PARTNERS HUMAN RESEARCH COMMITTEE
DETAILED PROTOCOL

PROTOCOL TITLE

A brain imaging study of opioid (morphine) and non-opioid (ketorolac) conditioning effects

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LIST OF ABBREVIATIONS

AC – Attenuation Correction
ACC – Anterior Cingulate Cortex
AI – Anterior Insula
ALT – Alanine Transaminase
AST – Aspartate Transaminase
ANOVA – Analysis of Variance
BMI – Body Mass Index
CNS – Central Nervous System
COPD – Chronic Obstructive Pulmonary Disease
COVAS – Computerized Visual Analog Scale Software
CRC – Clinical Research Core
CRC-BIC – Clinical Research Core - Biomedical Imaging Core
CT – Computerized Tomography
DNIC - Diffuse Noxious Inhibitory Control
DUTE – Duel-Echo Ultra-Short Echo Sequence
DVR – Distribution Volume Ratio
EKG – Electrocardiogram
EPI – Echo Planar Imaging
ERS – Expectation of Relief Scales
FDA – Federal Drug Administration
fMRI – Functional Magnetic Resonance Imaging
GLM – General Linear Model
GSES – General Self Efficacy Scale
IRI – Interpersonal Reactivity Index
IV – Intravenous
LMR – Longitudinal Medical Record
LOT-R – Life Orientation Test - Revised
MBq – Megabecquerel
mCi - Millicurie
MGH – Massachusetts General Hospital
mN - Millinewton
MRI – Magnetic Resonance Imaging
NEO-PI-S – NEO Personality Inventory - Short Form
N/P-VAS – Nausea/Pruritis Visual Analogue Scale
NSAID – Non-Steriodal Anti-Inflammatory Drug
OP-OSEM – Ordinary Poisson Ordered Subset Expectation Maximization
PADSS – Post-Anesthesia Discharge Scoring System
PAG – Periaqueductal Gray
PET – Positron Emission Tomography
PFC – Prefrontal Cortex
ROI – Region of Interest
SD – Standard Deviation
STAI – State-Trait Anxiety Inventory
TAS – Tellegen Absorption Scale
μSv – Microsieverts

I. BACKGROUND AND SIGNIFICANCE

Placebo effects are salubrious benefits attributable to the non-specific symbolic components of health care, which have profound implications for basic and clinical research, and medical practice . Placebo analgesia has demonstrated extremely robust placebo effects that both provide a unique example of how previous experience can modulate pain perception and open a new window into pain management. Previous studies have demonstrated that placebo analgesia derives from active neural process involving multiple brain regions. Studies also indicate that no single neurobiological mechanism can explain the general cause of this effect.

Although it is well known that both opioid and non-opioid mechanisms may underlie placebo analgesia , few studies have explored exactly what the difference is between these two mechanisms and under what circumstances they are activated. In one of the few studies that investigated the two mechanisms, Dr. Benedetti and colleagues found that morphine conditioning is naloxone reversible while ketorolac conditioning is not . This evidence provides an ideal model for tracing coherent but distinct placebo mechanisms.

To date, no neuro-imaging experiment has mapped these distinct pathways. We will adopt a paradigm developed and validated by Benedetti that separates out "pure conditioning" placebo effects of morphine and ketorolac from any "adulteration" of conscious expectancy using fMRI. Such a mapping would significantly elucidate and clarify critical placebo mechanisms. Furthermore, given that placebo effects are likely significantly configured by patients' previous drug experience, such an understanding would help move placebo studies closer to translational research.

II. SPECIFIC AIMS

Placebo effects are salubrious benefits attributable to the non-specific symbolic components of health care, which has profound implications for basic and clinical research, and medical practice . Placebo analgesia has demonstrated extremely robust placebo effects that both provide a unique example of how previous experience can modulate pain perception and open a new window into pain management. Previous studies have demonstrated that placebo analgesia derives from active neural process involving multiple brain regions. Studies also indicate that no single neurobiological mechanism can explain the general case of this effect.

Specific Aim: Characterize placebo analgesic response to morphine and ketorolac (conditioning) paradigms using an integrated MRI-PET system.
Hypothesis 1: Both morphine and ketorolac conditioning will produce significant decreases in pain rating compared to the control conditions.
Hypothesis 2: Morphine administration will work through and facilitate the opioid pain descending inhibition system ; thus, morphine conditioning will primarily rely on the pain descending inhibition system including pre-genual anterior cingulate cortex (pACC), periaqueductal gray (PAG), and lateral prefrontal cortex (LPFC). Morphine conditioning will produce fMRI signal increases in pACC, PAG, and LPFC, and fMRI signal decreases in insula and S2 in comparison to control conditions.
Hypothesis 3 Ketorolac is a nonsteroid anti-inflammatory drug that produces analgesia by inhibiting prostaglandins (compounds that can enhance the elicitation of pain input)

at both the peripheral and spinal level to decrease afferent noxious input . Studies have also suggested that spinal circuits have the capacity to respond to differential conditioning and environmental cues. Thus, the ketorolac conditioning effect will result from modulating spinal cord pathways that blunt the afferent noxious input from the spinal cord. Nevertheless, this decreased noxious input can be detected indirectly at the brain level and we will find fMRI signal decreases in the insula and S2 as an indication of reduced noxious signal input. There will be no significant [11C] diprenorphine binding potential (BP) reduction since ketorolac conditioning is not naloxone reversible [27].

III. SUBJECT SELECTION

Healthy, normal male and female adults who have no contraindications to fMRI scanning or to the study drugs will be recruited for this study. We will recruit a maximum of 80 subjects (until 52 complete the study) between the ages of 21 and 50 years old. From our experience in a previous study (Protocol 2004-P-000096), we have observed a high rejection rate for subjects due to inability to meet all continuation requirements (primarily the requirement of stable and reliable responses to pain stimuli necessary to perform the quantitative experiments). We typically require approximately twice the target number of subjects in order to complete a cohort. We will also recruit up to 8 additional "pilot subjects" as described below.

All subjects will be within 15% of normal body mass index (BMI). We will study both males and females for this study because we expect significant gender effects on measures of analgesia. We will study adult subjects of wide age range to investigate any significant age effects and to have appropriate controls for future clinical studies. Exclusion criteria will include current or past history of major medical, neurological, or psychiatric illness, the presence of indwelling ferromagnetic materials, pregnancy, contraindications to study drugs, claustrophobia, instability of responses to experimental pain (see below), and non-fluent English speaking and reading abilities.

Inclusion Criteria:
a) Healthy male and female adults, aged 21-50
b) No contraindications to fMRI scanning
c) Within 15% of normal BMI
d) Right handed (dominant hand is right hand)
e) Have taken an opioid drug at least once in the past (for example, after a surgery)

Exclusion Criteria:
a) Current or past history of major medical, neurological, or psychiatric illness
b) Women who are pregnant or breast feeding, have gone through menopause, or have irregular menstrual cycles (length of cycle must be within 26 to 32 days)
c) Contraindications to morphine administration:
    i) Hypersensitivity to morphine or other phenanthrene-derivative opioid agonists (codeine, oxycodone, etc.), morphine salts, or any component of the product
    ii) Concomitant use of other CNS depressants including antihistamines or alcohol
    iii) History of drug or alcohol abuse
    iv) History of head trauma
    v) History of liver problems
    vi) Pre-existing respiratory conditions (ex. COPD, asthma)

vii) Current use of any drugs that interact with morphine

d) Contraindications to ketorolac administration

i) Allergic-type reaction, or urticaria in response to exposure to aspirin or other NSAIDS

ii) Concomitant aspirin or NSAID use

iii) Hypersensitivity previously demonstrated to ketorolac tromethamine or to any product component

iv) Peptic ulcer disease (active or history), or other gastrointestinal problems

vi) Current use of any drugs that interact with ketorolac (ex. heparin/warfarin)

vii) History of bleeding disorder

viii) Low body weight, under 50 kg

e) Contraindications to fMRI scanning (including cardiac pacemaker, metal implants, claustrophobia, pregnancy)

f) Contraindications to either of the emergency medications (Ondansetron or Narcan)

g) History of head trauma

h) High blood pressure (>140 systolic, >90 diastolic)

i) History of impaired urinary elimination

j) Major kidney problems, bleeding problems, severe dehydration, or recovering from a recent surgery (within past year).

