Brain and Genetic Predictors of Individual Differences in Pain and Analgesia (Pain Genetics)

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I. OBJECTIVES

The goal of this study is to characterize the neurophysiological mechanisms that make an individual a strong or weak placebo responder, and provide behavioral and genetic markers for these individual differences. This would provide a tool for characterizing likely placebo responders in clinical settings and clinical trials, with two benefits. First, it could be used to better separate placebo vs. verum treatment effects, allowing for better targeting of the mechanisms of each type of effect. Second, it could be used to eliminate or control for placebo responses in clinical trials, increasing efficiency and trial success rates.

II. BACKGROUND AND SIGNIFICANCE

Throughout history, placebo effects have been variously considered as tricks played upon the gullible by medical practitioners and powerful but mysterious healing forces. With the advent of direct measurements of human brain function, modern science has shown that placebo effects are neither of these. Rather, they reflect the principled impact of psychological and brain processes on diseases of the brain and body. Placebo effects represent an opportunity because they provide a window into internal brain processes that influence health, and a challenge because many clinical trials have now failed due to large and durable placebo responses, at great cost to health care providers and consumers.

Definitive studies of the brain pathways involved in placebo responses—and the genetic, environmental, and neural factors that lead some individuals to respond more strongly than others—are critical to harnessing placebo effects, eliminating or controlling placebo responses in clinical trials, and understanding the psychological and brain factors that predispose one to successful treatment and "spontaneous" improvement. Placebo analgesia is the best-studied type of placebo effect, with well-developed paradigms and preliminary data on its brain mechanisms. This background provides a foundation for larger-scale, definitive studies.

In this project, we propose the first such large-scale study of brain mechanisms of placebo analgesia, combining neuroimaging, behavioral, and genetic approaches. It builds on 15 years' experience in PI Wager's laboratory on fMRI and placebo analgesia and 40 years of genetics research at the Institute for Behavior Genetics (IBG) at the University of Colorado, Boulder. We will use fMRI to characterize the neural bases of placebo effects in 600 twins recruited from the Colorado Twin Sample and predict individual differences in placebo effects across two forms of pain. In **Aim 1**, we will develop models that predict the magnitude of individuals' placebo effects in pain and pain neurophysiology based on a) fMRI activity, b) brain structure, and a combination of personality, behavioral, and cognitive measures that can be deployed clinically. In **Aim 2**, we conduct the first analyses of heritability of placebo effects and their neural predictors, and genetic correlations that can identify brain features whose relationships with placebo effects are genetic in origin. In **Aim 3**, we leverage the >50,000 person Enhancing Neuro-Imaging

Genetics through Meta-analysis (ENIGMA) consortium to identify genome-wide associations with placebo-linked brain features and develop polygenic risk scores for placebo effects. The research products from this endeavor will include data and models useful for characterizing and screening participants in clinical trials, assessing interactions between placebo responses and other treatments, and assessing placebo effects across disorders.

III. PRELIMINARY STUDIES

Large placebo responses occur in clinical trials of multiple mental health and neurological disorders, including Parkinson's[1-3], autism[4-7], epilepsy[8], schizophrenia[9, 10], migraine[11-13], other forms of chronic pain[14-17], and other brain disorders[18-21]. Fifteen years of research on brain mechanisms of placebo by our lab and others has found that placebo effects are reliably caused by *active neurobiological processes* that must be studied and understood in relation to health and disease[20, 22-26]. Placebo analgesia is the best studied area, but evidence suggests mechanistic overlap with placebo effects in Parkinson's disease[27-30] and depression[31-35]. These include prefrontal-striatal-brainstem interactions and involvement of endogenous neurochemicals (opioids, dopamine, cannabinoids, serotonin, and others) that may form a common substrate for influences of affective systems and context across disorders[22-24]. Understanding the neuroscience of placebo is a key to understanding endogenous brain contributions to disease vulnerability and resilience, and maximizing clinical benefit. It is also a key to increasing success rates in clinical trials. A growing number of trials are failing because of large placebo responses[9, 16, 18, 36], with large economic and public health costs. The ability to eliminate or control for placebo responses in clinical trials would help bring successful drugs to market.

IV. RESEARCH STUDY DESIGN

We will recruit 600 twins (300 same-sex pairs; 150 monozygotic and 150 dizygotic; ~50% female) from the Colorado Community Twin Sample (CTS). The CTS includes 727 same-sex twin pairs between 26-38 years of age (as of Spring 2017), originally derived from the Colorado Twin Registry, a population based registry, housed at the Institute for Behavioral Genetics (IBG) at the University of Colorado since 1984, with three previous waves of data collection (1997-2002, 2002-2008, and 2009-2014)[380]. The CTS participants are on average 37.8 years old (SD 2.5), ~50% female, mean education 14.5 years, 80% non-Hispanic Caucasian, 14% Hispanic, 4% African-American, 2% Asian, and < 1% Native American. ~75% of the sample still lives in Colorado. **Twin recruitment**: CTS pairs will be recruited via direct contact by IBG staff Corinne Gunn, who has worked with this sample for 15 years. The recruitment rate for repeat visits has been 87%; we need ~40% for the proposed study to be viable. Thus, we will be able to meet recruitment needs. An ongoing fMRI study of a separate sample of twins (target ~320 adult twin pairs) from our group (studying cognitive control; MH063207, PI Friedman, Co-I Wager) additionally demonstrates recruitment feasibility: to date we have tested 356 individuals and only 7 have declined to participate, 29 are not eligible for MRI, and 10 are deceased. **Recruitment for pain studies**: Extensive published and preliminary work by our group demonstrates that we can deliver intensities in the painful range that are safe and tolerable for virtually all participants. In fMRI studies we have run over the past

several years (*N* = 495 for heat and *N* = 81 for pressure), only 2 participants have dropped out due to pain intolerance.

A.2 Experimental design

A.2.1. Behavioral assessments. Before the main neuroimaging session, participants will complete a battery of online behavioral measures, including measures of (a) personality and (b) cognitive control (**Fig. 3**). At a six to twelve-month follow-up, participants will be contacted to complete a series of tasks, including tasks that measure (a) perceptual/decision biases and (b) affective biases. ***Personality and affective state measures*** include those with previous relationships with placebo effects in smaller studies (reviewed above), particularly the Life Optimism Test Revised (LOT-R[381]), Behavioral Inhibition/Behavioral Activation (BIS/BAS[382]), State/Trait Anxiety Inventory (STAI[59, 240, 383]), Beck Depression Inventory (BDI[384]), Fear of Pain (FOP[385]), Ego Resilience (ER-89[386]), and the "Big 5" brief inventory (neuroticism, openness, agreeableness, extraversion, conscientiousness[387]). ***Perceptual/decision bias tasks*** are adaptations of sensory suggestibility tasks predictive of placebo effects in past studies[388-390]. A series of perceptual judgments are made of the color, size, etc. of visible objects, and the test assesses whether judgments are biased by the identities of the objects. ***Affective bias measures*** use a well-validated task that assesses whether individuals consistently identify ambiguous faces and images as positive or negative in valence[390, 391]. ***Cognitive control measures*** include tasks studied extensively by our group[392-395], including measures of cognitive flexibility (letter/number

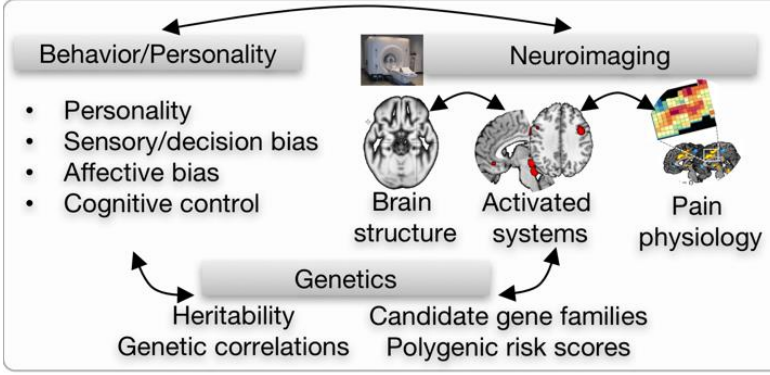


Figure 3. Overall design. *Before scan day*: Personality and online behavioral measures. *Scan day*: Placebo effects on thermal and mechanical pain, first with verbal suggestions only and then after conditioning. Measures of brain structure (T1 and diffusion tractography), activity increases (fMRI) and reductions in pain physiology (pain-related NPS, SIIPS patterns). Genetic assessments: Heritability of placebo-related brain features and behavioral measures, genetic correlations with analgesia. Polygenic risk scores (PRSs) estimated using ENIGMA consortium data.

task switching), response selection (go/no go, stop-signal, and antisaccade), and working memory (N-back and keep-track tasks). These tasks will be administered online in the month before scanning through the study website (www.paingen.science) in which all data will be stored in the secure RedCap system[396], with reaction time-based tasks implemented using Javascript embedding. *Prior data collection waves on the CTS sample* include neuroticism and extroversion[397], reward-dependence[398], harm-avoidance[398] and behavioral disinhibition[399], and standardized cognitive tests (PIAT-R, WISC-III), which we will also correlate with placebo outcomes. In order to test functionality and gather preliminary data for these online measures, we will recruit a pilot sample of 1000 'online only' participants using Amazon's Mechanical turk system. The link for mturk participants will be: www.paingen.science/mtruk.

An additional behavioral assessment titled **'Skin Prick Test (Allergy test)'** will be administered after the fMRI tasks on the day of testing at the Center for Innovation and Creativity (CINC). This task is modeled after previous research done in the Stanford Psychology department (Howe, Grover, Crum, 2017) in which 164 participants received the skin prick test with no adverse side effects. All subjects who are cleared for this task (based on a short in person screening procedure on the day of testing: see attachment 'skin prick test screening and allergy task instruction' document) will participate after the

fMRI scanning procedures. The allergy test will involve participants experiencing an induced itch through a standardized 'skin prick test' involving administration of a drop of 1mg/ml histamine gel (1% histamine dihydrochloride in 2.5% methylcellulose). Skin prick tests involving histamine are used as controls in routine allergy diagnosis tests. Typical allergy tests are done with actual (food or environmental) allergens. However, we will not be using any allergens in this procedure. Histamine is an organic compound naturally produced by the body and is classified as a monoamine neurotransmitter.

This test has been used in numerous studies to induce itching (e.g., Bromm, Scharein, Darsow, & Ring, 1995; Darsow, Ring, Scharein, & Bromm, 1996; Pfab et al., 2006, 2010). Further, this procedure induces an itching sensation, but did not cause pain or any other sensations to any participants in previous studies. Histamine will be applied to two skin sites on the participant's non-dominant volar forearm. Two inert creams will be administered to the participant with the suggestion that one will 'decrease the allergic reaction and itching' and the other will 'increase the allergic reaction and itching' (counterbalanced between subjects). Allergic reactions or 'wheals' will be measured for 30 minutes after application. This task is designed to measure participant's placebo response(s) in a non-painful scenario. This will allow us to compare different types of placebo responses, expectations, and genetic factors that contribute to the placebo effect.

