**AAO Foundation Final Report Form**
**(a/o 3/6/2015)**

**August 18, 2023**

Please prepare a report that addresses the following:

Type of Award: Biomedical Research Award

Name(s) of Principal Investigator(s): John K. Neubert, D.D.S., Ph.D.

Title of Project: Genetics of orthodontic pain

Period of AAOF Support: 07-01-15 to 06-30-23

Amount of Funding: $30,000

Summary/Abstract
Pain is a significant issue in terms of financial and emotional burdens for the overall population. Pain also can be a major contributor to decreased success in the orthodontic practice, as previous studies have implicated pain as a primary reason for discontinuing a patient's treatment. It is therefore critical that we understand the factors underlying pain sensitivity. We know that genetics play an important role in modulating pain, with several gene (e.g., mu opioid receptor) single nucleotide polymorphisms (SNPs) being associated with altered pain responses. Given the role of genetics on pain, the long-term goal is to investigate how different genes can modulate the orthodontic pain experience. For this project, our main objective was to characterize orthodontic-separator pain following cognitive suggestions and to then investigate genetic correlations for the pain responses. As such, we hypothesized that patient pain experience can be modulated with placebo/nocebo suggestions and that these responses will be genetically influenced. We used a novel expectation manipulation, which we expect will either decrease (placebo) or increase (nocebo) the pain response based on the suggestion provided. Our plan was to characterize pain-relevant genes and correlate them to the placebo and nocebo responses. We believe this collaborative project is significant because understanding how expectations work to change the pain experience can improve how we practice orthodontics. Additionally, understanding pain genetics may allow us to identify patients who are either more or less sensitive to pain and then modify how we treat them in practice. Lastly, by bringing together pain, genetic, and orthodontic expertise from the University of Florida's and University of Kentucky's orthodontics departments, this project will provide the foundation for future research studies related to the behavioral management of orthodontic patients.

Response to the following questions:

1. Were the original, specific aims of the proposal realized? **Yes**
2. Were the results published? **Not in a journal publication, but the results were used by 2 University of Florida orthodontic residents for each of their Master's thesis**
    a. If so, cite reference/s for publication/s including titles, dates, author or co-authors, journal, issue and page numbers. **N/A**
    b. Was AAOF support acknowledged? **N/A**
    c. If not, are there plans to publish? If not, why not? **The data generated was largely negative. We did find that individuals who are GG for the *OPRM1* rs1799971 SNP did appear to have the greatest difference in their pain as compared to other genotypes in a single SNP tested. The working hypothesis for future study is that the relatively few individuals who are GG for *OPRM1* rs1799971 are likely to experience greater pain from orthodontic separators.**
    d. If so and publishable data is generated, we will acknowledge the AAOF support. **N/A**
3. Have the results of this proposal been presented? **Yes**
    a. If so, list titles, author or co-authors of these presentation/s, year and locations.
        - **This data was used for Dr. Kenneth Gilbert and Dr. Sydney Jones in completion of their Master's thesis at the University of Florida (see attached).**
    b. Was AAOF support acknowledged? **Yes**
    c. If not, are there plans to do so? If not, why not? **N/A**
4. To what extent have you used, or how do you intend to use, AAOF funding to further your career?
    **This AAOF award has contributed to my academic advancement at the University of Florida as I was promoted from Associate to Full Professor. As stated above in question 3, two orthodontic residents were able to complete their master's research project with the support of this award. For the future, if I am awarded additional AAOF funding, I will continue to educate, train, and develop orthodontists. This has implications regarding advancing new advances in the field of orthodontics and health care in general.**

Please mail hard copy to AAOF and also send electronically
(as a Word document and e-mail attachment) to
aaofevp@aaortho.org

# Appendix 1: Kenneth Gilbert Master's thesis

EFFECTS OF NOCEBO SUGGESTIONS ON THE EXPERIENCE OF ORTHODONTIC PAIN

KENNETH C. GILBERT

A THESIS PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

2018

## Appendix 2: Sydney Greer Master's thesis

# EFFECTS OF PLACEBO SUGGESTIONS ON THE EXPERIENCE OF ORTHODONTIC PAIN

By

SYDNEY M. GREER

A THESIS PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
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# EFFECTS OF NOCEBO SUGGESTIONS ON THE EXPERIENCE OF ORTHODONTIC PAIN