k) Instability of responses to experimental pain (see Study Procedures Section)

l) History of asthma

m) History of diabetes

n) Liver Function Test results greater than 2.5 times the upper limit of normal (ULN) at Screening

o) History of smoking (past or current)

p) Use of psychotropic drugs, hormone treatments (including hormonal birth control) within 1 year

q) Non-fluent speaker of English

r) Positive urine drug screen (i.e. positive on any of the 10 measures tested, including cocaine, marijuana, opiates, amphetamines, methamphetamines, phencyclidine, barbiturates, benzodiazepines, methadone, and tricyclic antidepressants)

IV. SUBJECT ENROLLMENT

Subjects will be recruited by advertising through email, web, and bulletin board announcements posted within and outside of our hospital network community. Informed consent will be obtained from subjects after demonstration of the noxious stimuli by a licensed study physician investigator or licensed study nurse practitioner. This will be done in the Department of Psychiatry physiology laboratory, at the Clinical Research Center (CRC) Biomedical Imaging Core in the Charlestown Navy Yard, or at the CRC at the MGH main campus (White 13). Subjects who are consented by the nurse practitioner will have the option of also meeting with a study physician.

Subjects will be stratified by gender and randomly assigned, using a computerized random number generator, to one of the two arms of the study after the successful completion of Study Session 2 and before the start of Study Session 3.

V. STUDY PROCEDURES

Overview of Experimental Design

To accomplish the Specific Aims, a single experiment will be performed with up to 80 healthy subjects (until 52 complete the experiment) who will be divided into 2 treatment groups, one group receiving morphine and the other receiving ketorolac (n=26 in each group). Up to eight additional subjects will be considered “pilot subjects.” These subjects will complete only the MRI portion of the PET-MR scan during Study Session 5, as described below. All other study procedures will be identical. The purpose of these “pilot subjects” is to test all study procedures prior to exposing subjects to the radiation involved in PET scanning.

If during Study Session 5 technical complications prevent the PET portion of the PET-MRI scan from being completed, subjects will complete only the MRI portion of the PET-MRI scan. Like the “pilot subjects”, subjects who do not undergo the PET portion of the PET-MRI scan due to technical difficulties will undergo study procedures that are nearly identical to the full cohort on the day of the scan (as described below under *Study Session 5*),.

Calibrated mechanical pain stimuli will be delivered to the left lower leg (calf muscle) using a E20 Rapid Cuff Inflator (D.E. Hokanson, Inc, Biomedical Engineering # 1100865) This device is currently used in other protocols in our lab (2011P002217). All stimuli will be initiated using a cuff inflator. Each stimulus will last ~40 seconds with an inter-stimulus interval of about 30 seconds.

Punctate stimulation will be delivered to the skin of the dorsum of the middle finger of the right hand using custom-made weighted pinprick stimulators with a flat contact area of 0.2 mm diameter, exerting forces of 8 - 512 millinewtons (mN). These Weighted Brass Punctate Probes are utilized in the QST protocol designed by the German Research Network on Neuropathic Pain [67, 68]. This QST protocol has been used in a previously approved protocol in our lab (2009P000904), as well as in other Partners Healthcare studies [69]. Each stimulus is applied for 1 sec with an inter-stimulus interval of about 1 sec.

Gracely Sensory and Affective scales will be used to measure subjective pain ratings.

It will take up to 90 days to complete this study. All study procedures will take place in the MGH department of Psychiatry in Building 149 of the Charlestown Navy Yard Campus, the CRC at the Martinos Center for Biomedical Imaging (Charlestown Navy Yard Campus of MGH), or at the CRC at the MGH main campus (White 13). Each session will take place on a separate day, and the last four study sessions will be separated by 1 to 5 days. There will be one screening session followed by 5 study sessions. The consent and screening session will last about 2 hours and will be up to two weeks before the first study session. Study session 1 will be a training session (about 2 hours), study session 2 will be a testing session (about 2 hours), study sessions 3 & 4 will be conditioning sessions (about 3.5 hours each), and the last session will involve testing in the PET & fMRI scanner (about 6 hours). Pilot subjects and subjects who do not undergo the PET portion of the PET-MRI scan due to technical difficulties will only receive MRI scans, not PET scans concurrently with the MRI scans, on this day. Subjects will be debriefed by the principal investigator, a licensed physician investigator or a licensed study nurse practitioner at the end of their participation in this study. A licensed study nurse practitioner or physician investigator will follow-up with all subjects by telephone 24 hours after their study visit to confirm they are not experiencing any side

effects from the [11C]diprenorphine. No side effects are anticipated. There is no additional follow-up for this study.

Subjects who are unable to complete all study sessions within the 90 day period will be invited back to the CRC at the Martinos Center for Biomedical Imaging for a repeated screening visit (described in *Consent and Screening Visit* below) and/or a repeated study session 2 (described in *Study Session 2* below).

Pre-Screening:
All subjects will undergo a telephone or email prescreening to attempt to distinguish potential subjects from those not meeting eligibility criteria. The prescreening will be performed by a research assistant or coordinator and will cover the major inclusion and exclusion criteria. Likely candidates will undergo a full screening procedure by a study physician scientist investigators or study nurse practitioner after informed consent is obtained during the consent/screening visit. Subjects who are consented by the study nurse practitioner will also have the option to speak with a study investigator.

Consent and Screening Visit
Before this visit, a licensed physician investigator or study nurse practitioner will review the preliminary screening questionnaire performed by phone or email to determine if the subject meets the major eligibility requirements for this study.

Informed consent will be obtained from subjects after a description of the risks and benefits of the study. Consent will be obtained by one of the study licensed physician investigators or nurse practitioners. This will be done in the Department of Psychiatry physiology laboratory, the CRC Biomedical Imaging Core in the Charlestown Navy Yard, or the CRC at the MGH main campus (White 13). Up to eight subjects will be consented to participate as “pilot subjects.”

After subjects provide informed consent, a licensed physician investigator or an experienced licensed nurse practitioner will perform a clinical screening examination. This screening will serve to determine whether subjects meet all eligibility requirements and will include a review of subject medical history, concomitant medications, medication use within the last year, and alcohol and drug use. Particular attention will be paid to potential contraindications to the study drugs (morphine and ketorolac) and the emergency medications (ondansetron or narcan). Vital signs will be taken and en electrocardiogram (EKG) will be performed. Subjects will also have a blood draw (4mL of blood) for a complete metabolic panel and a CBC to check that the subject’s kidney function and liver enzymes are within the normal range. Subjects with liver function test results greater than 2.5 times the upper limit of normal will be excluded from further study participation. All female subjects will be asked to provide a urine sample for a urine pregnancy test to confirm that they are not pregnant. Female subjects will also be questioned about whether they are pregnant, think they could be pregnant, if they are breastfeeding, and when their last menstrual period was.

Measurements of height and weight will be obtained and BMI will be calculated. A urine sample will be obtained to perform a standard urine drug test to test for illicit drugs and specific medications that could interfere with, or cause adverse reactions when taken with, the study drugs.

Subjects who are unable to complete all study sessions within the 90 day period will be invited back for a modified second Screening Visit. This visit will be identical to the initial Screening Visit with the modification of the clinical screening examination and with the option of the EKG. The clinical screening examination will involve a review of any significant changes in health since the initial Screening Visit by the licensed physician investigator or an experienced licensed nurse practitioner. A second EKG will be performed at the discretion of the clinician following this review. This visit will take up to 45 minutes.

All subjects who successfully complete the preliminary screening questionnaire performed by phone or email will be scheduled for a screening visit. At least two business days in advance of the scheduled screening visit, subjects will be sent the informed consent form for review via mail or email. After the initial contact, subjects who decide not to participate will be dropped from the study with no adverse impact on the healthcare they receive whereas subjects who are unsure will be re-contacted after 1 and 2 weeks. Subjects who agree to participate will be scheduled for their first study visit.

