311-323.
- Beyer A, Koch T, Schroder H, Schulz S, Hollt V (2004) Effect of the A118G polymorphism on binding affinity, potency and agonist-mediated endocytosis, desensitization, and resensitization of the human mu-opioid receptor. *J Neurochem* 89:553-560.
- Bjork JM, Knutson B, Fong GW, Caggiano DM, Bennett SM, Hommer DW (2004) Incentive-elicited brain activation in adolescents: similarities and differences from young adults. *J Neurosci* 24:1793-1802.
- Bond C, LaForge KS, Tian M, Melia D, Zhang S, Borg L, Gong J, Schluger J, Strong JA, Leal SM, Tischfield JA, Kreek MJ, Yu L (1998) Single-nucleotide polymorphism in the human mu opioid receptor gene alters beta-endorphin binding and activity: possible implications for opiate addiction. *Proc Natl Acad Sci U S A* 95:9608-9613.
- Callaghan RC, Cunningham JK, Verdichevski M, MMath JS, Jaffer SR, Kish SJ (2012) All-cause mortality among individuals with disorders related to the use of methamphetamine: A comparative cohort study.
- Contet C, Kieffer BL, Befort K (2004) Mu opioid receptor: a gateway to drug addiction. *Curr Opin Neurobiol* 14:370-378.

- Daglish MR, Williams TM, Wilson SJ, Taylor LG, Eap CB, Augsburger M, Giroud C, Brooks DJ, Myles JS, Grasby P, Lingford-Hughes AR, Nutt DJ (2008) Brain dopamine response in human opioid addiction. *Br J Psychiatry* 193:65-72.
- Dahan A, Aarts L, Smith TW (2010) Incidence, reversal, and prevention of opioid-induced respiratory depression. *Anesthesiology* 112:226-238.
- Drakenberg K, Nikoshkov A, Horvath MC, Fagergren P, Gharibyan A, Saarelainen K, Rahman S, Nylander I, Bakalkin G, Rajs J, Keller E, Hurd YL (2006) Mu opioid receptor A118G polymorphism in association with striatal opioid neuropeptide gene expression in heroin abusers. *Proc Natl Acad Sci U S A* 103:7883-7888.
- FDA (2013) The Establishment and Operation of Clinical Trial Data Monitoring Committees for Clinical Trial Sponsors. In. Rockville, MD.
- Fillingham RB, Kaplan L, Staud R, Ness TJ, Glover TL, Campbell CM, Mogil JS, Wallace MR (2005) The A118G single nucleotide polymorphism of the mu-opioid receptor gene (OPRM1) is associated with pressure pain sensitivity in humans. *J Pain* 6:159-167.
- Franke P, Wang T, Nothen MM, Knapp M, Neidt H, Albrecht S, Jahnes E, Propping P, Maier W (2001) Nonreplication of association between mu-opioid-receptor gene (OPRM1) A118G polymorphism and substance dependence. *Am J Med Genet* 105:114-119.
- Glass PS, Hardman D, Kamiyama Y, Quill TJ, Marton G, Donn KH, Grosse CM, Hermann D (1993) Preliminary pharmacokinetics and pharmacodynamics of an ultra-short-acting opioid: remifentanyl (GI87084B). *Anesth Analg* 77:1031-1040.
- Grace PM, Stanford T, Gentgall M, Rolan PE (2010) Utility of saccadic eye movement analysis as an objective biomarker to detect the sedative interaction between opioids and sleep deprivation in opioid-naive and opioid-tolerant populations. *J Psychopharmacol* 24:1631-1640.
- Gunning-Dixon FM, Head D, McQuain J, Acker JD, Raz N (1998) Differential aging of the human striatum: a prospective MR imaging study. *AJNR Am J Neuroradiol* 19:1501-1507.
- Hagelberg N, Kajander JK, Nagren K, Hinkka S, Hietala J, Scheinin H (2002) Mu-receptor agonism with alfentanil increases striatal dopamine D2 receptor binding in man. *Synapse* 45:25-30.
- Heilig M, Goldman D, Berrettini W, O'Brien CP (2011) Pharmacogenetic approaches to the treatment of alcohol addiction. *Nat Rev Neurosci* 12:670-684.

- Holdstock L, de Wit H (1999) Ethanol impairs saccadic and smooth pursuit eye movements without producing self-reports of sedation. *Alcohol Clin Exp Res* 23:664-672.
- Jaffe JH (1985) Opioid Analgesics and Antagonists. In: Goodman and Gilman's pharmacological basis of therapeutics (Gillman AG, Goodman LS, Rall TW, Murad F, eds), pp 491-531. New York: MacMillan Publishing Co.
- Knutson B, Bjork JM, Fong GW, Hommer D, Mattay VS, Weinberger DR (2004) Amphetamine modulates human incentive processing. *Neuron* 43:261-269.
- Knutson B, Fong GW, Adams CM, Varner JL, Hommer D (2001) Dissociation of reward anticipation and outcome with event-related fMRI. *NeuroReport* 12:3683-3687.
- Knutson B, Fong GW, Bennett SM, Adams CM, Hommer D (2003) A region of mesial prefrontal cortex tracks monetarily rewarding outcomes: characterization with rapid event-related fMRI. *Neuroimage* 18:263-272.
- Knutson B, Westdorp A, Kaiser E, Hommer D (2000) FMRI visualization of brain activity during a monetary incentive delay task. *Neuroimage* 12:20-27.
- Kreek MJ, LaForge KS, Butelman E (2002) Pharmacotherapy of addictions. *Nat Rev Drug Discov* 1:710-726.
- LaForge KS, Yuferov V, Kreek MJ (2000) Opioid receptor and peptide gene polymorphisms: potential implications for addictions. *Eur J Pharmacol* 410:249-268.
- Lammertsma AA, Hume SP (1996) Simplified reference tissue model for PET receptor studies. *Neuroimage* 4:153-158.
- Landau R, Kern C, Columb MO, Smiley RM, Blouin JL (2008) Genetic variability of the mu-opioid receptor influences intrathecal fentanyl analgesia requirements in laboring women. *Pain* 139:5-14.
- Matthes HW, Maldonado R, Simonin F, Valverde O, Slowe S, Kitchen I, Befort K, Dierich A, Le MM, Dolle P, Tzavara E, Hanoune J, Roques BP, Kieffer BL (1996) Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the mu-opioid-receptor gene. *Nature* 383:819-823.
- Mitchell JM, O'Neil JP, Janabi M, Marks SM, Jagust WJ, Fields HL (2012) Alcohol consumption induces endogenous opioid release in the human orbitofrontal cortex and nucleus accumbens. *Sci Transl Med* 4:116ra6.
- Oertel BG, Preibisch C, Wallenhorst T, Hummel T, Geisslinger G, Lanfermann H, Lotsch J (2008) Differential opioid action on sensory and affective cerebral pain processing. *Clin Pharmacol Ther* 83:577-588.