By

Kenneth C. Gilbert

May 2018

Chair: John Neubert
Major: Dental Sciences – Orthodontics

Pain is a significant issue in terms of financial and emotional burdens for the overall population. Pain also can be a major contributor to decreased success in the orthodontic practice, as previous studies have implicated pain as a primary reason for discontinuing a patient's treatment. It is therefore critical that we understand the factors underlying pain sensitivity. For this project, our main objective is to characterize the role of a nocebo cognitive suggestion on the experience of orthodontic-separator pain. We hypothesized that patient pain experience could be modulated with nocebo suggestions. This study used a novel expectation manipulation, which we expected would increase (nocebo) the pain response based on the suggestion provided. A neutral-control group was also included that did not receive information about pain getting better or worse. Study subjects were instructed to rate their expected pain using a visual analog scale (VAS) and then rate their actual pain following separator placement. There were no significant differences (standard t-test) in VAS scores between the 2 study groups (nocebo, neutral). While we did not detect differences between these expectation manipulations, further studies are being considered that may enhance the cognitive

processes involved with the nocebo experience. This is important because it provides a novel model for acute pain in the clinical setting that may be used in more mechanistic studies related to pain control. In terms of clinical orthodontics, these mechanistic studies may lead to novel therapies or techniques that may reduce the pain and anxiety of our patients receiving orthodontic treatment.

# CHAPTER 1

# INTRODUCTION

The fear of pain has been found to be one of the primary factors discouraging patients from seeking dental care and orthodontics is no exception. Not only has pain been shown to be one of two primary factors deterring patients from seeking orthodontic treatment, it has also been reported as a major reason for discontinuing treatment (1,2). Although many orthodontic patients are highly motivated for treatment, pain continues to remain a hindrance to treatment. In regards to patient satisfaction during orthodontic treatment, pain has been rated as the single greatest dislike during active orthodontic treatment (3). One study population showed 8 percent of participant discontinued orthodontic treatment due to pain (4). It is therefore pertinent to both patients and clinicians to gain a more clear understanding of pain control as well as develop methods to identify individuals who may be more sensitive to pain.

Pain is an inherently difficult variable to measure and quantify because it is a subjective response with large individual variation that is dependent on numerous factors. These factors include age, gender, cultural differences, genetics, psychological state, and personal experience with pain (5-6). As it relates to orthodontic treatment, pain has been the subject of numerous studies. A common rather simple procedure orthodontists routinely perform that result in pain is the placement of orthodontic separators (7). Previous studies have shown initial pain induced by orthodontic separators occurred within 4 hours post-placement and proceeded to increase over a period of approximately 24 hours and fully subsided to baseline levels within 7 days (1, 8). A separate study reported the most significant pain was experienced on the second

day and almost fully subsided by day 5 (7). Pain experienced in association to separator placement and orthodontic tooth movement is modulated by a compression related inflammatory response causing direct stimulation of nociceptors within the compressed periodontal ligament (PDL) (9). However, even though the generation and mechanism of orthodontically related pain has been well studied, a large variation in individuals pain experience following orthodontic separator placement has been reported (10). This suggests that the aforementioned factors that are believed to be responsible for variation in pain response such as age, gender, race, previous experiences with pain, genetics, and psychological state are all involved in an individual's pain experience after placement of orthodontic separators. The focus of this study is to assess psychological state as a factor that has the potential to be manipulated in order to change an individual's pain experience. Furthermore, it is our future goal to assess genetics as another factor that could help pre-determine an individual's pain experience.

In regards to one's psychological state and how it relates to perceived pain, many are aware of what has become a relatively recent topic of interest called placebo effect. This reduction in pain that is not attributed to the placebo treatment itself has been shown to occur through activation of endogenous opioid systems (11). In relation to pain, the neurobiological effect to a placebo treatment has been shown to result in placebo analgesia (12). The focus of this study however is not on the placebo effect but on its generally lesser discussed negative counterpart, a phenomenon known as nocebo. In general, nocebo effect refers to symptoms related to negative expectations (13). More specific to our study on pain experience, the nocebo effect may be

described as an increase in pain resulting from a negative cognitive association with a specific treatment. Instead of placebo analgesia, a nocebo effect results in nocebo hyperalgesia. This increased expectation of pain has been shown to play a role regarding the significant amount of variation in actual pain experienced among individuals (14-15).