A.2.2. fMRI study design. The design of the proposed fMRI experiment is based closely on our work on fMRI of placebo analgesia over the last 15 years[221, 400-409], including recent studies comparing intensity-matched thermal and mechanical pain (*N* = 81 fMRI subjects run to date). There are *two main phases* during fMRI: In the *placebo administration phase*, participants are given two identical creams with different instructions: "Prodicaine, an effective pain-relieving drug" (Placebo) and "a control cream with no effects" (Control) applied to 4 fingers each on the left hand, with locations counterbalanced across subjects. Disclosure of side effects are presented on forms with realistic-looking drug company logos (see attachment 'drug_info_packet_script_PAIN-GEN'), and testing is done in a medical environment with medical equipment and related context cues. A 2-minute delay follows for the "cream to take effect", during which we will ask the participant to complete the survey "side effects questionnaire". Structural (T1-weighted) images will be collected prior to any of the placebo tests. A *post-conditioning test phase* tests both pain types on and off placebo treatment after reinforcement. We have successfully used similar procedures[414], as have others[222] (e.g., C. Büchel, *N* = 700 with suggestion-alone and post-conditioning, no imaging) *Rating scale*: Ratings of pain intensity and unpleasantness will be made after each trial on a 100-point generalized visual analogue scale[415-418] with anchors of "no pain" or "no unpleasantness" and "most intense sensation imaginable," or "Most unpleasantness imaginable", using an fMRI-compatible trackball[221].

Stimulation and trial details. A Control-Placebo-Control design (e.g., [222, 414]) in each stimulation phase permits assessments of placebo effects within individuals, without confounding placebo with habituation (or sensitization) to pain and avoiding order effects. Based on prior work, we expect little to no habituation of pain responses with this protocol[221, 288, 414, 419, 420]. Each trial will consist of: (1) A warning cue, followed by a variable-duration anticipation interval (4-16 sec with exponential distribution, 6 sec mean [73, 221]); (2) thermal or mechanical pain (10 sec plateau with 2 sec ramp up/down); (3) a 4-16 sec jittered delay followed by rating scales (4 sec each); (4) a 4-16 sec inter-trial interval. Ramps minimize head movement during stimulation to very low levels (0.07 mm average[37]). Standard stimulus intensities will permit assessment of individual differences in brain and behavior holding objective stimulus intensity constant[144, 421]. Based on previous calibration studies in our lab (heat *N* = 495, pressure *N* = 81), we will select *heat and pressure intensities matched for pain* in the

population. Max "high pain" intensities of 49° C and 7 kg/cm^2 are expected to be "very painful" for ~83% of the sample but *tolerable for nearly all (98%) of participants*[144, 270, 421]. A variable-duration anticipation period *separates anticipatory activity* from stimulus processing.

For the thermal stimuli, the temperatures will range from 43 to 49 °C, and the pressure will range from 4 to 7 kg/cm^2. As part of the screening process, we will perform a pain calibration procedure with the thermode (Medoc, Inc.) on each participant in order to ensure that all temperatures delivered during any subsequent procedures are within participants' tolerable range. Pain stimulation will be performed using a contact thermode (Medoc, Inc.) that is placed against the skin. The thermode is actively heated and cooled by the hardware of the device. Temperature values are controlled to within .1 degrees Celsius by a computer, with a safety shutoff at a level tolerable to some participants, and nondamaging to skin (the hardware will not allow stimulation at 50 degrees Celsius for longer than 6 seconds). Stimulation will not exceed 50 degrees Celsius in any of the studies covered under this protocol unless explicitly noted in a separate protocol.

The pain calibration procedure consists of applying temperatures to the subject's right index finger. Participants rate each stimulus on a visual-analog scale. Eligibility for participation in the behavioral or fMRI portion of this study involving pain requires that participants have pain tolerances that are not too high or too low—i.e., those that would require delivery of temperatures above the boundaries stipulated by the guidelines detailed below, or that do not allow for sufficient experimental variation of the temperatures delivered.

For pressure stimuli, the newly developed pressure pain device (Attachment 13) will be used within the safe range based on our pilot data and previous studies (Attachment 14: "Pressure pain pilot study results" and Attachment 15: "Pressure pain stimulation guideline").

During *post-conditioning test* phases, participants experience 32 medium-intensity trials divided equally among 4 conditions: Tests on Placebo and Control sites crossed with heat (47° C) vs. pressure (5.5 kg/cm^2), all on separate fingers. Our previous analyses of NPS "pain signature" responses indicate that 7 trials or more per condition will yield 90% classification accuracy for discriminating moderate (1 s.d.) differences in pain intensity[37, 38, 270]; thus, 8 trials per condition here is adequate. The *conditioning phase* is similar, except that stimulation will be applied at high intensities (49° C or 7 kg/cm^2) on Control sites and low intensities (45° C or 4 kg/cm^2) on Placebo sites, to associate placebo treatment with reduced pain. We will test different fingers during conditioning to avoid skin site-specific habituation effects from influencing test phases.

A2.3. Genetic assessments. IBG has over 40 years' experience as a leading center for behavioral genetics, and extensive experience collecting and analyzing Genome-Wide Association Study (GWAS) data. Our team also has substantial expertise in neuroimaging genetics (co-Is Friedman) and analysis of GWAS data (co-Is Keller). We will collect saliva samples during the fMRI scan day and genotype using the same facility (same laboratory, personnel, platform, and protocols), following standard GWAS quality control procedures[422] (e.g., for minor allele frequencies, Hardy-Weinberg equilibrium, call rates, etc.).

A.3 Analysis plan and hypotheses

Aim 1: Analyses 1a-1c identify brain and psychological predictors of placebo analgesia, respectively**.** Placebo effects will be defined as Control vs. Placebo ([C – P]) differences in pain report ("placebo analgesia") and two validated, pain-related brain measures: the NPS and SIIPS. These analyses will allow us to identify common and differential brain predictors of placebo effects on behavioral (pain report)

and neurobiological outcomes. They will permit three other novel tests: (1) Whether predictive patterns generalize across thermal and mechanical pain; (2) Whether they generalize across placebo effects in suggestion alone or post-conditioning; and (3) Whether we can identify latent factors across outcomes (thermal/mechanical, suggestion/conditioning), and develop predictors of those domain-general outcomes. Such factors are more likely to be genetically determined and useful in broader clinical contexts.

Multiple comparisons and power: This study is the first large-scale study powered for *whole-brain analyses* of structural and functional correlates of placebo analgesia with Family-Wise Error Rate (FWER) map-wise correction. We will use FWER correction as implemented in Statistical Nonparametric Mapping (SnPM)[423]. This correction is valid, makes minimal assumptions, and is not affected by recent findings of inflated false positive rates with some correction methods[424]. With our sample size (N = 600), we have 80% power with whole-brain FWER correction to detect correlations with placebo analgesia of $r \geq 0.22$, which is within the range of our previous cross-validated estimates [37, 268, 269, 353, 354, 369]. Previous studies have been substantially underpowered; with N = 50, which is larger than most placebo studies, correlations must be r = 0.65 or larger to be detectable with 80% power. Though this is the first study adequately powered for whole-brain correction, we will also focus on testing hypotheses derived from the previous literature, as described below.

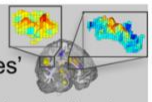
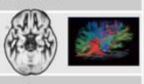
	Predictors	Outcomes: Placebo vs. control in
1a	fMRI before, during pain, placebo vs. control 	Placebo analgesia outcomes:  • Pain report • Pain-related fMRI 'signatures' (NPS, SIIPS) • Separately for thermal, mechanical pain • Separately for suggestion vs. conditioning • Factor scores across these outcomes
1b	Structural MRI 	
1c	Personality and behavior	
2a	Twin status: Monozygotic vs. Dizygotic vs. unrelated Structural model	• Heritability of pain report, NPS, SIIPS • Frontoparietal, Medial frontal-brainstem network increases
2b	Twin status (mono/dizygotic) Genetic correlations	Heritability of correlations between placebo outcomes and predictors from Aim 1
2c	SNPs from candidate gene families	• Placebo outcomes • Predictors from Aim 1
3a	Polygenic risk scores (PRS) in ENIGMA consortium	Regress placebo-linked structural features on SNPs in ENIGMA, yielding PRS. Test in Women's Health Initiative study.

Table 1. Overview of analyses

Analysis 1a identifies functional brain predictors of placebo analgesia. We focus on two systems related to placebo analgesia[35, 409] (**Fig. 1a**): A **fronto-parietal system** involved in executive control and cognitive appraisal of emotion[425-431], and a **medial cortical-striatal-brainstem system**—including mid-lateral OFC, vmPFC, VS/nAC, and PAG. We hypothesize that behavioral analgesia will be related to anticipatory fronto-parietal activity and down-regulation of SIIPS responses (related to endogenous brain contributors to pain), but not modulation of the NPS (related to nociceptive pain), indicating that reported analgesia is influenced by decision bias and providing brain correlates of this bias. Alternatively, analgesia may be correlated with NPS modulation and reductions in local targets of nociceptive afferents (S2, dpINS, mid-INS, aMCC), validating pain report as a predictor of neurobiological placebo effects. Increases in the medial system may also predict larger placebo effects on the NPS, linking this system to strong control of pain in placebo-responsive individuals.

Analysis 1b identifies structural brain predictors of placebo analgesia. We hypothesize that gray-matter density in the **medial cortical-striatal-brainstem system,** particularly nAC and vmPFC, will predict larger placebo responses in pain report, NPS, and SIIPS responses. Prior work suggests that gray-matter

density in the hippocampus[432, 433] and fronto-parietal system[273] may also be related to placebo effects. Diffusion-weighted imaging (DWI) will be used to assess the size/integrity of fiber tracts. We expect placebo effects to be associated with tracts connecting the vmPFC and OFC with the PAG[261]; but **alternatively**, connectivity in the fronto-parietal system (esp. dlPFC) and lateral frontal-brainstem connectivity may be more predictive. Such findings would connect placebo effects to systems involved in value and motivation, on one hand, or cognitive control on the other. This would permit evaluation of the parallel between pain regulation and other forms of cognitive regulation, and connect placebo effects with systems that degrade with age and dementia.

Analysis 1c will test whether placebo effects on behavioral and brain measures of analgesia are related to personality and behavioral measures that can be easily assessed clinically. We will apply confirmatory factor analysis to web-based assessments to identify latent factors[434] related to (a) susceptibility to perceptual/decision bias (i.e., influences of instructions on judgments of shape, color, etc.); (b) positive vs. negative interpretations of ambiguous events; (c) latent variables related to task switching, inhibition, and working memory identified in our previous work[331-336]; and (d) personality measures, focusing on an "optimistic/pro-engagement" style and an "anxious" style. We will conduct five-fold cross-validated analyses[435-437] developing an optimal combination of behavioral variables for predicting placebo analgesia. We expect that placebo analgesia will be related to **(a)** larger perceptual/decision biases[282], **(b)** high optimism[438], and **(c)** low anxiety and fear of pain[291-293]. We are agnostic about whether cognitive control measures will predict placebo analgesia, but it is important to know either way; some studies suggest placebo effects are related to cognitive control[438, 439], whereas others suggest they are distinct forms of motivation-based regulation[257, 440]. Positive results from Analysis 1c will provide a behavioral phenotype that can be readily assessed in clinical settings. We can then relate these behavioral measures to brain measures, as in Analysis 1a, to link them to neurobiology. Negative results will also be very important, as several companies are currently investing in personality-based predictors for clinical settings. The field needs to know whether or not such measures are viable predictors of placebo response magnitude.

