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7. **Raghuraman, N.**, Bedford, T., Tran, N., Haycock, N. R., Wang, Y., & Colloca, L. (2024). The Interplay Between Health Disparities and Acceptability of Virtual Reality: A Mixed-Method Study. *Cyberpsychology, Behavior, and Social Networking*, under review.

Book Chapters

1. Colloca L, **Raghuraman N**: Placebo Hypoalgesic Effects and Genomics, *Genomics of Pain and Co-Morbid Symptoms*, Springer, June 2020.

ABSTRACT

Title of Dissertation: The Relationship Between Socioeconomic Position and Endogenous Pain Modulation: A Quasi-Experimental Approach.

Nandini Raghuraman, Doctor of Philosophy, 2024

Dissertation Directed By: Luana Colloca, MD, PhD, MS

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Background: Differences in pain perception among racial and ethnic groups are well-documented and often attributed to underlying physiological mechanisms [1, 2]. However, emerging research highlights the importance of considering variations in pain experiences across different socioeconomic strata [3], alongside factors such as age, sex, and psychological traits, [4-6] which can influence the efficacy of placebo analgesia in diverse populations. Understanding these nuances is crucial for advancing personalized pain management strategies tailored to individuals from various backgrounds. Despite extensive studies on the impact of socioeconomic factors on chronic pain [7-9], there is a notable gap in research regarding their effects on experimental pain and placebo hypoalgesia. This quasi-experimental study aims to address this gap by investigating how individuals from diverse socioeconomic backgrounds respond to placebo hypoalgesia. By exploring the relationship between socioeconomic distress and placebo effects on pain perception, this study seeks to contribute valuable insights into the interplay of social determinants and pain modulation mechanisms. The significance of this research lies in

its potential to inform more effective and equitable pain management approaches that account for the diverse experiences of pain and placebo responses among different socioeconomic groups. By elucidating these relationships, this study may pave the way for targeted interventions that improve pain outcomes across various demographic categories.

Objective: My primary goal is to assess the influence of socioeconomic position (SEP) on endogenous pain modulation (EPM). Specifically, I aim to investigate, first the potential relationship between SEP and placebo hypoalgesia in individuals with chronic temporomandibular disorders (TMD) and those without pain. Second the association between SEP and specific genetic variations related to EPM among participants with TMD and pain-free individuals (AIM 2); and lastly the impact of SEP on the consistency of placebo hypoalgesia responsiveness in participants with TMD and pain-free subjects (AIM 3). My central hypothesis posits that SEP influences EPM levels, potentially explaining variations in placebo responses. These findings could enhance the effectiveness of novel therapeutic approaches and aid in identifying patients who are particularly susceptible to pain, guiding clinicians toward personalized and mechanism-based treatments.

Methods: A quasi-experimental approach was employed to assess the effect of socioeconomic distress on placebo hypoalgesia. We recruited 401 participants with TMD (306 females) and 400 pain-free participants (238 females) to complete a placebo experiment using the classical conditioning paradigm and verbal suggestion.

Participants first completed a quantitative sensory test to calibrate the temperatures for high- and low-pain stimuli for the conditioning phase. These temperatures were paired with a red and green cue, respectively, and participants were told that an analgesic trans-electrical intervention would have been turned on during the green cue to reduce pain. Pain intensities were set at the same level for both cues during the test phase.

The participants then rated their pain intensity levels for each stimulation on a visual analog scale (VAS) ranging from 0=no pain at all to 100=maximum imaginable pain. Operationally, we defined placebo effects as the difference between VAS scores collected for red- and green-paired cues during the test phase. Saliva samples were collected using Oragene-DNA (OGR-500) kits and were genotyped using the Illumina Human OmniExpressome array. Latent Class analysis was conducted to group participants into distinct SEP using individual markers namely income, education, occupation, and neighborhood marker namely Area Deprivation index (ADI) and Distressed community index (DCI). Linear mixed model was performed to compare the differences in placebo hypoalgesia among the SEP groups. TMD vs pain-free participants and the SEP groups were treated as two fixed factors. The interaction between these fixed factors was also modeled. Age, sex, race, and temperature used during the test phase were controlled as covariates. When a significant interaction was found, pairwise comparison applying the Bonferroni correction was performed.

Results: Both TMD and pain-free participants had similar placebo hypoalgesia levels ($F(1,4757.64) = 1.03, p = 0.30$). There was a significant main effect ($F(1,4757.64) = 7.62, p = 0.006$) and interaction of SEP, where among participants with TMD, those who were

SEP distressed had lower placebo hypoalgesia (15.03 ± 1.74) compared to SEP prosperous (19.79 ± 1.02 , $F(1,4757.64) = 14.67$, $p < 0.001$). Among participants with TMD with *OPRM1 rs1799971* G-carrier, those who were SEP distressed had lower placebo hypoalgesia compared to SEP prosperous ($F(1,4360.14) = 58.01$, $p < 0.001$). Similar results were found among those with *OPRM1 rs1799971* AA ($F(1,4360.14) = 4.36$, $p = 0.03$), *COMT rs4680* met/met ($F(1,4350.61) = 10.33$, $p = 0.001$), met/val ($F(1,4350.61) = 4.01$, $p = 0.04$), val/val ($F(1,4350.61) = 13.41$, $p < 0.001$) and *FAAH rs324420* pro/pro ($F(1,4359.12) = 20.59$, $p < 0.001$). On the contrary placebo hypoalgesia in pain-free participants did not differ among SEP distressed (20.47 ± 1.10) and SEP prosperous (20.05 ± 1.30 , $F(1,4757.64) = 0.03$, $p = 0.84$). Among participants with TMD ($F(1,150.9) = 2.24$, $p = 0.13$) and pain free participants ($F(1,130.9) = 0.83$, $p = 0.36$) there was no significant main effect of SEP on placebo reproducibility and the intraclass correlation coefficient for the test-retest reliability was $ICC = 0.21$, $p = 0.096$ and $ICC = 0.041$, $p = 0.39$ for participants with TMD and pain-free participants respectively.

Conclusions: Overall, these findings hold significant implications for the development of personalized and mechanism-based therapeutic approaches in pain management. By identifying vulnerable patient populations and elucidating the factors influencing placebo responses, this research contributes to the advancement of more effective and equitable pain management strategies. Further research in this area is warranted to validate and expand upon these findings, ultimately leading to improved pain outcomes across various demographic categories.

The Relationship Between Socioeconomic Position and Endogenous Pain Modulation: A
Quasi-Experimental Approach.

by
Nandini Raghuraman

A dissertation submitted to the Faculty of the Graduate School of the
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of the requirements for the degree of
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DEDICATION

To my beloved grandmothers Pattamal Kuppuswamy and Ganga Subramaniam, my uncle Krishnaswamy Subramaniam, and to my dearest parents Raghuraman Kuppuswamy and Vijayalakshmi Raghuraman.

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LIST OF ABBREVIATIONS

TMD	Temporomandibular Disorder
SEP	Socioeconomic Position
EPM	Endogenous Pain Modulation
CPM	Conditioned Pain Modulation
HWE	Hardy- Weinberg Linkage Equilibrium
<i>OPRM1</i>	μ -opioid receptor
<i>COMT</i>	Catechol-O-methyltransferase
<i>FAAH</i>	Fatty acid amide hydrolase
AIMs	Ancestry Informative Markers

DCI Distressed Community Index

ADI Area Deprivation Index

VAS Visual Analogue Scale

GCPS Graded Chronic Pain Scale

LMM Linear Mixed Model

LCA Latent Class Analysis

AIC Akaike Information Criteria

BIC Bayesian Information Criteria

aBIC Sample Size Adjusted Bayesian Information Criteria

ICC Intraclass Correlation Coefficient

CHAPTER 1: INTRODUCTION

Chronic pain is one of the most common reasons adults seek medical care [10], restricting their daily activities and increasing anxiety, depression, and dependence on opioids [11, 12]. Population-based estimates of chronic pain among adults in the U.S. in 2016 ranged from 11% to 40% [13] and chronic orofacial pain represents a burden in the U.S. and worldwide, affecting 5-12% of the population [14]. Pharmacological strategies for pain management have primarily focused on opioid receptor-mediated therapies [14], with a need to discover strategies that involve activating patients' endogenous pain modulation (EPM) systems. EPM refers to the mechanisms of pain reduction and outcome improvement related to the release of endogenous opioids [15], dopamine [16], oxytocin [17], vasopressin [18], and cannabinoids [19]. Placebo hypoalgesia is the result of top down EPM systems. Placebo hypoalgesia can be elicited in a laboratory setting using conditioning and verbally induced expectations. It can relieve [20-23] and can be used as a "powerful tool" to explore the contribution of EPM systems to the experience of pain. Using the opioid antagonist naloxone, previous studies have demonstrated that placebo hypoalgesia relies partially on releasing endogenous opioids [24-26]. These findings were further confirmed and extended by μ -opioid receptor (*OPRM1 rs1799971*) gene-association studies [27]. Other genetic studies have also determined that single nucleotide polymorphisms (SNPs) in catechol-O-methyltransferase (*COMT rs4680*) and fatty acid amide hydrolase (*FAAH rs324420*) genes are associated with EPM [28, 29]. Besides genetic variations, the sociodemographic perspective is highly relevant in accounting for placebo effects on chronic pain. One factor implicated in EPM and chronic pain disparities is socioeconomic position (SEP). Although SEP-related factors

have long been observed in epidemiological studies of pain [7-9], no studies have investigated how SEP groups might experience EPM. There is a mutual relationship between race and SEP, and race differences in placebo hypoalgesia have only recently been explored, where we found that white individuals reported greater placebo effects than their black counterparts [30]. All these studies aimed at characterizing pain and placebo differences often rely on self-reported race, and there is evidence suggesting that self-reported race can change over time. Scientists have coined term as ‘racial fluidity,’ and this phenomenon is most pronounced among minority and multiracial populations [31, 32]. In contrast, some studies have suggested utilizing ancestry informative markers scores (AIMs) as they can provide a biologically based measurable estimate of individual origin and potentially function as a surrogate for self-reported race [33, 34]. Currently, there are no studies exploring the association of genetic variants of EPM with ancestry markers and there is an emphasis on bringing together these biological aspects and the sociodemographic perspective to account for the variability in EPM responsiveness assessed in well-established experimental settings.

1.1 Specific Aims

Based on the background above, my overall objective is to determine the effect of SEP on EPM. Specifically, I aim to examine 1) how SEP could be associated with distinct magnitude of placebo hypoalgesia in participants with chronic temporomandibular disorders (TMD) as well as pain-free participants (AIM 1); 2) how SEP is associated with identified genetic variants related to EPM across TMD and pain-free participants (AIM 2), and finally, 3) whether SEP impacts the reproducibility of placebo hypoalgesia responsiveness in participants with TMD and pain-free participants (AIM 3). My central

hypothesis is that SEP is associated with distinct EPM magnitudes and potentially accounts for the variability in placebo phenotypes. These findings could inform the success of new therapeutic strategies. Furthermore, these findings will allow the identification of vulnerable patients and guide clinicians toward a personalized, mechanistic-based therapeutic approach.

Aim 1: To determine the relationship between SEP and EPM in participants with TMD and pain-free participants. I hypothesize that among TMD and pain-free participants, those who are socioeconomically distressed would elicit lower magnitude of EPM. The approach would be to quantify placebo hypoalgesia as an indicator of EPM through the classical conditioning paradigm [20] in TMD and pain-free participants. The primary outcome will be placebo hypoalgesia captured as a continuous variable. Secondary outcomes will be the quantitative pain sensory test, psychological components, pain severity, and interference in participants with TMD. First, a latent class analysis will be performed to classify participants into subgroups with distinct socioeconomic position. Self-reported individual-level markers (i.e., education level, income, occupation) and structural-level markers of SEP (i.e., distressed community index scores and area deprivation index scores) will be used as the indicator variables. The sequence of cousin models, starting with a two-class model, and then adding one class at a time [35] will be performed to determine the number of latent classes required. These models will be evaluated using the Bayesian information criterion [36] since it is a popular indicator of parsimonious model fit. Once the appropriate number of latent classes of SEP has been determined, linear mixed model (LMM) will be used to examine the effect of socioeconomic position on placebo hypoalgesia with the latent class of SEP

and group (TMD vs. pain-free participants) set as two fixed factors and the magnitude of the placebo hypoalgesia set as the outcome variable. Since SEP is also correlated with other demographic factors, such as sex, age, and race, these factors will be considered as a priori identified confounders and will be adjusted for accordingly in the model. Specifically, ancestry-informative marker scores (AIMs) will be used to confirm the self-reported race.

Aim 2: To determine the relationship between SEP and genetic variants related to

EPM. Mechanistic research is needed to corroborate the influence of SEPs [37]. I hypothesize that 1) the identified variants in *OPRM1* rs1799971, *COMT* rs4680, and *FAAH* rs324420 genes are associated with the magnitudes of placebo hypoalgesia among TMD and pain-free participants, and 2) SEP will interact with these SNP variations shaping placebo hypoalgesia phenotypes among TMD and pain-free participants. The independent variables will be the latent-class SEP and SNP variations, and the outcome will be placebo hypoalgesia, like in AIM1. The latent class SEP obtained for aim one will be used as the interaction variable to assess if the candidate genes were associated with the varied magnitude of EPM. LMM will be built with SEP, SNPs, and group (TMD vs. pain-free participants) as three fixed factors, and the magnitude of placebo hypoalgesia as the outcome. Self-reported race, along with other demographic factors such as sex and age and will be treated as a priori identified confounders similar to AIM1.

Aim 3: To assess the reproducibility of placebo hypoalgesia as a proxy for EPM.

Placebo hypoalgesia can potentially improve patient outcomes, and placebo effects have been shown to be reproducible [38, 39]. However, these studies were small in terms of sample size, making it difficult to draw definitive conclusions. Moreover, the

reproducibility of placebo hypoalgesia across SEPs remains unknown. Therefore, this non-hypothesis-driven exploratory study aimed to examine the internal reproducibility of behavioral responses to placebo hypoalgesia. I will utilize a subsample of well-phenotyped 82 participants with TMD and 70 pain-free participants from our parent sample of 401 participants with TMD and 400 pain-free participants and apply the intra-class correlation approach to assess the reproducibility of placebo hypoalgesia. I will further examine the reproducibility of placebo hypoalgesia across SEPs in TMD and pain-free participants. Moreover, I will use the permutation test [4] to study how the proportion of placebo responders varied across participants with TMD and pain-free participants.

1.2 Significance and Innovation

The research holds immense significance in its potential to revolutionize personalized, mechanism-based therapeutic approaches, thereby substantially improving the quality of life for individuals dealing with chronic pain. At its core, this study aims to investigate the impact of social disparities on placebo effects, offering profound insights into the intricate web of individual differences in pain perception and endogenous pain modulation (EPM) as manifested through placebo effects. Social disparities encapsulate variations in socioeconomic status, educational attainment, ethnic backgrounds, and access to healthcare resources, all of which can significantly influence an individual's health outcomes. By delving into these disparities, this research not only enhances our understanding of the mechanisms underpinning the placebo effect but also lays the foundation for the development of more targeted and effective pain management strategies that consider these social determinants.

Moreover, the findings from this study are poised to have critical clinical implications, potentially driving reforms in healthcare policies and clinical practices. Identifying specific social disparities that exacerbate pain symptoms or hinder the effectiveness of placebo effects could catalyze initiatives aimed at reducing these disparities, thereby fostering an environment conducive to improved healthcare outcomes overall. For instance, addressing educational inequalities might involve implementing community-based health education programs to empower individuals with knowledge about pain management, while mitigating the effects of socioeconomic disparities may necessitate structural changes in healthcare financing and access to ensure equitable care for all.

The exploration undertaken in Aim 1, which focuses on unraveling the influence of socioeconomic status (SEP) on EPM in participants with chronic temporomandibular disorders (TMD) and pain-free individuals, represents a crucial step toward understanding the disparities in pain experience and management. Through an in-depth analysis of how individual and structural SEP markers impact placebo hypoalgesia (as a proxy for EPM), this aim aims to provide invaluable insights into the role of socioeconomic factors in shaping pain perception. By clustering participants based on SEP, the study seeks to identify subpopulations most vulnerable to reduced EPM and those who could potentially benefit most from interventions aimed at enhancing endogenous pain modulation. This innovative approach addresses a significant gap in the existing literature, deepening our understanding of how socioeconomic factors contribute to variations in pain modulation and response to treatment.

Moving on to Aim 2, the investigation into the relationship between SEP and genetic variants associated with EPM will further enhance our understanding of the interplay between socioeconomic and genetic factors in pain modulation. By examining whether SEP interacts with variations in genes such as OPRM1 rs1799971, COMT rs4680, and FAAH rs324420 to influence placebo hypoalgesia, this aim seeks to uncover crucial genetic markers and their interactions with social conditions. Identifying these genetic factors and their interplay with socioeconomic factors can provide critical insights into the mechanisms of EPM and placebo hypoalgesia, ultimately guiding the development of personalized therapeutic strategies that account for both genetic predispositions and social contexts. This innovative approach expands our comprehension of individual variations in pain responses, paving the way for tailored interventions that address the complex interplay between genetic and social determinants of pain perception and management.

In Aim 3, the study delves into the reproducibility of placebo hypoalgesia, a fundamental aspect that sheds light on the consistency of this response across various socioeconomic backgrounds. By exploring how reliably placebo hypoalgesia can be elicited and assessing its practicality as an indicator of EPM and therapeutic technique, this investigation aims to deepen our understanding of pain perception and management within diverse demographic groups. The innovative aspect of this aim lies in its focus on examining the reproducibility of placebo hypoalgesia, a critical factor in pain research that has implications for its clinical relevance and utility as a therapeutic intervention. By

evaluating the consistency of behavioral reactions to placebo hypoalgesia, the study contributes valuable insights into the reliability and stability of this phenomenon, enhancing its potential as a tool in pain management and research.

In summary, this research endeavor has the potential to significantly advance our understanding of how socioeconomic status and genetic factors intersect to influence endogenous pain modulation. By shedding light on these complex interactions, this study could pave the way for the development of more effective, tailored, and equitable approaches to pain management, thus addressing the overarching goal of reducing disparities in pain treatment. In the context of the growing emphasis on personalized medicine and the imperative to mitigate health inequities, this study holds particular significance and relevance, positioning it as a crucial contribution to the evolving landscape of healthcare research and practice.

CHAPTER 2: BACKGROUND AND LITERATURE REVIEW

2.1 Chronic Pain and Alternative Pain Management Strategies

Chronic pain [10] interferes with daily activities and often escalates to drug abuse and dependence of opioids [11, 12] in an attempt to mitigate pain. Prevalence of chronic pain among adults in the U.S. ranged from 11% to 40% (2016 report) [13] and chronic orofacial pain in the form of Temporomandibular Disorder (TMD), represents an emerging pain disorder which affects 5-12% of the population [14]. TMD involves derangement of the jaw joint and/or muscles of mastication disproportionately affecting women (female-to-male ratio of 9:1[14, 40]), and it is a difficult pain disorder to treat [41-50].

Pain management has primarily focused on opioid receptor-mediated therapies [14]. One promising strategy to reduce chronic pain patients' needs for pharmacological therapeutics is to include non-pharmacological adjuvants, low costs interventions in the arsenal of pain management therapeutics [51, 52]. Often called mind-body therapies, these treatments are meant to target interactions among the brain, mind, body, and behaviour and aim to use the mind to affect physical functioning and promote health [53]. A recent systematic review indicated that mind-body interventions improved clinical chronic pain (moderate Cohen's $d = -0.43$) and resulted in small reductions of opioid doses[54].

2.2 Endogenous Pain Modulation and Placebo Hypoalgesia

One such non-pharmacological intervention would be the endogenous pain modulation (EPM). EPM has been well-documented in the pain literature [55-59] and can account for inter-individual variability in pain experience and chronicity [29, 60].