Just as there is a direct neurobiological mechanism to the placebo effect, increased pain experience due to nocebo can also be attributed to specific neurobiological processes. Such enhanced responses to painful stimuli are linked to increased stimulus of the anterior cingulate cortex as well as the parietal operculum and posterior insula regions of the brain, both of which have already been shown to play roles in regulating pain-dependent behavior (16). On a biochemical level, studies have shown a strong relationship between nocebo hyperalgesia and activation of endogenous opiodergic and cholesystokinin systems opposite to the endogenous opioids that are activated with placebo effect (17). Furthermore, nocebo cues can often result in increased levels of anxiety in addition to hyperalgesia suggesting that the same neurobiological pathways are involved in both processes (18).

Due to pain expectations playing a major role in the success of orthodontic treatment, understanding nocebo responses and characterizing the role of negative cognitive suggestions on pain experiences and ways in which that can be manipulated would be extremely beneficial to clinical providers. It is therefore the goal of this study to create a novel expectation manipulation model by providing nocebo suggestions to individuals prior to the placement of orthodontic separators and quantifying their pain

response in comparison to a control. We hypothesize that pain experience could be modulated with nocebo suggestions.

# CHAPTER 2
# MATERIALS AND METHODS

## Participant Recruitment

Participants were recruited from the general population at The University of Florida and greater Gainesville area. Recruitment was achieved through the posting of both physical and digital fliers via social media websites. Participants who showed interest had an initial screening over the telephone and then were further screened at their first appointment to ensure they the met inclusion criteria for the study as outlined in Table 2-1 along with exclusion criteria. Once selected, verbal and written informed consent was obtained from each participant.

## Initial Visit

### General

Upon their initial visit written informed consent was obtained and each subject completed a health history questionnaire. A focused history review was performed followed by a physical examination that included recording each participant's vitals. After medical history was reviewed, a standard evaluation of each participant's dentition was performed to ensure eligibility. Females were assigned to take pregnancy tests and the researcher conducting the study verified results.

### Nocebo Suggestion

Once it was determined that a participant was eligible to move forward in the study they were randomly assigned to either the *control* group or *nocebo* group. In an attempt to maintain consistency in the investigation, the investigator read word for word

a prepared standard script to each participant explaining the study procedures. Subjects were informed that they would be receiving orthodontic separators described in one of either two ways as follows:

1. “These separators are used in orthodontics to create small spaces between teeth and are thought to increase pain”—Nocebo Group
2. “These separators are used in orthodontics to create small spaces between teeth”—Control Group

**Separator Placement and Pain Ratings**

Prior to separator placement, each participants’ current perceived pain intensity was measured for baseline as well as expected pain intensity throughout the 48 hour duration of the study using a slide algometer visual analogue scale (VAS) (19). Such scales have been validated for use as a tool to quantify expected and current pain levels as well as anxiety in previous studies (20-21) When using the pain sensation intensity scale participants were instructed to place the middle sliding part of the device to the right, with the farther to the right indicating the greater the pain sensation expected. The arrow at the extreme left on the scale indicates “no pain at all” while the arrow at the extreme right indicates a pain sensation that is the “most intense pain sensation imaginable”. Eight standard orthodontic separators produced by Ortho-Direct were inserted via standard orthodontic procedure mesial and distal to all four first molars. Following the insertion of separators participants were then instructed to complete additional pain intensity rating scales at 4 hours, 24, hours, and at 48 hours post-separator placement.

**Collection of Saliva for Isolation of Genomic DNA and SNP Genotyping**

In coordination with the University of Kentucky and as part of a future second phase of this study, two to four milliliters of saliva were collected from all study participants using the Oragene-DNA Collection Kits (DNA Genotek Inc., Ottawa, Ontario, Canada). Samples were shipped to the University of Kentucky for genetic analysis. Genomic DNA will be isolated from the saliva/Oragene-DNA stabilized mixture by ethanol precipitation according to the manufacturer's instructions, and will be resuspended in 10mM Tris-HCl, 1mM EDTA pH 8.0. All DNA concentrations will be measured on the NanoDrop-1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE). The average DNA yield from the Oragene collection device is ~110 ug (with a range of >20ug to 300ug).