- Posner J (1991) Pain models in healthy volunteers. In: Clinical Measurement in Drug Evaluation (Nimmo WS, Tucker GT, eds), pp 73-90. Boca Raton: CRC.
- Posner J, Telekes A, Crowley D, Phillipson R, Peck AW (1985) Effects of an opiate on cold-induced pain and the CNS in healthy volunteers. *Pain* 23:73-82.
- Ramchandani VA, Umhau J, Pavon FJ, Ruiz-Velasco V, Margas W, Sun H, Damadzic R, Eskay R, Schoor M, Thorsell A, Schwandt ML, Sommer WH, George DT, Parsons LH, Herscovitch P, Hommer D, Heilig M (2011) A genetic determinant of the striatal dopamine response to alcohol in men. *Mol Psychiatry* 16:809-817.
- Ray R, Ruparel K, Newberg A, Wileyto EP, Loughhead JW, Divgi C, Blendy JA, Logan J, Zubieta JK, Lerman C (2011) Human Mu opioid receptor (OPRM1 A118G) polymorphism is associated with brain mu-opioid receptor binding potential in smokers. *Proc Natl Acad Sci U S A* 108:9268-9273.
- Roberts AJ, McDonald JS, Heyser CJ, Kieffer BL, Matthes HW, Koob GF, Gold LH (2000) mu-Opioid receptor knockout mice do not self-administer alcohol. *J Pharmacol Exp Ther* 293:1002-1008.
- Skarke C, Kirchhof A, Geisslinger G, Lotsch J (2004) Comprehensive mu-opioid-receptor genotyping by pyrosequencing. *Clin Chem* 50:640-644.
- Spreckelmeyer KN, Paulzen M, Raptis M, Baltus T, Schaffrath S, Van WJ, Zalewski MM, Rosch F, Vernaleken I, Schafer WM, Grunder G (2011) Opiate-induced dopamine release is modulated by severity of alcohol dependence: an [<sup>18</sup>F]fallypride positron emission tomography study. *Biol Psychiatry* 70:770-776.
- Stoops WW, Lofwall MR, Nuzzo PA, Craig LB, Siegel AJ, Walsh SL (2012) Pharmacodynamic profile of tramadol in humans: influence of naltrexone pretreatment. *Psychopharmacology (Berl)* 223:427-438.
- Szeto CY, Tang NL, Lee DT, Stadlin A (2001) Association between mu opioid receptor gene polymorphisms and Chinese heroin addicts. *NeuroReport* 12:1103-1106.
- Tan EC, Tan CH, Karupathivan U, Yap EP (2003) Mu opioid receptor gene polymorphisms and heroin dependence in Asian populations. *NeuroReport* 14:569-572.
- Tsuang MT, Lyons MJ, Eisen SA, Goldberg J, True W, Lin N, Meyer JM, Toomey R, Faraone SV, Eaves L (1996) Genetic influences on DSM-III-R drug abuse and dependence: a study of 3,372 twin pairs. *Am J Med Genet* 67:473-477.
- Tsuang MT, Lyons MJ, Meyer JM, Doyle T, Eisen SA, Goldberg J, True W, Lin N, Toomey R, Eaves L (1998) Co-occurrence of abuse of different drugs in men: the role of drug-specific and shared vulnerabilities. *Arch Gen Psychiatry* 55:967-972.

- Urban NBL, Kegeles LS, Slifstein M, Xu X, Martinez D, Sakr E, Castillo F, Moadel T, O'Malley SS, Krystal JH, Abi-Dargham A (2010) Sex differences in striatal dopamine release in young adults after oral alcohol challenge: a positron emission tomography imaging study with [<sup>11</sup>C]raclopride. *Biol Psychiatry* 68:689-696.
- Watabe H, Endres CJ, Breier A, Schmall B, Eckelman WC, Carson RE (2000) Measurement of dopamine release with continuous infusion of [<sup>11</sup>C]raclopride: optimization and signal-to-noise considerations. *J Nucl Med* 41:522-530.
- Weerts EM, McCaul ME, Kuwabara H, Yang X, Xu X, Dannals RF, Frost JJ, Wong DF, Wand GS (2012) Influence of OPRM1 Asn40Asp variant (A118G) on [<sup>11</sup>C]carfentanil binding potential: preliminary findings in human subjects. *Int J Neuropsychopharmacol* 16:47-53.
- Zacny JP, Conley K, Galinkin J (1997a) Comparing the subjective, psychomotor and physiological effects of intravenous buprenorphine and morphine in healthy volunteers. *J Pharmacol Exp Ther* 282:1187-1197.
- Zacny JP, Conley K, Marks S (1997b) Comparing the subjective, psychomotor and physiological effects of intravenous nalbuphine and morphine in healthy volunteers. *J Pharmacol Exp Ther* 280:1159-1169.
- Zacny JP, Lichtor JL, Flemming D, Coalson DW, Thompson WK (1994a) A dose-response analysis of the subjective, psychomotor and physiological effects of intravenous morphine in healthy volunteers. *J Pharmacol Exp Ther* 268:1-9.
- Zacny JP, Lichtor JL, Thapar P, Coalson DW, Flemming D, Thompson WK (1994b) Comparing the subjective, psychomotor and physiological effects of intravenous butorphanol and morphine in healthy volunteers. *J Pharmacol Exp Ther* 270:579-588.
- Zhang D, Shao C, Shao M, Yan P, Wang Y, Liu Y, Liu W, Lin T, Xie Y, Zhao Y, Lu D, Li Y, Jin L (2007) Effect of mu-opioid receptor gene polymorphisms on heroin-induced subjective responses in a Chinese population. *Biol Psychiatry* 61:1244-1251.
- Zhang H, Luo X, Kranzler HR, Lappalainen J, Yang BZ, Krupitsky E, Zvartau E, Gelernter J (2006) Association between two mu-opioid receptor gene (OPRM1) haplotype blocks and drug or alcohol dependence. *Hum Mol Genet* 15:807-819.