One form of EPM that depends on the activation of descending neural pathways that inhibit nociceptive signalling is placebo hypoalgesia [25, 61]. Placebo hypoalgesia is the result of top down EPM systems. Placebo hypoalgesia can be elicited in a laboratory setting using conditioning and verbally induced expectations of pain relief [20-23] and can be used as a powerful tool to explore the contribution of EPM systems to the experience of pain. Consequently, placebo hypoalgesia has emerged as a potent model for assessing EPM systems in humans [25, 26, 62], and we used laboratory assessments of placebo hypoalgesia as a proxy for EPM. Placebo analgesia depends on the release of substances such as endorphins/opioids [15], dopamine [16], oxytocin [17], vasopressin [18] and endocannabinoids [19] in descending pain modulation systems. Placebo effects are powerful and commonly encountered in clinical practice form of mind-body interventions. Through an understanding placebo mechanisms, strategies promoting placebo effects can optimize therapeutic outcomes in clinical practice [63].

2.3 Genetics of Placebo Hypoalgesia

Recent studies suggest that genetics plays an important role in pain sensitivity [64, 65] and in pain modulation, for example placebo effects (see reviews, [44, 66]). Not all individuals respond to placebo-induced hypoalgesia. Given that pain responses vary across individuals [64, 65] and are more than 50% heritable according to twin studies [67-69], it is plausible that specific genetic variations may contribute to EPM. In a recent review of the literature shows three single nucleotide polymorphisms (SNPs) that have been associated with placebo hypoalgesic effects such as those observed in well-controlled laboratory studies (e.g., natural history) performed in healthy participants and patients [66]. The opioid system plays a relevant role in driving placebo effects as

demonstrated by pharmacological and imaging studies [46, 47, 70-73]. Pecina and colleagues first examined the *OPRM1* A118G variant (Asn40Asp, rs1799971) which is thought to be expressed at low levels [74]. The rs4680 polymorphism in *COMT*, which encodes a valine to methionine amino acid substitution at codon 158 (Val158Met), reduces the enzymatic activity of the protein product of this gene by 3-4 fold [75]. This SNP has been associated with better outcomes in patients with Irritable Bowel Syndrome [76] and placebo analgesia in healthy subjects [77]. Specifically, met/met carriers for the *COMT* gene has been shown to have a significant positive association with clinical pain reduction in IBS patients [76] and placebo hypoalgesia in healthy subjects [77].

Interestingly, a recent study addressed the interaction between *OPRM1* rs1799971 and *COMT* rs4680 on placebo hypoalgesia and found that participants having the *COMT* met/met or val/met – *OPRM1* A/A carrier combination compared to those with the val/val – G combination, reported level of placebo induced pain reduction that was 4-6 times higher [78]. The cannabinoid system plays a role in placebo hypoalgesia [79, 80] and the functional variant in the fatty acid amide hydrolase (*FAAH*) rs324420 polymorphism encodes a Pro129Thr missense substitution that leads to distinct endocannabinoid levels [81], affects placebo hypoalgesia [19]. Dr. Colloca and team performed a replication of previous findings [19, 76, 78, 82, 83] and conducted a SNP-to-SNP by placebo procedure interactions study to determine the potential influence of combined genetic variants and psychological approach on hypoalgesic placebo effects. Colloca et al. demonstrated that the *OPRM1* rs1799971 by *COMT* rs4680 by *FAAH* rs324420 and placebo procedure interactions were predictive of distinct profiles of placebo responsiveness [28]. These SNP-based studies are in line with some pioneering

studies. Using the opioid antagonist naloxone, previous studies have in fact, demonstrated that placebo hypoalgesia relies partially on releasing endogenous opioids [24-26]. Brain imaging studies have also demonstrated that placebo hypoalgesia is linked to the release of endogenous opioids [84, 85].

2.4 Racial differences and Placebo Hypoalgesia

Studies have clearly demonstrated differences in the experience of clinical and experimental pain between racial groups [1, 86-90], and these differences often stem from various genetic [91, 92], cognitive-emotional [21], and sociocultural factors [93]. For example, Asians were found to more likely exhibit *OPRM1* A118G gene polymorphisms, causing a reduced response to opioids in G-alleles carriers when compared with A-alleles carriers, both endogenous and externally administered [92]. Another example of ethnic differences exists with Native Americans; this group have been shown to exhibit dampened pain responses and heightened pain tolerance when compared to Non-Hispanic Whites [94]. On the other hand, African Americans, Asians, and Hispanics have been found to show higher pain sensitivity, lower pain tolerance, higher pain ratings than Non-Hispanic Whites [86, 88, 89, 95, 96]. Studies with the goal of illuminating the racial differences of experienced pain often rely on measured outcomes include pain sensitivity, pain relief, and conditioned pain modulation (CPM). CPM refers to the mechanism by which the perceived pain of a noxious test stimulus is reduced through another noxious stimulus [97]. In general, African Americans and other minority groups experience greater degrees of pain sensitivity, as well as less efficient descending pain inhibition, evaluated through CPM, when compared to Whites [96, 98-102]. These differences in pain experiences may at least in part manifest in modified

EPM systems. Physiological mechanisms have been proposed to contribute to racial/ethnic differences [2, 103]. Dr. Colloca and team were the first to demonstrate that not only race influences pain threshold, pain tolerance, and chronic pain experiences, but also, they found participant's race did in fact provide measurable effects on placebo hypoalgesia, expectation ratings and conditioning strength. White participants reported greater conditioning strength effects, pain relief expectations, and placebo hypoalgesia when compared to their African American counterparts [30]. It is important to note that all these studies aimed at characterizing pain and placebo differences often rely on self-reported race, and there is evidence suggesting that self-reported race can change over time and bias the assumption that these variables remain fixed throughout life [104]. Scientists have coined the term 'racial fluidity' and this phenomenon is most pronounced among minority and multiracial populations [31, 32]. In contrast, some studies have suggested utilizing ancestry informative markers scores (AIMs) as they can provide a biologically based measurable estimate an individual's origin and potentially function as a surrogate for self-reported race [33, 34].

2.5 Summary

Despite the body of evidence suggesting notable differences in pain experiences between racial groups [86, 88, 89, 95, 96], previous research has not adequately taken into account the crucial interplay of EPM and chronic pain disparities in socioeconomic position (SEP). SEP is considered a multidimensional construct based on educational level, occupation, annual household income (e.g., individual levels), and access to education and employment across geographical areas or population groups (e.g., structural levels) [3, 105-107]. Although SEP-related factors have long been observed in epidemiological

studies of pain [7-9], no studies have investigated how SEP groups might experience EPM.

My research fills a critical gap in the larger body of pain and placebo research in understanding the role of disparities in the formation of placebo effects.

CHAPTER 3: RESEARCH DESIGN AND METHODOLOGY

3.1 Data Source and Study Design

Data for this study came from a grant from the National Institute of Dental and Craniofacial Research (1R01DE02594) titled "Chronic orofacial pain: genetics, cognitive-emotional variables, and endogenous modulator" systems." Luana Colloca, MD, PhD, MS is the principal investigator. The purpose of this grant was to identify the contribution of genetic variations to the behavioral, psychological, and neurological mechanisms of EPM in participants with TMD, with a particular emphasis on the deeper phenotyping of placebo response. Using a classical conditioning paradigm [108], a non-equivalent control quasi-experimental study was carried out to induce placebo hypoalgesia in participants with TMD and pain-free subjects. Between August 2016 and January 2021, 801 volunteers were recruited for the study, of which there were 401 participants with TMD and 400 pain-free participants from the DC-Maryland-Virginia region. All the participants were recruited by convenience sampling.

3.2 Study Participants

3.2.1 Recruitment

Distribution of flyers and brochures around the university campus and the greater Baltimore area, "word of mouth," social media postings, online advertisements, health and wellness fairs, written correspondence to dental clinicians, and letters to former Brotman Facial Pain Clinic University of Maryland Baltimore patients were among the recruitment techniques strategically used to enroll 401 participants with TMD and 400 pain-free participants. Before inviting potential participants for a more comprehensive in-

person screening, trained research personnel conducted a preliminary phone screening to determine their eligibility.

3.2.2 Eligibility Criteria

All participants aged 18 to 65 were screened over the phone with the DC/TMD screening instrument [109] to determine their potential eligibility as pain-free participants or participants with TMD. Personal screenings were conducted to guarantee eligibility. Every participant understood and spoke English (spoken and written). Degenerative neuromuscular diseases; cervical pain (i.e., stenosis or radiculopathy); a cardiovascular, neurological, kidney, or liver disease diagnosis; pulmonary abnormalities; cancer within the past three years; color-blindness; or uncorrected hearing impairment disqualified participants. In addition to these restrictions, pregnant or nursing women were not allowed to participate. Participants were disqualified if they had a history of alcohol or drug dependence in their lifetime or a history of alcohol or drug abuse in the previous year. In addition, individuals with a severe psychiatric disorder requiring medication or hospitalization within the past three years were excluded. If a subject had facial injuries within the past six weeks or a history of severe facial trauma within the past two to three months, they were excluded from the study.

3.2.3 Pain-free participants

400 volunteers were enrolled as pain-free subjects (162 men, 238 women). Participants without pain who had mania, schizophrenia, or other serious psychological illnesses were also excluded (i.e., anxiety disorder, depression, obsessive compulsive disorder, or attention deficit hyperactivity disorder). The final determination of eligibility was done in

person at the University of Maryland School of Nursing by a clinical member of the research team.

3.2.4 Participants with TMD

401 volunteers were found to be eligible and recruited as participants with TMD (95 men, 306 women). They were screened in person by a dental clinician at the Brotman Facial Pain Clinic of the University of Maryland School of Dentistry to confirm a self-reported history of at least three months of jaw, head, or facial pain and to determine the phenotypic classification and eligibility of their orofacial pain condition.

Myalgia and/or arthralgia in one or both temporomandibular joints contribute to TMD-related discomfort. Included in the study were participants who met the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) [110, 111]: a. Pain in the jaw, temple, in the ear, or in front of the ear; and b. Pain modified by jaw movement, function, or parafunction; and c. History of pain in the jaw, temple, ear, or in front of the ear within the previous 30 days; and d. a. Confirmation and duplication of pain location(s) in the temporalis, masseter, or other masticatory muscle(s); and/or b. Confirmation and duplication of pain in one or more temporomandibular joints. In contrast to pain-free participants, only a personal history of mania, schizophrenia, or other serious psychoses would disqualify a TMD participant due to the prevalence of concomitant emotional suffering in patients with chronic pain, such as anxiety and depression.

3.3 Experimental Procedures

After a full description of the experimental procedures, compensation, rights, risks, advantages, and disadvantages associated with their involvement, all eligible individuals

granted their signed consent to participate in this study. Due to the use of misleading information during the experimental method, participants were debriefed at the conclusion of the experiment using a study exit form (Appendix A) that described the nature and extent of deception. Participants who received a debriefing were given the option to withdraw their data from the study. Each participant was rewarded \$100 (through check or gift cards) upon completion of the complete research, with no withdrawals. The study was authorized by the Institutional Review Board of the University of Maryland Baltimore (IRB Protocol # HP-00068315) and conducted in line with the Declaration of Helsinki. The experiment took place at the University of Maryland School of Nursing clinical research space for a session that lasted approximately 4 hours. Vital signs i.e., heart rate, height, and weight were collected for monitoring purposes prior to testing.

3.3.1 Pain Sensitivity Assessment and Calibration

Individual pain measures of warmth, heat-pain threshold, medium pain, and maximum pain tolerance were taken to examine pain sensitivity and personalize the temperature settings utilized during the conditioning and testing phase (see below). This investigation was conducted using the procedures of limits [56]. Along with the PATHWAY (Pain and Sensory Evaluation System) device from Medoc Advanced Medical Systems in Rimat, Yishai, Israel, a 3x3cm thermode was utilized. To eliminate the potential confounding influence of regional variation in sensitivity, thermal stimuli were applied at the same location on the arm each time. The thermode was wrapped securely in an elastic self-adhering wrap on the participant's dominant forearm to provide stimulations ranging from 32 °C to 52 °C. At the start of the pain sensitivity test, the individual was handed a

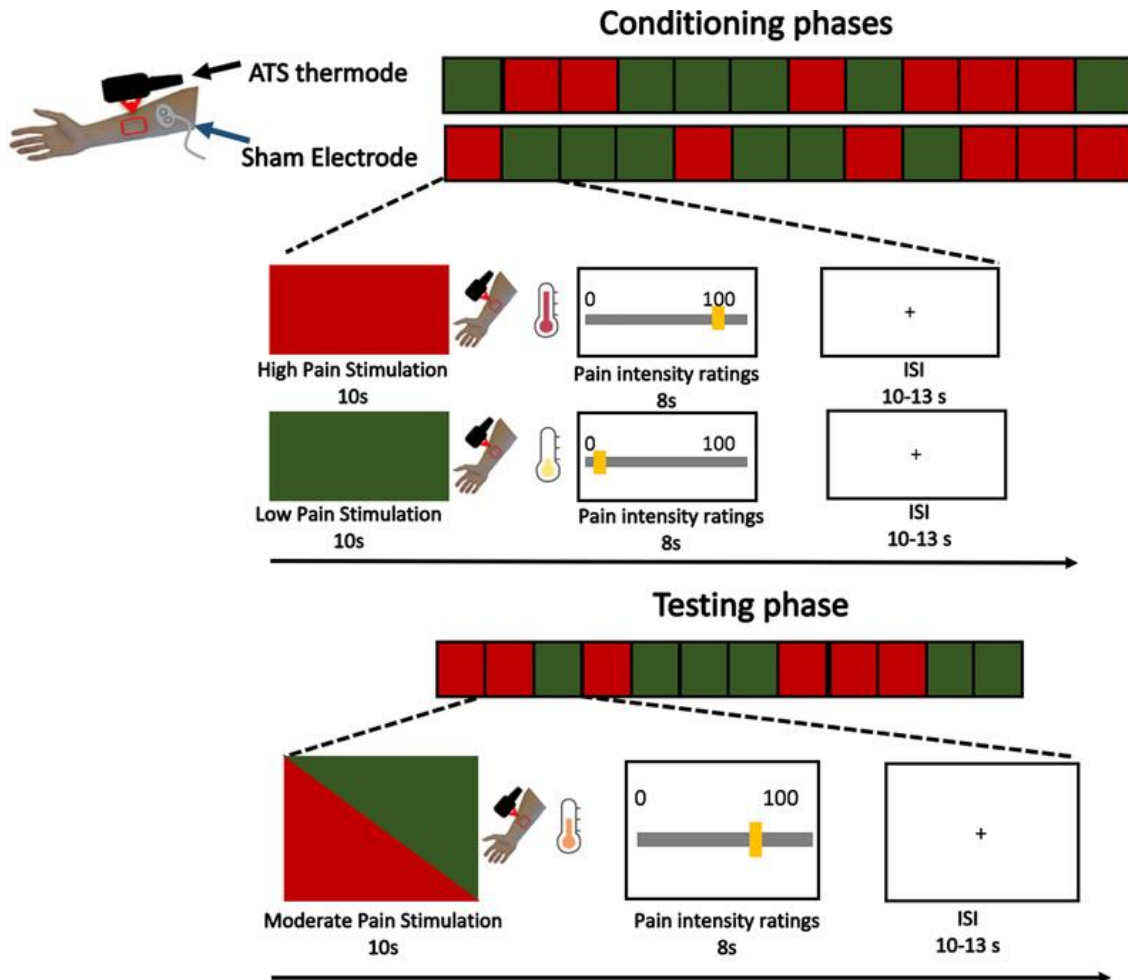
control (stop) button and instructed to press it when they first felt a warm, unpleasant thermal sensation. They were advised to press the stop button when they felt the least bit of heat pain. In addition, participants were asked to report their medium level of heat stimulation and their highest tolerable level of pain. The levels of warmth, heat-pain threshold, medium pain, and maximum acceptable pain were measured four times each. In addition, participants were asked to rate their pain on a scale ranging from 0 to 100, with 100 representing the most pain they could tolerate. The visual analogue scale (VAS) was employed for the pain sensitivity component to encourage interaction with the researcher and rapid cessation of stimulations when necessary. Using the average temperature of these four levels of heat intensities for the future conditioning and testing phases were then computed.

3.3.2 Conditioning Phase

We employed a well-established and validated model of conditioning and verbal suggestion for the placebo manipulation [108] (Fig. 1), in which two conditioning phases preceded the testing phase. During the two conditioning periods, each participant was informed that they would receive an examination of their endogenous pain modulation system. A sham electrode probe (the placebo intervention) was placed on the participant's arm above the thermode, and it was explained that it would administer subthreshold (imperceptible) electrical stimulation to alleviate the thermode-induced pain. Additionally, the subject was informed that a green screen would have been displayed while the electrode was active and a red screen when it was deactivated. The participants were informed that the temperature was the same for both the red and green screens, so that any change in pain intensity could be attributed to the intervention (the

placebo electrode). Based on the calibration phase, the temperatures were initially adjusted 2°C below the participant’s maximum tolerance for the red screens and 6 °C below the participant’s maximum tolerance for the green screens (conditioning phase). Subjective VAS evaluations were used to identify painful stimulations corresponding to about 20 (green) and 80 (red) on VAS.

Figure 1: Classical Conditioning Manipulation



3.3.3 Assessment of expectations

Participants rated their initial expectations of pain alleviation prior to the conditioning phase and their reinforced expectations of pain relief immediately following the conditioning phase. Using a script (ePrime, Inc.) and a hand-shape device (Celeritas, Inc.), which permits the movement of a square cursor on a 0–100 line in response to the query, "How much do you believe this procedure would reduce your pain?". VAS was utilized to assess baseline and reinforced expectations.

3.3.4 Testing Phase

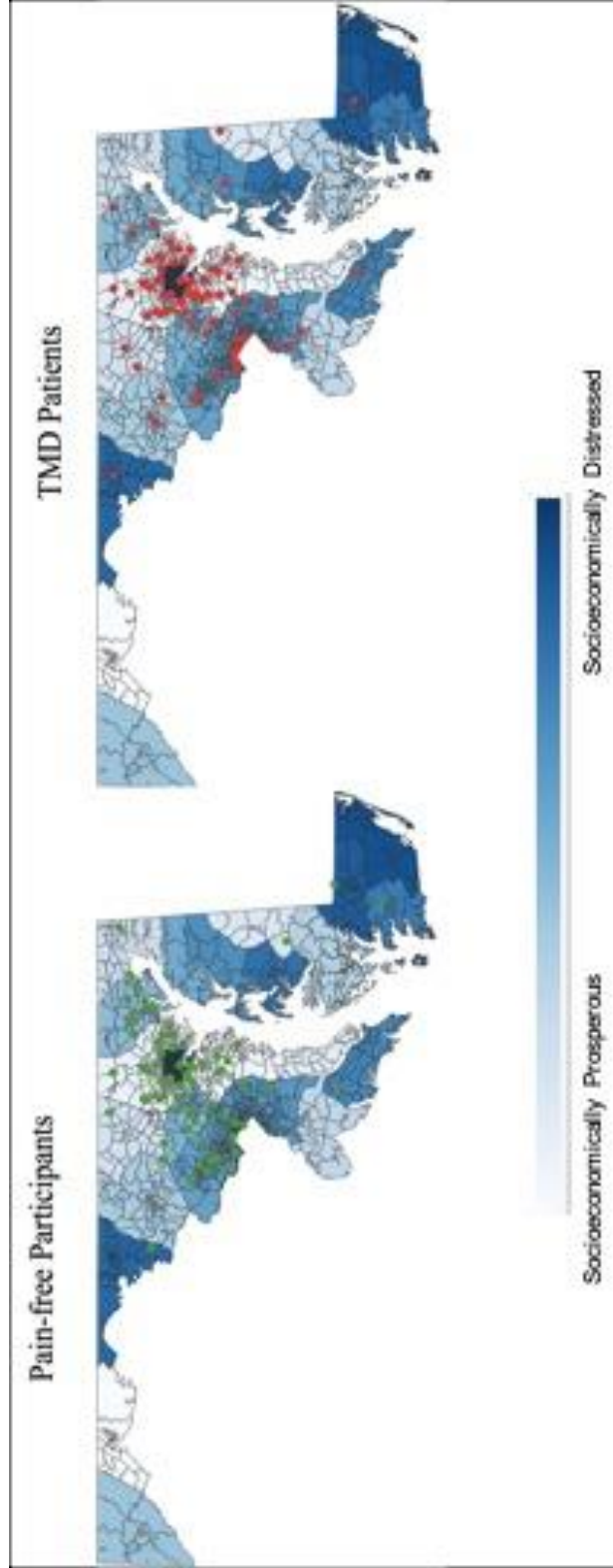
One block of alternating red and green screens constituted the testing phase (6 each according to the random sequence). Unbeknownst to the participant, both red- and green-paired intensities of stimulations were secretly set at the same level of heat thermal stimulations equating to 1°C below the temperature used for the red screens for each participant. Importantly, all participant procedures were standardized. To control for potential sequence effects, participants were assigned randomly to one of four produced screen color sequences.