**Take-Home Pain Assessment Logs**

In addition to completing pain intensity ratings using a VAS participants were also given forms to qualitatively describe their pain. At each 4 hour, 24 hour, and 48 hour time intervals participants were asked to describe their pain as either none, mild, moderate, or severe. Furthermore, participants completed the following questions as best they could:

1. Where do you have pain?
2. What does the pain feel like?
3. How long does the pain last?
4. When did you first have this pain
5. Other:

Furthermore, participants were asked to document the dosage, type, and time that any pain pain medication was taken during the 48 hour time period.

## Final Visit

Upon completion of the 48 hour time period participants presented back to The University of Florida Orthodontic Clinic for removal of separators and collection of take-home pain assessment logs. Separators that were missing were recorded and clinical inspection was performed to ensure missing separators were not gingivally displaced.

## Statistical Analysis

Once collected, all data was analyzed and standard T-tests were calculated to assess any statistical significant differences between nocebo and control groups.

Table 2-1. Outline of inclusion and exclusion criteria.

| | | |
|---|---|---|
| Inclusion criteria | 1. | Males or females between and including the ages of 18 and 40 years old. This age range is representative of an average adult patient seeking orthodontic treatment. |
| | 2. | Normal, healthy subjects, in good general health determined by medical history, ASA status 1 or 2 |
| | 3. | Adult dentition with fully erupted 1st and 2nd molars in all four dental quadrants |
| | 4. | Class 1 molar/canine dental classification with overbite and overjet within normal limits |
| | 5. | Mild to no crowding or spacing of dentition |
| | 6. | Normal pulp vitality and healthy periodontal tissues as determined by intraoral exam. |
| Exclusion Criteria | 1. | ASA status 3-5. Presence of chronic disease as assessed from medical history (e.g. cardiovascular disease, kidney disease, liver disease, diabetes) |
| | 2. | Pregnant or breast feeding mothers |
| | 3. | Active dental disease such as caries, periodontitis etc. |
| | 4. | Subjects who had taken any pain medications, either over the counter or prescription, within 48 hours prior to participating in study for either testing day (e.g. acetaminophen, ibuprofen, aspirin, steroids) |
| | 5. | No exclusions made based on race, sex, or religion |

# CHAPTER 3
# RESULTS

A total of number of 24 study participants met the necessary inclusion and exclusion criteria presented to The University of Florida Orthodontic clinic and were selected to proceed with in the study. Out of the 24 participants, 1 subject did not complete the study and was excluded from data analysis. In all, 10 study participants were randomly assigned to the control group and 13 participants were assigned to the nocebo group. An overview of demographic information including gender, age, and ethnicity for each randomly assigned study participant is provided in Table 3-1. The majority of participants were Caucasian (47.8%) females (65.2%) between the ages of 20 and 25 (69.6%).

A detailed overview of results comparing average pain-intensity ratings using VAS is reported in Table 3-2. No significant difference in reported pain intensity was seen at any of the three time points with p values all greater than 0.05 (Figure 3-1). Therefore, there was no significant difference in participants' perceived pain between the control group and nocebo group. Also as seen in Table 3-2, standard deviation measurements were very high as compared to the mean pain intensity scores within each group. Figure 3-2 shows that pain intensity in both study groups peaked at the 24-hour time interval and then trended downwards but remained more intense at 48 hours than at 4 hours post-separator placement.

Table 3-1. Subject demographics by gender, ethnicity, and age

| Demographics | Nocebo | Control | Total |
|---|---|---|---|
| | N=13 | N=10 | N=23 |
| **Gender** | | | |
| Females | 8 (61.5%) | 7 (70%) | 15 (65.2%) |
| Males | 5 (38.5%) | 3 (30%) | 8 (34.8 %) |
| **Ethnicity** | | | |
| Caucasian | 7 (53.8%) | 4 (40%) | 11 (47.8%) |
| Asian | 3 (23.1%) | 3 (30%) | 6 (26.1%) |
| Hispanic | 2 (15.4%) | 3 (30%) | 5 (21.7%) |
| Other | 1 (7.69%) | | 1 (4.3%) |
| **Age** | | | |
| 18-19 | 1 (7.69%) | 1 (10%) | 2 (8.7%) |
| 20-25 | 8 (61.5%) | 8 (80%) | 16 (69.6%) |
| 26-30 | 4 (30.8%) | 1 (10%) | 5 (21.7%) |
| Min | 19 | 19 | |
| Max | 27 | 28 | |
| Average | 24.2 | 22.9 | |
| SD | 2.28 | 2.73 | |