Study Session 1

The purpose of the first behavioral session is to familiarize subjects with the rating scales and determine appropriate stimulus intensities using methods employed in our previous studies . First, we will ask the subjects to undergo a mechanical temporal summation paradigm (punctate stimulation paradigm) [69, 70] using the Weighted Brass Punctate Probes. The lowest force probe to elicit a moderate pain rating (~11 on the Gracely 0-20 sensory scale), usually 128 or 256 mN for most subjects, will be determined and then applied in a sequence of 10 stimuli at the rate of 1 per second. We will ask the subject to rate the painfulness of the first, fifth, and tenth stimulus. Next, the Weighted Brass Punctate Probes and Calibrated Mechanical Pain Stimulus cuff will be employed concurrently to study the modulatory effects of one stimulus on the other, in a paradigm of diffuse noxious inhibitory control (DNIC) [70]. The Calibrated Mechanical Pain Stimulus cuff will be inflated around the subject's left calf muscle in a sequence of 30 second stimuli. We will begin at a low pressure of 60 mmHg (usually rated by subjects as a 0 on the Gracely scale) and ask the subject for a pain rating. We will increase the subsequent pressure stimuli in increments of 15 mmHg until the pressure stimulus that elicits a mild pain rating (4-5 on the Gracely 0-20 scale) is reached. Once this pressure stimulus is determined, the pressure cuff will remain inflated for 2 minutes. We will ask the subject to rate this pain stimulus every 30 seconds over the 2 minutes. At the conclusion of the 2 minutes the cuff will remain inflated until, we complete a repetition of the sequence of 10 punctate probe stimuli.

The remainder of the session will include 1-2 ascending stimuli sequences composed of 6-8 stimuli, each lasting about 40 seconds. One or two random stimuli sequences will also be run that use higher and lower levels of pressure based on subject responses from the ascending stimuli sequences. The pressure needed to evoke strong pain (14-15 on the Gracely 0-20 sensory scale) will then be calculated individually for each subject based on their pain ratings during the ascending and random sequences. Finally, an identical sequence (6 stimuli of equal intensity that evoke a STRONG rating) will be performed. The calibrated pressures determined for subjects in this session will be used for subsequent sessions. Subjects will be asked to complete a full set of psychological forms, including the Life Orientation Test-Revised, Tellegen Absorption Scale, NEO, State-Trait Anxiety Inventory, General Self-Efficacy Scale, Interpersonal Reactivity Index,

and Expectation for Relief Scale, during this session. Completing these forms will take about 40 minutes (see below).

Study Session 2
The second session is a response reliability session. Two pain sequences, each involving 6 strong cuff pain sequences (about 10 minutes per sequence) for a total of 12 painful stimuli, will be applied (about 20 minutes total). Subjects will then wait for one hour (the same amount of time they will be waiting during the treatment and scan sessions) and then we will repeat the same sequences. Subjects must reliably rate the pain stimuli within 15% in order to continue in this study.

Subjects who are unable to complete study session 2 within 1 – 5 days of study session 3 will be invited back to the MGH department of Psychiatry in Building 149 of the Charlestown Navy Yard Campus to repeat this study visit (approximately 2 hours).

Study Sessions 3 and 4
Subjects will be randomized to receive either morphine or ketorolac after the successful completion of Session 2 and prior to the start of Session 3. Each subject will receive the same drug in both session 3 and session 4 of this study.

Randomization: Subjects will be randomized using a permutated block randomization (with nine blocks of four and two blocks of eight) generated by R statistical package software. Randomization assignments for subjects who drop out of or do not complete the study (i.e. do not have complete data from the scan session) will NOT be reassigned. Instead, the next eligible subject will receive the subsequent randomization assignment.

At the start of these study visits a urine sample will again be obtained to perform a standard urine drug screening test to test for illicit drugs and specific medications that could interfere with, or cause adverse reactions when taken with, the study drugs. All female subjects will have 3mL of blood drawn for a serum pregnancy test to again confirm that they are not pregnant. During these study visits, we will repeat similar procedures to the second study visit. At the start of this visit a licensed CRC nurse will place an intravenous (IV) line into the subject's arm. First, subjects will receive the same 2 sets of 6 cuff pain stimuli as administered in study session 2. About one hour after the first pain administration, subjects will be given either morphine or ketorolac (according to randomization) through the IV line after a saline flush. Morphine sulfate will be manually administered at a dose of 0.07mg/kg (about 5mg for the average adult) infused within MGH Adult General Care Medication Guidelines. Subjects will receive one dose of morphine per session. Ketorolac tromethamine, a nonsteroid anti-inflammatory drug (NSAID) with no activity on opioid receptors, will be manually administered at a dose of 0.43 mg/kg up to (but not exceeding) a single dose of 48mg infused within MGH Adult General Care Medication Guidelines. Subjects will be given one dose of ketorolac per session.

The subject will be cued with an instruction before the injection starts. Immediately after drug administration subjects, will complete the drug response form to assess their symptoms. Ten minutes after drug administration (the amount of time for the drugs to take effect), 2 sets of 6 cuff pain stimuli, identical to those applied before drug administration, will be applied to test the analgesic effect of the drugs. After the pain

sequences have been completed, subjects will once again complete the drug response form. The IV will be removed at the end of this session.

Upon review of the pre- and post-drug administration pain ratings, the Principal Investigator will determine whether subjects whose pain rating difference is null will continue in the study.

A physician investigator, study nurse practitioner, or licensed CRC nurse will monitor subjects very closely during drug administration and the 10 minutes following before starting the pressure pain testing. During this time Ondansetron and Narcan will be available for emergency use. Nausea (~25%) and pruritis (~10%) are only infrequently reported in clinical populations treated with opioids [63, 64]. Subjects who experience excessive nausea will be given the antiemetic Ondansetron (4mg IV) [65, 66] at the discretion of a physician investigator or nurse practitioner. Narcan is used for the complete or partial reversal of opioid depression, including respiratory depression and will be given to subjects who experience severe side effects as a result of morphine (0.4 mg to 2 mg) at the discretion of a physician investigator or study nurse practitioner.

Subjects will continue to be monitored by a physician investigator, study nurse practitioner, or CRC nurse for at least 2 hours after administration of the morphine or ketorolac. Before being permitted to leave, a physician investigator, study nurse practitioner, or trained CRC nurse must deem it safe for the subject to leave MGH based on the following criteria:

- Orientation to person, place and time
- Stable vital signs
- Nausea and dizziness are minimal (as assessed by N/P-VAS)
- Hydration is adequate
- At least one urination has occurred (to ensure no urinary retention)

Prior to the start of study visits 3 and 4, subjects must confirm that they will be escorted with a ride home under the care of a competent adult. If the subject is unable to provide this confirmation they will be unable to participate in that particular study visit. At the conclusion of these visits, written instructions will be given to the subject that include an explanation of potential or anticipated post sedation effects and limitations on activities and behavior. Specifically, subjects will be strongly advised to refrain from operating heavy machinery, drive a car, consume alcohol, and make important decisions for 12 hours. A 24-hour emergency contact telephone number will also be provided.

Study Session 5

As previously indicated, a serum pregnancy test will be performed on all female subjects completing the PET scan on the day of the scan.
Female “pilot subjects” will be asked to provide a urine sample for a urine pregnancy test.

In this session, fMRI and PET data will be collected at the Martinos Center for Biomedical Imaging using an integrated MRI-PET system, a dedicated brain PET scanner (BrainPET) inserted into the bore of the Siemens Medical MAGNETOM Trio (TIM system 3T MRI scanner) located at Building 149 in the Charlestown Navy Yard. This system has been installed and been running in the Martinos Center for Biomedical Imaging for more than a year and will allow us to collect fMRI and PET data simultaneously. “Pilot subjects”, subjects who do not undergo the PET portion of the

PET-MRI scan due to technical difficulties, and subjects completing the PET scan will be scanned on this machine, however, the PET portion of the scanner will not be turned on and PET scans will not be collected for the “pilot subjects” and subjects who do not undergo the PET portion of the PET-MRI scan due to technical difficulties. All other procedures will be identical between the pilot subjects, subjects who do not undergo the PET portion of the PET-MRI scan due to technical difficulties and non-pilot subjects.