3.3.5 Demographic assessments

Race, ethnicity, marital status, education, annual income, and occupation was determined by self-report via a demographic questionnaire. The participants also provided their residential address from which the *zip codes* were utilized to assign the appropriate Area Deprivation Index (ADI) score[112] and Distressed Community Index (DCI) score[113]. ADI is based on a metric developed by the Health Resources & Services Administration (HRSA) and modified by a University of Wisconsin-Madison research team for use at the census block group neighborhood level. It makes it possible to rank communities in an area of interest according to socioeconomic disadvantage (e.g., at the state or national level). It takes into account factors like employment, education, income, and the standard of housing. The national percentile rankings of the ADI range from 1 to 100 for the country. The lowest level of "disadvantage" in the country is represented by a block group with a ranking of 1, and the highest level of "disadvantage" is represented by block group with a ranking of 100. For every state, ADIs are also provided in deciles ranging from 1 to 10. The least disadvantaged number on the ADI scale is 1, while the most disadvantaged number on the ADI scale is 10. DCI, which was created by the economic innovation group, divides zip codes, counties, and congressional districts into five quintiles of well-being: prosperous, comfortable, mid-tier, at-risk, and distressed. It utilizes the U.S. Census Bureau's American Community Survey (ACS) 5-Year Estimates for each state. The index is calculated by considering variables like the rate of adults without a high school education, the number of vacant homes, the percentage of adults without jobs, the rate of poverty, the median income ratio, shifts in employment, and

changes in business locations. I mapped the geographic location in Maryland State for the TMD and Pain-free participants (Fig. 2).

Figure 2: State of Maryland Socioeconomic Position Census Tract Heat Map



3.3.6 Clinical Pain assessments

Clinical pain was also assessed with Axis II evaluation via the Graded Chronic Pain Scale (GCPS) [114], Jaw Function Limitation Scale [115], and the Oral Behaviors Checklist for parafunctional behaviors [116]. The differences in the severity and disability of participants with TMD [110], [114],[117],[95] assessed was used in the sub-group analyses as a secondary outcome.

3.3.7 Saliva Sample and DNA Genotyping

Using Oragene-DNA (OGR-500) kits, saliva samples were collected. On the consent form, participants were given the option of providing a saliva sample for DNA extraction. Only 735 participants were genotyped out of the 801 participants in the parent sample. Due to sample deterioration, genotyping errors, or participant refusal to provide a saliva sample, 66 participants (32 participants with TMD) had missing data. On the day of the experiment, participants were instructed to rinse their mouths with water for ten minutes before spitting saliva into the OGR-500 container, per the instructions supplied for saliva self-collection. The saliva self-collection process was non-invasive, and saliva samples were maintained at -20°C until DNA extraction at the Laboratory of Neurogenetics at the National Institute on Alcohol Abuse and Alcoholism. All the samples were genotyped on the Illumina Human OmniExpressome array. Using the GeneMapper program, genotypes were determined (version 4.0; Applied Biosystems).

3.3.8 Ancestry Informative Markers

Ancestry Informative Markers (AIMs) was extracted using the ADMIXTURE software[118]. For the ADMIXTURE analyses a range of values of the ancestral populations, denoted as K, was run to explore different population models and the performance of the model was evaluated using cross-validation. Varying number of ancestral populations, and the corresponding cross-validation error rates were used to determine the best-fitting model.

3.4 Methods for Aim 1:

3.4.1 Independent variable Assessment (Socioeconomic Position)

The goal of the LCA model was to discover classes of similar socioeconomic position among participants with TMD and Pain-free participants. Using Mplus, the self-reported education, annual income, occupation, DCI and ADI scores were used to determine the probable socioeconomic classes [119]. A Lo-Mendel-Rubén Likelihood Ratio Test (LMR-LRT), a measure of fit[120, 121], was utilized as part of the LCA model to determine the optimal group separation. Using entropy, Akaike information criteria (AIC), Bayesian information criteria (BIC), the distinction between classes were validated. Entropy[122] is a measure of group separation, with higher values indicating a greater degree of differentiation between defined classes. The BIC is selected as the model fit criterion, with a lower value indicating a better model fit [121]. Specifically, the optimal number of classes was determined by considering the following criteria: 1) The number of classes (n) was determined when the LMR-LRT is significant ($p \leq 0.05$) for the n-class model but not for the next level of classes (i.e., the n+1-class model)[120,

121]; 2) The entropy value was greater than 0.75[122]; 3) The classes model which had the smallest BIC value; and 4) Each identified class contained more than 15 participants.

3.4.2 Dependent Variable Assessment (Placebo Hypoalgesia)

From the classical conditioning paradigm, the difference in VAS ratings between red- and green-paired stimuli during the test phase was operationally described as "placebo hypoalgesia."

3.4.3 Statistical Methods

AIMs and Self-reported Race: The self-reported race was validated using AIMs, and the strong predictive power of self-reported race over AIMs scores was confirmed by Multivariate Pillai's Trace analysis. We included self-reported race as a covariate in the analysis to prevent multicollinearity because of the strong correlation between AIMs and self-reported race.

Latent class SEP and group differences in demographic, clinical and psychological factors: First, an independent t-test and χ^2 analyses was performed to examine how the latent class SEP is associated with the demographic characteristics such as age, sex, and self-reported race within participants with TMD and pain-free participants. Next, an univariate ANCOVA with group (TMD vs. Pain-free participants) and latent class SEP set as between-subjects factors was conducted to test the SEP and group differences in heat-pain threshold, maximum pain tolerance, and the psychological factors.

Demographic variables age, sex, and self-reported race was controlled for accordingly. Post-hoc analyses was conducted, and Bonferroni corrections was applied for multiple comparisons. A separate univariate ANCOVA was also conducted within participants

with TMD to explore the effect of SEP on the duration of pain, clinical pain intensity and pain interference assessed by GCPS, jaw function limitation, and oral behavior.

Latent class SEP and group differences in conditioning strengths: To determine the latent class SEP and group differences in conditioning strengths, Linear mixed model (LMM) analysis was conducted in red-green differences of VAS pain intensity ratings during the conditioning phase. SEP and group (TMD vs. Pain-free participants) was treated as fixed factors. Age, sex, self-reported race, and heat stimulus temperature administered for the conditioning phase were set as covariates. The LMM was chosen method of analysis because it provides an advantage in dealing with the unbalanced data structure[123]. Bonferroni corrections for multiple comparisons was applied.

Latent class SEP and group differences in expectations: LMM analysis was conducted for the participants' expectation ratings, treating group (TMD vs. Pain-free participants), SEP, and time (baseline vs. reinforced expectations) as fixed factors. Age, sex, and self-reported race were treated as covariates. Bonferroni corrections for multiple comparisons was applied.

Latent class SEP and group differences in placebo hypoalgesia: LMM analysis was conducted to compare the SEP and group differences in placebo hypoalgesia (red-green differences of pain ratings during the testing phase), with SEP, group (TMD vs. Pain-free participants) and trials set as fixed factors. Age, sex, self-reported race, and heat stimulus temperature used in testing phase was set as covariates. Regardless of the significance, all the interactions will remain in the model [124]. Planned pair-wise comparisons was conducted between the latent classes of SEP within each group and at each trial [124-126]). Bonferroni corrections for multiple comparisons was applied.

A linear relationship among SEP, conditioning strength, reinforced expectations, and placebo hypoalgesia, was assumed in order to conduct a mediation analysis [127, 128] to explore the potential driving force of the SEP differences in placebo analgesic effects. Therefore, in the mediation model, SEP was treated as the independent variable (X), while red-green difference of pain intensity ratings during the testing phase was set as the dependent variable (Y). Conditioning strength (the mean red-green difference of pain intensity ratings during the conditioning phase) and reinforced expectations was considered as the two mediators (M1, and M2). The indirect effects was tested with a bias-corrected bootstrapping method based on resampling of 5,000 times. If the 95% bootstrapped confidence interval (BCI) did not contain zero, the indirect effects was considered significant. A mediating effect was significant when the indirect effects and the total effects was significant.

3.4.5 Power Calculations

Power calculation was performed based on the 2 (Latent classes of SEP) by 2 (TMD vs. pain free participants) LMM statistical analyses plan. As far as we know, no study has been done to systematically assess the effect of SEP on placebo effects. Therefore, I followed our previous study that examined racial disparities in placebo effects to estimate the effect size [30]. I expected to observe a medium effect size for the SEP by Group interaction on the repeatedly measured placebo effects with Cohen's $f=0.25$. Power analysis indicated a minimal number of 184 participants for each cell would be sufficient to detect a medium size effect (Cohen's $f=0.25$) with 0.8 statistical power at the alpha level of 0.05. Noted that no validated power and sample size methods are available for mixed models study design. I used previous studies recommendations to apply power

analysis for repeated measure ANOVA using G*Power [129]. Further information regarding power calculations can be found in Appendix B.

3.5 Methods for Aim 2

3.5.1 Hardy–Weinberg equilibrium (HWE)

The three SNPs was tested for HWE using Haploview 4.2 [130] which controlled for type I error in relatively small sample size [131].

3.5.2 Independent Variable Assessment (Socioeconomic Position)

The latent class analysis performed to determine the Socioeconomic position in Aim 1 was used as the independent variable for Aim 2 as well.

3.5.3 Dependent Variable Assessment (Placebo Hypoalgesia)

Similar to AIM 1 placebo hypoalgesia was the dependent variable and was computed using the difference in VAS ratings between red- and green-paired stimuli during the test phase.

3.5.4 Statistical Methods

Latent class SEP, group, and *COMT* rs4680 genotype differences in placebo hypoalgesia: The *COMT* rs4680 was genotyped as met/met, val/met and val/val. LMM analysis was conducted with placebo hypoalgesia as dependant variable and *COMT* rs4680 genotype, latent class SEP, and group (TMD vs. Pain-free participants) were set as fixed factors, while age, sex, self-reported race, and heat stimulus temperature used in testing phase were considered as a covariates. Bonferroni corrections for multiple comparisons was applied.

Latent class SEP and group and *OPRM1* rs1799971 genotype differences in placebo hypoalgesia: *OPRM1* rs1799971 was genotyped as A/A and G carriers (G/A and G/G). LMM analysis was conducted with placebo hypoalgesia as dependant variable and *OPRM1* rs1799971 genotype, latent class SEP, and group (TMD vs. Pain-free participants) were set as fixed factors, while age, sex, self-reported race, and heat stimulus temperature used in testing phase were considered as a covariates. Bonferroni corrections for multiple comparisons was applied.

Latent class SEP and group and *FAAH* rs324420 genotype differences in placebo hypoalgesia: The *FAAH* rs324420 was genotyped as pro/pro and thr carriers (thr/pro and thr/thr). LMM analysis was conducted with placebo hypoalgesia as dependant variable and *FAAH* rs324420 genotype, latent class SEP, and group (TMD vs. Pain-free participants) were set as fixed factors, while age, sex, self-reported race, and heat stimulus temperature used in testing phase were considered as a covariates. Bonferroni corrections for multiple comparisons was applied.

3.5.5 Power Calculations

Similar to Aim1, the power analysis for repeated ANOVA was applied to calculate sample size for Aim 2 mixed model study design following previous studies recommendations [42; 28]. Power calculation was conducted separately for each of the genotypes including *OPRM1* rs1799971 (A.A. vs. G carriers), *COMT* rs 4680 (met/met vs. val carriers) and *FAAH* rs 324420 (pro/pro vs. thr carriers). Based on previous studies on gene by environment interactions on pain and placebo analgesia [28, 132], we expected to observe a small to medium effect size for the 2 (SEP levels) by 2 (group) by 2 (genotype) interaction. We conducted a power calculation with 6VAS individual

measurements, effect size set at $f=0.20$ and power (1- β probability) set at 0.8., A minimum of $n=47$ in each cell was needed to detect a significant effect. G*Power 3.0 software was used to conduct this calculation. Further information regarding power calculations can be found in Appendix B.

3.6 Methods for Aim 3

3.6.1 Independent Variable Assessment (Socioeconomic Position)

The latent class analysis performed to determine the Socioeconomic position in Aim 1 was again used as the independent variable for my Aim 3 as well.

3.6.2 Dependent Variable Assessment (Placebo Hypoalgesia)

Among participants with TMD, placebo hypoalgesia was computed using the difference in VAS ratings between red- and green-paired stimuli during the test phase for both the test and retest setting. While among pain-free participants, for the test setting placebo hypoalgesia was computed using the difference in VAS ratings between blue- and green-paired stimuli during the test phase, since placebo hypoalgesia was induced using the social observational learning paradigm. For the retest setting placebo hypoalgesia was computed using the difference in VAS ratings between red- and green-paired stimuli during the test phase.

3.6.3 Placebo Responsiveness

Placebo-responsiveness status via permutation tests as previously done [28, 133] was conducted on the repeated measures of pain intensity ratings during the testing phase. For the permutation test, first the observed difference T_{obs} as the average pain ratings differences of the was calculated. Next, resampling was conducted 1000 times from the

pooled pain ratings to create a new distribution of possible difference scores under the null hypothesis that pain ratings for each condition were exchangeable (i.e., came from the same distribution). The p value was calculated based on the observed difference T_{obs} and the new resampled distribution. Wherever the T_{obs} was contained within the 95% of the resampled possible differences scores, the null hypothesis was not rejected, and the permutation test was considered as non-significant. If the absolute value of T_{obs} was greater or equal to the 95% of the resampled possible difference scores, the null hypothesis was rejected and the permutation test was considered significant, indicating that pain ratings were different for each condition. The significance level was set as $p=0.05$. An individual was classified as placebo responder when the observed difference is positive and the permutation test was significant.

3.6.4 Statistical Methods

Stratified statistical analysis was conducted for this Aim. For both participants with TMD and pain-free participants LMM analysis was conducted to determine difference in placebo hypoalgesia based on latent class SEP. Latent class SEP and test modality was set as fixed factor, while participants' age, sex, self-reported race, heat stimulus temperature used in testing phase, and the time duration between the test and retest setting was set as covariate. Intraclass correlation coefficients (ICCs) and cohen's kappa was computed to examine the test–retest reliability of the placebo hypoalgesia and placebo responsiveness respectively among participants with TMD and pain-free participants.

3.6.5 Power Calculations

Within the TMD sample, a medium effect size for the rest-retest ICC ($\rho=0.40$) was assumed given that identical placebo manipulation procedure was utilized for. The test retest setting. Based on the effect size estimation, the power calculation indicated a minimum of 45 participants would be sufficient to detect medium effect size for ICC with 0.8 statistical power at the alpha level of 0.05. For pain-free participants, a smaller ICC was assumed given different placebo manipulation procedure were adopted. With an estimation of $\rho=0.35$, a minimum of 60 participants was needed to have 0.8 statistical power at the alpha level of 0.05 to detect a significant effect. Power calculation was conducted using R package “ICC.Sample.Size” ([134], see Appendix B).

CHAPTER 4: RESULTS

4.1 Latent Class Analysis

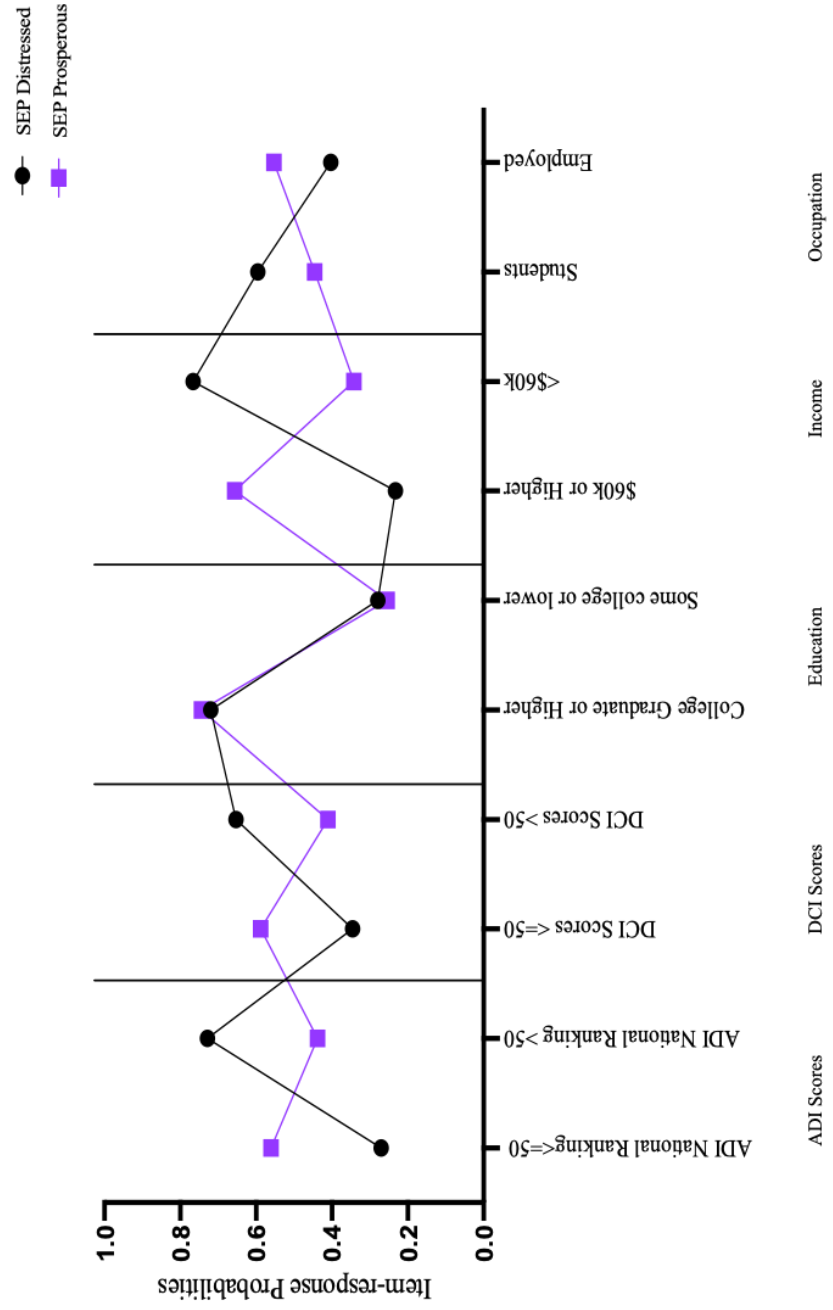
To evaluate the latent SEP variable, models with one through four latent classes were compared. For each model, Table 1 displays the degrees of freedom, the G^2 test statistic, and the p-value, which indicates the test statistic's level of significance. Test statistics for models with one and two latent classes were significant ($p < 0.001$ for one-class and $p = 0.0021$ for two-classes). Table 1 also displays the information criteria's values. The two-class model was more strongly suggested by the BIC and aBIC (BIC=5754.03 for two-classes vs. BIC= 5775.40 for three-classes; aBIC= 5712.74 for two-classes vs. aBIC= 5716.89 for three-classes), but the AIC suggested that the three-class model was marginally superior (AIC= 5681.69 for three-classes v. AIC= 5693.11 for two-classes). Following a thorough analysis of the solutions for the two- and three-class models, the two-class model was chosen since it was easier to identify, and its parameter estimates provided a solution with a substantive interpretation. A relatively high degree of classification quality was indicated by the entropy value of 0.78, which implies that the two-class model successfully captures the underlying heterogeneity in the data.