Extensions and alternative plans. (1) We are developing new large-sample predictors of pain, and will test those as they become available. (2) Another important extension relates to individual differences in pain sensitivity. While not the main focus here, this dataset and our approaches are well-suited to predicting individual differences in pain sensitivity—a core feature of many pain conditions[441-443] and a risk factor for later chronic pain [444-446]. Models of risk for chronic pain following surgery (unfortunately common) and other procedures would be a crucial advance in precision medicine[447-452]. Risks can be assessed at both phenotypic (predicted by evoked pain sensitivity) and genetic[453] levels. Exploratory analysis will allow us to develop a foundation for understanding the neurobiology of individual differences in pain sensitivity.

Aim 2: Analyses 2a-2c will assess the heritability of placebo effects and placebo-predictive brain features, and relate these features to candidate gene families. **Analysis 2a (Aim 2.1)** identifies the heritability of placebo effects in pain report and brain responses for the first time. We hypothesize that heritability of pain reports will be modest, but heritability of placebo modulation of specific pain-related brain measures (the NPS and SIIPS) will be stronger, as these reflect component systems that are less etiologically complex and less context-sensitive that overall pain report. We also expect heritability to be high for gray-matter density in nAC and hippocampus[454], and mPFC-brainstem pathways. The **model** for analysis 2a is a standard univariate ACE model[455,456], which decomposes twin covariances for a trait of interest into additive genetic (A), shared environmental (C), and non-shared environmental (E) variance

components (**Fig. 5**). This model will be applied to individual placebo measures (e.g., thermal and mechanical pain), and also to latent variables (i.e., using a common factor model with thermal and mechanical pain loadings constrained to equality to identify the factor).

Analysis 2b (Aim 2.2) identifies genetic correlations between placebo effects and brain features that predict them. It tests whether individuals who are more genetically related show higher cross-twin, cross-trait correlations. The **model** for this analysis is a bivariate extension of the ACE model[455, 456] (**Fig. 5b**). The covariance between two measures is decomposed into genetic and environmental components with a Cholesky decomposition. The ACE variables for the first measure (e.g., placebo analgesia) are allowed to predict the second trait, and the second trait is allowed to have unique ACE influences. Of primary interest for **Aim 2.2** is the genetic correlation (*r*A) of the two phenotypes, which indicates the extent to which the genetic influences overlap across the two traits: *r*A = a12/√(a12^2 + a2^2); *r*C and *r*E can also be computed analogously. We will apply this model to relationships between analgesia and pain neurophysiology (i.e., NPS and SIIPS), and functional and structural features that predict placebo effects (e.g., nAC density, fronto-striatal activity). We expect the strongest genetic correlations in structural MRI measures that are likely highly genetically determined[457]. Structural features that are both placebo-linked and genetically associated with placebo (e.g., nAC gray-matter density) are good candidates for polygenic risk scores in Aim 3. **Power**: Assuming a moderate E variance of 30%, we will be able to detect genetic variance as low as 34% and shared environmental variance as low as 36% with >80% power. With an E variance of 40%, we can detect an A of 45% and C of 40% with >80% power. *Genetic correlations*: We will have power to detect either an *r*A or *r*C in the presence of an *r*E: Assuming 40% A and 30% C and E variances for both variables, we will be able to detect *r*As of at least .36 and *r*Es as low as .22 with >80% power. Assuming 40% C and 30% A and E variances for both variables and correlations between both Cs and Es, we will be able to detect an *r*C of .34 and *r*E of .19 with >80% power.

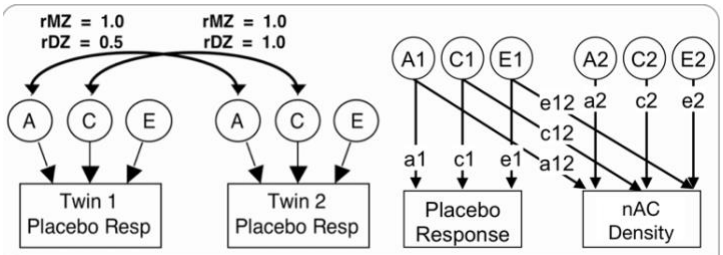


Figure 5. Structural ACE model for heritablility. *Circles*: latent variables for the genetic (A), shared environmental (C), and unique environmental (E) influences for each twin in a pair. MZ: monozygotic; DZ, dizygotic. The correlation between the genetic effects (A) is set to 1.0 in MZ twins and 0.5 for DZ twins (who share on average half their segregating genes). All twins are reared together in our sample, so C is set to 1.0 in both groups, and E is set to zero because unique influences are unshared, by definition. *Right*: Bivariate extension for estimating genetic correlations. nAC: nucleus accumbens, an example candidate region.

In **Analysis 2c**, we will attempt to replicate previously identified associations between candidate genes and placebo effects (reviewed in [376]). We expect the most meaningful results to come from polygenic risk scores (see below), but it is important for the field to know whether the candidate genes already identified predict placebo responses or not. Based on previously reported associations, our study is well powered to determine this. We focus on SNPs within (+/- 10 kb of gene boundaries) several neurotransmitter/ neuropeptide gene families previously linked with placebo analgesia in pharmacological and molecular imaging studies, including **catecholamine and monoamine clearance** (COMT, MAO-A), **opioids** (OPRD1, OPRM1, OPCML, SIGMAR1, OPRK1, PDYN, OGFR), **dopamine** (DBH, DRD5, PPP1R1B, DRD1, SLC6A3, DRD4, DRD2, DRD3, CALY), **serotonin** (5-HT; HTR[1-3]A/B, HTR[1-3]B, HTR1[D-F], HTR2C, HTR3C/D/E, HTR5A/B, HTR[4, 6, 7], MAOA, SLC6A4), **cannabinoids** (CNR1, CNR2, CNRIP1), and **cholecystokinin** (CCK; CCKAR, CCKBR, CCK, DBI), and BDNF. We will test for statistical enrichment in SNP associations within these gene families using the MAGMA

[doi:10.1371/journal.pcbi.1004219] and VEGAS [http://dx.doi.org/10.1016/j.ajhg.2010.06.009] software tools. This set comprises 48 genes with an estimated 320 genotyped SNPs and 4800 imputed SNPs with MAF > .005. Most of these SNPs do not exist on the Affymetrix Axiom Precision Medicine chip, but they can be imputed with excellent fidelity from genotyped SNPs using the Haplotype Reference Consortium reference sequence database [doi:10.1038/ng.3643], which has replaced the 1kG reference database as the gold standard. This approach is now standard for GWAS. We estimate imputation of ~97% of candidate SNPs with ~99% accuracy using the *MACH* program[458]. **Power**: Assuming correction across ~50 candidate genes, we will have 80% power to detect effects of d = 0.24 ("small") or greater, substantially smaller than most previously reported associations. **Hypotheses**: Previous studies reported that opioid genes were related to fMRI signal in PAG and vmPFC[459-461]; dopamine genes to enhanced placebo after conditioning, compared to suggestion alone[462, 463]; and serotonin and CCK genes to (a) anticipatory activity in vmPFC, which correlates negatively with placebo analgesia[63, 70, 464] and positively with anxiety[408], and (b) activity in the serotonergic dorsal raphe nucleus (adjacent to the PAG), which is activated in placebo studies[162, 403, 465, 466]) and is linked to threat and analgesia in animal models[467-469].

Aim 3: Analysis 3. These analyses will develop Polygenic Risk Scores (PRSs) for placebo-linked brain structural features in the ENIGMA consortium dataset **(Fig 6).** The ENIGMA consortium is the world's largest study of relationships between brain structure and GWAS[470] . ENIGMA contains no personally identifiable information; investigators share summary statistics from analyses back to the requestor, in this case the Wager Lab. As such, it is a meta-analysis and does not involve sharing of identifiable data which could be linked to participants. In **Aim 3**, we leverage the information provided by the smaller, pain- and placebo-phenotyped twin sample to take advantage of the information in the larger, but less extensively phenotyped, consortium sample. Structural brain features with the highest genetic correlations with placebo response in our study—e.g., gray-matter density in nAC, vmPFC, and hippocampus—will be extracted from T1 data and regressed on GWAS SNP data in the ENIGMA sample., We estimate including at least *N* = 10,000 individuals, with a target of *N* = 30,000. PRSs will be developed by regressing individual SNPs on structural features using linkage disequilibrium (LD) regression[471, 472] as implemented in the PLINK package [472]. Regression weights on SNPs comprise a model that can be applied to new GWAS data to calculate an individual's aggregate genetic risk[130]. We employ several choices shown to be advantageous, including using a wider set of SNPs in the model[472, 473] and pruning SNPs in linkage with the strongest signals (e.g., r^2>0.05)[130, 474]. Finally, to estimate the effects of this PRS on placebo responses, we will correlate PRSs with clinical placebo responses in the Women's Health Study (*N* = 5,800

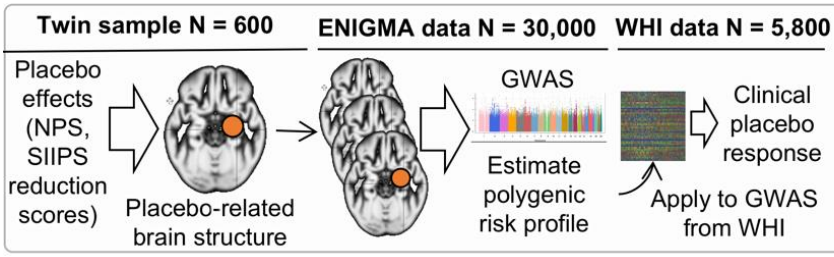


Figure 6. Developing and testing polygenic risk scores (PRSs; Aim 3). Placebo-predictive structural features are identified in T1 images in the twin sample. The ENIGMA sample has T1s and GWAS on all participants. Target brain structures will be regressed on GWAS SNPs in ENIGMA to develop weights for PRSs. We will apply weights to calculate PRSs in the Women's Health Initiative (WHI) study and correlate them with clinical placebo responses.

with GWAS data) in collaboration with Consultant K. Hall. Testing aggregate risk involves only a single test, with >80% power to detect even extremely small relationships (e.g., r^2 = 0.002) between PRS scores and placebo responses.