Table 3-2. Comparison of Average Visual Analog Scores

| Study Group | 4 hours | 24 hours | 48 hours |
|---|---|---|---|
| Nocebo Avg. VAS | 1.2 | 2.0 | 1.8 |
| SD | 1.3 | 1.9 | 1.7 |
| SEM | 0.4 | 0.5 | 0.5 |
| Control Avg. VAS | 2.0 | 3.6 | 2.8 |
| SD | 3.1 | 2.7 | 1.6 |
| SEM | 1.0 | 0.9 | 0.5 |
| **P Values** | P=0.4535 | P=0.1037 | P=0.1651 |

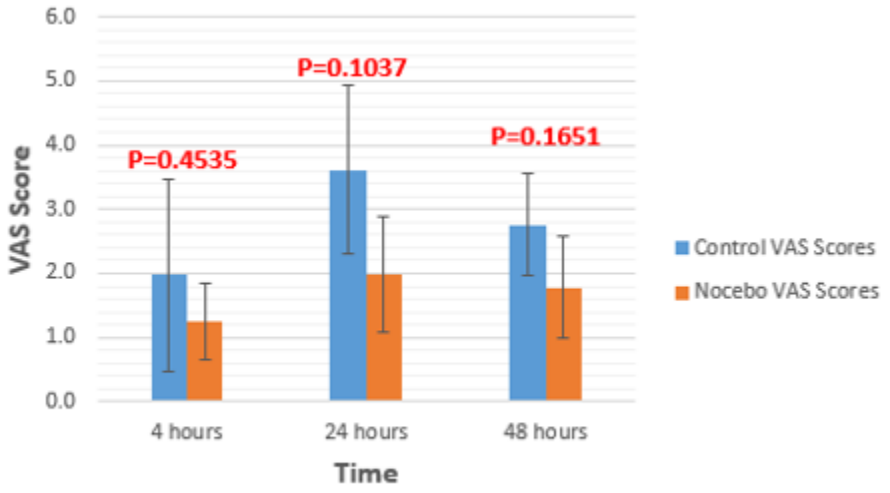


Figure 3-1. Comparison of Average Visual analog scores over 48 hour time period

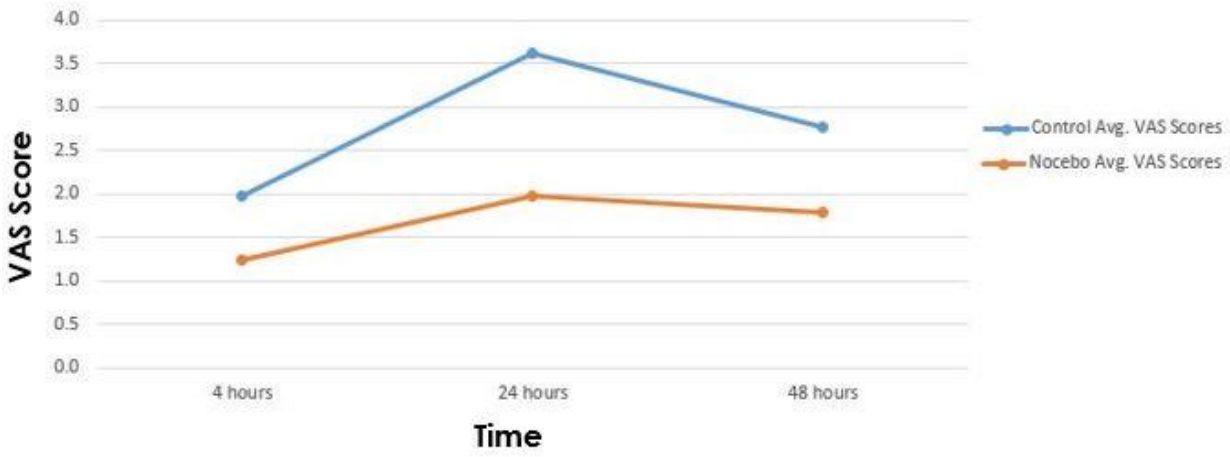


Figure 3-2. Comparison of Overall Pain Experiences

# CHAPTER 4
# DISCUSSION

Our attempt to quantify an individual's pain experience and to detect differences in each person's experience resulting from a nocebo suggestion proved to be a difficult task. As can be seen from the high standard deviation values within each group at every time point, we confirmed what is already known, that regardless of cognitive suggestions pain is a subjective response with large individual variation that is dependent on numerous factors. Furthermore, these high standard deviation values suggest that a larger sample size is needed if we wish to detect any sort of significant difference between study groups. As this was a pilot study we did not have a large sample size of participants, but future studies could add to this sample size.

Although the differences were insignificant with p values all less than 0.05, it is worth noting that all nocebo pain-intensity ratings were recorded at slightly lower values than the control group. This is opposite to what we had expected and allows us to further reject our studies hypothesis that an individual's pain experience could be modulated with a simple nocebo suggestion prior to placement of orthodontic separators.

There are a number of possible factors that may have contributed to the lack of response from the nocebo suggestion that was anticipated. One possibility is that the content of the nocebo prompt was not strong enough to elicit a nocebo effect. The content in the script that we hypothesized would elicit a nocebo effect in study participants was simply the addition of the words "thought to increase pain" at the end of defining what orthodontic separators are and used for. There was no emphasis placed

on the words “increase pain” and every subject listened to the same script. It is possible that these four words were just not enough or were insignificant in comparison of the larger script to trigger a nocebo effect in study participants. In future studies the nocebo prompt could be improved upon by adding either more emphasis on the thought of increased pain or the addition of audio or visual aids. For example, a picture of an individual in obvious discomfort could be shown to the participants while explaining what orthodontic separators are. This in theory could trigger a stronger nocebo response from study participants.