Table 1: Indicators of fit for models with one through four latent classes

Number of Class	Degrees of Freedom	G ²	p-value	AIC	BIC	aBIC	Entropy
1	41	220.09	<0.001	5844.64	5872.76	5853.70	
2	34	62.41	0.0021	5693.11	5754.03	5712.74	0.78
3	27	37.26	0.090	5681.69	5775.40	5716.89	0.61
4	20	25.22	0.214	5683.18	5809.70	5723.96	0.62

The parameter estimates depicted in Fig. 3 provide the necessary information for interpreting and labelling of the two latent classes. For instance, the first latent class includes both TMD and pain-free participants, who were highly likely to hail from distressed neighborhoods since their ADI national score and DCI score were higher than 50 (0.729 and 0.654, respectively), Furthermore these participants were extremely likely to report having a college degree or higher level of education, but they were also highly likely to report having an annual income below \$60,000 and to be students (0.720, 0.767, and 0.596, respectively). Hence I classified this subgroup as SEP distressed. The second latent class comprised of both TMD and pain-free participants who were likely to hail from prosperous neighborhoods, since their ADI national score and DCI scores were less than 50 (0.561 and 0.589, respectively). Additionally, these participants were highly likely to report college graduate or higher level of education, an annual income greater than \$60,000, and were employed (0.745, 0.657, and 0.554 respectively). Hence I classified this group as SEP prosperous.

Figure 3: Item-response probabilities for the two-class model



4.2 ADMIXTURE Analysis

The ADMIXTURE calculates the value of the K ancestral population using a cross-validation process. Compared to other K values, a good K value will have a low cross-validation error. Fig. 4 displays the cross-validation error for the K=1 to K=8 ancestral population. K=4 was shown to be a reasonable selection for the ancestral population.

Each individual participant's AIMs scores for the four populations—European, African, East Asian, and South Asian—are shown in Figure 5. Results of univariate ANOVA indicated that self-reported race significantly predicted Europe ($F(4,760)=1084.38$, $p < 0.001$), Africa ($F(4,760)=1382.36$, $p < 0.001$), East Asia ($F(4,760)=269.01$, $p < 0.001$), South Asia ($F(4,760)=56.22$, $p < 0.001$) AIMs scores (Fig. 6). AIMs was significantly predicted by the self-reported race through the Multivariate Pillai's trace analysis ($V=1.71$, $F(12,2280) = 250.3$, $p < 0.001$)

Figure 4: ADMIXTURE Cross-validation error and K ancestral population

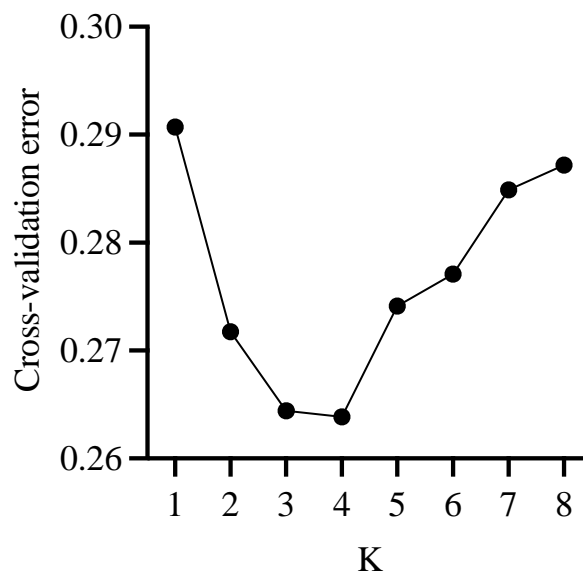


Figure 5: ADMIXTURE Ancestral Informative Markers (AIMs)

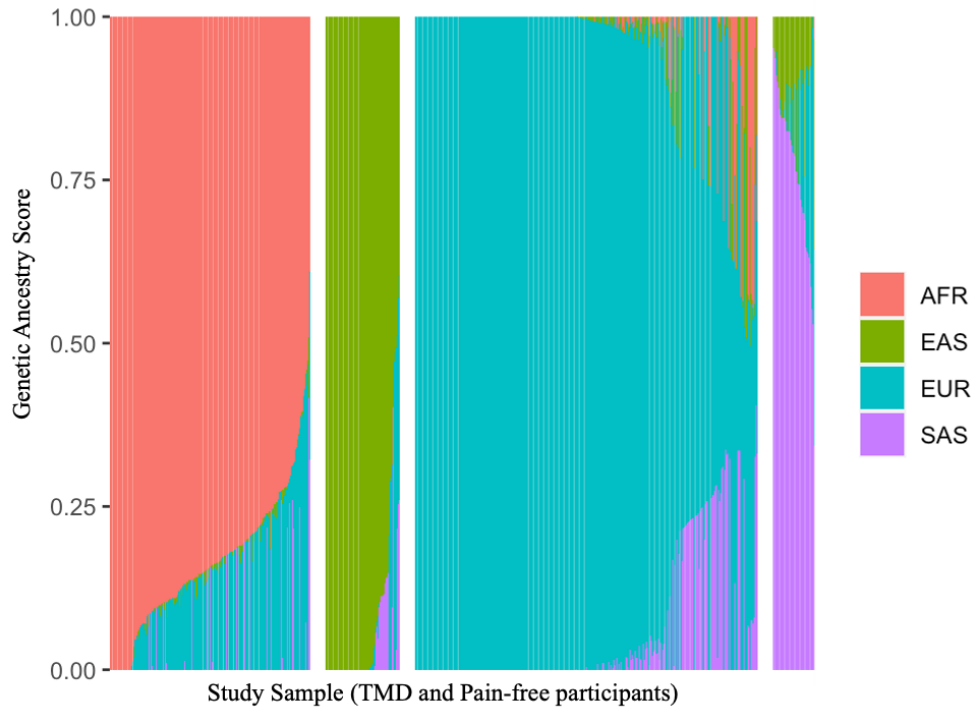
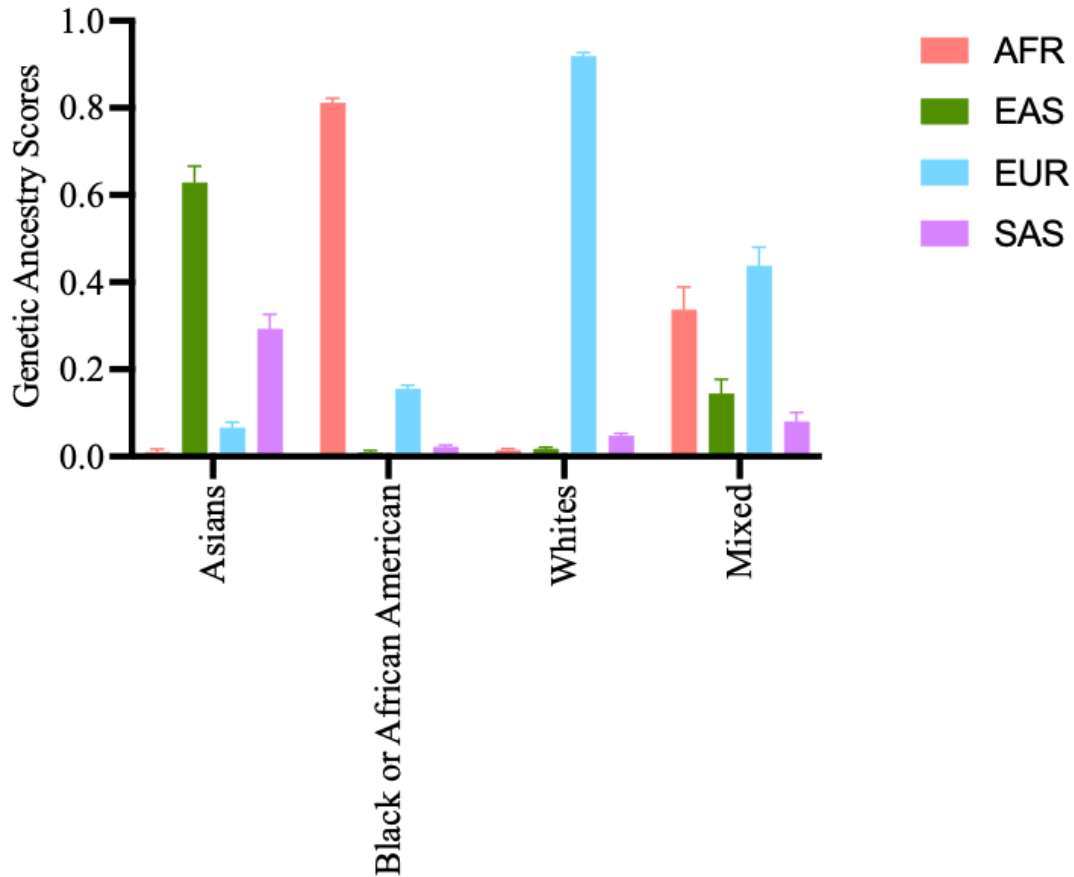


Figure 6: Figure 4C: Ancestral Informative Markers (AIMs) and self-reported race



4.3 Study Participant Demographic Characteristics

TMD and pain-free participants were characterized by distinct SEP. Namely, among the TMD participants, those clustered as SEP prosperous were younger (mean=39.6, sem=0.8) compared to those clustered as SEP distressed (mean=46.1, sem=1.3, $t(183.5)=4.2$, $p<0.001$), While among pain-free participants those clustered as SEP distressed (mean=30.3, sem=0.8) or prosperous (mean=28.8, sem=0.6) had a similar age range ($t(321.1)=-1.4$, $p=0.16$). A significant difference was observed in sex distribution between SEP distressed and SEP prosperous TMD participants ($\chi^2=5.3$, $p=0.02$), but not in pain-free participants ($\chi^2=1.4$, $p=0.24$). Similarly, a significant difference was observed

in self-reported race distribution between SEP distressed and prosperous TMD participants ($\chi^2=65.9$, $p<0.001$), but not in pain-free participants ($\chi^2=3.4$, $p=0.49$). SEP distressed TMD participants had significantly higher ADI scores (mean=53.4, sem=2.6) compared to SEP prosperous TMD participants ((mean=37.7, sem=1.3), $t(154.1)=5.2$, $p<0.001$). A similar pattern was observed among pain-free participants (SEP distressed mean=45.9, sem=1.5; SEP prosperous mean=36.4, sem=1.7, $t(357.5)=4.1$, $p<0.001$). Again SEP distressed TMD participants also had higher DCI scores (mean=70.7, sem=2.6) compared to SEP prosperous TMD participants ((mean=49.8, sem=1.8), $t(206.1)=6.4$, $p<0.001$) and a similar pattern was observed among pain-free participants (SEP distressed mean=58.3, sem=1.9; SEP prosperous mean=43.7, sem=2.2; $t(361.8)=4.8$, $p<0.001$) (see Table 2). SEP distressed participants had greater BMI (mean=29.8, sem=0.78) than SEP prosperous (mean=27.9, sem=0.39) among TMD participants ($t(155.7)=2.2$, $p=0.03$). Demographic characteristics are presented in Table 2.

Table 2: Demographic distribution among TMD and Pain-free participants

	TMD Participants (N= 401)			Pain-free participants (N= 400)		
	<i>Distressed</i> N=103	<i>Prosperous</i> N=298		<i>Distressed</i> N=233	<i>Prosperous</i> N=167	
Age (years, $\mu \pm$ sem)	46.1 \pm1.3	39.6 \pm0.8	$t_{183.5}=4.2,$ p<0.001	30.3 \pm 0.8	28.8 \pm 0.6	$t_{321.1}=-1.4,$ p=0.16
Sex (women)	70	236	$\chi^2=5.3,$ p=0.02	133	105	$\chi^2=1.4,$ p=0.24
Race (whites)	24	178	$\chi^2=65.9,$ p<0.001	103	88	$\chi^2=3.4,$ p=0.49
Ethnicity (non-Hispanic)	94	275	$\chi^2=0.12,$ p=0.94	214	156	$\chi^2=1.6,$ p=0.45
Education (college graduate or higher)	65	203	$\chi^2=0.86,$ p=0.35	152	120	$\chi^2=1.9,$ p=0.16
Annual Income (\$60k or higher)	48	122	$\chi^2=1.01,$ p=0.31	84	75	$\chi^2=3.2,$ p=0.07
Occupation (employed)	52	172	$\chi^2=55.9,$ p<0.001	82	84	$\chi^2=9.2,$ p=0.01
ADI scores ($\mu \pm$ sem)	53.4 \pm2.6	37.7 \pm1.3	$t_{154.1}=5.2,$ p<0.001	45.9 \pm1.5	36.4 \pm1.7	$t_{357.5}=4.1,$ p<0.001

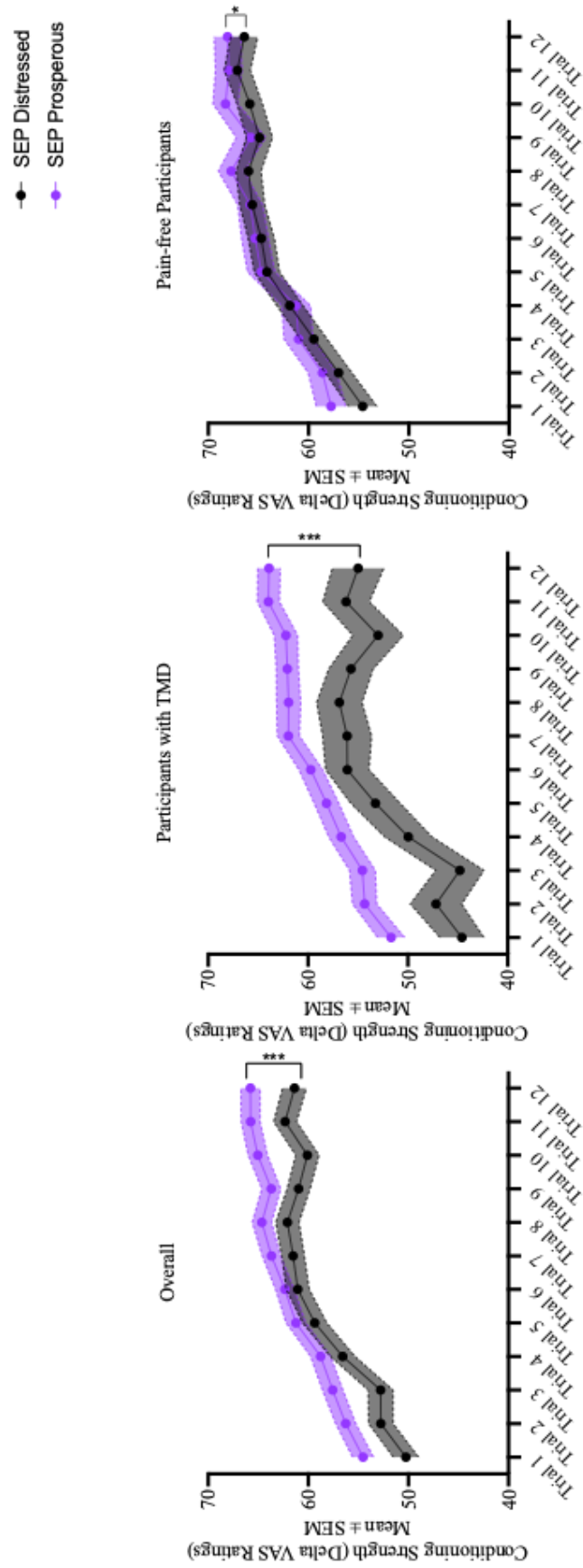
Table 2 Continued: Demographic distribution among TMD and Pain-free participants

	TMD Participants (N= 401)			Pain-free participants (N= 400)		
	<i>Distressed</i> <i>N=103</i>	<i>Prosperous</i> <i>N=298</i>		<i>Distressed</i> <i>N=233</i>	<i>Prosperous</i> <i>N=167</i>	
DCI scores (μ \pm sem)	70.7 \pm2.6	49.8 \pm1.8	t_{206.1}=6.4, p<0.001	58.3\pm1.9	43.7\pm2.2	t_{361.8}=4.8, p<0.001
Blood Pressure (mmHg, μ \pm sem)						
Systolic	143.2 \pm 12.8	126.6 \pm 0.93	t _{103.1} =1.2, p=0.21	119.8 \pm 0.85	119.8 \pm 1.1	t _{347.4} =- 0.02, p=0.98
Diastolic	81.7 \pm 1.1	79.5 \pm 0.53	t _{148.9} =1.7, p=0.09	74.1 \pm 0.63	73.6 \pm 0.78	t _{343.7} =0.42, p=0.67
Heart Rate (beats/min, μ \pm sem)	78.9 \pm 9.1	72.1 \pm 0.63	t _{103.1} =0.7 4, p=0.45	70.5 \pm 0.77	68.7 \pm 0.93	t _{350.9} =1.5, p=0.12
BMI (μ \pm sem)	29.8 \pm0.78	27.9 \pm0.39	t_{155.7}=2.2, p=0.03	25.1 \pm 0.35	25.5 \pm 0.39	t _{357.2} =- 1.01, p=0.31

4.4 Results for AIM 1

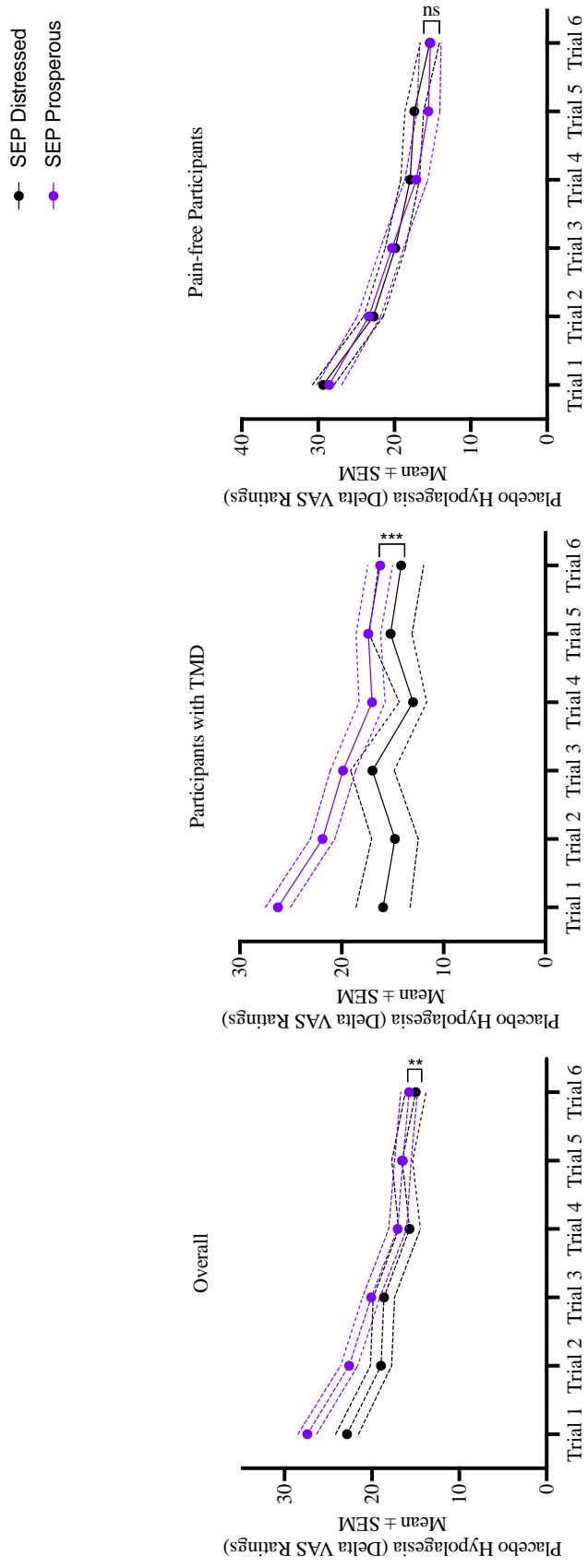
Latent class SEP and group differences in conditioning strength: Controlling for the individual painful stimulation intensities used for the conditioning, age, sex, and self-report race the LMM indicated a significant main effect of SEP ($F(1,9512.3)=46.86$, $p<0.001$) and group ($F(1,9512.3)=136.49$, $p<0.001$) on the delta scores of red-minus-green pain intensity ratings during the conditioning phase. Specifically, participants who were SEP prosperous had greater conditioning strength (mean=61.93, sem=0.28) than those participants who were SEP distressed (mean=58.81, sem=0.35, $p<0.001$). Moreover, pain-free participants displayed stronger conditioning strength (mean=63.34, sem=0.31) than TMD participants (mean=57.40, sem=0.36, $p<0.001$). There was a significant SEP and group interaction on conditioning strength ($F(1,9512.3)=16.45$, $p<0.001$). The differences between SEP distressed and prosperous participants within TMD (mean=4.98, sem=0.69) were larger than the differences within pain-free participants (mean=1.28, sem=0.59, Fig. 7).