Prior to the start of this scan session, all subjects will have the details of the study and scanning procedures explained to them. Before the scan, study staff will complete the MRI safety screening form with each subject. Negative pregnancy tests results will be confirmed for all female subjects. They will also be trained to rate the intensity of each pain stimulus while in the scanner by pressing buttons on a button box when the Gracely rating scale subsequently appears on a screen. This software is coded with E-prime (and has been used in our previous studies [26, 91-94]) and with Presentation (and is currently used in other protocols in our lab (2011P002217 and 2010P001368). Subjects will be randomized to receive either the group receiving the pain only scan followed by the pain+glucose solution scan or to the group receiving the pain+glucose solution scan followed by the pain only scan.

The whole scanning session will last up to approximately 6 hours. Before the scan, an IV line will be placed in the subject’s arm and will be used for injection of the radiotracer and glucose solution (approximately 10mL of solution containing 5% dextrose in water). [11C]diprenorphine is the radiolabeled opioid PET ligand that will be used as the radiotracer in this study. [11C]diprenorphine is produced at the Martinos Center for Biomedical Imaging Nuclear Pharmacy and the use of this radiopharmaceutical has been provisionally approved by the MGH Radioactive Drug Research Committee. [11C]diprenorphine will be used to find where in the brain pain changes the amount of endogenous opioids that bind to opioid receptors. [11C]diprenorphine will be given in very low amounts so that subjects should not feel any physiological effects. The maximum mass of [11C] diprenorphine injected into a subject will be 0.5 μg/kg per scan, ensuring that the compound will be administered in trace quantities (i.e., subpharmacological doses). It is estimated that the mean effective dose will be approximately 5 microsieverts per megabecquerel (μSv/MBq). The maximum injected radiation dose will be 15 millicurie (mCi) (555 MBq), an exposure of approximately 2.8 mSv per scan. The whole body exposure for the entire study will therefore be about 5.6 mSv. The radiotracer will be injected by a trained nuclear medicine technologist from the Martinos Center. “Pilot subjects” and subjects who do not undergo the PET portion of the PET-MRI scan due to technical difficulties will not receive the radiotracer as they will not be participating in the PET scan. These subjects will still have the IV placed for administration of the glucose solution.

The purpose of this session is to elucidate the different pharmacological learning effects. At the start of this study visit, all female subjects will have a blood draw (3mL) for a serum pregnancy test to confirm that they are not pregnant. Next, all subjects will be told that we will scan their brain activity during three conditions (1) pain only, 2) pain+drug, and 3) pain+glucose solution) and that to avoid the residual effects of the drug, we will apply the pain only and pain+glucose solution conditions first. They will also be **told** that due to time constraints (in certain circumstances) we may not be able to complete all three scans. **In reality**, we will only run two scans for every subject: one for the pain only condition and one for the pain+glucose solution condition. Thus, subjects will not receive

morphine or ketorolac during this visit. The pain only and pain+glucose solution scans will be conducted in random order and subjects will be given a visual cue to inform them of the condition.

This design excludes the accumulated expectancy (cognitive) effects of previous drug administration but maintains the effects of conditioning alone (injection). Thus, during the pain+glucose solution scan, the expectation effect is washed out and only “pure” conditioning effects are left. Because subjects will be told if they are about to receive the pain only condition, or the pain+glucose solution condition, there is no placebo condition for this study. This study will utilize a glucose solution, instead of a saline solution, to circumvent potential preconceived associations between the terms “saline solution” and “placebo” as such associations would confound study results.

No study drug (morphine or ketorolac) will be administered during the scanning session.

Once in the scanning room, a licensed CRC nurse will place physiological monitoring equipment, such as an MRI compatible oxygen partial pressure (pO2) fingerclip, on subjects for continuous monitoring during the scan session. Each PET/fMRI scan will begin with up to two 6-minute resting state fMRI scans (subjects will be instructed to keep their eyes opened and to concentrate on a fixation cross projected on the screen behind them). Subjects will then receive the ‘cue’ for either “pain only” or “pain+glucose solution.” For the pain+glucose solution scan, subjects will then receive an injection of glucose solution by IV (equivalent to the volume of drug they received in each of Sessions 3 and 4. Ten minutes after the cue/injection of the glucose solution, we will then perform 20 minutes of pressure pain (the same sets of 6 identical pressure stimuli from previous sessions), followed by structural and diffusion tensor imaging scans (about half an hour). The two PET/fMRI scans will be separated by 20 minutes to 2 hours to allow for tracer decay. Scan order will be randomized prior to the start of Session 5 as detailed above.

PET data acquisition

[11C]diprenorphine will be synthesized based on previous literature [42]. The maximum mass of diprenorphine injected will be 0.5 μg/kg per scan, ensuring that the compound will be administered in trace quantities (i.e., subpharmacological doses). The maximum injected radiation dose will be 15 mCi. On the BrainPET scanner, coincidence emission data are acquired and stored in list-mode format. A set of 1399 span-9 sinograms – trues, prompts and randoms – are obtained after data rebinning and histogramming. The estimation of random coincidences in each frame is performed through delayed coincidence histogramming. The normalization sinogram is obtained by taking the inverse of the sensitivity data acquired with a plane source scanned in 16 positions, 4 hours per position. The head attenuation map is obtained using a recently implemented MR-based attenuation correction (AC) method. Briefly, this AC method relies on novel dual-echo ultra-short echo sequences (DUTE) to segment the head into three compartments (i.e. soft tissue, bone tissue, and air cavities); this head map is then combined with the RF coil attenuation map obtained from a CT scan of the coil. The complete attenuation map thus obtained is forward projected to obtain the attenuation correction sinogram. The scatter sinogram is obtained using a model-based approach. Head motion will be corrected either based on the PET data or using a newly implemented MR-assisted PET motion correction algorithm [43]. The images are reconstructed using the Ordinary Poisson Ordered Subset Expectation Maximization (OP-OSEM; 6 iterations, 16 subsets) 3D algorithm from prompt coincidences,

normalization, attenuation and expected random and scatter coincidences. The reconstructed volume consists of 153 slices with 256×256 pixels (1.25×1.25×1.25 mm3). The spatial resolution at 8 cm radically from the center of the field of view is ~3 mm.

The two PET scans, each lasting up to 90 minutes (separated by at least 20 minutes to 2 hours for tracer decay), will be performed and analyzed using similar methods previously described [12, 17, 20, 35]. In each case, [11C]diprenorphine, prepared at Martinos Center for Biomedical Imaging Nuclear Pharmacy, will be administered intravenously through a previously placed line. The emission data will be acquired in list-mode for 90 minutes and frames will be subsequently generated for time-activity curve analysis. The use of list-mode acquisition allows post-hoc rebinning of PET data into time frames of interest based on the results of fMRI data. Twenty minutes of intermittent cuff pain will be applied during each PET scan.

"Pilot subjects" and subjects who do not undergo the PET portion of the PET-MRI scan due to technical difficulties will not participate in this portion of the study.

fMRI data acquisition

fMRI data will be acquired using gradient echo T2*-weighted sequence (TR/TE=2000/30 msec, flip angle=90º, slice thickness=3mm) during the 20 minutes of pain stimulation (two ten minutes fMRI runs will be collected during the application of the two pain sequences). In addition, the following MRI scans will also be performed during the PET data acquisition: 1) High resolution 3D MPRAGE sequences with 1 mm^3 isotropic resolution for co-registration and anatomic localization in data analysis, 2) one six minute long resting state fMRI scan with eyes open, 3) Dual echo ultra-short echo time (DUTE) scan for PET attenuation correction, 4) Time-of-flight MR angiography, and 5) EPI series for tracking the movements of the subject's head and motion correction of the PET acquired between 60-90 minutes post tracer administration.