Lastly, even though we did not observe any significant difference between study groups, it is important to assess the separator pain model overall and compare our findings to those of previous studies. Just as previous reports such as those conducted by Ngan et al., we found that pain induced by separator placement peaked at 24 hours and then proceeded to decrease over the next 24 hour time interval (2). This is important as it shows that our separators model for studying pain perception not only worked, in that the separators induced pain, but that it mirrors current knowledge of the pain experience generated from orthodontic separators as well as orthodontic archwires and can be a valid model for studying orthodontic pain in future studies.

# CHAPTER 5
# CONCLUSIONS

For this study, our main objective was to characterize the role of a nocebo cognitive suggestion on the experience of orthodontic-separator pain with the hypothesis that participant's pain experience could be modulated with a nocebo suggestion. According to our data we did not meet our objective in creating a nocebo cognitive suggestion as no significant difference was seen in pain experience between the nocebo and neutral study groups. There are a number of factors that may have contributed to these results as discussed.

While a significant difference between study groups was not observed this remains a pilot study with further studies being considered that could enhance the cognitive processes involved with nocebo experience. This study did validate a novel model for studying acute pain in a clinical setting as pain was experienced by study participants and we were able to successfully quantify that experience with VAS. It is the hope that further studies will be conducted that may lead to novel therapies and techniques to reduce patient pain and anxiety throughout orthodontic treatment.

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## BIOGRAPHICAL SKETCH

Kenneth C. Gilbert was born in Reno, Nevada to Gregory and Becky Gilbert. His father was a certified public accountant who owned his own firm while his mother kept busy raising the family. Kenneth was raised in Reno, Nevada with his two sisters, Melissa and Danielle, and his brother, Mitchell, and upon graduation from Bishop Manogue High School he continued his education at The University of Nevada, Reno (UNR).

Kenneth received a BS in biochemistry from UNR and after his undergraduate training went on to attend Oregon Health and Science University School of Dentistry (OHSU) where he was awarded his DMD. While training to be a dentist at (OHSU) Kenneth served various leadership positions including class president, school of dentistry treasurer and vice president, as well as OHSU student body president. Furthermore, Kenneth was the recipient of the MODA/ODS scholarship, faculty nominated Pierre Fauchard Foundation Dental Student Scholarship Award, OCTRI Fellowship Award for Research, the National ADA Foundation Scholarship, and the college of dentistry's Oral Biology Award, Leadership Award.