Figure 7: SEP and group trial-by-trial assessment of Conditioning Strength



Latent class SEP and group differences in expectations and placebo hypoalgesia: Controlling for age, sex, self-report race, and individual painful stimulation intensities used during the test phase there was a significant main effect of SEP on placebo hypoalgesia ($F(1,4775.2)=8.22$, $p=0.004$, Fig. 8). Overall, SEP prosperous participants reported greater placebo hypoalgesia (mean=19.89, sem=0.41) compared to SEP distressed participants (mean=18.01, sem=0.51, Fig. 8). The main effect of group on placebo hypoalgesia was not significant ($F(1,4765.7)=1.19$, $p=0.27$), suggesting that the placebo analgesic effects for participants with TMD was comparable to the analgesic effects observed in pain-free participants. The SEP by group interaction was significant ($F(1,4765.7)=9.68$, $p=0.002$), where among participants with TMD, those who were SEP prosperous (mean=20.51, sem=0.51) had greater placebo hypoalgesia compared to those who were SEP distressed (mean=16.58, sem=0.87). While among pain-free participants there was no significant difference in placebo hypoalgesia among those who were SEP prosperous (mean=19.27, sem=0.66) or distressed (mean=19.42, sem=0.58, $p=0.86$).

Figure 8: SEP and group trial-by-trial assessment of Placebo Hypoalgesia

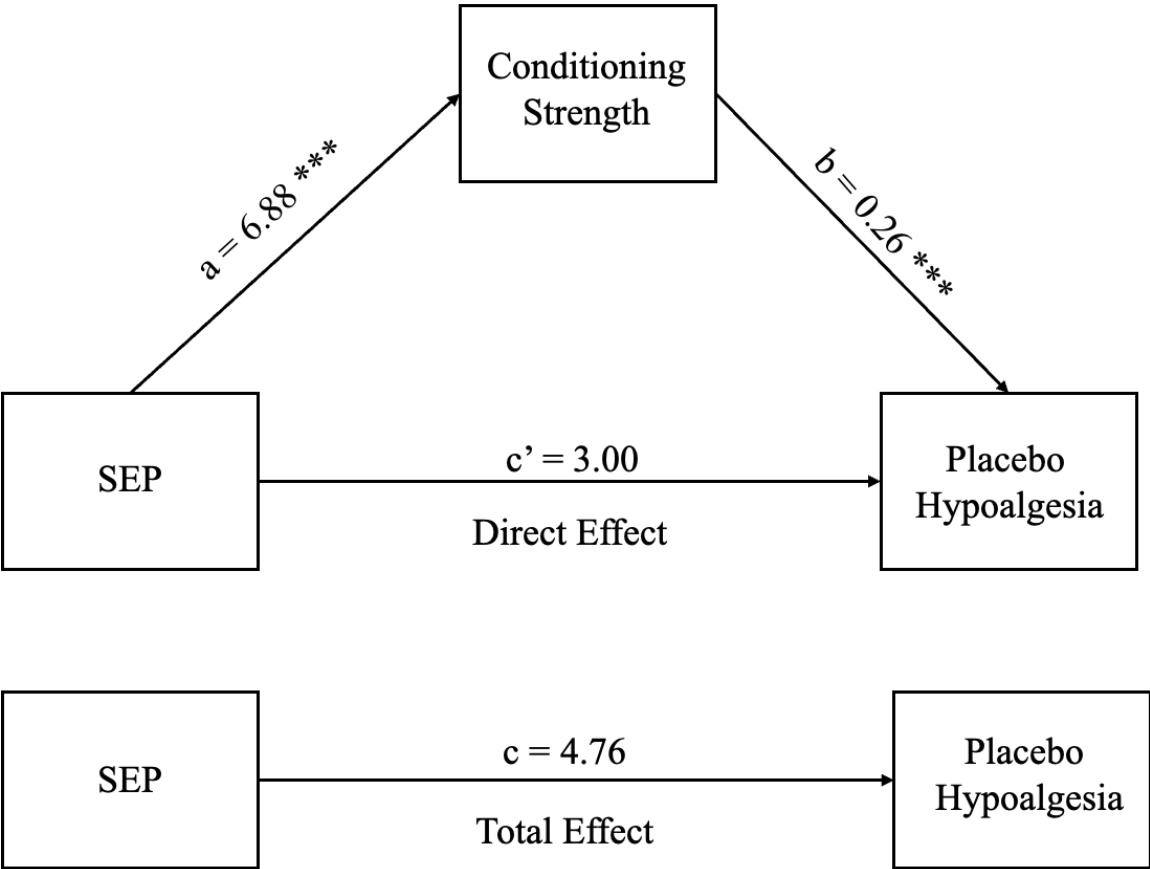


We tested for extinction of placebo effects over the entire experimental testing phase. There was a significant main effect of trials on placebo hypoalgesia ($F(5,1383.54)=27.01$, $p<0.001$). The first trials were significantly larger than the remaining trials (all $p<0.001$). However, there was no significant differences in placebo hypoalgesic ratings from trial 3 to trial 6 (all $p>0.11$), indicating that placebo effects gradually decreased over time but did not extinguish by the end of the testing phase. Moreover, the placebo extinction pattern was similar between SEP prosperous and distressed participants (SEP by trial interaction: $F(5,1383.54)=0.27$, $p=0.92$).

Surprisingly, SEP ($F(1,1007.18)=1.23$, $p=0.26$) and group ($F(1,1007.18)=1.14$, $p=0.28$) did not influence the baseline and reinforced expectation of benefits.

Mediation model: Given that among participants with TMD, SEP prosperous participants displayed larger conditioning strength, greater placebo hypoalgesia during testing phase, we conducted a mediation analysis to determine whether conditioning strength mediated the observed SEP differences in placebo hypoalgesia. While the strength of conditioning was significantly linked to placebo hypoalgesia (path $b=0.26$, $p<0.001$), the mediation results indicated that this did not mediate the SEP difference in placebo hypoalgesia (total effect $c=4.76$, 95% CI=0.80 to 8.72; indirect effect $a*b=1.76$, 95% CI=0.70 to 3.02). In sum, the SEP effects on placebo hypoalgesia were independent of the strength of the conditioning among participants with TMD.

Figure 9: Mediation Model of SEP, Conditioning Strength, and Placebo Hypoalgesia among Participants with TMD



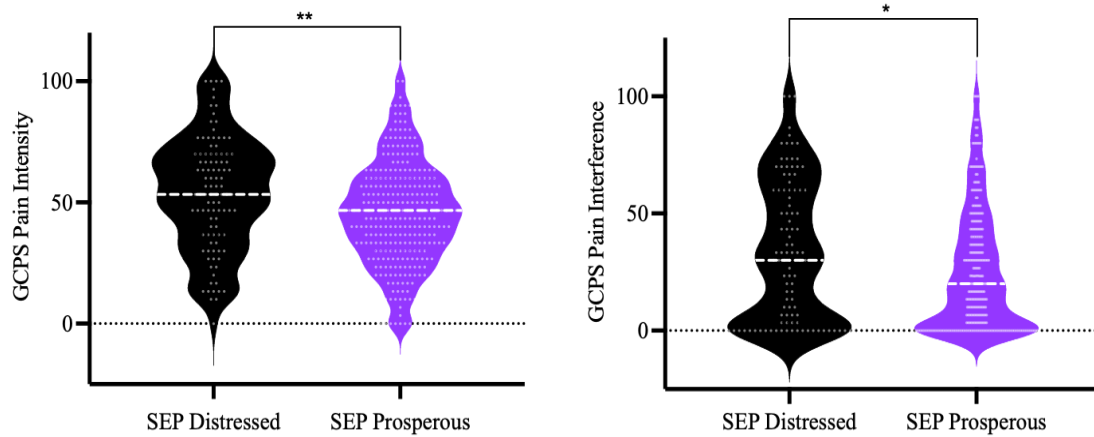
Latent class SEP and group differences in painful threshold and tolerance: Controlling for age, sex, and self-report race, the ANCOVA with group and SEP revealed a significant main effect of group on painful threshold ($F(1,740)=10.94, p<0.001$). Overall, pain-free participants had significantly higher level of heat painful threshold (mean=39.07 °C, sem=0.18) than participants with TMD (mean=38.06 °C, sem=0.22). Neither the main effect of SEP ($F(1,740)=0.005, p=0.94$) nor its interaction with group was significant ($F(1,740)=0.00, p=0.99$), suggesting that SEP did not influence heat painful threshold within either TMD or pain-free participants.

In terms of heat painful tolerance, the main effect of group was significant ($F(1,698)=14.60, p<0.001$) with pain-free participants displaying higher heat painful tolerance (mean=48.14 °C, sem=0.12) than participants with TMD (mean=47.37°C, sem=0.14) after controlling for age, sex, and self-report race. We found no significant main effect of SEP on painful tolerance ($F(1,740)=2.45, p=0.12$), although we found a trend in SEP by group interaction ($F(1,740)=3.21, p=0.073$). Among participants with TMD, those who were SEP prosperous (mean=47.68°C, sem=0.14) displayed higher painful tolerance compared to those who were SEP distressed (mean=47.07°C, sem=0.24, $p=0.02$), while no significant difference in painful tolerance was observed among pain-free participants who were SEP prosperous (mean=48.12°C, sem=0.18) or distressed (mean=48.16°C, sem=0.15, $p=0.86$).

Additionally, we examined the influences of SEP on the severity of TMD disease. The significant main effect of SEP ($F(1,400)=8.71, p=0.003$) on GCPS indicates that SEP distressed participants with TMD had higher chronic pain severity (mean=53.30, sem=2.19) than SEP prosperous participants with TMD (mean=45.70, sem=1.2, Fig.

10A). This effect was independent of participants' age, sex, self-reported race. Also SEP distressed participants with TMD reported higher levels of pain interference (mean=33.43, sem=2.7) than SEP prosperous participants (mean=25.43, sem=1.5, Fig. 10B) as revealed by the significant main effect of SEP on pain interference ($F(1,400)=6.19, p=0.013$) independent of participants' age, sex, and self-report race.

Figure 10A-B: GCPS Pain intensity and Interference and SEP among participants with TMD



Post-hoc power calculations: Through latent class analysis, I identified two distinct clusters representing different levels of socioeconomic position (SEP). Subsequently, a post-hoc power calculation was conducted based on a 2 (Latent classes of SEP) by 2 (TMD vs. pain-free participants) Linear Mixed Model (LMM) statistical analysis plan. An effect size for the SEP by Group interaction on the repeatedly measured placebo effects was observed, with Cohen's $f=0.11$. The post-hoc power analysis revealed a statistical power of 0.75 at the alpha level of 0.05 for an effect size of Cohen's $f=0.11$. It's important to note that validated power and sample size methods for mixed models study designs are currently unavailable. Therefore, I followed recommendations from previous studies and utilized G*Power [129] for post-hoc power analysis tailored for repeated measure ANOVA. Additional details regarding the post-hoc power calculations can be found in Appendix B.

4.5 Results for Aim 2

Pain-free participants and participants with TMD underwent separate HWE tests for the three SNPs. The genotypes of *OPRM1* rs1799971, *COMT* rs4680, and *FAAH* rs324420 were found to be in HWE ($p=0.015$, $p=0.143$, and $p=0.126$, respectively) for pain-free participants reported in Table 3. The genotypes of *OPRM1* rs1799971, *COMT* rs4680, and *FAAH* rs324420 were also found to be in HWE ($p=0.073$, $p=0.772$, and $p=1.00$, respectively) for the participants with TMD reported in Table 4.

Table 3: Hardy–Weinberg equilibrium tests for Pain-free participants

Gene	Chromosome	SNP	Position	Genotype	Minor Allele Frequency	p-value
<i>OPRM1</i>	6	rs1799971	154039662	AA	0.20	0.015
				AG/GG		
<i>COMT</i>	22	rs4680	19963748	Met/Met	0.42	0.143
				Met/Val		
				Val/Val		
<i>FAAH</i>	1	rs324420	46405089	Pro/Pro	0.21	0.126
				Pro/Thr, Thr/Thr		

Table 4: Hardy–Weinberg equilibrium tests for participants with

Gene	Chromosome	SNP	Position	Genotype	Minor Allele Frequency	p-value
<i>OPRM1</i>	6	rs1799971	154039662	AA	0.11	0.073
				AG/GG		
<i>COMT</i>	22	rs4680	19963748	Met/ Met	0.40	0.772
				Met/Val		
				Val/Val		
<i>FAAH</i>	1	rs324420	46405089	Pro/Pro	0.23	1.000
				Pro/Thr, Thr/Thr		

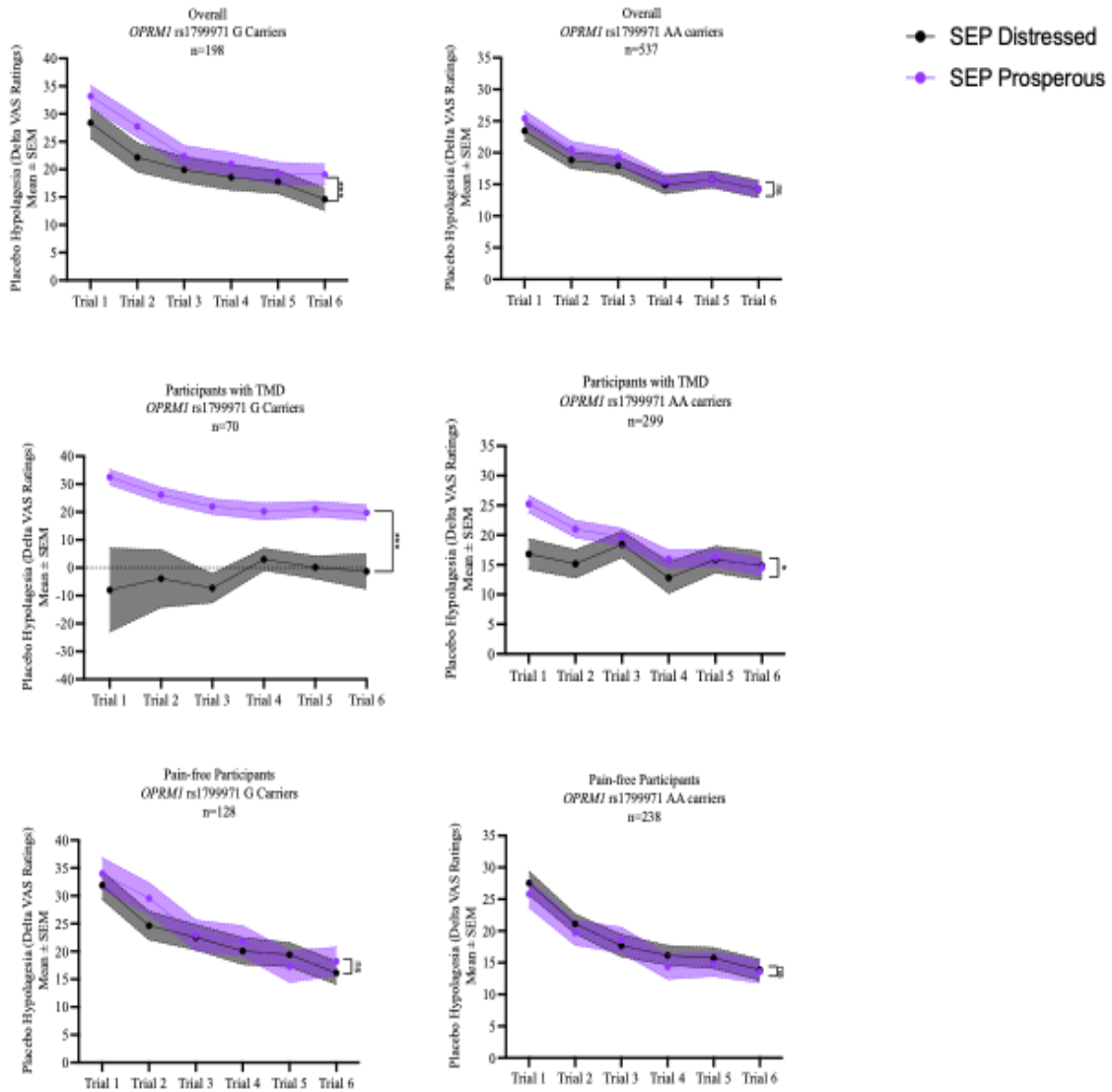
Latent class SEP, group, and *OPRM1* rs1799971 genotype differences in placebo hypoalgesia: Controlling for the individual painful stimulation intensities used for the test phase, age, sex, and self-report race the LMM indicated a significant main effect of SEP ($F(1,4360.14)=51.27, p<0.001$), group ($F(1,4360.14)=21.83, p<0.001$), and *OPRM1* rs1799971 ($F(1,4360.14)=4.73, p=0.03$) on the delta scores of red-minus-green pain intensity ratings of placebo hypoalgesia.

There was a significant interaction between SEP and *OPRM1* rs1799971 ($F(1,4630.1)=38.62, p<0.001$), where among participants who were *OPRM1* rs1799971 G carriers, those who were SEP prosperous (mean=22.45, sem=0.79) had a greater placebo hypoalgesia compared to those who were SEP distressed (mean=8.98, sem=1.68, $p<0.001$). While among participants who were *OPRM1* rs1799971 AA carriers there was no significant difference in placebo hypoalgesia among those who were SEP prosperous (mean=18.43, sem=0.52) or distressed (mean=17.44, sem=0.58, $p=0.21$).

Similarly, there was a significant interaction between group and *OPRM1* rs1799971 ($F(1,4360.14)=28.14, p<0.001$), where among participants with TMD, those who were *OPRM1* rs1799971 AA carriers (mean=18.16, sem=0.58) had a greater placebo hypoalgesia compared to those who were *OPRM1* rs1799971 G carriers (mean=10.57, sem=1.68, $p<0.001$). While among pain-free participants the reverse was observed, where those who were *OPRM1* rs1799971 AA carriers (mean=17.71, sem=0.57) had a lower placebo hypoalgesia compared to those who were *OPRM1* rs1799971 G carriers (mean=20.86, sem=0.81, $p=0.001$).