At the end of this study visit, the principal investigator, a licensed physician investigator or a study nurse practitioner will complete a debriefing with the subject. The principal investigator, physician investigators and study nurse practitioner have the research experience and expertise to appropriately inform the subjects and explain study procedures, and also to answer any questions about the study or study drugs. Subjects will be told that the aim of the study was to assess the conditioning effects of the study drugs (morphine and ketorolac), rather than to assess the analgesic effects of the drugs. We will explain that we did not complete all three scans as discussed in the consent form because we were actually interested in their responses to pain after the injection of glucose solution, and with no injection. The principal investigator, physician investigator or study nurse practitioner will answer any additional questions from the subject and the subject will be provided with a copy of the debriefing form.

Follow-Up Telephone Call:

A licensed study nurse practitioner or physician investigator will follow-up with all subjects by telephone 24 hours after their study visit to confirm they are not experiencing any side effects from the [11C]diprenorphine. No side effects are anticipated. Subjects who do not receive [11C]diprenorphine will not receive this call. Subjects that were randomized to the ketorolac group will receive a follow-up phone call after 1 week of drug administration to ensure the subject is not experiencing potential adverse effects of ketorolac on the kidney.

Psychological and Symptom assessments
During Session 1, subjects will complete the Tellegen Absorption Scale, the Spielberger State-Trait Anxiety Inventory, the NEO Personality Inventory- Short Form, the Life Orientation Test-Revised, the General Self Efficacy Scale, and Interpersonal Reactivity Index. It will take about 40 minutes to complete the questionnaires. In sessions 3 and 4 (when drug is administered), subjects will complete the Drug Response Form immediately after drug administration and after the second set of painful stimuli (about half an hour after drug administration). After the second set of painful stimuli subjects will complete the Nausea/Pruritis Visual Analogue Scale (N/P-VAS) as a measure of specific drug-induced symptoms. They will complete this scale again before leaving.

Procedures for Psychometric Measurement
During Session 1 or 2, subjects will take about 40 minutes to complete the following scales, forms, and behavioral assessments. The STAI will be administered again at the beginning of the scan session.

Tellegen Absorption Scale (TAS): The TAS measures individual differences in absorption, a trait that involves an openness to experience and emotional and cognitive alterations across a range of situations [46]. It is a 34-item true or false scale that asks participants to rate the degree to which they become absorbed in everyday imaginative experiences, like viewing a sunset. In an early study, Neff et al. [47] reported that vascular headache patients with a high or low absorption score showed different responses to relaxation and biofeedback training. Owens et al. [48] found that absorption correlates with the use of greater numbers of complimentary medicine modalities in patients with chronic pain and in those with cancer. More recently, Bell and colleagues [49] found that high absorbers respond better than low absorbers to both the specific (active) and nonspecific (placebo) components of homeopathic therapy.
State-Trait Anxiety Inventory (STAI): The STAI consists of two 20-item self-report inventories. It is a rapid but detailed assessment that distinguishes between basal and reactive anxiety [50]. Scores range from 20 to 80 with higher scores indicating a greater level of anxiety.
NEO Personality Inventory-Short Form (NEO-PI-S): The 60 item NEO-PI-S is one of the most widely-used measures of personality [51]. It assess the five domains of personality: neuroticism, extraversion, openness, agreeableness, and conscientiousness [52]. In a study, Oswald et al [53] showed that less openness was associated with lower cortisol responses to challenges, suggesting that personality traits traditionally associated with greater psychopathology are also associated with blunted HPA axis responses to stress.
The Life Orientation Test-Revised (LOT-R): The LOT was developed to assess individual differences in generalized optimism versus pessimism. This measure is commonly used in research on the behavioral, affective, and health consequences of personality variable [54]. The revised scale focuses more exclusively on expectations of good versus bad outcomes and has a high correlation with the original LOT questionnaire.
General Self Efficacy Scale (GSES): This is a 10-item psychometric scale that is designed to assess optimistic self-beliefs to cope with a variety of difficult demands in life. In contrast to other scales that were designed to assess optimism, this one explicitly refers to personal agency, i.e., the belief that one's actions are responsible for successful outcomes [55].

Interpersonal Reactivity Index (IRI): This instrument contains four seven-item subscales, each tapping a separate facet of empathy. The perspective taking (PT) scale measures the reported tendency to spontaneously adopt the psychological point of view of others in everyday life. The empathic concern (EC) scale assesses the tendency to experience feelings of sympathy and compassion for unfortunate others. The personal distress (PD) scale taps the tendency to experience distress and discomfort in response to extreme distress in others. The fantasy (FS) scale measures the tendency to imaginatively transpose oneself into fictional situations [56].
North American Adult Reading Test (NAART): The North American Adult Reading Test is a 35-item inventory designed to measure cognitive ability [71].
Cognitive Reflection Test (CRT): The Cognitive Reflection Test is a 3-item questionnaire designed to measure cognitive impulsivity [72].
Monetary Questionnaire: The Monetary Choice Questionnaire is a task-based assessment of the degree to which people discount future outcomes (delay discounting). This assessment can be used as a measure of impulsivity and reward seeking behavior [75].

Behavioral Assessments:
Task-based assessments will be collected as exploratory mechanistic indicators of neural processing. Over the course of the study, all subjects will be asked to complete these computer-based assessments designed to measure inhibitory behaviors, belief adjustment, risk-seeking behaviors and impulsive behaviors. In total, the computer-based assessments should take approximately 15-20 minutes. All computer-based tasks will be presented to the subject on a computer screen using the software packages Eprime and Presentation.
Framing Task: The Framing Task is a computer-based task designed to measure the degree to which subjective decisions are affected by a framing manipulation. This will be used as an assessment of risk seeking behaviors [73].
Event Estimation Learning Task: The Event Estimation Learning Task is a computer-based task designed to measure optimism and the degree to which an individual adjusts their beliefs based on novel information [74].
Stop Signal Task: The Stop Signal Task is a computer-based task designed to measure response inhibition and cognitive control [76].

During treatment sessions, the Expectations for Relief Scale, Drug Response Form, Nausea/Pruritis Visual Analogue Scale, and Post Anaesthesia Discharge Scoring System will be administered.
Expectations for Relief Scale (ERS): During treatment sessions subjects will complete a form about how they expect the treatment will work. The ERS is a 0-10 scale used to measure the expectation of pain relief after drug treatment. In each treatment session, after drug administration, subjects will be asked to use this scale to rate how much pain relief they expect from this particular treatment with 0 indicating a very negative expectation of “does not work at all” and 10 indicating a very positive expectation of “complete pain relief.” Studies have shown that expectation can significantly influence an individual’s perception of pain relief [4-8, 57].
Drug Response Form: This scale, adapted from that used in protocol 2008P000282 (PI: Karleyton Evans) will be used immediately after drug administration and about 30 minutes after drug administration to monitor common symptoms.
Nausea/Pruritis Visual Analogue Scale (N/P-VAS): The N/P-VAS is an 11-point visual-analogue scale with verbal anchors used to rate either nausea or puritis (itchy skin). The

scale is derived from similar VAS scales used for rating opioid side effects [46, 47]. Subjects will be instructed to rate their discomfort on the following scale: none (0), slight (1-3), moderate (4-6), severe (7-9), and extreme (10).

Post Anaesthesia Discharge Scoring System (PADSS): The PADSS is a 5 item scoring system used to assess objective discharge criteria after anaesthesia (opioid drug administration). The assessment incorporates measures of vital signs, mental status, ambulation, etc. The PADSS has been validated in clinical settings [62].

VI. BIOSTATISTICAL ANALYSIS

Primary endpoints: Subjective pain rating and PET/fMRI signal differences between pain only and pain+glucose solution.