Kenneth is a member of Omicron Kappa Upsilon dental honor fraternity as well as Delta Sigma Delta dental fraternity. Under the guidance of Dr. Curtis Machida at OHSU, Kenneth was a primary author on an article published in the Journal of Oral Microbiology, titled *Children with severe early childhood caries: streptococci genetic strains within carious and white spot lesions,* and was primary author on a published

textbook chapter, titled *Mutans Streptococci Genetic Strains in Children with Severe Early Childhood Caries: Implications for Caries Incidence and Treatment Outcome*.

After completion of dental school at OHSU, Kenneth pursued specialty training in orthodontics and matched at the University of Florida orthodontic residency program. It was there, in Gainesville Florida, where Kenneth met and married his wife Erika Gilbert who is currently completing training at the University of Florida College of Veterinary Medicine and will soon earn her DVM. In May of 2018, Kenneth will graduate from the University of Florida with a certificate in orthodontics and a Master of Science degree. Kenneth and his wife will move to his hometown Reno, Nevada in June 2018 to start their lives together and enjoy their numerous hobbies including hiking, skiing, rock climbing, wakeboarding, and baking.

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To God for giving me the strength and capability to make it this far and to my husband and family for supporting me every step of the way

ACKNOWLEDGMENTS

Thank you to my mentors, Dr. John Neubert, Dr. Susan P. McGorray, and Dr. Robert Caudle, for your guidance through this process. Thank you to my husband and family for supporting me throughout dental school and residency. Thank you to my instructors, Dr. Calogero Dolce and Dr. Wellington Rody for giving me the opportunity to become an orthodontist and for sharing their knowledge with me these last three years.

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By

Sydney M. Greer

May 2018

Chair: Calogero Dolce
Major: Dental Sciences – Orthodontics

Orthodontic separators are one of many things associated with pain in the dental office. The placebo effect is a beneficial effect that can help decrease pain due to the subject's belief or expectations of a given procedure. Orthodontic separators were placed in 20 subjects (17 females and 3 males), 18-40 years old for 2 days after they were randomly divided up into two different groups, the placebo group and the control group. Subjects reported their expected pain as well as their current level of pain using a Visual Analog Scale before the separators were placed as well as at 4 hours, 24 hours, and 48 hours after placement. The placebo group did have a lower pain expectation than the control group, although not clinically significant ($p=0.1128$). The placebo group also had a lower recorded amount of pain at the 4 hour, 24 hour, and 48 hour time points, but again there were no significant differences at any of these time points between the two groups. Saliva samples were taken for further studies to see if there is a genetic component in subjects who had a large amount of pain versus the subjects who had minimal or no pain.

# CHAPTER 1
# INTRODUCTION

Pain is frequently related to dentistry by patients all over the world and presents itself differently in each unique individual [1]. While some dental patients complain of sensitivity to cold or uncomfortable tactile sensations, others may be more concerned with the stretching of their soft tissues, pressure on their gingiva, or soreness during tooth movement [2, 3]. This pain during treatment is one of the most common reasons that patients do not commit to orthodontic treatment, end their treatment before completion, or have poor cooperation throughout their treatment [4, 5]. During orthodontic treatment, not only do some patients struggle to adjust to their changing bite, mastication patterns, and speech impairment, but they often become self-conscious of the unaesthetic appliances and the attention they draw [4]. When it comes to orthodontics, pain can result during certain procedures such as initial arch wire placement, arch wire adjustments, separator placement, or during the removal of braces [1]. There have been numerous studies completed that have investigated pain resulting from orthodontic treatment, including studying the pain caused by orthodontic separator placement [2]. The type of pain from orthodontic separators is most often described to be mild to moderate and of short duration. However, some patients claim to have severe pain that even prevents them from being able to brush or chew in their normal fashion [1]. Multiple studies on separators found that patients typically begin to feel discomfort about 4 hours after placement, increased over the next 24 hours, decreased between 24-48 hours, and then diminished within one week after placement [6]. Separator pain is a result of the inflammatory cascade being activated by stimulation of nociceptors during periodontal ligament compression.