Extensions and alternative plans. **(1)** While we expect ENIGMA to be an outstanding resource for these analyses, other options exist. The UK Biobank recently released its first 10,000 participants[475], and

it includes structural and diffusion imaging and GWAS; similar to the ENIGMA consortium, UK Biobank data is de-identified and cannot be linked to individual participants. The ENIGMA consortium have access to this data. **(2)** In addition to LD models for PRS development, we will explore using the machine learning models used for the main fMRI analysis, adapted for binomial allelic outcomes (5-fold cross-validation stratified by data-collection site, modeling covariance across twins). This will allow us to train models on a subset (4/5) of the twin sample, train PRSs on the ENIGMA sample, and then "back-validate" the relationships with placebo effects on an independent (1/5) of the twin sample. This will also let us identify psychological/behavioral correlates the PRSs in the twin sample. **(3)** Finally, new informatics methods are actively being developed to conduct more elaborate analyses on the ENIGMA data. As these methods become available, another extension is to run cross-validated analyses identifying more complex patterns of structural changes in the twins, identifying PRSs in ENIGMA, and back-validating in independent subsets of the twin data. **(4)** We will conduct standard GWAS with whole-genome correction ($p < 5e-8$) further corrected for number of brain variables, with 80% power to detect SNPs correlated at $r^2 > .006$, as well as the 6 gene families discussed above, with virtually perfect power to detect SNPs with even extremely small effects ($r^2 > .006$).

A.4 Data acquisition and model specifics

fMRI acquisition and analysis. Images will be acquired using the University of Colorado's 3T Prisma scanner with 32-channel parallel imaging. Structural T1 (0.7 mm isotropic) will follow the 10,000-person Adolescent Brain Cognitive Development (ABCD) study protocol; CU Boulder is a project site and PI Wager and Co-I Friedman are members of the study team. Functional T2*-weighted echo-planar imaging (EPI) data will be acquired using a multiband sequence (MB factor 8, TR=460ms, 56 slices, 2.7 x 2.7 x 2.7 mm, TE=29 ms, FA=52˚) aligned to the plane of the OFC. Head movement will be minimized by soft restraint as in the ABCD study; we are the study site with the best quality control measures among the 18 sites in tests to date. We anticipate <1 mm displacement / 1.5° rotation within-run in ~95% of participants. State-of-the art image preprocessing techniques (SPM12; Wellcome Department of Cognitive Neurology, UCL), use procedures detailed in our published work [406, 408, 476-480], with improvements described below. Diffusion-weighted imaging (DWI) for tractography will follow the UK Biobank protocol (100 diffusion-encoding directions, 2 shells, 7-min acquisition; [481, 482]). *Preprocessing of structural images*: inhomogeneity correction, coregistration to the mean realigned functional image, enhanced nonlinear *normalization* to standard brain space using SPM12 plus a custom genetic algorithm[406]. *DWI images*: DWI analysis will follow the UK Biobank pipeline protocol[483] implemented in FSL[484, 485], including distortion correction, fractional anisotropy, tensor mode and mean diffusivity estimation (DTIFIT), Neurite Orientation Dispersion and Density Imaging modelling(NODDI)[485, 486], registration to standard space using Tract-based Spatial Statistics (TBSS), and probabilistic tractography with crossing fiber modelling (bedpostx/probtrackx)[487-490]. Outputs will be used to run tractography from seed regions of interest (dlPFC, vmPFC, PAG, mid-lateral OFC), and from each brain region in standard parcellations[491, 492] for pattern-recognition based analyses. *Preprocessing of functional images*: Time series *outlier/artifact assessment*; *realignment/motion correction*; application of *normalization* parameters, *high-pass filtering* with an optimized cutoff. *Head movement*: High motion (0.3 mm) and high image-to-image root mean square successive differences ($q < 0.05$ FDR) modeled as nuisance covariates. Additionally, 24 movement-related regressors per run in first-level GLM[493, 494]. First-level functional models: Two-level General Linear Model (GLM). *First level*: locally smoothed AR(2) model, linear and higher-order movement-related covariates[493, 494], flexible hemodynamics optimized for

pain[406, 495-498]. Event timing optimized using a genetic algorithm (GA)[499], design efficiency evaluated for each individual. *Second level*: (1) Voxelwise hierarchical mixed-effects modeling with restricted iterative generalized least squares (rIGLS)[500] accounting for correlated errors across DZ/MZ twins; or (2) multivariate machine learning models.

The scanning portion of the study will take place at the Intermountain Neuroimaging Consortium at the Center for Innovation and Creativity at 1777 Exposition Dr., Boulder, CO 80301. The MRI device for these scans is FDA approved for research with human subjects and has all the safety inherent in a clinical MRI scanner. The radio frequency fields conform to guidelines determined by the FDA and the FDA has designated MRI scanners to be a non-significant risk device. MR techniques non-invasively produce images and measurements from tissues in the intact, living human.

Statistical learning framework for fMRI and molecular genetic analyses. In contrast to typical voxel-wise mapping fMRI analyses and SNP-by-SNP GWA analyses, multivariate predictive models predict outcomes as a combination of (pattern across) multiple voxels/SNPs. This model yields an aggregate predictive score based on all available brain/SNP information, which is validated by applying it to new individual test subjects. Placebo analgesia ([C – P] in pain report) and changes in pain-related NPS and SIIPS responses ([C – P]) are the outcomes to be explained. We use LASSO-PCR[408] or Least Absolute Shrinkage and Selection Operator[501] Principal Components Regression (**Fig. 2;** this compares favorably with popular support vector regression). *Data reduction*: principal components analysis retaining *N*-2 orthogonal predictors; *Penalized regression* of component scores onto the outcome uses LASSO shrinkage, simplifying the model. *Cross-validation* is five-fold, balanced cross-validation[502] in discovery sample (N = 500), with *N* = 100 reserved as a final test set[435]. These methods provide stable, robust estimates when there are more predictor variables (voxels or SNPs) than observations, and account for covariance among predictors (i.e., linkage disequilibrium in SNPs[503]). In addition, feature (variable) selection using our prior work on placebo analgesia[408] will simplify the models and increase interpretability. Tests of voxel and SNP importance will be accomplished using a null-hypothesis permutation test[408, 423, 504] ($p < 0.05$ FWER). In *molecular genetic* analyses, feature selection will be accomplished by (a) focusing on polymorphisms related to six key placebo-linked gene families, or (b) optimizing the inclusion threshold and sparsity[503] via nested cross-validation.

Study timeline

Data collection and analysis will take place in each year of this 5-year project. We anticipate testing ~10 subjects/month, for target totals of 115 in Year 1, 135 in Year 2, 120 in Year 3, 120 in Year 4, and 115 in Year 5. We project 3 months of development and pilot fMRI scanning time to ensure that the paradigm and image acquisition are optimized for the main study. Prior arrangements with the Brain Imaging Center ensure that adequate time will be available for testing. fMRI and genetic data analysis will be conducted on a rolling basis. Preliminary reports will be written and submitted for publication in Years 2-4 that focus on group fMRI effects of placebo and methodology of machine learning applications to fMRI and genetic data. Final analysis and a report on the full sample will be prepared in Months 7-12 of Year 5.

V. ABOUT THE SUBJECTS

Subject Population(s)	*Number to be enrolled in each group*
Pilot Participants	*35 (30 behavioral only + 5 fMRI)*
Colorado Community Twin Sample (CTS)	*~700*
MTurk Pilot Participants	*1000*

All CTS participants will be screened over the phone by Corinne Gunn. Corinne will walk through the attachment 'fmri twin recruitment script' which will lead to the 'fmri safety screening' (attachment '18_twin_fmri_safety_screening (1).doc') questions if the potential participant is willing to be in the study. We will primarily recruit individuals aged 18-55 years who are fMRI compatible (passed the 'fMRI safety screening'), capable of performing experimental tasks (e.g., are able to read), are fluent or native speakers of English, and have no current or recent history of reported neurological disorders. Participants less than 18 years old will be excluded because of population vulnerability issues. Participants over 55 years of age will be excluded based on a diminished sensitivity to pain and changes in brain structure that require special studies of older populations, which is outside the scope of this study.

In addition, we will also exclude people who cannot tolerate the maximum level of thermal pain stimuli (for thermal stimuli: 49 °C).

Participants who have any contraindication to magnetic resonance scanning (e.g., metal in body, claustrophobia, pregnant) will be excluded from the study. These exclusions are specific to MRI and are consistent with most studies involving MRI. Potential participants will be screened for the presence of any of these exclusion criteria prior to participating in this MRI study (see attachments 18 and 19).

For M-Turk Participants: Recruitment will only target workers who are above the age of 18 (all Mturk works are already required to be fluent in the English language). We will also only recruit MTurk workers with the "master worker" designation and with a 95% previous work approval rate, to increase the likelihood of obtaining good quality data. No other inclusion/exclusion criteria will be used. The website link for Mturk participants will be www.paingen.science/mturk.

VI. VULNERABLE POPULATIONS

We will not exclude anyone based on class or income so there is a possibility that economically disadvantaged individuals will be enrolled in this study, but no other vulnerable populations will be included, and recruitment will not target vulnerable populations.

VII. RECRUITMENT METHODS

We will recruit 700 twins (350 same-sex pairs; 175 monozygotic and 175 dizygotic; ~50% female) from the Colorado Community Twin Sample (CTS). The CTS includes 727 same-sex twin pairs between 26-38 years old (as of Spring 2017), originally derived from the Colorado Twin Registry, a population based registry, housed at the Institute for Behavioral Genetics (IBG) at the University of Colorado since 1984, with three previous waves of data collection (1997-2002, 2002-2008, and 2009-2014)[380]. The CTS participants are on average 37.8 years old (SD 2.5), ~50% female, mean education 14.5 years, 80% non-Hispanic Caucasian, 14% Hispanic, 4% African-American, 2% Asian, and < 1% Native American. ~75% of the sample still lives in Colorado.

Twin recruitment and screening: Colorado subjects are drawn exclusively from the samples of past participants in the CTS. They will be contacted by telephone and invited to learn more about the study (See Attachment #17). If they agree to participate, the interview will be scheduled and instructions for filling out the online consent form and questionnaires will be conveyed over the phone. CTS pairs will be recruited via direct contact by IBG staff Corinne Gunn, who has worked with this sample for 15 years. Corinne will also conduct the fmri safety screening (attachment 18) over the phone. All participants who express willingness to participate in this study will then be given a Docusign consent form by Corinne via email. Once a participant has signed the consent form, Corinne will mail them a brochure (see document: 'pain-mailer10.02'). This brochure will be used as additional recruitment material. The brochure contains information about the study (includes some deception to maintain the notion that this is a drug study), Corinne's contact information, and a link to the website which they will be filling out the online tasks (www.paingen.science). Since participants are able to stop and start the online surveys at any time, we hope that this brochure will serve as a physical reminder for participants.

MTurk recruitment: Mturk participants will be recruited through Amazon's Mechanical Turk. Workers that fill out surveys will be able to see the web based task as a HIT (Human Intelligence Task; title: **Brain and Genetic Predictors of Individual Differences in Pain and Placebo Analgesia**) on a posting board including thousands of other HITs.
Pain study screening: Pain screening will be conducted when participants arrive at CINC (see Procedures: pain calibration task).

List recruitment methods/materials and attach a copy of each in eRA
1. 17_fmri_twin_recruitment_script.docx
2. Pain-mailer10.02

VIII. COMPENSATION

Laboratory participants will be given $200, in cash or electronically, at the end of the study. We will also give participants an image of their MRI scan, and a 3-D model of their brain created on a 3-D printer. Participants who withdraw prior to study completion will be paid pro-rated for their time to the nearest hour.