# Appendix I - Questionnaires

Previous experience with pain pills:

Drug taken (please check those you have taken:

- Codeine
- Demerol
- Lorcet
- Vicodin
- Lorcet Plus
- Vicodin ES
- Norco
- Lortab
- Vicodin HP
- Percocet
- Percodan
- Oxycontin 40
- RMS MS Contin
- Duragesic
- Fentora
- Actiq
- Dilaudid (tabs)
- Palladone (caps)
- Opana
- Opana ER
- Oxycotin
- oxycodone
- Other \_\_\_\_\_

Please indicate why you were prescribed this medication:

---

---

---

---

When did you take this medication (month, year)? \_\_\_\_\_

How long did you take this medication? \_\_\_\_\_ days \_\_\_\_\_ months \_\_\_\_\_ years

When did you stop taking this medication? \_\_\_\_\_

Please rate whether you experienced the following effects when you have used pain pills in the past and how much you felt this effect. List is adapted from Apfelbaum et al. (2004)

Relaxed	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
	No effect								very relaxed	
Drowsiness	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
	Not drowsy								Fell asleep	
Dizziness	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
	Not dizzy								Felt very dizzy	
Nausea	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
	Not nauseous								Vomited a lot	
Pain Relief	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
	No relief								Pain gone	
Constipation	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
	None								Needed Medication	
Trouble breathing	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
	No trouble breathing								very short of breath	
Itching	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
	No itching						couldn't stop scratching			
Dry mouth	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
	Not experienced								very dry mouth	

Now, please rate whether you considered this effect good, bad or of no concern

Relaxed	good	no concern	bad
Drowsiness	good	no concern	bad
Dizziness	good	no concern	bad
Nausea	good	no concern	bad
Pain Relief	good	no concern	bad
Constipation	good	no concern	bad
Trouble breathing	good	no concern	bad
Itching	good	no concern	bad
Dry mouth	good	no concern	bad



The DEQ consists of four visual analog scales used to describe current drug effects. Subjects mark on 100-mm lines whether they

(1) feel drug effects

0	x	x	x	x	x	x	x	x	x	100
None										a lot

(2) like the effects they feel

0	x	x	x	x	50	x	x	x	x	100
Dislike					neutral					like extremely

(3) feel high

0	x	x	x	x	x	x	x	x	x	100
not at all										a lot

(4) would like more

0	x	x	x	x	x	x	x	x	x	100
not at all										a lot

Likert-like visual analog scale (VAS) of common side effects of opiate administration.

Sedation	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	
	No effect								very relaxed		
Drowsiness	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	
	Not drowsy								Fell asleep		
Dizziness	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	
	Not dizzy								Felt very dizzy		
Upset stomach		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
	Not nauseous								Vomited a lot		
Trouble breathing	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	
	No trouble breathing								very short of breath		
Itching	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	
	No itching						couldn't stop scratching				
Dry mouth	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	
	Not experienced								very dry mouth		
Headache	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	
	No effect								bad headache		
Euphoria	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	
	No effect								very high		
Anxiousness	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	
	No effect								very anxious		
Depression	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	
	No effect								felt very sad		
Tremors	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	
	No effect								shaking a lot		

## Abbreviated Comprehensive Psychopathology Rating Scales (NIAAA/LCTS version)

Patient's Name:

Patient's MRN:

Date and Time:

### 1. *Reported sadness*

Representing reports of depressed mood, regardless of whether it is reflected in appearance or not. Includes low spirits, despondency or the feeling of being beyond help and without hope. Rate according to intensity, duration and the extent to which the mood is reported to be influenced by events.

0 = Occasional sadness in keeping with the circumstances.

1

2 = Sad or low but brightens up without difficulty.

3

4 = Pervasive feelings of sadness or gloominess. The mood is still influenced by external circumstances.

5

6 = Continuous or unvarying sadness, misery or despondency.

### 2. *Inner tension*

Representing feelings of ill-defined discomfort, edginess, inner turmoil, mental tension mounting to either panic, dread or anguish.

Rate according to intensity, frequency, duration and the extent of reassurance called for.

0 = Placid. Only fleeting inner tension.

1

2 = Occasional feelings of edginess and ill-defined discomfort.

3

4 = Continuous feelings of inner tension or intermittent panic which the patient can only master with some difficulty.

5

6 = Unrelenting dread or anguish. Overwhelming panic.

### 3. *Hostile feelings*

Representing anger, hostility and aggressive feelings regardless of whether they are acted or not.

Rate according to intensity, frequency and the amount of provocation tolerated.

0 = Not easily angered. Inability to feel anger.

1

2 = Easily angered. Reports hostile feelings which are easily dissipated

3

4 = Reacts to provocation with excessive anger and hostility

5

6 = Persistent anger, rage or intense hatred which difficult or impossible to control

### 4. *Inability to feel*

Representing the subjective experience of reduced interest in the surroundings, or activities that normally give pleasure. The ability to react with adequate emotion to circumstances or people is reduced.

0 = Normal interest in the surroundings and in other people.

1

2 = Reduced ability to enjoy usual interests.

3

4 = Loss of interest in the surroundings. Loss of feelings for friends and acquaintances.

5

6 = The experience of being emotionally paralyzed, inability to feel anger, grief or pleasure and a complete or even painful failure to feel for close relatives and friends.

5. *Pessimistic thoughts*

Representing thoughts of guilt, inferiority, self-reproach, sinfulness, remorse and ruin.

0 = No pessimistic thoughts.

1

2 = Fluctuating ideas of failure, self-reproach or self-depreciation.

3

4 = Persistent self-accusations, or definite but still rational ideas of guilt or sin. Increasingly pessimistic about the future

5

6 = Delusions of ruin, remorse or unredeemable sin. Self-accusations which are absurd and unshakable.

6. *Suicidal thoughts*

Representing the feeling that life is not worth living, that a natural death would be welcome, suicidal thoughts, and preparations for suicide. Suicidal attempts should not in themselves influence the rating.

0 = Enjoys life or takes it as it comes.

1

2 = Weary of life. Only fleeting suicidal thoughts.

3

4 = Probably better off dead. Suicidal thoughts are common, and suicide is considered as a possible solution, but without specific plans or intention.

5

6 = Explicit plans for suicide when there is an opportunity. Active preparations for suicide.

7. *Hypochondriasis*

Representing exaggerated preoccupation or unrealistic worrying about ill health or disease. Distinguish from worrying over trifles and aches and pains.

0 = No particular preoccupation with ill health

1

2 = Reacting to minor bodily dysfunction with foreboding. Exaggerated fear of disease.

3

4 = Convinced that there is some disease but can be reassured, if only briefly.

5

6 = Incapacitating or absurd hypochondriacal convictions (body rotting away, bowels have not worked for months)

8. *Worrying over trifles*

Representing apprehension, and undue concern over trifles, which is difficult to stop and out of proportion to the circumstances. Distinguish from inner tension, pessimistic thoughts, hypochondriasis and phobias.