Moreover, there was a significant three way SEP by group by *OPRM1* rs1799971 interaction ($F(1,4360.14)=29.75$, $p<0.001$, Fig. 11). Among participants with TMD and were *OPRM1* rs1799971 G carriers, those who were SEP prosperous (mean=23.44, sem=1.07) had greater placebo hypoalgesia compared to those who were SEP distressed (mean=-2.29, sem=3.20, $p<0.001$). While among participants with TMD and were *OPRM1* rs1799971 AA carriers, similar results were observed where those who were SEP prosperous (mean=19.32, sem=0.61) had greater placebo hypoalgesia compared to those who were SEP distressed (mean=17.01, sem=0.95, $p=0.037$). On the contrary, among pain-free participants who were *OPRM1* rs1799971 G carriers, there was no significant difference in placebo hypoalgesia observed among SEP prosperous (mean=21.47, sem=1.17) or distressed participants (mean=20.25, sem=1.04, $p=0.42$). Similar results were observed among pain-free participants who were *OPRM1* rs1799971 AA carriers, there was no significant difference in placebo hypoalgesia observed among SEP prosperous (mean=17.55, sem=0.87) or distressed participants (mean=17.87, sem=0.73, $p=0.77$).

Figure 11: SEP by group by OPRM1 rs179971 interaction on Placebo Hypoalgesia



Post-hoc Power Calculations: Similar to Aim 1 where I employed latent class analysis to discern two distinct clusters denoting varying socioeconomic positions (SEP). Following this, a post-hoc power calculation was executed for each SNP variation using a 2 (SEP latent classes) by 2 (TMD vs. pain-free participants) by 2 (*OPRM1* rs1799971 G carriers vs. AA carriers) Linear Mixed Model (LMM) statistical analysis framework. An effect size reflecting the interaction between SEP, Group, and *OPRM1* rs1799971 genotype on placebo effects was identified, yielding a Cohen's f value of 0.13. Subsequent post-hoc power analysis indicated a statistical power of 0.75 at an alpha level of 0.05 for an effect size equivalent to Cohen's f of 0.13. It's essential to mention that validated power and sample size methods specifically designed for mixed models in research are currently lacking. As a result, I followed recommendations from prior research and utilized *G*Power* [129] for post-hoc power analysis tailored for repeated measure ANOVA. Further elaboration on the post-hoc power calculations can be found in Appendix C.

Latent class SEP, group, and *COMT* rs4680 genotype differences in placebo hypoalgesia: Controlling for the individual painful stimulation intensities used for the test phase, age, sex, and self-report race the LMM indicated a significant main effect of SEP ($F(1,4350.61)=12.47, p<0.001$), group ($F(1,4350.61)=6.67, p=0.01$), and *COMT* rs4680 ($F(1,4350.61)=5.13, p=0.006$) on the delta scores of red-minus-green pain intensity ratings of placebo hypoalgesia.

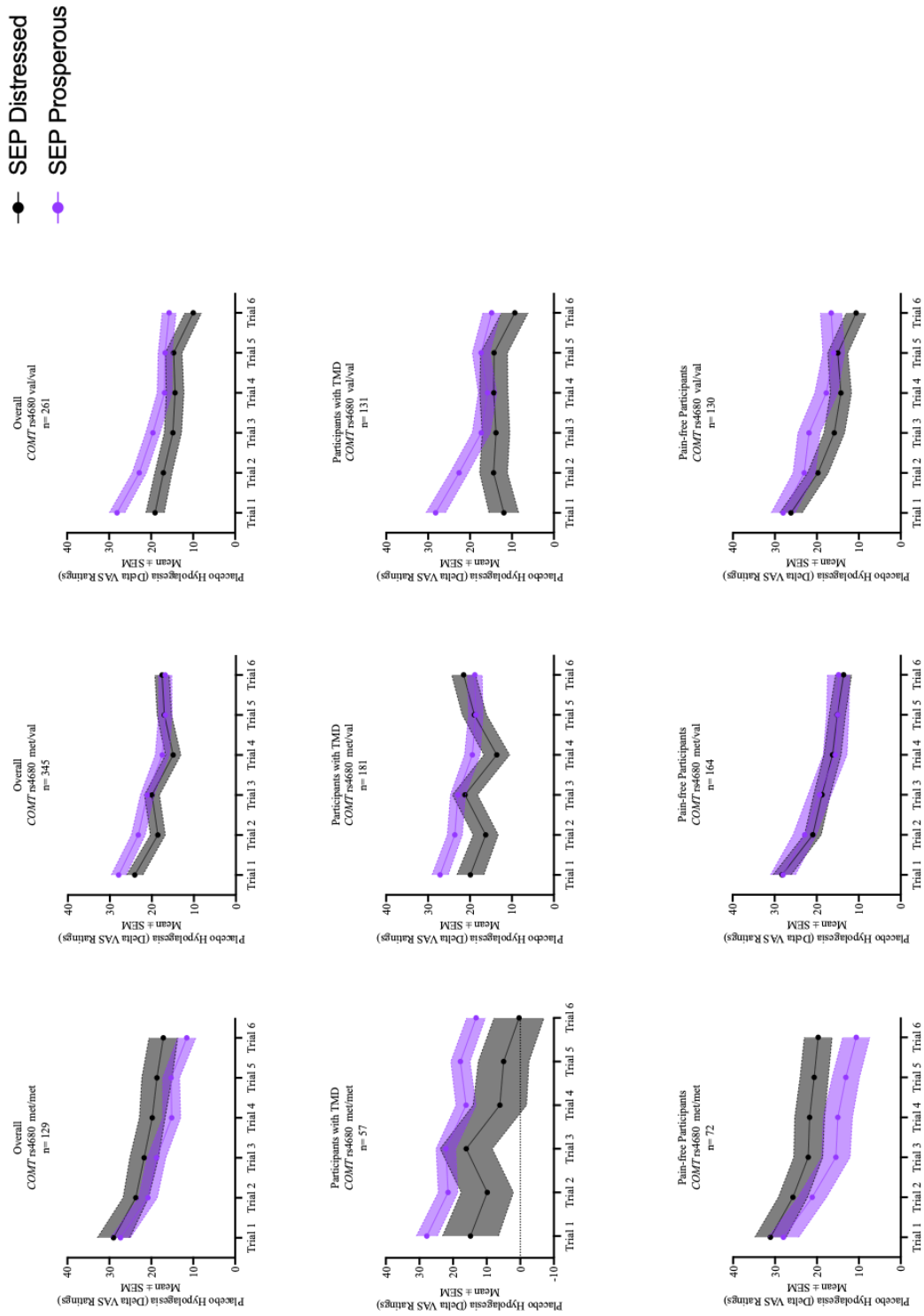
There was no significant interaction observed between SEP and *COMT* rs4680 ($F(2,4350.61)=2.23, p=0.10$), rather there was a significant interaction between group and *COMT* rs4680 ($F(2,4350.61)=6.92, p<0.001$). Among participants with TMD those who were *COMT* rs4680 met/val carriers (mean=20.07, sem=0.74), had greater placebo hypoalgesia compared to those participants with TMD who were *COMT* rs4680 met/met carriers (mean=13.92, sem=1.72, $p=0.003$) or *COMT* rs4680 val/val carriers (mean=16.01, sem=0.83, $p<0.001$). On the contrary there was no significant difference in placebo hypoalgesia observed among pain-free participants with *COMT* rs4680 met/met, met/val, or val, val carriers.

Moreover, there was a significant three way SEP by group by *COMT* rs4680 interaction ($F(2,4350.61)=6.33, p=0.002$, Fig. 12). Among participants with TMD and were *COMT* rs4680 met/met carriers, those who were SEP prosperous (mean=19.43, sem=1.21) had greater placebo hypoalgesia compared to those who were SEP distressed (mean=8.40, sem=3.22, $p=0.001$). While among participants with TMD and were *COMT* rs4680 met/val carriers, similar results were observed where those who were SEP prosperous

(mean=19.32, sem=0.61) had greater placebo hypoalgesia compared to those who were SEP distressed (mean=17.01, sem=0.95, $p=0.045$). Again, among participants with TMD and were *COMT* rs4680 val/val carriers, similar results were observed where those who were SEP prosperous (mean=19.01, sem=0.90) had greater placebo hypoalgesia compared to those who were SEP distressed (mean=13.03, sem=1.38, $p<0.001$).

On the contrary, among pain-free participants who were *COMT* rs4680 met/met carriers, those who were SEP prosperous (mean=16.74, sem=1.40) had lower placebo hypoalgesia compared to those who were SEP distressed (mean=23.18, sem=1.44, $p=0.001$). There was no significant difference in placebo hypoalgesia observed among SEP prosperous (mean=18.89, sem=1.14) or distressed (mean=18.40, sem=0.84, $p=0.72$) in pain-free participants who were *COMT* rs4680 met/val carriers. However, among pain-free participants who were *COMT* rs4680 val/val carriers, those who were SEP prosperous (mean=20.21, sem=1.10) had greater placebo hypoalgesia compared to those who were SEP distressed (mean=16.56, sem=1.03, $p=0.015$).

Figure 12: SEP by group by COMT rs4680 interaction on Placebo Hypoalgesia



Post-hoc Power Calculations: Following a similar process to Aim 1 where I employed latent class analysis to discern two distinct clusters denoting varying socioeconomic positions (SEP). I conducted a post-hoc power calculation for each SNP variation using a 2 (SEP latent classes) by 2 (TMD vs. pain-free participants) by 3 (*COMT* rs4680 met/met carriers vs. met/val carriers vs. val/val carriers) Linear Mixed Model (LMM) statistical analysis framework. An effect size reflecting the interaction between SEP, Group, and *COMT* rs4680 genotype on placebo effects was identified, yielding a Cohen's *f* value of 0.11. Subsequent post-hoc power analysis indicated a statistical power of 0.49 at an alpha level of 0.05 for an effect size equivalent to Cohen's *f* of 0.11. It's essential to mention that validated power and sample size methods specifically designed for mixed models in research are currently lacking. As a result, I followed recommendations from prior research and utilized G*Power [129] for post-hoc power analysis tailored for repeated measure ANOVA. Further elaboration on the post-hoc power calculations can be found in Appendix C.

Latent class SEP, group, and *FAAH* rs324420 genotype differences in placebo hypoalgesia: Controlling for the individual painful stimulation intensities used for the test phase, age, sex, and self-report race the LMM indicated a significant main effect of SEP ($F(1,4359.1)=12.86, p<0.001$) and *FAAH* rs324420 ($F(1,4359.1)=5.03, p=0.025$) but not group ($F(1,4359.1)= 0.629, p=0.42$) on the delta scores of red-minus-green pain intensity ratings of placebo hypoalgesia.

There was a no significant interaction between SEP and *FAAH* rs324420 ($F(1,4359.1)=0.83, p=0.36$), rather there was a significant interaction between group and *FAAH* rs324420 ($F(1,4359.1)=5.88, p=0.015$), where among participants with TMD, between those who were *FAAH* rs324420 pro/pro carriers (mean=17.69, sem=0.74) or Thr carriers (mean=17.82, sem=0.76 $p=0.90$) did not have any significant difference in placebo hypoalgesia. While among pain-free participants those who were *FAAH* rs324420 pro/pro carriers (mean=20.01, sem=0.59) had greater placebo hypoalgesia compared to those who were *FAAH* rs324420 Thr carriers (mean=16.76, sem=0.76, $p<0.001$).

Moreover, there was a trend observed in the three way SEP by group by *FAAH* rs324420 interaction ($F(1,4359.1)=3.66, p=0.056$, Fig. 13). Among participants with TMD and were, *FAAH* rs324420 Thr carriers I observed a trend where those who were SEP prosperous (mean=19.16, sem=0.85) had greater placebo hypoalgesia compared to those who were SEP distressed (mean=16.49, sem=1.24, $p=0.07$). While among participants with TMD and were *FAAH* rs324420 pro/pro carriers, stronger association were observed

where those who were SEP prosperous (mean=20.99, sem=0.67) had greater placebo hypoalgesia compared to those who were SEP distressed (mean=14.4, sem=1.305, $p=0.037$). On the contrary, among pain-free participants who were *FAAH* rs324420 Thr carriers, there was no significant difference in placebo hypoalgesia observed among SEP prosperous (mean=17.32, sem=1.15) or distressed (mean=16.19, sem=0.97, $p=0.45$). Similar results were observed among pain-free participants who were *FAAH* rs324420 pro/pro carriers, there was no significant difference in placebo hypoalgesia observed among SEP prosperous (mean=19.87, sem=0.87) or distressed (mean=20.13, sem=0.76, $p=0.81$).

Post-hoc Power Calculations: Following a similar process to Aim 1 where I employed latent class analysis to discern two distinct clusters denoting varying socioeconomic positions (SEP). I conducted a post-hoc power calculation for each SNP variation using a 2 (SEP latent classes) by 2 (TMD vs. pain-free participants) by 2 (*FAAH* rs324420 pro/pro carriers vs. Thr carriers) Linear Mixed Model (LMM) statistical analysis framework. An effect size reflecting the interaction between SEP, Group, and *FAAH* rs324420 genotype on placebo effects was identified, yielding a Cohen's f value of 0.09. Subsequent post-hoc power analysis indicated a statistical power of 0.43 at an alpha level of 0.05 for an effect size equivalent to Cohen's f of 0.09. It's essential to mention that validated power and sample size methods specifically designed for mixed models in research are currently lacking. As a result, I followed recommendations from prior research and utilized G*Power [129] for post-hoc power analysis tailored for repeated measure ANOVA. Further elaboration on the post-hoc power calculations can be found in Appendix C.

4.6 Results for AIM 3

For aim 3, I conducted the analysis on a subsample of 86 participants with TMD and 72 pain-free participants, their demographic characteristics are provided in Table 5. Namely, The TMD participants, were older (mean=44.9, sem=1.5) compared to the pain-free participants (mean=25.4, sem=0.75). A significant difference was observed in sex distribution between participants with TMD and pain-free participants ($\chi^2=5.01$, $p=0.025$). Similarly, a significant difference was observed in self-reported race distribution between participants with TMD and pain-free participants ($\chi^2=13.4$, $p=0.004$), lastly a significant difference was observed in the SEP distribution among participants with TMD and pain-free participants ($\chi^2=35.7$, $p<0.001$).

For participants with TMD, the LMM analysis yielded a no significant main effect of SEP ($F(1,150.91)=2.24$, $p=0.13$) on test re-test placebo hypoalgesia while controlling for participants' age, sex, self-reported race, heat stimulus temperature used in testing phase, and the time duration between the test and retest setting. Intraclass correlation coefficient was calculated to be 0.214, $p=0.096$ and cohen's kappa of 0.036, $p=0.73$. The 2x2 table for placebo responsiveness is provided in Table 6. Also for the pain-free participants, the LMM analysis yielded a no significant main effect of SEP ($F(1,130.9)=0.83$, $p=0.36$) on test re-test placebo hypoalgesia while controlling for participants' age, sex, self-reported race, heat stimulus temperature used in testing phase, and the time duration between the test and retest setting. Intraclass correlation coefficient was calculated to be 0.042, $p=0.39$ and cohen's kappa of -0.096, $p=0.35$. The 2x2 table for placebo responsiveness is provided in Table 7.

Table 5: Demographic distribution of study sample for test re-test reliability

	TMD Participants N= 86	Pain –free Participants N= 70	
Age (years, mean ± sem)	44.9 ± 1.5	25.4 ± 0.75	t_{120.81} = 9.39, p<0.001
Race^a	48	31	χ² = 13.48, p=0.004
Sex^b	68	44	χ² = 5.01, p=0.025
Duration between session (Months, mean ± sem)	38.67 ± 1.4	1.87 ± 0.40	t_{103.09} = -25.47, p<0.001
SEP^c	16	46	χ² = 35.76, p<0.001
<p>Race^a: The number of white participants was reported for each subgroup.</p> <p>Sex^b: The number of women was reported for each subgroup.</p> <p>SEP^c: The number of distressed SEP participants was reported for each subgroup.</p>			

Table 6: 2x2 Table for Participants with TMD placebo responsiveness across test re-test

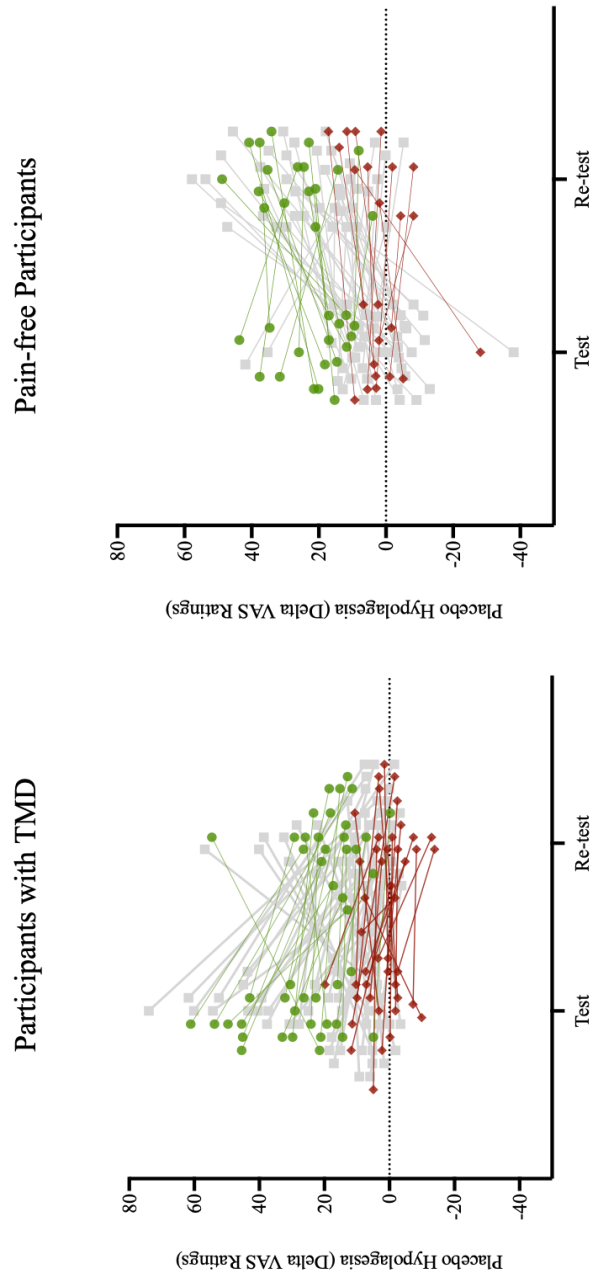
		Conditioning re-test		
		Responders	Non-responders	Total
Conditioning test	Responders	22	26	48
	Non-responders	16	22	38
Total		38	48	86

Table 7: 2x2 Table for Pain-free participants placebo responsiveness across test re-test

		Conditioning re-test		
		Responders	Non-responders	Total
Social Learning test	Responders	18	11	29
	Non-responders	31	12	43
Total		49	23	72

Figure 13: Placebo responsiveness patterns across test re-test conditions among Participants with TMD and Pain-free participants

- Placebo Responder for either one test
- Placebo Responder for both tests
- ◆ Placebo Non-Responder for both tests



CHAPTER 5: DISCUSSION

5.1 Discussion for Aim 1

This quasi-experimental study investigated the association among disparities, pain features and placebo hypoalgesia. Based on LCA, participants were clustered into socioeconomically prosperous versus distressed. Interestingly, SEP clusters were not associated with heat pain threshold and tolerance. However, the combination of pain status and SEP determined distinct patterns of placebo effects. In particular, participants suffering from chronic pain and were socioeconomically distressed showed lower placebo hypoalgesia, higher severe pain intensity and interference. Socioeconomically prosperous versus distressed condition was not associated with placebo hypoalgesia in pain-free participants.

Despite the body of evidence suggesting notable differences in pain experiences between racial groups [86, 88, 89, 95, 96], previous research has not adequately considered the crucial interplay of EPM and chronic pain disparities. SEP is considered a multidimensional construct based on educational level, occupation, annual household income (e.g., individual levels), and access to education and employment across geographical areas or population groups (e.g., structural levels) [3, 105-107]. Although SEP-related factors have long been observed in epidemiological studies of pain [7-9], no studies have investigated how SEP clusters might affect the ability to activate the EPM and experience placebo hypoalgesia.