Some participants in the CTS may be residing outside of the state of Colorado or the city of Boulder. Participants will be reimbursed for all travel costs (airfare or car travel over 1 hour) and a room will be purchased at the Best Western Hotel in Boulder, CO (770 28th St, Boulder, CO 80303) by the Wager lab for the time needed to complete this study.

Twin participants who participate in completing the follow up surveys will be paid an additional $25, which will be sent to them via mail.

M-Turk Participants will be compensated with $0.1 per minute. We worked out this pay rate because higher pay rate makes respondents repeat the survey, which ends up creating useless data. Participants who do not complete the study will not be paid. Participants who provide poor quality data demonstrating careless responding (i.e. too many surveys open at the same time; or improper data responding using certain data quality question checks) will not be paid. Participants will be notified in advance that data quality measures are in place, and participants with poor data will not be paid.

IX. CONSENT PROCESS

Corinne Gunn will contact participants on the CTS list to describe the study (attachment 17) over the phone and check for interest in participation, then conduct an fMRI safety screening if they agree to participate. Then, we will obtain an additional experiment specific consent (Attachment 2), via Docusign, provided by Corinne after the initial phone screening. The online consent form will have the Wager lab's email and phone number contact information if they have any questions. When the participant arrives at CINC the day of the fMRI scan, they will meet a research coordinator who will remind them of the study's procedures. The research coordinator will have a copy of the consent form to re-confirm the participant's willingness to participate and go through the procedures of the study. The participant's voluntary participation is stressed in that they are informed, both verbally and in writing, that they can discontinue the study at any time.

To study the effects of individual differences in placebo responding, some deception is required. Specifically, we will inform participants that a cream will be applied to the skin sites that will receive heat and pressure pain stimulation in order to test the effects of a pain relieving topical treatment, Prodicaine. We believe that our experiment qualifies for a Waiver of Some Elements of consent. As is detailed in the "Risks to Participants" section, substantial regular testing of our laboratory equipment and standardized safety procedures for the use of stimulus delivery systems ensure that the risk of injury to participants is minimal. The use of deception in this way will not adversely affect the welfare of the subjects in this experiment, as our use of placebos here is designed to attenuate the experience of pain, not increase it. Deception is inherently necessary to induce placebo effects, and research in this area cannot be performed without it. A debriefing session will be held (Attachments 11 and 12) upon termination of the study, where participants will be informed of all aspects of the deception used, and be able to voice any questions or concerns they have.

As a part of the consent form, participants will indicate whether they wish to be contacted for future studies. If so, a member of the research team will store contact information for this participant in a secure location, outlined in the "Data Management" section, so that this participant may be contacted in the future.

For M-Turk Participants: Informed consent will be administered as the first page of the survey. Participants will be required to answer a yes/no question, "Do you consent to being part of this study?". Only those who answer 'yes' will be advanced to the next page of the survey. We reformatted the consent form template provided by the IRB to be more suitable for the online environment. All the information from the IRB template was included in the reformatted (condensed) consent from attached to this protocol (see attachment - Mtruk consent online). Mturk participants will see this consent form after being directed to their specific link: www.paingen.science/mturk.

X. PROCESS TO DOCUMENT CONSENT IN WRITING

As noted above, digital documentation of consent will be obtained for laboratory and online sessions via docusign (or through the study website for Mturk participants), and a trained experimenter will review the consent document and procedures with the prospective participant before informed consent is obtained.

XI. PROCEDURES

1. **Online Behavioral Assessments through the study website (**www.paingen.science or www.paingen.science/mturk **) –all data stored on Redcap:** After agreeing to participate in the study and completing the consent form (to be completed within one month of fMRI scanning).
    a. Personality and affective state measures (Surveys a-l in the table below)
    b. In the event that an Mturk participant comes across an issues they will be given the following email to contact: paingene.canlab@gmail.com. Only research personnel listed will have access to the email account. Xiaochun Han, co-investigator, will be managing the email and responding to participants' inquiries.
2. **Online Cognitive Tasks (also via study website):** Participants will perform the following tasks implemented in an online system (i.e., using JavaScript) that will collect reaction times and error rates. Protected information (e.g., names and contact information) will not be collected in this system. Instead, participants' data will be coded with a unique alphanumeric code. Data will be collected on and stored in Redcap.
    a. Cognitive Control Measures:
        i. **Letter/number task:** Participants will be presented with a set of numbers and letters. Their task is to classify the letter as either consonant or vowel and whether the number is even or odd.
        ii. **Go/no-go task:** Participants will be presented with the word "go" or "no-go" and will be asked to press a certain key when the "go" signal appears and to avoid pressing the key when the "no-go" signal is present.

iii. **Stop Signal task:** Participants will perform a choice reaction time in which they have to respond as quickly as possible to a particular stimulus feature (e.g. colour, shape, identity, or location). On a minority of the trials, the go stimulus is followed by an additional signal (e.g. an auditory tone), which instructs participants to withhold their planned response.

iv. **N-back Task:** Participants will be presented with letters one at a time and they must identify each item that repeats relative to the item that occurred "n" items before its onset.

v. **Keep track task:** Participants are first shown a set of categories to keep track of for a particular trial (e.g., animals, colors, and countries). They are then presented with words (including words from each category), and must remember the last word that was presented from each of the categories and recall those words at the end of the trial.

3. **Intro Survey (CINC room 174):**
Within one month of completion of the online behavioral/cognitive tasks (and fMRI eligible) the participant arrives at CINC they will meet a research coordinator who will have a copy of the consent form in order to walk them through the experiment details and remind them of procedures for this study. Then, they will be asked to complete the Pain Genetics intro survey in room 174.

   i. **Antisaccade task:** After the intro survey, in room 174 participant will complete the anti-saccade task in the same room. Participants view a fixation point and a visual target (e.g. a black circle) is presented. Subjects are instructed to look away from the target (antisaccade) and respond with what number they saw on the screen.

   ii. **Saliva Sampling:** Prior to the fMRI scanning session, participants will be asked to give a saliva sample. This will involve spitting into a tube to the desired amount of fluid needed.

4. **Pressure Pain Calibration task (Room 174 and room 173):** Then, they will be asked to conduct the 'pressure pain calibration' task. Participants will receive mechanical pain stimuli (between 4 kg/cm^2 and 8 kg/cm^2, max duration = 10 seconds), and will provide their pain ratings verbally between 0 and 10. If the participant is unable to tolerate the pressure stimulations, they will be deemed ineligible for this study.

5. **Intensity and Unpleasantness scale practice (Room 173):** Participants will then complete the "Scale-practice survey" in order to get more familiar with using our rating scales.

6. **Thermal Pain Calibration task (room 173):** Then, they will be asked to conduct the 'thermal pain calibration' task. Participants will receive different levels of thermal stimuli in a random order (between 45.5 and 48.5 °C, max duration = 12 seconds), and will provide their pain ratings verbally between 0 and 10. If the participant is unable to tolerate the maximum thermal stimulations, they will be deemed ineligible for this study.

7. **Pain tasks outside the fMRI scanner (Room 173):** There are *three main phases* during behavioral testing: In the *placebo administration phase*, participants are given two identical creams with different instructions: "Prodicaine, an effective pain-relieving drug" (Placebo) and "a control cream with no effects" (Control) applied to 2 fingers on the left hand (two sites for thermal stimuli only), with locations counterbalanced across subjects. Disclosure of side effects are presented on forms with realistic-looking drug company logos (see attachment 'drug_info_packet_script_PAIN-GEN'), and testing is done in a medical environment with medical equipment and related context cues. A 2-minute delay follows for the "cream to take effect", during which we will ask the participant to complete the survey "side effects questionnaire". A *suggestion-only test phase* in which participants will view pseudo-randomly generated pain ratings given by "prior" participants. The suggestion only test does not use any thermal or mechanical stimulation but tests participant's ability to track images of "pain" ratings in order to generate an expectation that the placebo cream will work. Next, is a *conditioning phase* in which delivery of high- and low-intensity stimuli (thermal only) on placebo- and control-treated skin sites, respectively[383, 410-412]. This reinforcement strengthens expectations[383, 410-412] and induces non-conscious learning[24, 234, 413]. Ratings of pain intensity and unpleasantness will be made after each trial of the conditioning phase on a 100-point generalized visual analogue scale[415-418] with anchors of "no pain" or "no unpleasantness" and "most intense sensation imaginable" or "Most unpleasantness imaginable", using an fMRI-compatible trackball.

8. **Preparation for MRI scanning:** Following the calibration task, but prior to going into the MRI scanner, the MRI technologist on duty will ask participants to remove all jewelry and metal objects from their pockets. Participants will be required to change into scrubs to prevent any possible risk from metallic objects or decorations in their clothing. Participants will complete the required fMRI and CINC safety screening forms (Attachments 18-19) prior to entering the scanning room.

   In an MRI scan the subject lies down on a table and is placed into a long donut-shaped magnet. A specially designed coil will be placed around the head to provide better images (as is done with standard clinical examinations). As the MRI scan is performed, the subject will hear loud rapping and knocking noises that are normal for a MRI scan.

9. **Pain tasks in the fMRI scanner:** During the MRI scan, multiple pain tasks described in the "Research Design" section will be conducted. All scanning will be conducted on a research-dedicated Siemens 3T MRI scanner at the Center for Innovation and Creativity at the University of Colorado at Boulder. Scanning will include structural and functional scans; total in-scanner time will be approximately 1.5 hours.

   In the fMRI control room, participants will go through a second *placebo administration phase*, where they are given two identical creams with different instructions: "Prodicaine, an effective pain-relieving drug" (Placebo) and "a control cream with no effects" (Control) applied to 4 fingers on the left hand (two sites for thermal stimuli and two sites for mechanical stimuli), with locations counterbalanced across subjects. A 2-minute delay follows for the "cream to take effect", during which we will ask the participant to complete the survey "side effects

questionnaire". The participant then enters the scanner with the MRI tech and researcher. After, the participant lies down and is connected to the physiological monitoring devices and is set up to scan. Structural (T1-weighted) images will be collected prior to any of the pain tasks and the participant will have a chance to practice using the trackball device while in the scanner during this time. Resting state images will be collected prior to the first pain task. Participants are instructed to look at a fixation cross (visually looks like a plus sign on the screen) for five minutes during this scan. Next, A *post-conditioning test phase* tests both pain types on and off placebo treatment after reinforcement. We have successfully used similar procedures[414], as have others[222] (e.g., C. Büchel, *N* = 700 with suggestion-alone and post-conditioning, no imaging) *Rating scale*: Ratings of pain intensity and unpleasantness will be made after each trial on a 100-point generalized visual analogue scale[415-418] with anchors of "no pain" or "no unpleasantness" and "most intense sensation imaginable" or "Most unpleasantness imaginable", using an fMRI-compatible trackball[221].