0 = No particular worries

1

2 = Undue concern, worrying that can be shaken off

3

4 = Apprehensive and bothered about trifles or minor daily routines

5

6 = Unrelenting and often painful worrying. Reassurance is ineffective

9. *Phobias*

Representing feelings of unreasonable fear in specific situations (such as buses, supermarkets, crowds, feeling enclosed, being alone) which are avoided if possible.

0 = No phobias

1

2 = Feelings of vague discomfort in particular situations which can be mastered without help or by taking simple precautions like avoiding rush hours when possible

3

4 = Certain situations consistently provoked marked discomfort, and are avoided without impairing social performance

5

6 = Incapacitating phobias which severely restrict activities, for example completely unable to leave home

10. *Lassitude*

Representing a difficulty getting started or slowness initiating and performing everyday activities.

0 = Hardly any difficulties in getting started. No sluggishness.

1

2 = Difficulties in starting activities

3

4 = Difficulties in starting simple routine activities which are carried out with effort.

5

6 = Complete lassitude. Unable to do anything without help.

11. *Concentration difficulties*

Representing difficulties in collecting one's thoughts amounting to incapacitating lack of concentration.

Rate according to intensity, frequency, and degree of incapacity produced.

0 = No difficulties in concentrating.

1

2 = Occasional difficulties in collecting one's thoughts.

3

4 = Difficulties in concentrating and sustaining thought which reduces ability to read or hold a conversation.

5

6 = Unable to read or converse without great difficulty.

12. *Reduced appetite*

Representing the feeling of a loss of appetite compared with when well. Rate by loss of desire for food or the need to force oneself to eat.

0 = Normal or increased appetite

1

2 = Slightly reduced appetite.

3

4 = No appetite. Food is tasteless.

5

6 = Needs persuasion to eat at all.

13. *Reduced sleep*

Representing the experience of reduced duration or depth of sleep compared to the subject's own normal pattern when well.

0 = Sleeps as usual.

1

2 = Slight difficulty dropping off to sleep or slightly reduced, light or fitful sleep.

3

4 = Sleep reduced or broken by at least two hours.

5

6 = Less than two or three hours sleep.

14. *Autonomic disturbances*

Representing descriptions of palpitations, breathing difficulties, dizziness, increased sweating, cold hands and feet, dry mouth, indigestion, diarrhea, frequent micturition. Distinguish from hypochondriasis, autonomic disturbance (item 18), and muscular tension.

0 = No autonomic disturbances

1

2 = Occasional autonomic symptoms which occur under emotional stress

3

4 = Frequent or intense autonomic disturbances which are experienced as discomforting or socially inconvenient

5

6 = Very frequent autonomic disturbances which interrupt other activities or are incapacitating.

15. *Aches and pains*

Representing reports of bodily discomfort, aches and pains. Rate according to intensity, frequency and duration, and also request for relief. Disregard any opinion of organic cause. Distinguish from hypochondriasis, autonomic disturbance, and muscular tension.

0 = Absent or transient

1

2 = Occasional definite aches and pains

3

4 = Prolonged and inconvenient aches and pains. Requests for effective analgesics

5

6 = Severely or crippling pains

16. *Muscular tension*

Representing observed muscular tension as shown in facial expression, posture, and movements.

0 = Appears relaxed

1

2 = Slightly tense posture and face.

3

4 = Moderately tense posture and face (easily seen in jaw and neck muscles). Does not seem to find a relaxed position when sitting. Stiff and awkward movements

5

6 = Strikingly tense. Often sits hunched and crouched, or tense and rigidly upright at the edge of the chair

17. *Apparent Sadness*

Representing despondency, gloom and despair, (more than just ordinary transient low spirit) reflected in speech, facial expression, and posture. Rate by depth and inability to brighten up.

0 = No sadness.

1

2 = Looks dispirited but does brighten up without difficulty.

3

4 = Appears sad and unhappy most of the time.

5

6 = Looks miserable all the time. Extremely despondent

18. *Autonomic disturbances*

Representing signs of autonomic dysfunction, hyperventilation or frequent sighing, blushing, cold hands, enlarged pupils and dry mouth, fainting.

0 = No observed autonomic disturbances

1

2 = Occasional or slight autonomic disturbances such as blushing or blanching, or sweating under stress

3

4 = Obvious autonomic disturbance on several occasions even when not under stress

5

6 = Autonomic disturbances which disrupt the interview

*Items used in scoring Depression (MADRS) and Anxiety (BSA)*

Depression

- Reported sadness (Item #1)
- Inner tension (Item #2)
- Inability to feel (Item #4)
- Pessimistic thoughts (item #5)
- Suicidal thoughts (item #6)
- Lassitude (item #10)
- Concentration difficulties (item #11)
- Reduced appetite (item #12)
- Reduced sleep (item #13)
- Apparent Sadness (CPRS item #17)

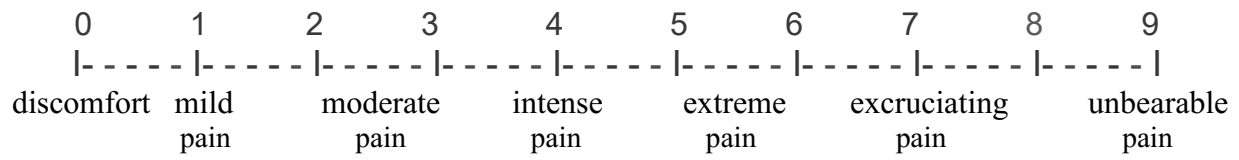
Anxiety

- Inner tension (Item #2)
- Hostile feelings (Item #3)
- Hypochondriasis (item #7)
- Worrying over trifles (item #8)
- Phobias (item #9)
- Reduced sleep (item #13)
- Autonomic disturbances (reported) (item #14)
- Aches and pains (item #15)
- Muscular tension (item #16)
- Autonomic disturbances (observed) (item #18)

Note that items #2 (inner tension) and #13 (reduced sleep) contribute to the score for both indexes (MADRS and BSA)

### Cold Pressor Test – Pain scoring

How does your hand in the water bath feel right now?



Hand withdrawn: \_\_\_min \_\_\_sec (Max 3 min)



## Appendix II. Recruiting advertisements

### 1) Kimes\_Opioid Pills Protocol\_DRAFT Ad

The ad will include a standard NIDA graphic and the following text.

If you ...