Power Analysis: In total, 52 subjects will be scanned, 26 in the morphine conditioning group and 26 in the ketorolac conditioning group. We will examine differences between subjective pain ratings between the pain only and pain+glucose solution conditions. Assuming a similar SD of 1.7 observed in our previous study , and noting that we will target a mean subjective pain rating of 15 for the pain only condition, we will have 82% power to detect a difference in pain rating change of 1.0 or greater between the pain only and pain+glucose solution conditions (paired t-test at the 0.05 two-tailed significance level). In the study by Dr. Benedetti and colleagues , they observed a 29% change in pain tolerance for the same paradigm as in the proposed experiment 1. Although we recognize that the outcomes are different, we note that the percent change already observed in pain tolerance is almost four times the magnitude of pain rating (1/15 = 6.7%) difference for which we expect 80% power. Therefore, we believe that our sample size is adequate for the pain-rating outcome for the proposed experiment. For fMRI, based on our previous studies , we extracted the beta values of three representative brain regions for placebo (gACC, anterior insula (AI) and LPFC). Of these brain regions, we used the median SD of three regions (AI), 0.85, for power analysis. With 26 subjects per group, we will have 80% power to detect a beta value change of 0.66 or more based on a two-sided paired t-test with an alpha value of 0.005 (threshold for whole brain fMRI analysis). In order to determine the corresponding power for PET, we use a SD of 7% for BP changes, obtained from a published study ([25], Table 1, median SD for opiod). With this SD, we anticipate 80% power to detect an absolute difference of 7.7% or more in BP changes based on a two-sided paired t-test with an alpha value of 0.0001 (threshold for whole brain PET analysis). Because several published fMRI or PET studies [17, 18, 22, 25, 26] have reported significant fMRI and PET findings with smaller sample sizes, we believe that our sample size is adequate for the fMRI/PET outcome measures.

Data Analysis

Subjective pain rating change analysis

The primary outcome of this study is the subjective pain rating changes in session 5.

The primary endpoint is the morphine and ketorolac conditioning effects as indicated by subjective pain rating changes within each group (pain only versus pain+glucose). A paired t-test will be applied for the comparison. Additional analyses, such as regression analysis, may be conducted as necessary.

Secondary endpoints include the difference between the morphine and ketorolac conditioning effects (morphine (pain+ morphine - pain) versus ketorolac (pain+ morphine - pain). A two sample t-test will be used for analysis.

Exploratory endpoints include the correlation between the conditioning effects and psychometric measurements (TAS, STAI, NEO, LOT-R, GSES, IRI,ERS, NAART, CRT, Monetary Questionnaire, Framing Task, Event Estimation Learning Task, Stop Signal Task). A regression analysis will be applied separately for each measurement.

PET/fMRI Statistical analysis

PET data analysis

The primary endpoint for this study is the brain opioid binding potential changes evoked by morphine and ketolorac conditioning. To investigate this, PET image data will be transformed on a voxel-by-voxel basis into 2 sets of parametric maps, a tracer transport measure (K1 ratio) and a receptor-related measure (distribution volume ratio [DVR] at equilibrium), using data from 45- to 90-minute post-tracer administration. To avoid the need for arterial blood sampling, these measures will be calculated by means of a modified Logan graphical analysis [12, 17, 20, 35] using the occipital cortex (an area devoid of μ-opioid receptors) as reference region. The slope of the Logan plot will be used for the estimation of the distribution volume ratio (DVR), a measure equal to the (Bmax/Kd) + 1 for this receptor site and radiotracer. Bmax/Kd (or DVR-1) is the receptor related measure (μ-opioid receptor availability, or binding potential). DVR images will be spatially smoothed with a 6-mm isotropic Gaussian filter.

Whole brain analysis will be performed using Statistical Parametric Mapping software (SPM8, Wellcome Trust Centre for Neuroimaging, London, UK) with general linear model. Only regions with specific μ-opioid receptor binding will be included in the analyses (voxels with DVR>1.1 or BP>0.1) [17, 20]. Within each group, a paired t test will be calculated (pain only versus pain+glucose) for each voxel using the pooled variance across voxels. Similar to a previous study [20], a threshold of $P<0.0001$ will be used for pre-defined ROI. A threshold of a threshold of $P<0.0001$ and $p<0.05$ corrected for multiple comparison at the cluster level will be used for non-ROI brain region.

An ROI-based analysis will be performed for the regions of interest (ROI). ROIs will be determined using the MPRAGE high-resolution data for each subject and projected to the co-registered PET image for analysis using the toolbox Freesurfer (http://surfer.nmr.mgh.harvard.edu/) developed at the Martinos Center. The BP values for percentage of change calculations will be extracted from image data by averaging the values of voxels contained in an area where significant differences are obtained in the ROI analysis using a threshold of $P<0.01$ after correcting for multiple comparisons. “Pilot subjects” and subjects who do not undergo the PET portion of the PET-MRI scan due to technical difficulties will not be included in the PET analysis.

The secondary endpoints of this study will be analyzed as follows: 1) The brain opioid binding potential change difference between the morphine conditioning and ketorolac conditioning ((morphine (pain+ morphine - pain) versus ketorolac (pain+ morphine - pain)). A two-sample t-test will be applied for the comparison. 2) The association between subjective pain rating change and the brain opioid binding potential changes. A regression analysis will be applied for the analysis.

The exploratory outcomes include the correlation between the brain opioid binding potential change evoked by morphine and ketorolac conditioning, and psychometric

measurements (TAS, STAI, NEO, LOT-R, GSES, IRI, and ERS). A regression analysis will be applied separately for each measurement and conditioning effect (morphine and ketorolac).

fMRI data analysis

The primary endpoint is the brain fMRI signal changes evoked by morphine and ketorolac conditioning. To investigate this, fMRI Data analysis will be performed using previously described methods using the Statistical Parametric Mapping software (SPM8, Wellcome Trust Centre for Neuroimaging, London, UK). Pre-processing includes motion correction, spatial normalization to the MNI template, spatial smoothing with an 8 mm Gaussian kernel, and high-pass temporal filtering (cut-off 128s). The univariate data analysis will be performed using the general linear model (GLM). The individual design matrix for each subject (first-level matrix) will include a total of six regressors (pain only and pain+glucose solution by anticipation, pain administration and pain intensity rating compared with the fixation (baseline). A file containing the movement parameters for each individual (6 directions) obtained from the realignment step will also be included in the model. Regression coefficients for all regressors will be estimated using least squares within SPM8. Specific effects will be tested by creating contrasts of the parameter estimates, resulting in a t-statistic for each voxel.

Second-level analyses will be performed using one sample t-tests, representing different temporal stages of the experiment: for pain and pain+glucose solution condition during (1) anticipation of pain, (2) pain application, and (3) pain intensity rating. Then, a separate paired t-test will be applied to compare the pain only and pain+glucose solution condition in the morphine and ketorolac groups so as to elucidate the fMRI signal changes evoked by morphine and ketorolac conditioning. Similar to previous studies , a threshold of voxel wise $p<0.005$ with 20 contiguous voxels will be used for pre-defined regions of interest (ROI). For other brain regions, a threshold of voxel wise $p<0.005$ with 20 contiguous voxels and cluster level $p<0.05$ corrected will be used.

The secondary endpoints include: 1) The fMRI signal change difference between the morphine conditioning and ketorolac conditioning ((morphine (pain+ morphine - pain) versus ketorolac (pain+ morphine - pain)). A two-sample t-test will be applied for the comparison. 2) The association between subjective pain rating change and the brain fMRI changes. A regression analysis will be applied for the analysis.