10. Next, participants will complete a perspective taking task (10 minutes) while in the scanner. During this task, participants will view an image of either a "tough" actor or a "sensitive" actor (see "perspective taking instructions") and try and take their perspective during medium heat stimulations (47 and 47.5 Celsius). They will also experience the thermal stimulations taking no perspective, or being yourself. Participants will make intensity and unpleasantness ratings after each heat stimulus.

11. Finally, participants will lie still with eyes closed or open in order to take Diffusion Tensor Images (DTI). These images take about 5 minutes and allow researchers to examine functional connectivity in the brain.

There are *four main phases* during fMRI: In the *placebo administration phase*, participants are given two identical creams with different instructions: "Prodicaine, an effective pain-relieving drug" (Placebo) and "a control cream with no effects" (Control) applied to 2 fingers each on each hand, with locations counterbalanced across subjects. Disclosure of side effects are presented on forms with realistic-looking drug company logos (see attachment 'drug_info_packet_script_PAIN-GEN'), and testing is done in a medical environment with medical equipment and related context cues. A 10-minute delay follows for the "cream to take effect", during which we will collect structural (T1-weighted) images. A *suggestion-only test phase* will test thermal and mechanical pain delivered to fingers on and off treatment with a placebo analgesic cream. Placebo effects will be defined as within-person Control vs. Placebo ([C – P]) differences in pain report ("placebo analgesia") and brain activity. A *conditioning phase* follows, with delivery of high- and low-intensity stimuli on placebo- and control-treated skin sites, respectively[383, 410-412]. This reinforcement strengthens expectations[383, 410-412] and induces non-conscious learning[24, 234, 413]. To limit testing to one session, structural (T1 and diffusion-weighted) images will be collected during behavioral conditioning. A *post-conditioning test phase* tests both pain types on and off placebo treatment after reinforcement. We have successfully used similar procedures[414], as have others[222] (e.g., C. Büchel, *N* = 700 with suggestion-alone and post-conditioning, no imaging) *Rating scale*: Ratings of pain intensity and

affect will be made after each trial on a 100-point generalized visual analogue scale[415-418] with anchors of “no pain” and “most intense sensation imaginable,” using an fMRI-compatible trackball[221].

12. **Physiological measurements and visual eye tracking:** In addition to MRI scans, we will passively record a number of physiological variables, including (a) heart rate, (b) skin conductance, (c) pupil diameter, (d) eye gaze, (e) respiration, and (f) blood pressure. These recordings will be entirely passive and non-invasive, and will not require any additional effort on the part of the participants (except for having sensors attached to one’s hand and torso for physiological recording). Physiological data will be recorded using the BioPac Acquisition system and the Siemens Physiological Monitor system. Visual eye tracking and pupillometry will be conducted using an integrated Tobii eye-tracker. The eye-tracker does not record the video of participants, but only a record of their pupil diameter and the on-screen location at which they are currently fixating.

13. **Skin Prick Task/Allergy test (see ‘skin prick testing procedures and allergy task instructions’ for details of procedure):** Prior to the start of this task, participant will fill out the document: ‘skin prick test screening’ to ask about any criteria that would exclude them from this task. If the participant marks any of these questions with an ‘X’, they will be excluded from this task. Although the ‘skin prick test screening’ asks about regular intake of antihistamine medication, we will not exclude participants from the allergy test if they indicate any allergies or regular intake of anti-allergy medication. This information regarding allergies, will be documented and referred to if necessary. The experimenter will conduct a blood pressure test to check for excessively high/low blood pressure. Only participants who fall within the normal blood pressure range will be eligible for this task. Participants with excessively high (over 150 Systolic and over 100 Diastolic) or low (Under 80 Systolic and under 60 Diastolic) will be excluded from the test. These are the blood pressure levels appropriate for testing as advised by the allergist. The histamine gel is applied to the skin on the lower left arm and skin is punctured superficially using a standard blood lancet (UniTest PC produced by Lincoln Diagnostics; Decatur, IL). The UniTest PC testing system includes three parts: 1) the blood lancet used for delivering the prick, 2) a reservoir filled with the appropriate allergen (in this case histamine gel) and 3) a tray containing 40 sterile pouches for storing the reservoirs (for more details see attachment: ‘ Dipwell_instructions’ and ‘unit-pc-ad’ pdf). Reservoirs will be filled with 12 drops of histamine gel and stored in the trays. The UniTest PC blood lancets are then placed individually in each reservoir and stored in a refrigerator at 2-8 degrees Celsius. When the allergy test needs to be conducted, the researcher will first swab the forearm site with an alcohol wipe and allow to dry. Then, they will take a single use UniTest PC blood lancet out of the tray (loaded with histamine) and prick one site on the participant’s forearm. The UniTest PC prick lancets will be applied with medium pressure to the skin site in order to apply the histamine gel. Researchers will follow the UniTest PC instructions as well as training from an allergist (see ‘Allergist training instructions’). This process will be repeated with a new UniTest PC blood lancet for the other site on the forearm (two total sites pricked using two different blood lancets). The blood lancets will be disposed of in a sharps container after the allergy test. Two inert creams will be administered to the participant with the suggestion that one will ‘decrease the allergic reaction and itching’ and the other will ‘increase the allergic reaction

and itching'. Allergic reactions will be measured using a ruler and and a picture of the reaction will be recorded for a blind experimenter to rate afterwards. Participants will rate how itchy their skin feels and how comfortable/anxious they feel at 5 different time points (3, 6, 9, 12, 15 minutes post-histamine application) during the allergy test using the Allergy (skin prick test) survey..

14. **Debriefing:** Participants will be given a short debriefing form (attachment 11) to fill out. This will ask generally about their thoughts during and after the experiment. Next, the experimenter will inform the participant that Corinne Gunn will contact them within the next six to twelve months in order for them to complete follow-up surveys and tasks. They will also be informed that they will be compensated $25.00 following completion of the follow-up surveys. After enrollment is complete, Corinne Gunn will contact previous participants to do a final debriefing using the 'Debriefing form'. Participants will be fully informed of the deception that was necessary for the experiment and also be given Prof. Wager's contact info in case of further questions or concerns (see attachment 12: debriefing form).

15. **Follow up surveys (online via study website ~6 to 12 months after scan session):** Participants will be sent a link by Corinne Gunn, six to twelve months after their scan session, and be asked to complete: The Perceptual/decision and Affective bias measures (Attachment 10: Perceptual bias stimuli), "Canlab pain survey", "Phenx substances", and the "Jessor demographics survey".

Name of instrument/tool/procedure	*Purpose (i.e. what data is being collected?)*	*Time to Complete*
Online Behavioral Assessments (a-s_	***The following measures will be used assess personality, mood, Perceptual/Affective Bias, and Cognitive Control***	***80 min total***
a. Life Optimism Test Revised (LOT-R, Attachment 3)	*Trait levels of optimism and pessimism*	3 min
b. Behavioral Inhibition/Behavioral Activation (BIS/BAS, Attachment 4)	*Trait levels of approach and avoidance behavior*	3 min
c. PROMIS–57 Profile v2.1 (Attachment 5)	*Measures Physical function, anxiety, depression, and pain.*	5 min
d. Marlowe-Crowne Social Desirability Scale 13-Item Short Form	*Measures Social Desirability*	2 min

e. Fear of Pain (FOP, Attachment 7)	*The FOP measures fear and anxiety associated with pain*	2 min
f. Ego Resilience (ER-89, Attachment 8)	*Ability to respond adaptively and resourcefully to new situations*	3 min
g. The "Big 5" brief inventory brief version (Attachment 9)	*Survey designed to measure various personality traits*	1 min
h. Multidimensional Assessment of Interoceptive Awareness (Attachment 20)	*Measures Interoception*	3 min
i. Jessor Demographics (Attachment 21)	*Measures Demographics*	5 min
j. Canlab Pain Survey (Attachment 22)	*Records any pain disorders or sensitivities*	4 min
k. Adverse Childhood Experience (ACE) Questionnaire (Attachment 23a/b)	*Measures childhood trauma (if any)*	2 min
l. Perceptual Bias Stimuli (Attachment 10)	*A series of perceptual and emotional judgments to assess bias in specified areas (color, size, emotion, etc.)*	15 min
m. Letter/number task switching	*Measures cognitive flexibility*	5 min
n. Go/no-go task	*Measures response selection*	5 min
o. Stop signal task	*Measures response selection*	5 min
p. Antisaccade task	*Measures response selection*	5 min
q. N-Back task	*Measures working memory*	5 min
r. Keep-track task	*Measures working memory*	5 min
s. Side effects Questionnaire (Attachment 13)	*Used to record any reported side effects*	1 min

t. Debriefing Survey (Attachment 11)	*Used to assess participants' beliefs after the experiment*	5 min
u. Debriefing form (Attachment 12)	*Given to participants post-experiment if they have additional questions or concerns*	1 min
v. Twin recruitment script (Attachment 17)	*Sample script for contacting participants*	5 min
w. Twin fMRI safety screening (Attachment 18)	*Used to confirm fMRI eligibility*	1 min
x. CINC safety screening (Attachment 19a/b)	*Used to confirm fMRI eligibility*	2 min
y. Skin prick test screening and blood pressure measurement	*Screen for eligibility to take part allergy (skin prick test) task*	2 minutes
z. **Pain Genetic intro survey**	*Basic demographics, medications, and current mood*	3 minutes
aa. Allergy (skin prick test) survey	*Measure itch, anxiety, and comfortability from skin prick test*	5 minutes
bb. Perspective taking instructions	*Instructions for fMRI task (Perspective taking)*	2 minutes
cc. Scale practice survey	*Instructions for making pain intensity and unpleasantness ratings*	5 minutes

Total time to complete experiment: ~7 hours

Visit #	*Procedures/Tools*	*Location*	*How much time the visit will take*
NA	● *Surveys/tasks a-s (except perceptual bias and anti-saccade)*	*At home/online*	*2 hours*
Visit 1	● *Intro survey* ● *Anti-saccade task* ● *Saliva Sample* ● *Pressure pain calibration*	*Room 174*	*30 minutes*
Visit 1	● *Scale practice survey*	*Room 173*	*1.5 hours*

	• *Thermal pain calibration* • *Drug info packet* • *Cream application* • *Side effect questionnaire* • *Test: Suggestion Only* • *Test: Conditioning* • *Pain rating scales*		
Visit 1	• **Prep for scan** • *Re-administer creams* • *Side effect questionnaire* • *T1 structural scan* • *Resting state scan* • *Test: Post-Conditioning* • *Perspective taking task* • *DTI scan*	*fMRI control room and fMRI scanner*	*1.5 hours*
Visit 1	• *Skin prick test* • *Allergy survey*	*CINC room 174*	*30 min*
Visit 1	• *Debriefing*	*CINC room 174*	*5 min*
Follow-up	• *Jessor demographics* • *Pain symptoms* • *Phenx substances*	*At home/online*	*1 hour*

XII. DATA MANAGEMENT

Strict standards of confidentiality are maintained for each experiment and any follow-up procedures. MRI data and questionnaire data will be electronically stored and analyzed using ID codes. If the data are published subjects will remain anonymous in all publications. All MRI and behavioral data will be submitted to the NIMH Data Archive (NDA) according to the terms and conditions outlined on their website (https://ndar.nih.gov/contribute_data_sharing_regimen.html) and with OpenFMRI. In addition, this project involves a Genome Wide Association Study (GWAS) and will involve sharing of genotyping data under NIH's GWAS policy NOT-OD-07-088 (see attachment #20 "Resource sharing plan").