- Are healthy and between 21 – 55
- Have ever used pain pills for a medical condition such as a broken bone or dental surgery
- Are a non-smoker

... we need YOU for a research study in East Baltimore.

The purpose of this research is to test the effect of genetics and a pain reducing medication on receptors for a brain molecule called dopamine.

There is no cost to participate

- If selected, you will be compensated for your participation in this study.

Call today for a confidential screening.

## Appendix III. Screening questionnaires for Patient Recruitment Office

The following provides the stepwise participant screening procedure for this protocol. Advertisements for this protocol are placed under the MMG contract. The prospective participants call a phone number that is answered by MMG Recruitment staff. The standard, approved phone screening consent form is used. If the initial phone screen indicates that the prospective participant is eligible for participation in this study, he is invited to NIDA for the rest of the screening procedure. Participant is excluded if he is younger than 21 yrs or older than 55 yrs.

The prospective participant comes to the MMG recruitment reception area. One of the recruiters explains the screening process to the person and obtains his consent for the screening process. . All clinical assessments/procedures will be carried out by an appropriately trained staff member.

### 1) Psychological and Psychosocial Testing

- a. Shipley Institute of Living Scales or Vocabulary module of WASI (Wechsler Abbreviated Scale of Intelligence).
- b. All subjects must have a stable point of contact.
- c. Edinburgh Handedness Inventory – Subjects undergoing PET scans must be right handed.
- d. *SCID Screen Patient Questionnaire – Extended (SSPQ-X)* Prospective participants are excluded if they present with any current DSM-IV axis I diagnosis, including substance use disorders (except nicotine dependence for either group). Subjects may not be dependent on alcohol or caffeine. Occasional marijuana use is allowed and is defined as less than or equal to two cigarettes or blunts/month.
- e. Participant is excluded if there is claustrophobia. To further exclude this possibility, all potential participants will be placed in the mock scanner for at least 5 min.

### 2) Clinical Laboratory Testing

A trained medical staff must perform the blood collection. The collection site will generally be the middle arm veins. Notify the MAI of any values outside the normal range. The MAI will make determination of participant's suitability for the study.

#### 1. Chemistry

- a. Liver function: The acceptable upper limits are as follows:
  - i. AST, ALT > 100
  - ii. GGT twice the upper limit of normal

#### 2. Exclusionary Criteria for Other Laboratory Studies

- a. Potassium < 3.5 > 5.1
- b. BUN > 35
- c. Creatinine > 1.5
- d. Glucose > 125 fasting
- e. Total bilirubin > 2.0
- f. Calcium < 8.0 > 10.5

#### 3. Complete blood count - Results should be generally within or close to the normal ranges – Notify MAI of any values outside the normal range. MAI will make determination of participant's suitability for the study.

- a. Hematocrit: < 39% exception: hemoglobin thalassemia trait is present where hematocrit of 35% would be minimum acceptable value.
- b. White blood cell count must be between 3,500 and 11,500.

4. Urinalysis - Results should be generally within or close to the normal ranges – Notify MAI of any values outside the normal range. MAI will make determination of participant's suitability for the study.
    - a. Protein of greater than or equal to 1+
    - b. Glucose of greater than or equal to 1+
  5. Urine toxicology – There should be no positive results.
  6. Tuberculin skin testing (unless documented prior positive response, see Tuberculosis Policy). – Test results must be negative.
  7. HIV, Hepatitis B SAg, & hepatitis C –Results should be negative.
- 3) History and Physical: A complete history and physical examination must be obtained on all applicants who wish to participate in this protocol. Presentation of false medical information may be grounds for dismissal from study participation. History to include the following – Except those items for which exclusion criteria are noted, MAI to review results and decide if participant must be excluded.
- A. History
1. General:
    - a. Recent unexplained weight loss greater than 10 lbs. in a 3-month period
    - b. Fever of unexplained origin or a fever of greater than a one-month duration
    - c. Unexplained night sweats
    - d. History of malignancy
    - e. Participant is excluded if he has ferromagnetic metal in the body or heart pacemaker
    - f. Participant is excluded if he weighs more than 300 lbs.
    - g. Participant is excluded if veins are inaccessible
    - h. Radiation exposure from research studies exceeding 5.0 rem in the past 12 months
    - i. Known chronic renal or hepatic dysfunction
  2. Cardiac: Participant excluded if he has a history of:
    - a. Known arrhythmia
    - b. Symptomatic or known coronary artery disease
    - c. History of endocarditis, cerebral embolism
  3. Pulmonary: Participant excluded if he has a history of:
    - a. Adult asthma – unless mild and occasional.
    - b. History of obstructive pulmonary disease
  4. Neurological: Participant excluded if he has a history of:
    - a. Structural brain abnormalities
    - b. Infectious disease
    - c. Head trauma resulting in unconsciousness greater than 5 minutes or hospitalization
    - d. History of seizures as an adult
    - e. Sleep apnea
    - f. Tic disorder
    - g. Autoimmune disease involving CNS
  5. Endocrine: Participant excluded if he has a history of:
    - a. Hyperthyroidism
    - b. Hypothyroidism
    - c. Diabetes mellitus
    - d. Endocrine disease

6. Current or Past Medication use: Volunteers may not be currently using chronic prescription or over the counter medications.

B. Physical Examination:

1. Vital Signs

- a. Participant excluded if: Systolic blood pressure over 135 mm Hg or less than 95 mm hg; diastolic blood pressure over 90 mm Hg, recorded on at least 3 different occasions.
- b. Participant excluded if: Pulse rate over 105 or less than 50 on a consistent basis. Irregular pulse rate should be evaluated with a 12 lead ECG and 3-5 minute rhythm strip that will become a part of the permanent record.
- c. Participant excluded if: Respiratory rate over 24

2. Cardiac: Participant excluded if he has:

- a. Jugular venous distention
- b. Carotid bruits
- c. S3 and S4 heart sound
- d. Displaced point of maximal impulse, beyond the anterior axillary line
- e. Valvular disease
- f. Palpable thrill
- g. Peripheral edema

3. Pulmonary: Participant excluded if he has:

- a. Wheezes, rales or rhonchi
- b. Cyanosis

4. Diagnostic Studies - MAI to review results and decide if participant must be excluded.

- a. Electrocardiogram:

All participants screened for non-therapeutic studies will have a 12 lead EKG and a 3 to 5 minute rhythm strip done that will become a part of the permanent medical record.

Participant is excluded if any of the following conditions are documented on an EKG:

- i. Wolfe Parkinson White syndrome
- ii. Myocardial infarction
- iii. Left bundle branch block, complete or partial
- iv. PR interval of less than .12 seconds or over .20 seconds
- v. Prolonged QT interval (corrected) greater than 0.43 seconds

MMG Recruiting staff assigns ARC #s for the participant, prepares the charts and informs the MAI of the prospective participant.