The exploratory outcomes include:
1) Resting state functional connectivity of brain networks (as elucidated by both PET and fMRI imaging tools) involved in the morphine and ketorolac conditioning. To investigate this, resting state fMRI data will be analyzed using a method used in previously published studies from both our group [21, 36, 58, 59] and other groups [60, 61]. Briefly, functional data will be preprocessed to decrease image artifacts and between-slice timing differences, and to eliminate differences in odd/even slice intensity. Data will then be spatially smoothed using a Gaussian kernel of 6mm full-width at half-maximum and temporally filtered ($0.009Hz<f<0.08Hz$). Next, several spurious or nonspecific sources of variance will be removed by regression of the following variables: (1) six movement parameters computed by rigid body translation and rotation during preprocessing, (2) mean whole brain signal, (3) mean brain signal within the lateral ventricles, and (4) the mean signal within a deep white matter ROI. Next, a functional connectivity analysis will produce coefficients for each previously defined seed-voxel correlation. Fisher's r-to-z

transformation will be used to convert correlation maps into z maps. Group effects will be tested with a random-effects analysis using a one-sample t-test. The threshold will be set at voxel-wise p<0.005 uncorrected with a minimum cluster extent of 20 contiguous voxels and cluster level p<0.05 corrected. Seeds (2-3 mm around the peak) will be chosen based on the PET and fMRI data analyses results. We will pick represented brain regions observed in PET analysis, fMRI analysis and in both of PET and fMRI data analysis (overlaid on each other). We believe that this exploration will further elucidate different networks linked with endogenous opioid-mediated brain regions, and non-opioid mediated brain regions during pain perception and different conditioning effects.
2) The correlation between the fMRI signal change evoked by morphine and ketorolac conditionings and psychometric measurements (TAS, STAI, NEO, LOT-R, GSES, IRI, and ERS). A regression analysis will be applied separately for each measurement and conditioning effect (morphine and ketorolac).

A priori brain regions

Specific fMRI signal changes involved with opioid and non-opioid conditioning are predicted to occur in several areas of the brain. Based on previous studies and our hypothesis, the region of interest will include pACC, LPFC, PAG, insula, and S2.

VII. RISKS AND DISCOMFORTS

Risks associated with answering screening questions

All subjects will complete a set of psychological assessments. Subjects may feel uncomfortable completing some of the questions. In the analysis of these measures, the investigators may find evidence of psychological disorder.

There is a small risk that the subjects' confidential medical information could be revealed or discovered by mistake (due to human error). The results of the urine toxicology screen testing nor the results of the research will not be placed in the subjects' medical record. However, the CRC nurses will be documenting each subject's assessment, interventions and outcomes in the longitudinal medical record (LMR) during the Screening Visit, Visit Three, Visit Four, and the Scan Visit, and the study MD and/or NP will also file a note. LMR, the Partners Healthcare electronic medical record technology, is secure..Information that could identify a specific individual will not be released or published any way.

Risks associated with cuff pain stimuli:

The application of mechanical stimuli may cause a bruise. However, in this study the maximum pressure values to be utilized are below those associated with tissue injury. All subjects will undergo repeated assessment of subjective response to brief (~40 sec) noxious ischemic stimuli. The application of the pressure cuff may cause minor tingling in the skin.

Risks associated with punctate stimulation:

There is a very slight risk that the applications of the Weighted Brass Punctuate Probes could puncture the skin (if, for example, someone has particularly fragile skin). This risk is avoided by adhering to similar safety precautions taken by other IRB-approved Protocols employing the Weighted Brass Punctate Probes (2008P002222, 2008P001385, 2010P001061, 2010P000978, 2009P001020, 2009P000667, 2008P001019, 2007P001047). Study staff will customize the intensity of the probe

(there are 6 probes of varying pressures from which to choose) so that for each subject we use the probe that exerts the minimal amount of pressure needed to reach the subject's individual mild or moderate pain rating. The study staff will assess the area before applying this device to inspect if the skin appears to be susceptible to an accidental puncture. We will also sterilize the punctuate probes between subjects to ensure that there is minimal risk of infection. In the event that the skin is punctured, we will follow standard clinical procedures to clean the site of puncture in order to prevent infection and assess the site to determine if follow up care is needed.

Risks associated with drug (morphine or ketorolac) administration:
The morphine and ketorolac will be administered by well-trained licensed physician investigators or a study nurse practitioner with the assistance of experienced licensed nurses from the CRC in the Martino's center of biomedical imaging core facility.

The side effects/risks of morphine include:

- Pain at the intravenous access site (common)
- Dry skin, skin rash, urticaria (common)
- Decreased oxygen saturation (common)
- General muscle weakness (very common)
- Xerostomia – dry mouth (very common)
- Vomiting (very common)
- Drowsiness (very common)
- Dizziness (very common)
- Fever (very common)
- Confusion (very common)
- Bradycardia (common)
- Hypotension (common)
- Heart attack (uncommon)
- Respiratory arrest (uncommon)
- Allergic reaction (anaphylaxis, bronchospasm) (uncommon)
- Nausea (common)

There is a risk of allergic reaction to morphine, including:

- Anaphylaxis (uncommon)
- Bronchospasm (uncommon)

The risks to embryo, fetus, or breastfeeding infant of a woman who is taking morphine. Newborn babies born to mothers receiving morphine during pregnancy should be monitored for neonate withdrawal syndrome. Treatment of mother with single doses of morphine is not expected to cause adverse effects in nursing infants. Breast-feeding should be delayed for 48 hours after morphine injection.

After morphine injection, subjects will be strongly advised to refrain from operating heavy machinery, driving a car, consuming alcohol, or making important decisions for 12 hours, as the side effects from morphine may impair reactions and cognitive functioning.

The side effects/risks of ketorolac include:

- Pain at the intravenous access site (common)
- Pruritus, purpura, skin splotches/rash (common)

- Gastrointestinal pain, dyspepsia, nausea (common – very common)
- Constipation, flatulence (very common)
- GI bleeding, fullness, perforation, ulcer, heartburn (common)
- Swollen mouth (common)
- Abnormal renal function (common)
- Increased liver enzymes (common)
- Increased bleeding time (common)
- Headache (very common)
- Dizziness (common)
- Drowsiness (common)
- Cardiovascular edema (common)
- Hypertension (common)
- Chest pain (uncommon)
- Respiratory depression (uncommon)
- Renal failure (uncommon)
- Allergic reaction (anaphylaxis, bronchospasm) (uncommon)

Risks to embryo, fetus, or breastfeeding infant of a woman who is taking ketorolac include:

- Heart defects
- Cleft palate
- Kidney dysfunction
- Low blood pressure
- May inhibit contractions during labor
- May cause miscarriage

Ketorolac is found in breast milk in nursing mothers in small amounts after oral dosing. Ketorolac is considered compatible with breast-feeding by the The American Academy of Pediatrics, however nursing mothers are advised not to use ketorolac because of the potential adverse effects of prostaglandin-inhibiting drugs on newborns.

The side effects/risks of Ondansetron include:

- Diarrhea (common)
- Headache (common)
- Constipation (common)
- Fever (uncommon)

The side effects/risks of Narcan (for abrupt reversal of opioid depression, or post-operative) include:

- Nausea
- Vomiting
- Sweating
- Tachycardia
- Increased blood pressure
- Tremulousness
- Seizures
- Ventricular tachycardia and fibrillation
- Pulmonary edema
- Cardiac arrest (rare and reported in post-operative patients)

Risks/Discomfort associated with PET imaging
[11C] diprenorphine PET imaging involves a certain level of exposure to radiation. No side effects from the radiopharmaceutical [11C] diprenorphine are expected as the dose of [11C] diprenorphine being administered in this study is below that at which we expect any physiological effect. It is estimated that the mean effective dose will be approximately 5 μSv/MBq, Subjects will be injected with no more than 15 mCi (555 MBq) of [11C] diprenorphine, and thus subjects will be exposed to approximately 2.8 mSv per scan. The total amount of radiation subjects will receive from participation in this study is equal to a whole body exposure of approximately 5.6 mSv. This amount of radiation is equivalent to 12 months of average background radioactive exposure (~6 mSv). This amount of radiation exposure has been provisionally approved by the MGH Radiation Safety Committee and is comparable to radiation exposure during other PET ligand binding studies.

Risks/Discomfort associated with intravenous lines/blood draw:
Subjects will have an intravenous line for administration of drug (morphine/ketorolac) and the glucose solution (and for emergency drugs if necessary). Local infection, swelling, and redness could occur at the sites of placement of intravenous lines. This area may have a bruise or feel uncomfortable for 2-3 days after the catheter is removed. The risks associated with having blood drawn include: bruising, local discomfort, or infection at the site of the needle puncture. A licensed physician investigator, study nurse practitioner, or licensed nurse will draw about 4mL of blood during the screening session. An additional 2mL of blood may be drawn from subjects, who are amenable to a follow-up blood draw, who have abnormal results on the complete metabolic panel and CBC performed during screening session. During Sessions 3 and 4, all female subjects will have an additional 3mL of blood drawn for a pregnancy test. During Session 5, female PET subjects will also be asked for an additional 3mL of blood drawn for a pregnancy test.