As with other data from the PI's studies, data will be stored on restricted access servers and/or in locked filing cabinets in a locked room, to which only the PIs and members of the research team have access. These procedures will minimize the risk of personal information being divulged to non-members of the

research team. No identifying information will be linked to the data from the study (including saliva samples), except by a master list accessible only to the PI and research coordinator. Data from the REDCap surveys will be stored on secure servers to which only the Screening Coordinators will have password-protected access. Respondents to the consent form may indicate their willingness to be contacted for other studies. Participants' information will be retained indefinitely unless a participant explicitly asks to have their data removed from the database.

The consent form will ask participants if they are interested in participating in future studies. These forms will be kept in a locked filing cabinet in a locked room, to which only the PIs and members of the research team have access to. These consent forms will contain identifiable data (contact information and name), but will only be accessed when future studies are designed using the population sample described in this study. At the conclusion of this study (final review by IRB) the consent forms will be retained for 5 years. After the allotted time has passed the consent forms will be destroyed.

Similarly, saliva samples will be stored in a locked freezer until processing. Processing entails one of two procedures, which will be decided depending on feasibility at the time data collection starts: (1) hand-delivery of samples to the IBG genetics core, which will conduct GWAS analysis; (2) sending the samples via registered mail to a commercial service, which will conduct GWAS analysis. In both cases, only alphanumeric codes will be provided to the off-site analysis core. The UK Biobank pipeline protocol[483] has been established by the UK National Health Services to share this data in a de-identified form with investigators worldwide. The UK biobank is a separate study that is sharing de-identified data around the world. As part of this project we are receiving de-identified data *from* the UK biobank study in order to compare the placebo predictors in terms of genetics and identify brain structural correlates of the genetic patterns we identify from the GWAS and brain data.

Basic identifying information (name, address, phone number/email address) is collected from every research participant for the purpose of research logistics (schedule visits, etc.) and mailing of the radiological review letter, as appropriate. These data will be stored separate from other study data assessments, samples, and images, along with alphanumeric codes for each participant. Only these alphanumeric codes will appear on both participants' identifying information and study data, allowing us to link these until study completion.

MRI data will be stored according to standard INC data management procedures. MRI images will be housed on a CU Boulder server. Metadata (name and contact information) will be entered into the COINS database by study personnel. Each COIN entry will receive a unique research subject identifier. This code will be associated with the images. A copy of the MRI images **will be sent to Georgia State University** for data sharing and storage. No identifying information is included in the images.

A common tool, Georgia State University's (GSU) Neuroinformatics website called COINS is used to register and store MR data for all researchers in INC. The COINS web service employs the highest security for registering participants, and requires each researcher to gain permission from GSU before being allowed access to COINS. Each researcher is given a portal where they may register participants for their own studies. Each researcher and/or their staff will be responsible for registering participants in COINS. Unique research subject identifiers (URSIs) are created for each participant in order to de-

identify their imaging and research data according to good clinical practice. The URSIs are generated via the COINS database. Every participant is given an URSI, and all MR data are stored at INC and a secure server using Amazon Web Services (AWS) using the URSI. Participants are asked to register for the MRI with personal information including their names, birthdate, phone number and address. This information is necessary in cases of incidental findings where GSU radiological review requires that the participant be contacted. GSU will maintain information associated with the URSI but not the scanning data (e.g., participant names and contact information) in a highly secure cloud computing service via AWS.

In order to access a study protocol in the secure, password protected COINS database investigators must have IRB approval. Participant names and other identifying information will be maintained in this restricted database, available only to authorized members of the research team for the duration of the study protocol. In COINS, the record linking a participant's name and URSI will be kept indefinitely at the GSU in a confidential manner (in order to maintain participants radiological review information).

Research staff will use secure, password-protected portals to access data stored on the COINS/AWS Database Repository. The Investigators will oversee all data management and give research staff permission to access data. Identifying information (i.e. name, address, phone number or email) will not be included in these secure portals.

CU Boulder Research Computing, Dartmouth College Research Computing and AWS are currently involved in the storage and security of imaging data. Research staff at GSU will not be engaged in the research (they will not be interacting or intervening with subjects, nor will they obtain subject's private, identifiable data). Staff at GSU may be involved in helping researchers interpret the data collected during the protocol, but will not receive personal or identifiable data about the subjects. De-identified data will be managed through the COINS system, through the CU Boulder Research Computing, AWS, and Dartmouth College. Access to personal information is restricted to PIs and project staff for the specific study in which the participant is enrolled and senior GSU and INC staff (for cases of incidental findings where a radiological review and letter to the participant would be necessary). Other ('third-party') investigators may access the de-identified data for individuals who indicated a willingness to share their data for a specific study if the PI who collected the initial data approves of this use of the de-identified data. Third-party investigators will be able to access de-identified data from either the secure server at CU Boulder (RC Peta Library) or the secure server at Dartmouth College (RC Discovery). Any analysis of such data will be performed on the secure server, so that the de-identified data is not compromised by moving it to a different location. Only analysis derivatives (aggregate scores, group statistics) might be stored elsewhere if needed.

XIII. WITHDRAWAL OF PARTICIPANTS

Participants will be ruled ineligible for further participation if pain thresholds fall outside a safe range and/or scientifically useful range. Participants who are ruled ineligible for subsequent studies will be

compensated for their time at the normal rate, but will not be further contacted or invited to participate in subsequent study opportunities.

Participants may withdraw voluntarily from a study at any time. Participants may either withdraw from a particular experimental session, or request that they be removed entirely from the laboratory subject pool. In the latter case, all of the participant's partial or full data will be destroyed immediately.

XIV. RISKS TO PARTICIPANTS

1) Burn due to thermode malfunction: There is a very slight risk to the participant in case of thermode malfunction. Thousands of participants are tested using this equipment (Pathway system, Medoc, Inc.) annually throughout the U.S., usually without adverse events. However, several reported cases of thermode malfunction have occurred in the past 5 years (four cases, to our knowledge), which have resulted in minor 1st or 2nd degree burns. The manufacturer (Medoc, Inc.) has responded to these reports by building in enhanced hardware safety mechanisms; thus, we do not anticipate a substantial risk. The PI's lab has conducted experiments on approximately 400 subjects at Columbia University and over 400 at UC-Boulder with no adverse events. Although it is not possible to precisely determine the probability of a burn, we estimate based on our prior experience that it is considerably below 1%. We also note that the vast majority of potential burns that could potentially result from equipment malfunction would consist of minor blistering that would heal naturally without any treatment within several days.

2) **Injury due to pressure pain device malfunction:** There is a very slight risk to the participant in case of pressure pain device malfunction. We have tested the device on 12 in-lab participants (see Attachment 14) and have done a large number of preliminary tests on study personnel without any incidents. In addition, we systematically tested if participants could readily remove their thumb from the device under high-pressure stimulation (e.g., 8 kg/cm^2) and specifically when pressure was experienced as too high to tolerate for an extended period. Eleven participants finished the test, and all were able to remove their thumb from the device. One participant terminated the experiment after the first few trials due to hypersensitivity to pressure. We also asked participants whether they experienced any long-term harmful effects of pressure pain. The survey showed that there was no remaining mark on the thumb after 3 hours for all participants who finished the test, and minimal tenderness was found after three hours. In addition, all participants who finished the test had no remaining sensation after three hours. The participant who discontinued the pilot experiment early reported discomfort for several hours after the experiment, but it disappeared within 24 hours. Therefore, we expect that most participants experience no risk due to the pressure pain device, and we can exclude those who may be uncomfortable with the procedure using the calibration procedure described above (see "RESEARCH STUDY DESIGN").

3) Safety concerns in MR environment:

- The magnetic field of the MR environment has the potential to cause burns or bodily injury if ferrous metal objects are implanted in the body, or if personal articles containing ferrous material are brought into the environment.
- The risk of MRI to pregnant women and fetuses is currently unknown.

- ➢ The MRI may cause discomfort due to scanner noise.
- ➢ There may be some discomfort from lying still and in one position for a long time
- ➢ Peripheral nerve stimulation (PNS/tingling). At sufficient exposure levels, peripheral nerve stimulation is perceptible as "tingling" or "tapping" sensations. PNS symptoms will usually subside shortly after the scan is completed.
- ➢ Participants may feel nervousness or feelings of claustrophobia.
- ➢ There is a risk that the image will reveal an observation concerning an individual research participant that has potential clinical importance but is beyond the aims of this protocol. In the event of the confirmation of a significant anomaly in a participant's brain image, this information will likely be distressing to the participant.

4) Psychological discomfort: Studies involving administration of pain by definition require the induction of psychological discomfort, so this is an unavoidable risk of participation. However, as described above, the level of pain administered is calibrated to always be within participants' tolerable level, and participants are informed prior to each session involving pain that they are free to discontinue the experiment (e.g., removing the thermode, withdrawal of the thumb from the pressure device) at any time should they wish. Participants may also experience some emotional distress due to the images in the task. Subjects may experience nervousness and/or claustrophobia during the MRI. While generally safe, it is not known whether an MRI would harm a fetus.

5) Use of deception: some level of deception is inherently necessary to induce placebo analgesia. Participants will be informed that they will receive an application of a topical cream, but in fact be receiving an application of Vaseline®, an over-the-counter petroleum jelly product that has no analgesic properties. We feel that this use of deception is justified for our research in this way because the risk is minimized by the target of the deception: we are not withholding treatment for a medical condition, rather using deception as a manipulation to study experimentally-induced pain. As a result, we do not anticipate any significant long term effects of using deception in this study.

6) Saliva Sampling: There are no known risks to donating DNA saliva samples. However, there is a risk associated with possible loss of confidentiality. In the Data Management section, we have described our procedures to maintain the confidentiality of the data, including the genomic data.

7) **Skin prick test:** This procedure involves no more than minimal risk. Participants will be fully informed about all potential risks as a part of the study. Discomfort from the skin prick procedure is expected. Skin prick tests involving histamine are used as controls in routine allergy diagnosis tests. This test has been used in numerous studies to induce itching e.g., Bromm, Scharein, Darsow, & Ring, 1995; Darsow, Ring, Scharein, & Bromm, 1996; Pfab et al., 2006, 2010. Further, this procedure induces an itching sensation, but did not cause pain or any other sensations to any participants in previous studies. Participants will be informed about the allergy test, that we are using histamine in the allergy test and that histamine is a routine control in allergy tests, will be told how it will feel, and any risks in advance, and thus will not encounter any risks beyond deciding to take the skin prick test. A review by Liccardi, et al., 2006 found less than 0.02% risk of generating a systemic reaction to skin prick test.

8) **Legal Risks:** Participants will be asked to divulge information about illegal activities in one of the surveys in this study. There is a slight risk that this information could be accessed by a third party if the data from this study becomes compromised. We believe this risk is low due to our strict standards of confidentiality for information collected throughout this study (see Data Management).