Unraveling the influence of SEP on EPM in participants with TMD and pain-free participants is an essential step in understanding the disparities in pain experience and management. EPM has been well-documented in the pain literature [55-59] and can account for inter-individual variability in pain experience and chronicity [29, 60]. One form of EPM that depends on the activation of descending neural pathways that inhibit nociceptive signaling is placebo hypoalgesia [25, 61]. Placebo hypoalgesia is the result of top down EPM systems [63, 135]. Placebo hypoalgesia can be elicited in a laboratory setting using conditioning and verbally induced expectations of pain relief [20-23] and can be used as a powerful tool to explore the contribution of EPM systems to the experience of pain. Consequently, placebo hypoalgesia has emerged as a potent model for assessing EPM systems in humans [25, 26, 62], and we used laboratory assessments of placebo hypoalgesia as a proxy for EPM. Placebo analgesia depends on the release of substances such as endorphins/opioids [15], dopamine [16], oxytocin [17], vasopressin [18] and endocannabinoids [19] in descending pain modulation systems. Placebo effects are powerful and commonly encountered in clinical practice in the form of mind-body interventions. Through an understanding placebo mechanism, strategies promoting placebo effects can optimize therapeutic outcomes in clinical practice [63].

In this study, we used the LCA model to understand the contribution of SEP influences on the magnitude of EPM among TMD participants and pain-free participants. By determining the appropriate number of latent classes of SEP, and using self-reported race, ancestry-informative marker scores (AIMs) as a surrogate indicator(s), we clustered participants into sub-groups with distinct placebo effect magnitudes. By analyzing the effects of individual and structural SEP markers on placebo hypoalgesia (as a surrogate

for EPM), this study provided critical insights into the role of socioeconomic factors in pain perception. By stratifying participants based on SEP, we identified the subpopulations most vulnerable to reduced EPM and those that could potentially benefit most from strategies enhancing endogenous pain modulation. These findings could lead to more personalized pain management approaches and could serve as a catalyst for interventions aimed at mitigating health disparities.

Chronic pain [10]interferes with daily activities and often escalates to drug abuse and dependence of opioids [11, 12] in an attempt to mitigate pain. Prevalence of chronic pain among adults in the U.S. ranged from 11% to 40% (2016 report) [13] and chronic orofacial pain in the form of Temporomandibular Disorder (TMD), represents an emerging pain disorder which affects 5-12% of the population [14]. TMD involves derangement of the jaw joint and/or muscles of mastication disproportionately affecting women (female-to-male ratio of 9:1[14, 40]), and it is a difficult pain disorder to treat [29, 41-43, 46-50, 64]. Pain management has primarily focused on opioid receptor-mediated therapies [14]. One promising strategy to reduce chronic pain participants' needs for pharmacological therapeutics is to include non-pharmacological adjuvants, low costs interventions in the arsenal of pain management therapeutics[51, 52]. Often called mind-body therapies, these treatments are meant to target interactions among the brain, mind, body, and behavior and aim to use the mind to affect physical functioning and promote health [53]. A recent systematic review indicated that mind-body interventions improved clinical chronic pain (moderate Cohen's $d = -0.43$) and resulted in small reductions of opioid doses [54]. In this study, we demonstrated that SEP is linked to reduced placebo effects and more severe pain-related outcomes. This approach deserves

more research because it indicates that disparities can affect pain and the ability to tackle into the inner pharmacy and EPM. Future studies should determine how SEP influence other forms of EPM such as conditioned pain modulation (CPM), temporal summation (TS) and other procedures to induce placebo effects.

5.2 Discussion for Aim 2

This aim delves into the intricate interplay between socioeconomic status (SEP), genetic variations, pain conditions, and their combined effects on placebo hypoalgesia, shedding light on the complexity of pain modulation mechanisms. In aim 2 I employed a Linear Mixed Model (LMM) analysis to investigate the influence of SEP, genetic variants (OPRM1 rs1799971, COMT rs4680, FAAH rs324420), and pain conditions (TMD) on placebo-induced pain relief. Controlling for individual pain stimulation intensities, age, sex, and self-reported race, the analysis revealed several significant main effects and interactions. One key finding was the interaction between OPRM1 rs1799971 and SEP on placebo hypoalgesia. Among OPRM1 rs1799971 G carriers, participants from prosperous SEP backgrounds exhibited greater placebo-induced pain relief compared to those from distressed SEP backgrounds. This effect was prominent in individuals with TMD. However, among OPRM1 rs1799971 AA carriers, no significant difference in placebo response was observed between prosperous and distressed SEP groups. Similarly, the study highlighted interactions involving COMT rs4680, TMD, and SEP. Participants with TMD who were COMT rs4680 met/val carriers experienced enhanced placebo hypoalgesia in prosperous SEP environments compared to distressed SEP environments. This pattern was consistent across different COMT rs4680 genotypes, indicating a nuanced relationship between genetic makeup, pain condition, and

socioeconomic context in shaping placebo responses. In contrast, FAAH rs324420 showed a less pronounced impact on placebo hypoalgesia. While a trend was observed in the interaction between FAAH rs324420, TMD, and SEP, significant differences were not consistently observed across all genotype and pain condition combinations. The significant main effects and interactions underscore the multifactorial nature of placebo responses. SEP emerged as a potent modulator of placebo hypoalgesia, aligning with previous research highlighting socioeconomic disparities in pain perception and treatment outcomes. The differential impact of SEP on placebo responses among individuals with varying genetic profiles (OPRM1 rs1799971, COMT rs4680, FAAH rs324420) suggests that gene-environment interactions play a crucial role in pain modulation mechanisms. Understanding the factors influencing individual variability in placebo responses has significant clinical implications. Tailoring pain management strategies based on genetic profiles, socioeconomic contexts, and pain conditions could optimize treatment outcomes and mitigate health disparities. One of the previous studies conducted in the lab delved into the intricate relationship between genetic variants – specifically OPRM1 rs1799971, COMT rs4680, and FAAH rs324420 – and their interactions with placebo procedures in influencing pain perception. The findings revealed distinct profiles of placebo responsiveness based on genetic combinations, challenging previous assumptions and highlighting the potential predictive power of these genetic markers in understanding placebo effects. One of the novel aspects of that study is the exploration of gene-to-gene interactions in predicting placebo responsiveness. Contrary to expectations, they found significant placebo effects in individuals with specific genetic profiles, such as those with OPRM1 rs1799971 AA combined with FAAH rs324420 Pro/Pro [4], and COMT rs4680

met/met along with FAAH rs324420 Pro/Pro [30]. Moreover, participants with COMT met/val alleles also exhibited significant placebo effects independently of other genetic combinations [62]. Interestingly, G_val/val_Thr carriers also showed notable hypoalgesia [25]. These findings suggest that genetic interactions are crucial in modulating placebo responses and highlight the complexity of genetic contributions to pain modulation.

While genetic variants play a significant role, several other factors can influence placebo hypoalgesia. Age, sex, type of pain stimulation, placebo procedure, and levels of empathy are among the factors that may impact placebo responses [45][55]. Previous research has noted an inverse correlation between placebo hypoalgesia and aging [26], with older individuals potentially exhibiting reduced placebo responsiveness [60]. However, the role of sex in influencing placebo effects remains inconsistent [45][55], likely due to variations in gonadal hormone levels and interactions between the sex of the experimenter and participants. Other studies have demonstrated that *OPRM1* rs1799971 has been linked to mu-opioid and dopamine release in the nucleus accumbens [46], influencing pain modulation and psychological traits related to pain perception [59][51]. Similarly, *COMT* rs4680 variants have been associated with dopamine metabolism and fear of medical pain [25], although its role in placebo hypoalgesia is less consistent [3][24][30][62]. *FAAH* rs324420 carriers have shown larger molecular changes and improved mood [47], indicating potential interactions between endocannabinoid pathways and placebo responses. Future research should also explore gene-environment interactions and the impact of encounter factors on placebo effects to provide a more comprehensive understanding of pain modulation mechanisms. This knowledge has promising implications for personalized pain management strategies and may contribute

to more effective, targeted treatments in clinical settings. Continued research in this area is crucial for unravelling the complexities of placebo effects and their neurobiological underpinnings.

5.3 Discussion for Aim 3

The results presented for Aim 3 focused on analyzing a subsample of participants with temporomandibular disorder (TMD) and pain-free individuals to investigate the impact of socioeconomic position (SEP) on test re-test placebo hypoalgesia. The demographic characteristics of the participants revealed significant differences in age, sex distribution, self-reported race, and SEP between the TMD and pain-free groups. For participants with TMD, the linear mixed model (LMM) analysis did not yield a significant main effect of SEP on test re-test placebo hypoalgesia. This result suggests that SEP did not significantly impact the consistency of placebo responses over time among individuals with TMD. The analysis controlled for various factors, including age, sex, self-reported race, heat stimulus temperature, and the time duration between the test and retest settings. The intraclass correlation coefficient (ICC) calculated for test re-test placebo hypoalgesia in TMD participants was 0.214, although it did not reach statistical significance ($p=0.096$). Similarly, Cohen's kappa coefficient was 0.036, which also did not reach statistical significance ($p=0.73$). These coefficients indicate the low degree of agreement or consistency in placebo responsiveness between the test and retest sessions, with lower values indicating mediocre consistency.

The 2x2 table for placebo responsiveness provided additional insight into the distribution of participants based on their consistency in placebo response between the test and retest

sessions. However, the lack of statistical significance in the main effect of SEP suggests that other factors or individual variability may play a more substantial role in determining placebo responsiveness over time among individuals with TMD. Similarly, for pain-free participants, the LMM analysis did not reveal a significant main effect of SEP on test re-test placebo hypoalgesia. This finding indicates that SEP did not significantly influence the consistency of placebo responses over time among pain-free individuals. The analysis was controlled for demographic factors and other relevant variables similar to those of the TMD group. The ICC for test re-test placebo hypoalgesia in pain-free participants was calculated to be 0.042, with a non-significant p-value of 0.39. Cohen's kappa coefficient was -0.096, also with a non-significant p-value of 0.35. These coefficients suggest a low level of agreement or consistency in placebo responsiveness between the test and retest sessions among pain-free participants. The 2x2 table for placebo responsiveness in pain-free participants provided a visual representation of the distribution of participants based on their placebo response consistency. However, the lack of significance in the main effect of SEP implies that other factors, rather than socioeconomic position, may have a stronger influence on the stability of placebo responses over time in pain-free individuals. Placebo hypoalgesia can potentially improve patient outcomes, and *experimental* placebo effects have been shown to be reproducible [38, 39]. Since the published studies have small sample size, my study will help elucidating reproducibility of placebo effects. This aim was innovative because it was the first time that I explored the reproducibility of placebo effects in chronic pain participants with high translational value. It has in fact demonstrated that placebo effects in experimental settings are of larger magnitude than placebo effects in Randomized

Clinical Trials (RCT) [136]. A pioneering meta-analysis of clinical analgesic trial studies had indicated low magnitudes of placebo analgesia [137]. Hrobjartsson and Gotzsche explored the evidence supporting efficacy of placebo responses across diseases. They conducted a systematic review of clinical trials in which patients were randomly assigned to no-treatment or placebo. The placebo was a tablet, physical manipulation, or psychological intervention. In the 130 trials identified trials, placebo had no significant effects on objective outcomes, but there were small effects in studies with continuous subjective outcomes and for the treatment of pain. On a follow-up study, Vase et al. conducted two meta-analyses, one in which 23 studies used only placebo as a control in RCTs, and one in which 14 studies investigated experimental placebo analgesic mechanisms [136]. Vase et al demonstrated that magnitudes of placebo analgesic effects are higher in the *experimental* settings (mean effect size=0.95) as compared to effects observed in RCTs (mean effect size=0.15) and were significantly different (P=0.003). These differences within studies of placebo mechanisms and RCTs can be due the level of expectations for pain reductions elicited by placebo suggestions and by conditioning. More recently, a recent meta-analysis reported greater benefit from placebo treatment in patients than in pain-free participants [138]. Therefore, I focused on placebo effects reproducibility in both TMD and pain-free participants. Therefore, my experimental study determined the occurrence of, magnitude of, and reproducibility over time to placebo effects comparatively in TMD and pain-free participants. I aimed on addressing the question do participants with chronic orofacial pain respond consistently (magnitude and duration) to placebo effects than pain-free participants?

5.4 Limitations and strengths

The study's strengths lie in its innovative approach to investigating the complex interplay between SEP and EPM, marking a significant advancement in the field of pain research. One of the notable strengths is the use of Latent Class Analysis (LCA) to generate a composite measure for SEP. This methodological approach allows for identifying underlying patterns in socioeconomic variables, creating a more comprehensive understanding of how different socioeconomic factors interact and influence placebo responses. However, it's essential to note that while LCA provides a nuanced view of SEP, it lacks a direct measure and may rely on proxy indicators, potentially introducing measurement biases or limitations.

The study's large sample size is another strength, particularly concerning assessing placebo reliability across multiple modalities. A substantial sample size enhances statistical power, improves the generalizability of findings, and enables more robust conclusions regarding the impact of SEP on placebo responses. Additionally, the study employs linear mixed modeling to account for unequal subgroup sample sizes. This statistical approach is crucial in ensuring that the analysis appropriately addresses any imbalance in sample sizes between different subgroups, minimizing biases and improving the accuracy of the results.

Despite these strengths, the study faces several weaknesses that necessitate careful consideration and interpretation of the results. One notable weakness is the inadequate power to explore interactions between genetic factors and SEP. This limitation hinders the depth of analysis and may limit the ability to draw definitive conclusions regarding the combined influence of genetic markers and socioeconomic status on placebo

responses. Future research with larger sample sizes or targeted genetic analyses may help overcome this limitation and provide more insights into gene-SEP interactions.

Another challenge is managing type 1 and type 2 errors in statistical analysis. Given the complexity of the relationship between SEP and EPM, there is a risk of both false positives (type 1 errors) and false negatives (type 2 errors) in identifying significant associations. Robust statistical methods, careful control of variables, and replication studies are essential to mitigate these errors and ensure the validity of the findings.

The study also faces the risk of ecological fallacy due to using structural level indicators in generating latent class SEP. Ecological fallacy occurs when group-level data are used to make inferences about individual-level characteristics, potentially leading to erroneous conclusions. While LCA offers a comprehensive view of SEP patterns, researchers must interpret the results cautiously and avoid making direct individual-level inferences based solely on group-level data.

Moreover, the limited generalizability of the findings is a concern, primarily because the study's use of laboratory-controlled conditions may further restrict the results' applicability to real-world settings. Larger replication studies with diverse participant populations and settings are necessary to validate the findings and assess their external validity.

Additionally, the study may have unaddressed potential confounders that could influence placebo responses. Factors such as comorbid conditions, specific types of temporomandibular disorder (TMD), and other behavioral, psychological, or physiological variables were not fully controlled for in the analysis. While including secondary outcomes like quantitative sensory tests, pain severity, and interference for

TMD participants partially addresses these confounders, a more comprehensive control of variables is essential for future studies.

5.5 Conclusion

In conclusion, social disparities encompass differences in socioeconomic status, education level, ethnicity, and access to healthcare resources, which all have considerable potential to individual's health outcomes. Exploring these disparities is not just essential for understanding the mechanisms underpinning the placebo effect but also for developing more effective, tailored pain management strategies that take these social factors into account.

The significance of this research lies in its potential to contribute to personalized, mechanism-based therapeutic approaches, ultimately improving the lives of those living with chronic pain. Specifically, the exploration of social disparities and their influence on placebo effects has significant potential to deepen our understanding of individual differences in pain perception and EPM. Further, such research has clinical implications that are critical for healthcare policies and clinical practices. Identifying specific social disparities that exacerbate pain symptoms or hinder the effectiveness of the placebo effect could spur reforms aimed at reducing these disparities, thereby improving the overall quality of healthcare. For example, addressing educational disparities might involve implementing community-based health education programs, while mitigating the effects of socioeconomic disparities may require changes in healthcare financing and access.

APPENDICES

Appendix A

Subject # _____ Protocol # _____ Date _____

Study Exit Form

When you enrolled in the study, we explained that this study uses deception because we were going to provide you with misleading information about parts of the study. When we use deception in a study, we always explain the nature and purpose of the deception after participation.

The purpose of this form is to disclose to you the full nature of the study and to answer any questions that you may have. You were told that the purpose of the study is to test the role of candidate genes on pain experience (Experiment 1) and brain responses (Experiment 2). You were told that you would receive two levels of painful stimulation – low and high – given immediately after a green and red light, respectively.

However, we did not tell you, that during the last series of stimulations we set the intensity to the same painful level only. Using the same painful stimulation for the green and red lights, allows us to study the way in which pain perception is influenced by expectations. In fact, we can assume that any change in how you experienced pain would be due to your expectations of low and high pain rather than the pain per se.

We were not able to tell you about the change in the intensity of stimulation beforehand, because your knowledge would have affected your responses and perceptions. Some people do not want to have any further involvement in a study once the deception is described. Please answer the question below to let us know if you would like us to remove your data from the study.

Please check the box and sign below:

You may use my study data:

Yes _____

No _____

If you felt concerned or uncomfortable about the fact that you have been intentionally deceived, we will be happy to discuss this with you.

Please contact the principal investigator Luana Colloca
(Phone 410-706-8422; Floor 7th, Room 729A).