## **Appendix IV. Investigator's Brochure**

- none available for [<sup>11</sup>C]raclopride
- See attached file of Morphine Package insert

## **Appendix VI. Other – Evaluation to sign consent and expected answers**

**NIDA/NIAAA Evaluation of Potential Research Participants' Ability to Consent**

ARC#: \_\_\_\_\_ Protocol #: \_\_\_\_\_ Subject Initials: \_\_\_\_\_ Date: \_\_\_\_/\_\_\_\_/\_\_\_\_

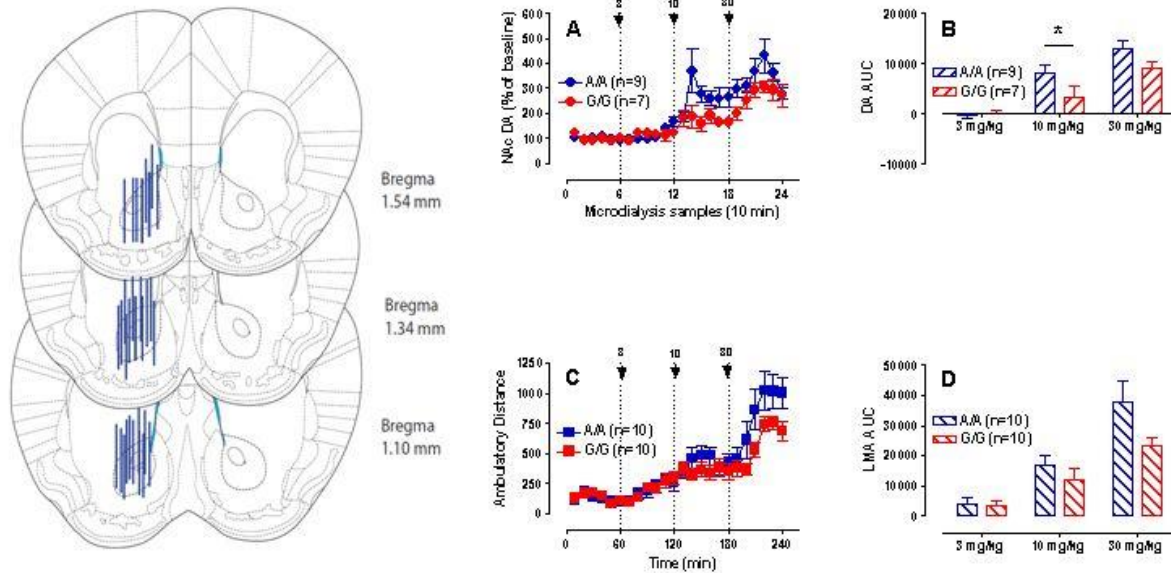
For each question, circle the score that most closely reflects your evaluation.  
You may explain or rephrase questions to help the potential participant understand them. Individuals who do not pass the first time may be re-evaluated once.

Is the potential participant able to communicate, oriented, and not under the influence of drugs or alcohol? [if presently not capable, may be re-assessed later]	0= Not Capable	1= Capable
1. What will you be asked to do if you enroll in the study? [must understand primary procedures]	0 = no or insufficient understanding	1 = sufficient understanding
2. What are the most significant risks to you of being in the study? [must recognize the most significant risks]	0 = no or insufficient understanding	1 = sufficient understanding
3. What happens if you don't want to be in the study? [must understand that can decline without penalty]	0 = no or insufficient understanding	1 = sufficient understanding
4. If you enroll in the study, can you later change your mind and stop participating? [must understand that can stop participating at any time]	0 = no or insufficient understanding	1 = sufficient understanding
5. What are the chances that being in the study will benefit you personally? [must understand the potential benefits, including the possibility that will not benefit personally]	0 = no or insufficient understanding	1 = sufficient understanding
6. What would you do if you experience pain or distress while in the study? [must be willing to tell team]	0 = no or insufficient understanding	1 = sufficient understanding
7. What things could you do (for your condition) besides being the study? [must recognize available alternatives]	0 = no or insufficient understanding	1 = sufficient understanding
8. How do the risks and potential benefits of being in the study compare to the risks and benefits of your every day life. [must understand comparative risks and potential benefits]?	0 = no or insufficient understanding	1 = sufficient understanding
9. For what reasons could we decide that you will not finish the study?	0 = no or insufficient understanding	1 = sufficient understanding

I hereby certify that the potential participant has the ability to consent and scored 1 on all questions

\_\_\_\_\_ (Evaluator Signature)    \_\_\_\_/\_\_\_\_/\_\_\_\_ (Date)

## Appendix VII. Results from mouse study



Individual probe placements in the nucleus accumbens (NAc) and dialysate DA levels in response to systemic morphine (3' 10 and 30 mg/kg, i.p.) in h/mOPRM1-118AA and h/mOPRM1-118GG mice.

Repeated measures ANOVA (with genotype as a between-factor and drug doses and time as within-factors) conducted for DA time course data showed significant main effect of dose ( $F_{(3,6)} = 34.6$ ;  $p < 0.001$ ) and genotype ( $F_{(1,6)} = 8.98$ ;  $p = 0.024$ ), indicating that NAc DA increased dose-dependently following morphine injections and overall response was different between two genotypes. Repeated measures ANOVA (genotype – between-factor, dose – within-factor) conducted for area under the curve (AUC) DA data revealed significant main effect of genotype ( $F_{(1,14)} = 4.38$ ;  $p = 0.05$ ) and dose x genotype interaction ( $F_{(2,14)} = 4.06$ ;  $p = 0.037$ ), as well as significant simple effect of genotype for DA response to the second drug dose ( $F_{(1,14)} = 7.31$ ;  $p = 0.02$ ). Overall, these results indicate that NAc DA response to systemic morphine was significantly reduced in h/mOPRM1-118GG genotype as compared with h/mOPRM1-118AA genotype.

Repeated measures ANOVA (genotype – between-factor, dose and time – within-factors) conducted for LMA time course data revealed significant main effect of dose ( $F_{(3,18)} = 62.5$ ;  $p < 0.001$ ) and dose x genotype interaction ( $F_{(3,18)} = 3.62$ ;  $p = 0.05$ ). Repeated measures ANOVA (genotype – between-factor, dose – within-factor) conducted for LMA AUC data also showed significant main effect of dose ( $F_{(2,18)} = 68.9$ ;  $p < 0.001$ ) and dose x genotype interaction ( $F_{(2,18)} = 4.5$ ;  $p = 0.035$ ); however, simple effects of genotype for each dose separately were not significant. Overall, these results indicate that systemic morphine administration dose-dependently increased LMA and these increases were reduced in h/mOPRM1-118GG genotype as compared with h/mOPRM1-118AA genotype.