Discomfort associated with MRI scanning:
fMRI is not associated with any known adverse effects except in people with metal or magnetic implants (such as metallic clips in the brain or cardiac pacemakers). Subjects will be excluded from this study if they have any of these devices. The high speed, high field MRI system has been approved by the FDA and will be operated within the parameters reviewed and accepted by the FDA. Subjects will be required to lie still on the imaging table with their heads in the scanner. Some subjects find it unpleasant or feel anxious when confined in the enclosed space of the scanner. If this happens, the study will be aborted. Subjects will be offered earplugs to decrease the noise in the scanner.

This is a voluntary study in normal adults. The alternative is that the subject does not have to participate. Unwillingness to participate or complete the study will not affect future patient care.

Drug storage
Morphine and ketorolac will be kept and dispensed according to the policies and requirements of the MGH research pharmacy (MGH main campus, 55 Fruit St.) and the CRC of building 149 of the Charlestown Navy Yard (CRC-BIC). Ondansetron and Narcan will be available if necessary.

Protection Against Risks

To prevent/reduce the side effects of morphine and ketorolac, we will ask subjects during the recruitment screening if they have an allergy to opioid or non-opioid analgesics or have ever experienced any of the above symptoms when using a product containing morphine or ketorolac, subjects who report a history of adverse reaction will be excluded from the study. The clinical screening performed by a licensed physician investigator or study nurse practitioner will include more details and questions about the subject's medical history to screen out subjects with any possibility of an adverse reaction to morphine, ketorolac, or the emergency medications.

In addition, during the screening visit, we will perform a urine toxicology screen to exclude any subjects with potential substance abuse or dependence, and to check for the presence of some medications that interact with the study drugs. We will also collect two blood samples during this visit to test for liver and kidney function. A complete metabolic panel (including a hepatic panel) and CBC will be collected and sent to LabCorp for analysis. Lab results will be obtained so that subject eligibility can be determined before contacting the subject to confirm his or her continued interest in the study. We will exclude all subjects who return abnormal liver function tests in order to exclude subjects with liver disorders. Additionally, subjects with other abnormal results on the complete metabolic panel and/or CBC may be asked to have additional blood drawn to determine whether the abnormal results are a normal variant. We will exclude all subjects whose follow-up bloodwork is determined to be clinically significant by the licensed physician investigator or nurse practitioner.

Ondansetron and Narcan will be available during all treatment visits (3 and 4) as emergency medications. A licensed physician investigator or nurse practitioner will determine whether to administer either medication. We anticipate that the tests and clinical screening we perform will exclude subjects with potential adverse reactions, however, subjects will be monitored at the CRC after drug administration and before they are allowed to leave in the rare event of an adverse reaction.

The intensity of pain stimulation will be determined for each subject individually to minimize potential for tissue damage. The maximum pressure to be utilized is below that associated with tissue injury. We will assess subjects as each visit to determine if they experienced any tissue injury at the site of noxious stimulation. If there is any evidence that the protocol causes more than minor, rapidly reversible sensitization, the protocol will be amended to decrease the frequency and intensity of the noxious stimuli.

During Session 1, subjects will complete a set of psychological assessments. Subjects will be instructed to complete the questionnaires to the best of their ability, but will have the option to leave any question(s) blank. In the unlikely event that evidence of psychological disorder is found (the healthy subjects are screened for psychiatric disorders before entering the study), the principal investigator, Dr. Randy Gollub (a board-certified psychiatrist with 15 years of experience in Acute Psychiatric Service) will be consulted immediately to direct care as needed.

During the administration of morphine or ketorolac by a licensed physician, study nurse practitioner, or nurse, subjects will be closely monitored and drug administration will stop immediately if a subject develops severe symptoms. If necessary, a physician investigator or nurse practitioner will send the subject to the emergency room and the subject will be withdrawn from the study. All subjects will be instructed to stay in the lab

for at least 2 hours after initial administration of the drug before they are released. Subjects will also be required to guarantee that a responsible adult will take them home in order to complete these sessions.

Subjects will be debriefed at the end of study visit 5 by the principal investigator, a physician investigator or a study nurse practitioner and informed that the aim of the study was to assess the conditioning effects of the study drugs (morphine and ketorolac) rather than to assess the analgesic effects of the drugs. The principal investigator, physician investigator or nurse practitioner will explain that only two scans were collected, not all three scans discussed in the consent form, because we were actually interested in comparing responses to pain after the injection of glucose solution to pain response without an injection.

VIII. POTENTIAL BENEFITS

Subjects will be paid for their participation but will derive no other direct benefit. However, it is hoped that through improved understanding of the neurobiological mechanisms underlying pain, this study will benefit future patients with acute pain. This research will also expand the body of knowledge about functional neuroimaging of pain and the role of conscious and unconscious learning.

Remuneration
Subjects will be paid $25 for the consent/screening visit, $25 for the behavioral sessions (2), $100 for the drug conditioning sessions (2), and $325 for one PET/MRI scanning session. Subjects who complete all study procedures will be paid a total of $625.

Subjects will be paid $25 for a repeated screening visit and $50 for a repeated behavioral session.

If subjects drive (are driven during the drug administration sessions), we will provide them with a validation sticker to cover the cost of parking at the Martinos Center Garage – 149 13th St. Charlestown, MA 02129.

Subjects will be paid by check at the completion of all study procedures.

This level of compensation is deemed appropriate given the time commitment, drug administration, and willingness to tolerate noxious stimuli.

IX. MONITORING AND QUALITY ASSURANCE

Data Quality and Management
The Principal Investigator and supervised study staff will review all data collection forms on an ongoing basis for completeness and accuracy, as well as protocol compliance. Data quality inspection will occur at every study visit. In addition, the Principal Investigator will review subject accrual, status of enrolled subjects, adherence data regarding study visits and intervention, and adverse event rates on at least a quarterly basis. Please see the Data Safety Monitoring Plan for additional information.

Data Safety Monitoring Board (DSMB)

As per NIH guidelines, a Data Safety Monitoring Board (DSMB) will be utilized in this study. The DSMB will be a three-member multidisciplinary group whose members include a brain imaging scientist, a biostatistician, and a physician experienced in both internal and emergency medicine. All adverse events will be reported to the DSMB in addition to the Partners IRB.

The DSMB will meet bi-annually to review and evaluate patient safety data and any preliminary study results. In the event of a serious adverse event (please see below), the committee will immediately review the case and decide whether to call an emergency meeting of the DSMB to revisit study procedures.

Adverse Events
An adverse event is any untoward medical occurrence in a subject hat is temporally associated with participation in the study. A serious adverse event is any adverse event that results in one or more of the following outcomes: death, a life threatening event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, a congenital anomaly or birth defect, and/or an important medical event based upon appropriate medical judgment.

Serious adverse events are not expected as a result of the study procedures. If one occurs, it will be reported to the Principal Investigator and the Partner's Human Research Committee within 24 hours. Copies of the follow-up report will also be sent to the Partner's Human Research Committee. Additional follow-up evaluations required by the event will also be sent to the Partner's Human Research Committee. Subjects who develop an SAE will be followed-up until the resolution of the event and for one month thereafter. The Principal Investigator will also report any SAE that occurs within one month after a subject has either discontinued or completed the study.

All adverse events will be categorized based on severity and relatedness to study procedures and will be reported to the Human Research Committee promptly in accordance with guidelines. All adverse events will also be promptly reported to the DSMB, as detailed above.

Privacy and Confidentiality
HIPAA compliant methods, as specified by the Partners Research Administration, will be followed for collection, storage and analysis of all study data. All information regarding experimental subjects will be deidentified and coded with a unique study ID. Similarly, all study documents will be kept in the offices of the Principal Investigator. All data for presentation will be deidentified and represented only by unique study code numbers.

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