Appendix B

Figure 14: Power Calculations for Aim 1

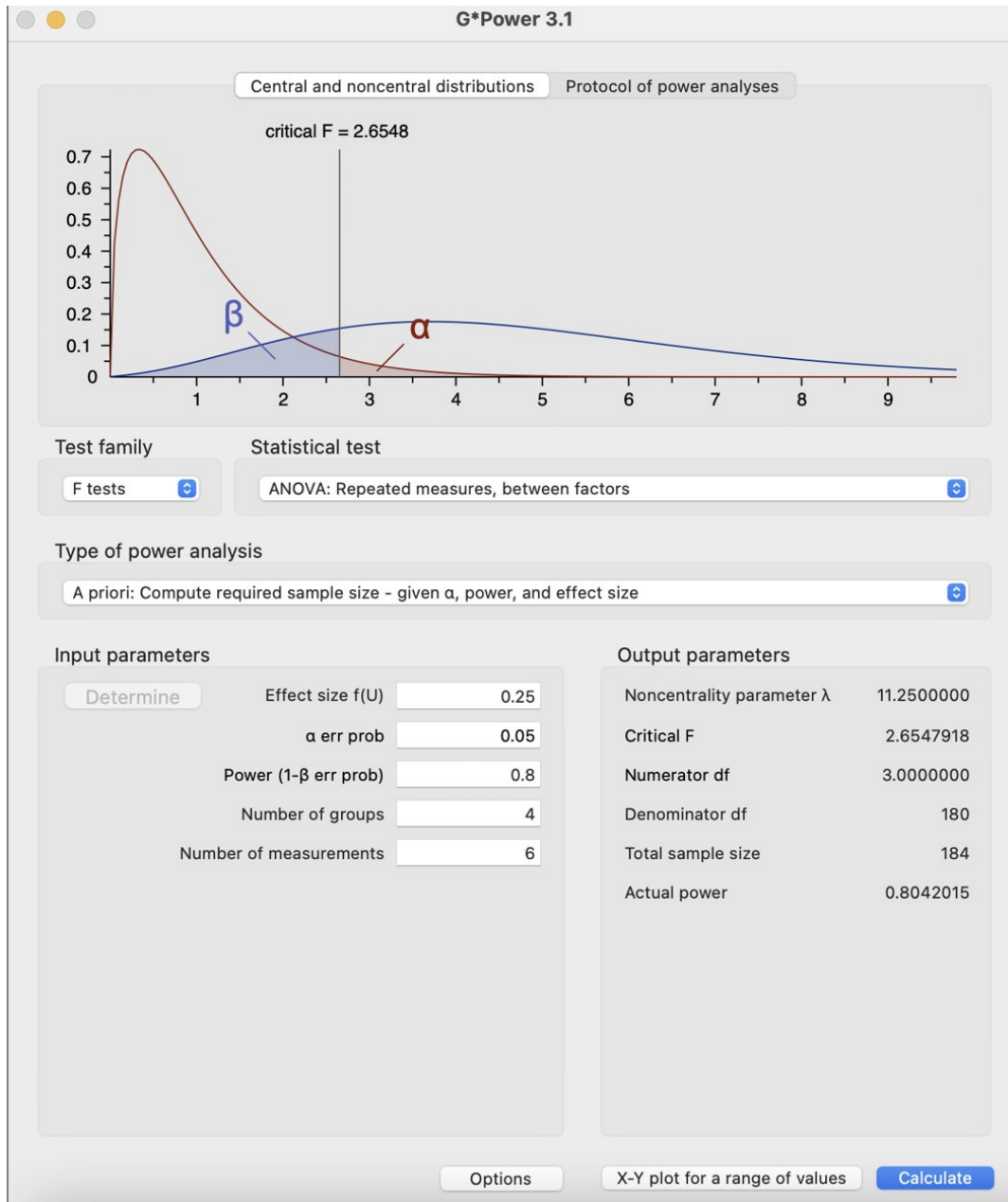


Figure 15: Power Calculations for Aim 2

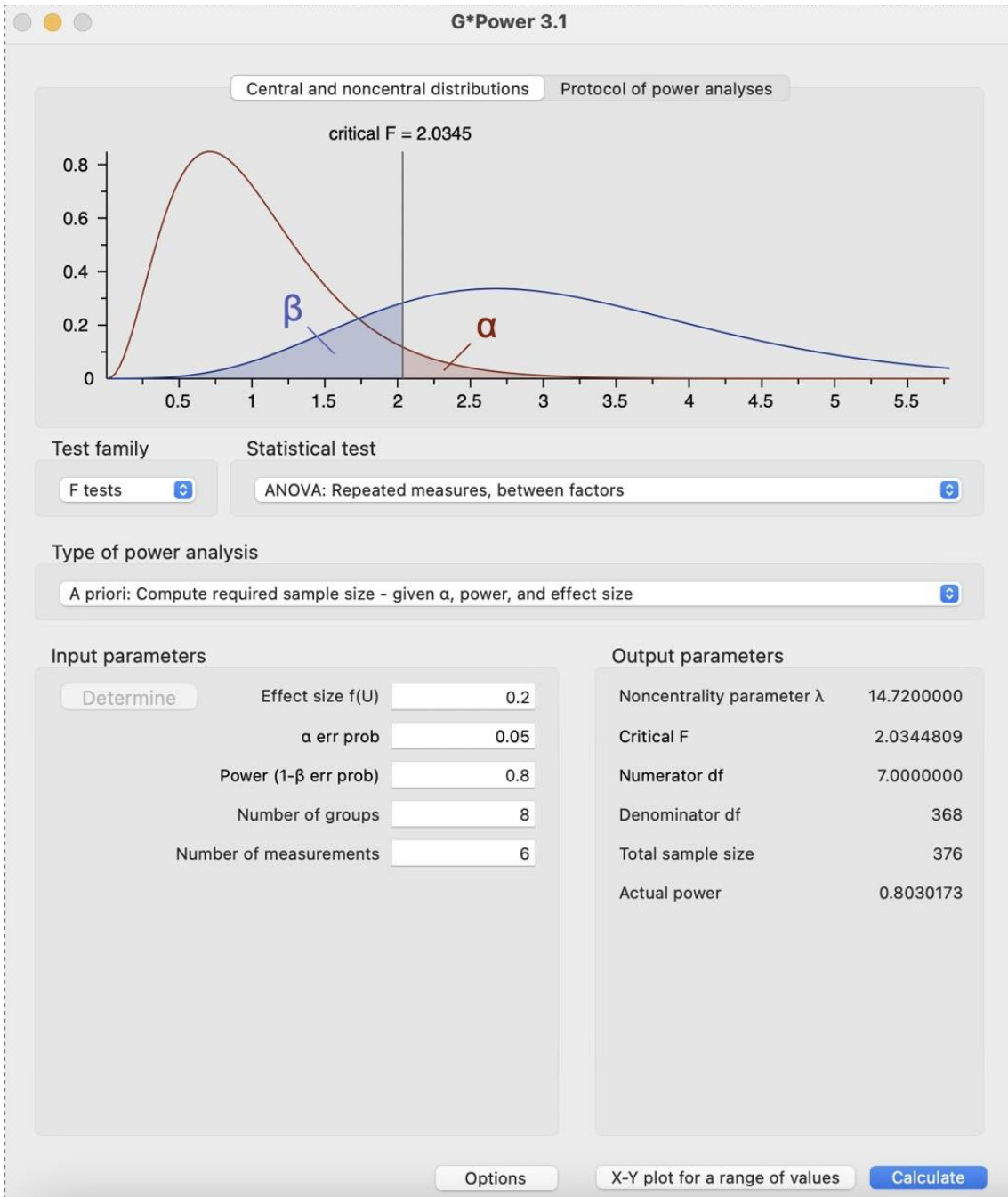


Figure 16: Power Calculation for Aim 3

ICC.Sample.Size (R package)

Description

Calculates a sample size for given values of p , the null hypothesis p_0 , number of ratings (k), desired power and alpha. Can also generate sample sizes for different values of p , p_0 or combinations of p and p_0 from 0-1.

Usage

`calculateIccSampleSize(p,p0,k,alpha,tails,power,by,step)`

Arguments

- p The hypothesized value of p . **Hypothesized based on previous data**
- p_0 The null hypothesis value of p . **Set as 0 in the current proposal.**
- k The number of ratings of each subject.
- α The desired alpha for hypothesis testing. **Set as 0.05 in the current proposal.**
- tails The number of trails for hypothesis test. **Set as 2 in the current proposal.**
- Power The desired power of the hypothesis test. **Set as 0.80 in the current proposal.**

Appendix C

Figure 17: Post-hoc Power Calculation for Aim 1

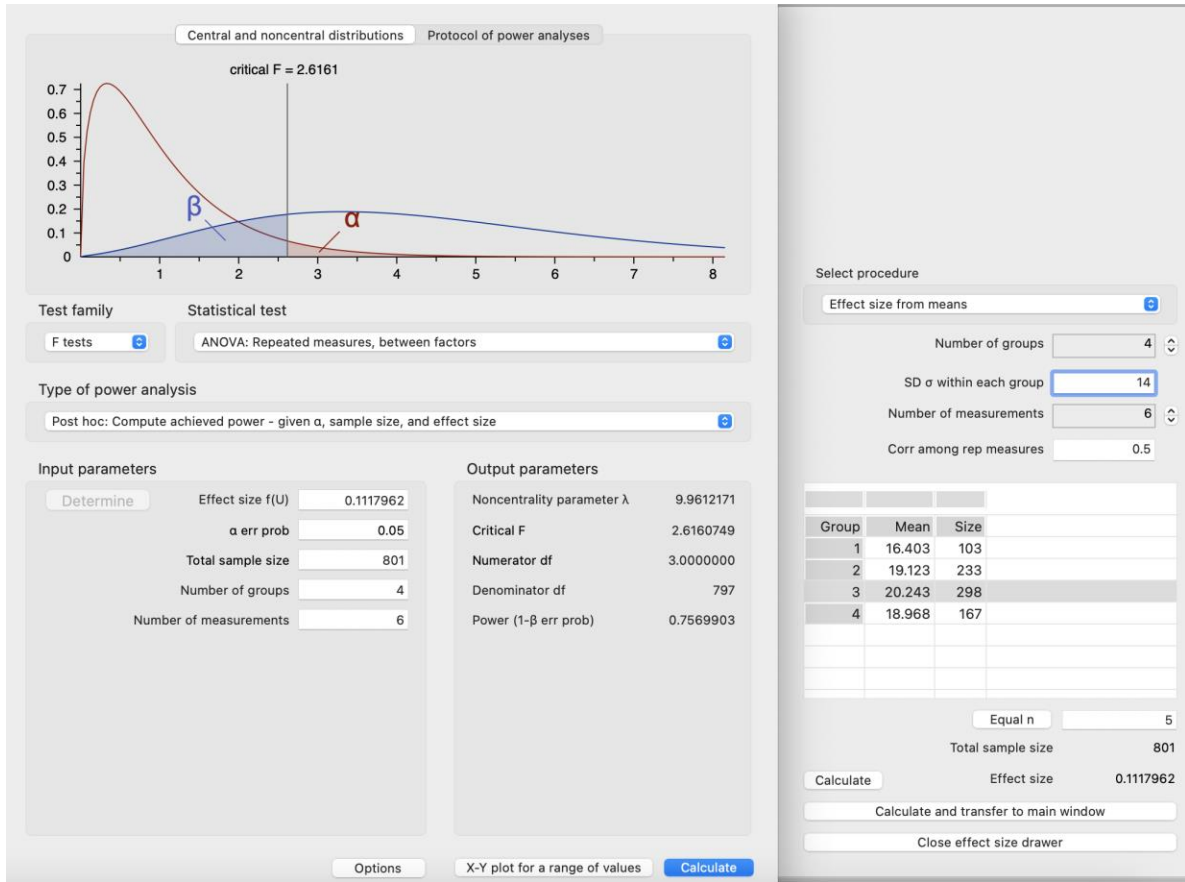


Figure 18: Post-hoc Power Calculation for Aim 2 – OPRMI rs1799971

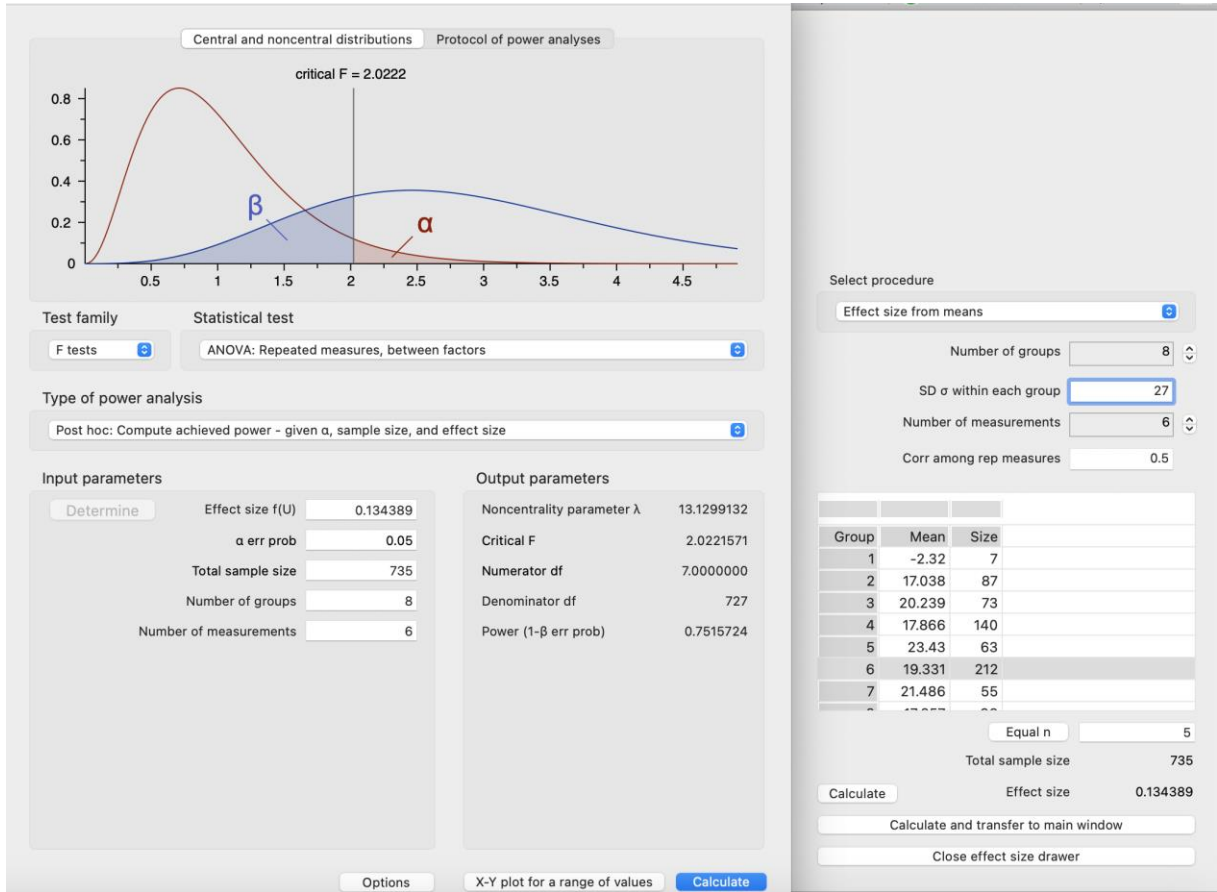


Figure 19: Post-hoc Power Calculation for Aim 2 – COMT rs4680

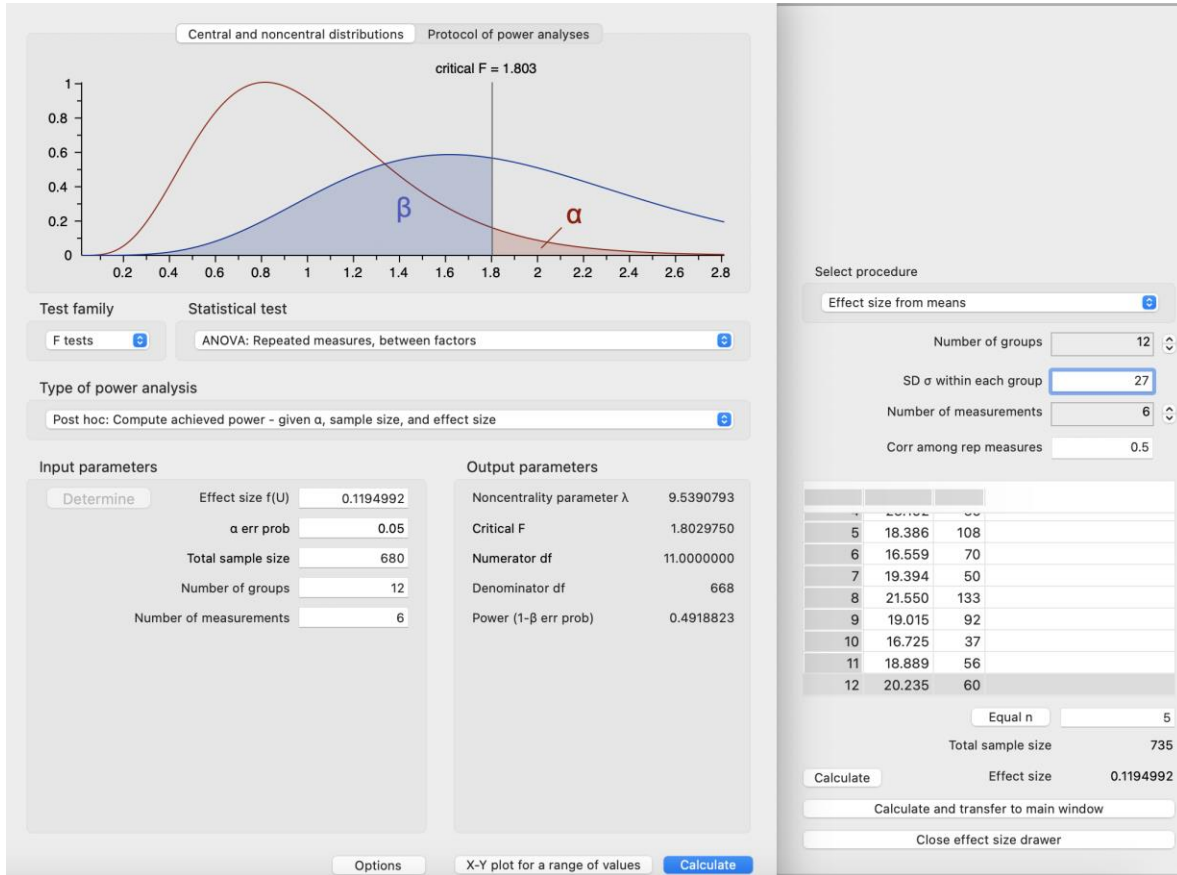
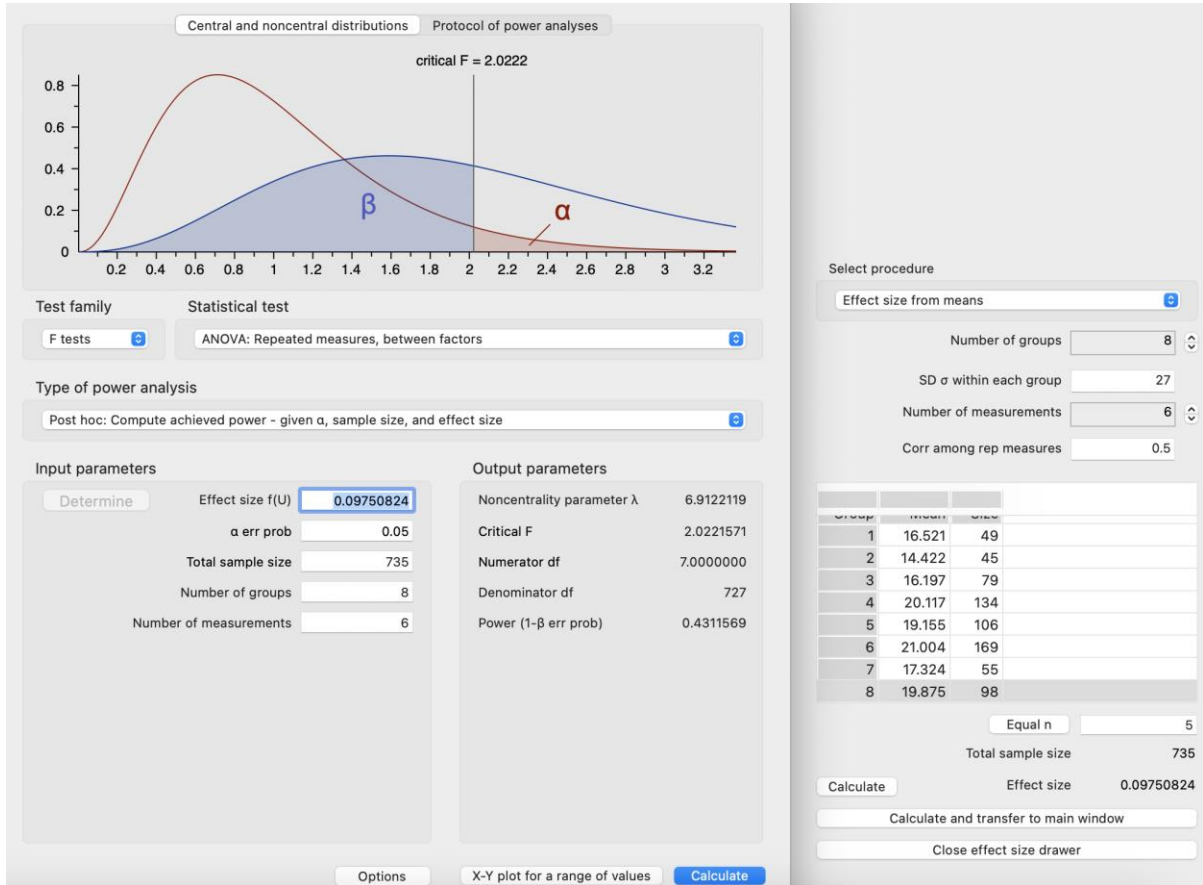


Figure 20: Post-hoc Power Calculation for Aim 2 – FAAH rs324420



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