# **Appendix IX. 1SE Inpatient Nursing Information Sheet**

## **Welcome To 1SE**

### **Non-Treatment Seeking Participant**

Welcome to 1 Southeast (1SE), a locked behavioral health inpatient research care unit at the National Institutes of Health (NIH) Clinical Center in Bethesda, Maryland. Individuals admitted to (1SE) take part in clinical research studies with the National Institute on Alcohol Abuse and Alcoholism (NIAAA) and the National Institute on Drug Abuse (NIDA). This information has been prepared for you to help you adjust to our inpatient research setting. You will also receive a general Welcome Book when you are admitted to 1SE to help address any other questions you may have concerning your stay with us. Your specific protocol consent form will identify and explain what research activities you will be involved in. Thank you for helping to advance our understanding of health and illness.

### **General Unit Information**

#### **Overview**

(1SE) is set up as a therapeutic alcohol treatment program. This treatment program is approximately a month long and involves many structured activities that are focused on alcoholism recovery. As a non-treatment seeking research participant, you are a guest in this community, and will not be participating in the recovery program.

You will be on 1 SE as an inpatient for a series of short research visits to successfully complete your research protocol. Your research procedures and activities are explained in your consent form. Beyond your research schedule, you will have time to fill. Please think ahead and bring items (for example, personal computers and DVD players) to use this time comfortably. Our Recreational Therapy staff may provide some diversional activities.

You will be accompanied by a member of our research or nursing team at all times while off the unit. We provide you with this extra attention to make sure that the research is done the same way with each of you. This allows the scientists to compare your results with confidence to that of all of the other participants, thus maintaining the integrity of our research protocols.

#### **Medications from Home**

As part of our safety routines, we ask all research participants to tell us what medications that they are taking on admission. If you bring any of your medication with you, your nurse will collect it from you and store it in a secure location until your discharge. While you are an inpatient, all of your medications will be dispensed by your nurse, including your own medications as well as the study medication(s) which may be part of your research protocol. Because of our hospital policy, you will not be allowed to keep any medication in your room.

#### **Sharps Objects and Toiletries**

Upon admission your personal belongings will be searched. For everyone's safety, no sharp objects such as razors, nail clippers, knives or keys can be kept in your room. Personal care items containing alcohol such as after-shave lotions, colognes, and perfumes also will be stored in the nurses' station. You may ask for them when you need them for short-term use, and return them to a nurse after each use. Your belongings will be returned to you upon discharge.

#### **Activity/Privilege Levels**

Because (1 SE) is a locked unit, your ability to leave the unit is dependent on your physical/mental status and the requirements of your research protocol. The 1SE privilege levels are as follows: Level 1 – Restricted to Unit, Level 2 – Staff Accompanied while off the unit,

Level 3 – May leave the unit alone in the CRC or on the NIH Grounds. All patients are required to sign out and sign back in upon return to the unit.

### **Smoking Policy**

NIH is a smoke free campus. However, (1 SE) patients are permitted to smoke if they have a Physician's order, in a restricted area designated only for smoking. Your Nurse will show you where this is located should you be interested. Please be aware that your research protocol requirements may limit your ability to smoke.

### **Food**

For regular meals, you will have a choice of menus. You may eat your meals in your room or at the communal tables in the day room area. Visitors may bring food items to you but they must be checked by the nursing staff. Please be aware that certain research protocols have dietary requirements that impact the type of diet you might be on as well as the times that you may eat. This type of specific dietary information is covered in your protocol consent form. Research staff will also review this information with you individually. Generally, meals are served at the following times: Breakfast: 7:30 AM -8:00 AM, Lunch: 11:30 AM – 12:15 PM, Dinner: 4:45 PM – 5:15 PM.

### **Cell Phones and Laptops**

You may use your cell phone on the unit. However, we ask that you do not talk on your cell phone in the day room area where other patients might be involved in treatment or recreational activities. Additionally, we ask that you do not bring your cell phone to any research related activities. You may use your laptop in your room and in the common areas.

### **Rooms**

1SE has two single rooms and six double rooms. The single rooms are generally reserved to accommodate patients with specific protocol and/or clinical requirements. Therefore, we cannot guarantee that you will be able to have a single room. Each room is equipped with a full bathroom, a comfortable bed, dressers and closet space. The staff of 1SE will make every effort to maintain your privacy. However, due to unit safety requirements, 1SE nurses make safety rounds on patients every 30 minutes during the day and every hour during the night to make sure all patients are safe and accounted for. If you have any additional questions or concerns please ask your nurse.

We thank you in advance for your participation and welcome you to our research care unit. With 24 -hour Nursing care and Research team members available to you; we are working to offer you a safe, comfortable and meaningful stay with us.

## **Appendix X. Plan for clinical monitoring of participants receiving morphine at NIDA:**

Note: Equivalent medications and equipment may be substituted with those listed here based on need and availability. This document is a guideline and all numbers provided herein are guidelines and must be accompanied by sound clinical judgment when decisions are made.

Equipment/medications to be available:

1. Crash cart with multiple doses of naloxone
2. 1mg/ml Naloxone hydrochloride injection USP, for intravenous, intramuscular and subcutaneous administration
  - a. Ten 2 mL single dose disposable prefilled syringes, in the MIN-I-JET® system with 21 G. x 1 1/2" needle. Boxes of 25. NDC 76329-1469-5, Stock No. 1469 (contains no preservative), or
  - b. Ten 2 mL single dose disposable Luer-Jet™ Luer-Lock Prefilled Syringe. Shrink Wrapped Packages of 10. NDC 76329-3369-1, Stock No. 3369 (contains no preservative)
3. Epinephrine IM (EpiPen, AdrenaClick, Twinject, etc.)
4. Diphenhydramine 25-50mg PO or 10-50mg IV/IM
5. Ranitidine 150mg PO or 50mg IV/IM
6. Ondansetron ODT (Orally Disintegrating Tablets) 4mg
7. Solumedrol 8mg PO or prednisone 10mg PO
8. Supplemental oxygen tank with tubing
9. Nasal cannula
10. Venturi mask
11. Pulse oximeter
12. Portable blood pressure/heart rate monitor
13. Normal saline (3 bags)
14. Electrocardiogram machine

Plan:

1. Personnel with NIH medical credentials (MD, PA, or RN) and ACLS certification as well as the investigator administering the tests will be in the room with the participant from 5 minutes before the injection until 30 minutes after the injection.
2. Participant's respiratory rate and oxygen saturation will be continuously monitored by pulse oximetry.
3. If participant's oxygen saturation level drops below 94% for >1-2 minutes, he will be given supplemental oxygen. If the oxygen saturation level improves, the participant will be allowed to continue the study.