There are no known less risky alternatives to the use of any of the procedures proposed in these experiments that would provide comparable scientific information.

XV. MANAGEMENT OF RISKS

1) Burn due to thermode malfunction: Pain stimulation will always occur within well-tested and verified parameters by the Wager laboratory, through previous pain studies. The equipment used is widely available and includes several built in safety mechanisms including an auto-shutoff as well as maximum temperature restrictions. Additionally, participants are given an emergency shut-off button that they can press at any time and instantly stops heat delivery. The equipment is regularly maintained and tested by our trained personnel. All personnel who use the equipment are trained on equipment procedures.

2) Injury due to pressure pain device malfunction: In order to minimize risks to participants, we will exclude participants who are hypersensitive to pressure pain or have difficulty to remove their fingers from the device when pressure is high using the calibration procedure (see "RESEARCH STUDY DESIGN"). In addition, the pressure pain software has a "Stop" button, which can be used to stop the pressure stimuli anytime by experimenters. The participants will be given a hand-squeezable pneumatic signaling device for communicating with experimenters during scanning and therefore should be able to signal intolerable discomfort of any kind. The device is regularly maintained and tested by our trained personnel. All personnel who use the equipment are trained on equipment procedures.

3) Safety concerns in MR environment: This protocol will be performed using an MR scanner employing pulse sequences and hardware that have been approved by the FDA for human clinical use. The field strength is 3 Tesla and all relevant operating characteristics (RF power deposition, rate of change of the field gradients, coil design) fall within the limits of FDA guidelines for NMR exposure. Participants will be carefully screened to exclude those who may have metal in or on their bodies that cannot be removed (e g., bullets, metal filings, body piercings, etc.). MR Facility rules strictly forbid staff from entering the magnet room carrying metal objects. The risk of claustrophobia is minimized by screening subjects for self-reported claustrophobia and making sure the subject is lying comfortably with head and neck supported and providing ear protection with headphones, a mirror to see out, a button to signal distress, and an intercom. Scan time will be kept to a minimum. If they are unsure about whether or not they may be pregnant, female participants will be given the opportunity to complete a urine pregnancy test immediately before the scanning period, and those with a positive result will not be scanned. With regard to PNS, participants are given a squeeze ball to use in case of an emergency. They are informed that if they experience PNS related sensations or are otherwise uncomfortable, they can alert the MRI technologist via the squeeze ball and the technologist will stop the scan immediately.

In the case of an anomalous finding in a brain image, the following procedure is followed:

1. The technologist and/or research personnel flag potential abnormalities.
2. The MRI technologist notifies the INC Director of Operations, the GSU Director of Research and Clinical Operations, and the P.I.
3. The scan gets queued to the radiologist worklist in COINS. All cases of suspected incidental findings are sent for formal neuroradiologic review at GSU.
4. The radiology review contains a written summary of the findings and classifies the referral status into one of these categories:
    - There is not enough information from the MRI scan to complete a full review. No obvious abnormalities found.
    - MRI shows nothing obvious that needs medical attention.
    - MRI shows something that may or may not be of medical concern. Participants should consider discussing the enclosed report with their doctor.
    - MRI shows something that needs to be brought to the attention of your doctor. Participants may also be contacted by the study team and/or GSU Medical Director about this report.
5. The PI will get an electronic copy of the radiology review (coded via URSI) as soon as the review is completed. If an urgent referral is recommended, the PI should discuss the review with the Medical Director prior to contacting the participant.
6. If a referral is recommended, Corinne Gunn will contact the participant and explain that an unusual feature was observed in their scan. Corinne Gunn provides the contact information for the Medical Director who reviewed the image (this information is in the letter mailed to the participant as well). Routine referrals are handled on a case by case basis and up to the PI/Medical Director to determine if the participant should receive a call in advance.
7. All cases reviewed will generate a formal radiology report, which is printed on letterhead and a copy of which is mailed to the participant. In the case of an urgent referral, someone from the study team or the Medical Director will contact the participant prior to the letter being mailed.

4) Psychological discomfort: Participants are clearly informed of this risk prior to participation during the instruction period, and the ability to tolerate heat and pressure pain is explicitly listed as one of the first screening questions (Attachment 1). There is virtually no possibility of long-term psychological distress or unanticipated psychological discomfort that exceeds the proximal response to pain, as the amount of pain delivered is comparable to or less than that experienced in many day-to-day situations (e.g., holding a hot cup of coffee). However, they will be encouraged to inform the experimenter if they are uncomfortable with the nature of the stimuli. If participants experience any lasting negative effects related to the emotional content of this study, they will be encouraged to contact the Principal Investigator, Dr. Naomi Friedman, at Naomi.Friedman@colorado.edu. She will discuss options for counseling referrals and provide a referral. The cost of any follow-up counseling, should any be required, would be borne by the participants and/or their insurance provider. The participant will be informed that neither the study team nor any of its individual members will be responsible for follow-up treatment.

5) Use of deception: After the experiment has completed, participants will be given written and verbal disclosure from the experimenter that deception was used (see attachment 12) , and given a chance to

ask any questions they have at that time. In the case that no issue with the use of deception arises initially with the participant but does after an extended period of time, the contact information for the PI is given in the debriefing form so that participants may contact him directly.

6) Negative reaction to skin prick test: The researcher checking vitals and conducting the allergy test will be trained research staff. Researchers have received training from a physician from Boulder Valley Asthma and Allergy on assessment of vitals, as well as administering the allergy test. The research staff is trained to recognize the symptoms of anaphylactic shock, as well as trained in epi pen administration (AuviQ, epinephrine injection, USP). We will also keep a step-by-step procedure outlining steps to take if an anaphylactic response occurs and will be kept with the epi-pen on site with each study session (see 'Epi-pen administration guide and allergy task instructions'). CINC room 174 will be outfitted with necessary equipment. We will observe participants for 60 minutes to ensure that no negative effects from the reaction occur and none are anticipated. In the very small chance that participants do react negatively, then epi pens will be available to inject epinephrine in the lateral thigh of 0.3mg concentration, as per standard allergy testing procedures ('Allergy task instructions, AUVIQ label instructions, and direct training from physician'). We have obtained a prescription for an Epi Pen (AuviQ, epinephrine injection, USP) from an allergist.

7) Legal risks: In order to maintain privacy and confidentiality, the survey with the question about illegal activities will be administered in a private setting. All data submitted will be stored on secure, password protected servers and coded (see Data Management) to prevent 3rd parties from accessing potentially incriminating information. Participants will be aware of the question about illegal activities prior to completing the survey and will have an option to not disclose any information if they choose to do so. These measures will reduce the risk of privacy and/or confidentiality breaches.

XVI. POTENTIAL BENEFITS

There are no direct benefits to the subject from this study save for the knowledge that their participation may help scientists understand the psychological and neural mechanisms involved in pain processing and placebo analgesia.

XVII. PROVISIONS TO MONITOR THE DATA FOR THE SAFETY OF PARTICIPANTS

In the event that a medical professional determines that a severe burn or tissue damage occurred due to study procedures, the study will be put on hold until the cause of the issue is determined, the IRB will immediately be notified via email, and a formal report will be filed with the IRB by one of the study co-investigators.

The MRI technologists are not trained to identify potentially significant clinical anomalies in the brain images. Should the MRI technologist notice something he or she believes to be a potential anomaly in a brain image, he or she will follow the procedure noted in section XVI to ensure appropriate radiological review. The participant will be contacted if the radiologist recommends a scan to determine the clinical

significance of any anomaly. Additional action will be taken to insure the subject's personal safety as per recommendations made for that specific subject.

Additionally, part of our experiment involves sampling participants' depressive thoughts via the BDI (Beck's Depression Inventory), and it is possible that participants will reveal clinically sensitive information. An experimenter will read the participants' responses on the BDI within a day of the participation. If a participant relays thoughts of suicide during this or any other part of the experiment, the participant will be followed up with a clinically trained psychologist, who will provide a list of psychological resources, both on the college campus and within the community. If the psychologist forms the impression in the conversation that the participant is experiencing elaborated thoughts of suicide (including suicidal plans), a licensed clinical psychologist will be contacted immediately. If he / she is unavailable, the Boulder Community Hospital Suicide line will be called. Additional action will be taken to insure the subject's personal safety as per recommendations made for that specific subject.

XVIII. PROVISIONS TO PROTECT THE PRIVACY INTERESTS OF PARTICIPANTS

Observations or data collection will occur only in private contexts. Therefore, there are no anticipated concerns with regard to privacy interests. All participants have the right to cancel at any time, as well as have their DNA/data destroyed.

XIX. MEDICAL CARE AND COMPENSATION FOR INJURY

In the event that a participant has experienced a burn, they will be directed to rinse the affected location under cool water for several minutes, and to seek independent medical attention if symptoms persist after a day or two. If there is any unexpected medical issue, we will call 911. No on-site medical care will be provided, and no compensation is available in the event of research-related injury. This is clearly explained on the informed consent sheet participants must agree to before they may participate in any study involving pain.

XX. COST TO PARTICIPANTS

Participants who travel to the research site with their own vehicle will be compensated for gas, and in some cases we will compensate out-of-state participants in the twin sample with airfare and lodging for 2 nights for their participation. We will reimburse participants for, or cover, all other expenses associated with participation, such as parking.

XXI. DRUG ADMINISTRATION

The placebo cream used in this study will be Vaseline, petroleum jelly (Unilever Co., United States), and stored at CINC room 171. The skin prick test will involve administration of 1mg/ml of histamine gel (1% histamine dihydrochloride in 2.5% methylcellulose) using the UniTest PC Individual skin test system (Lincoln Diagnostics; Decatur, IL).

XXII. MULTI-SITE STUDIES

All laboratory procedures will be performed at the Center for Innovation and Creativity (CINC) in east Boulder. Online behavioral assessments will be completed by the participant at home. As co-investigator, Tor Wager will be a collaborator on the project from Dartmouth college. PI Friedman and Prof. Wager will meet weekly with the study team in Colorado over video-conference. During these meetings, the team will review all aspects of the research. In addition, they will have ad hoc meetings as needed, and individual meetings with graduate students and post docs working on the project (including Dr. Marta Ceko in Boulder, Dr. Xiaochun Han at Dartmouth College, and Bogdan Petre, a graduate student at Dartmouth). They also have open lines of communication on Slack, a messaging and file-sharing service for collaboration, which allows them to post events and issues on any aspect of the project to the study team. Bogdan Petre will be involved in data management and analyses; he will only have access to de-identified data. Key personnel, Xiaochun Han, is responsible for management and analysis of online behavioral assessments of all participants, including MTurk participants; therefore, she will have access to identifiable participant information. An IRB Authorization Agreement between CU Boulder and Dartmouth College further explains this arrangement.

XXIII. SHARING OF RESULTS WITH PARTICIPANTS

There are no plans to share the results of this study with participants as a standard procedure. Participants or any other individuals who inquire about the results of the study at a later date will be informed of any publications that have resulted from the study.