4. If participant's oxygen saturation level drops and remains below 90% for >1-2 minutes or his respiration rate drops below 8 breaths/min, or s/he becomes sedated and difficult to arouse the following will be done:
  - a. Discontinue morphine.
  - b. Administer supplemental oxygen by Venturi mask
  - c. If no improvement, administer Naloxone 0.2-0.4mg IV/IM/SC .  
Additional Naloxone 0.2-0.4mg IV/IM/SC may be administered every 2-3 minutes if no response. Given the short half-life of naloxone (30-90 mins), naloxone will be repeated if vital signs or participant condition indicates re-sedation prior to arrival of emergency personnel.
    - 1.If participant does not respond to above measures, call 911 and transport to the Johns Hopkins bayview Emergency Room
    - 2.Participant will be disqualified from the study.
5. If participant vomits or feels excessively nauseous, he will be disqualified from the study.

## **Appendix XI. Plan for clinical monitoring of participants receiving morphine at the NIH Clinical Center in the Course of PET sessions**

Note: Equivalent medications and equipment may be substituted with those listed here based on need and availability. This document is a guideline and all numbers provided herein are guidelines and must be accompanied by sound clinical judgment when decisions are made.

1. Rapid Response Team (ICU staff) receive a courtesy call notifying them in advance of the time and location for this study session by the Research Coordinator.
2. The ACLS certified physician responsible for the participant and drug administration has called the RRT and given a brief clinical report and confirmed dosing time.
3. A full stocked Code Cart remains in the designated area in Interventional Radiology (including a suction device), 40 Seconds walking distance from the PetScan room.
4. A full stocked, ACLS Code Cart Tray provided by Pharmacy will remain in the PetScan room for the duration of the session, by the Clinical Nurse manager
5. Emergency drugs, prepared for immediate administration have been provided to our ACLS physician to be held by this M.D. on hand for the duration of the study session, including Naloxone, Atropine and Ondansetron.
6. A bell will be given to the participant once in the PET Scanner with proper instructions to use at any point in time during the study session as a means to signal distress in addition to free verbal expressions.
  
7. Code cart with multiple doses of naloxone
  - a. 1mg/ml Naloxone hydrochloride injection USP, for intravenous, intramuscular and subcutaneous administration. Initial naloxone dose should be 0.4mg, and subsequent doses 0.4-2.0mg. The rationale for starting with the lower dose is to avoid precipitating an opioid withdrawal reaction should the subject have a high degree of tolerance due to the use of some synthetic opioid that has remained undetected by our biochemical tests.
  - b. Ten 2 mL single dose disposable prefilled syringes, in the MIN-I-JET® system with 21 G. x 1 1/2" needle. Boxes of 25. NDC 76329-1469-5, Stock No. 1469 (contains no preservative), or
  - c. Ten 2 mL single dose disposable Luer-Jet™ Luer-Lock Prefilled Syringe. Shrink Wrapped Packages of 10. NDC 76329-3369-1, Stock No. 3369 (contains no preservative)
8. Atropine sulphate IV: 1mg/ml  
Dose of 0.5 ml IV push PRN for acute symptomatic bradycardia. This dose may be repeated every 3 to 5 minutes up to a maximum total dose of 3 mg
9. Epinephrine IM: (EpiPen, Adrenalick, Twinject, etc.)
10. Diphenhydramine: 25-50mg PO or 10-50mg IV/IM
11. Ranitidine: 150mg PO or 50mg IV/IM
12. Ondansetron: 2mg/ml. Dose 4 mg IV push PRN nausea and/or vomiting
13. Solumedrol 8mg PO or prednisone 10mg PO
14. Supplemental oxygen with tubing, in the PetScan room attached to the wall.

15. Nasal cannula
16. Venturi mask
17. Pulse oximeter
18. Portable blood pressure/heart rate monitor
19. Normal saline (3 bags)
20. Electrocardiogram machine

**Plan:**

1. Personnel with NIH medical credentials (MD, NP, or RN) and ACLS certification as well as the investigator administering the tests will be in the room with the participant from 5 minutes before the injection until 60 minutes after the injection.
2. Participant's respiratory rate, oxygen saturation, heart rate and blood pressure will be continuously monitored.
3. If participant's oxygen saturation level drops below 94% for >1-2 minutes, he will be given supplemental oxygen which will be adjusted to reach a target saturation of 94% or higher. If the oxygen saturation level improves, the participant will be allowed to continue the study.
4. If participant's oxygen saturation level drops and remains below 90% for >1-2 minutes or his respiration rate drops below 8 breaths/min, or s/he becomes sedated and difficult to arouse the following will be done:
  - a. Discontinue drug administration if still ongoing, terminate study session, and rapidly remove subject from the scanner bed to a position that allows adequate observation and access for procedures.
  - b. Administer supplemental oxygen by Venturi mask, adjusting it to achieve a target saturation of 94% or higher. If no improvement, administer Naloxone 0.4mg IV/IM/SC and call the Code team.
  - c. Observe for signs of precipitated withdrawal due to use opioid use that has been undetected by drug screens. If no signs of withdrawal, additional Naloxone 0.4-2.0 mg IV/IM/SC may be administered every 2-3 minutes if no response. Given the short half-life of naloxone (30-90 mins), naloxone will be repeated if vital signs or participant condition indicates re-sedation prior to arrival of emergency personnel.
5. If participant displays clinically significant bradycardia, hypotension or a clinically significant combination thereof, Initiation of the standard ACLS algorithm and the Code team will be called for treatment of these conditions, i.e. atropine 0.5mg IV push, may repeat up to a total dose of 3mg. If there is not an adequate response to this intervention, consider initiating the next step of the standard ACLS algorithm, i.e. administration of epinephrine, and initiating IV fluid.
6. If participant displays any of the problems above, vomits or feels excessively nauseous, or displays any other adverse reactions such as e.g. excessive sedation, anxiety or allergic symptoms and these cannot be adequately managed, (the subject will be removed from the scanner [note: Clinical Center version]) any ongoing research procedures will be discontinued, and subject will be placed in a position that ensures free airways, adequate observation, and access for procedures.

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