Pain is a very subjective feeling and different individuals express their responses differently. Previous pain experiences, sex, age, individual pain threshold, stress level, psychology, genetics, and pain expectation are several of the factors that define an individual's pain experience [2, 7-10]. Pain expectation and genetics are the two variables that we are focusing on in this study. Identifying individuals that are more susceptible to pain and the reason behind that could be the key to improving methods for pain control, leading to benefits for both the doctor and the patients.

An example of the placebo analgesic effect is when a patient is told that they have been given a strong painkiller, but instead are administered a non-analgesic like saline, but they still experience pain relief. There are multiple theories to explain this phenomenon. It has been shown that cognitive factors, like when a patient is expecting pain relief to occur, can trigger the central nervous system to release endogenous opioids [11]. The second theory is the classical conditioning mechanism. When a patient has continually been told they are taking pain medication and then it results in pain relief, they will typically have the same response when they are given the placebo medication due to their expectations [12]. The placebo effect has been a hot topic among researchers interested in controlling pain, and it involves neurological processes that diminish pain, including decreasing excitatory pathways and increasing inhibitory neural synapses to produce analgesia. Preliminary experiments investigating the placebo effect and the neurobiology behind it have started to incorporate neuropharmacological studies on pain [13-15]. In 1978, Levine et al. demonstrated that the placebo effect is due to endorphins that are released in the CNS in the group of participants that were placebo responders [16]. When the placebo drug was combined

with naloxone, the analgesic placebo response was eliminated due to the inhibition of endorphins [15, 17-19]. This outcome suggests that endorphin opioids and placebo analgesia share a common neural mechanism. Placebo analgesia, and the decrease in pain-related neural activity associated with it, has shown to reduce activity within the ascending pain processes [19, 20]. When the placebo drug and naloxone are taken simultaneously, it is obvious that the placebo response greatly effects how an individual processes pain and that both the ascending and descending pain pathways are involved. The placebo effect not only alters the activity of the ascending and descending pain pathways, but it also has effects on certain areas of the brain that are involved with emotions and expectations [21, 22]. An example of this would be when a trusted acquaintance or medical doctor gives advice or treatment, and expectations are changed by a higher cognitive process, leading to the placebo effect and a higher chance of success. The last few decades have seen major progression in proving that placebo effects and expectancy effects use much of the same brain circuitry. Brain-imaging techniques are now being performed in humans to verify this concept.

Because expectations can influence pain and how a patient handles treatment, we emphasize that understanding the placebo response in the orthodontic office can provide valuable insight for handling different types of patients. The goal of this study is to create a placebo effect using orthodontic separators and a suggestive instruction set, and evaluate this effect on the patient's experience of pain. A secondary study will be completed to then evaluate the genetic components of these individuals to see if any correlations can be made.

# CHAPTER 2
# MATERIALS AND METHODS

## Study Design

This is a pilot study with the main objective being to characterize orthodontic separator pain following cognitive suggestions to induce the placebo effect. Genetic correlations for the pain responses will then be analyzed in a secondary study. IRB approval was obtained to conduct a clinical trial at the University of Florida Graduate Orthodontic Clinic, and the secondary genetic analysis will be conducted at the University of Kentucky. The subjects participating in the study were ages of 18-40, in good general health, and had no dental pain. There were two groups of subjects with ten participants in each group that were randomly assigned to either the placebo or control group. Othodontic separators were placed around all subjects first molars with pain ratings recorded at 0 hours, 4 hours, 24 hours, and 48 hours.

## Enrollment and Study Participation

Participants were recruited from the University of Florida campus and orthodontic clinic. Initially each subject was screened via telephone to confirm that they fit within the inclusion and exclusion criteria, which is listed in Table 2-1. If they qualified, they were assigned a study number and scheduled for the first of two visits.

Written informed consent was obtained at the beginning of the first appointment, along with the medical history questionnaire, vitals, pregnancy test (if female), oral examination, and a pain rating. The slide algometer Visual Analog Scale was used routinely throughout the study to gather pain intensities from the participants [23, 24]. The left side of the VAS says “no pain sensation at all” and the right end says “the most

intense pain you could possibly imagine". They chose a spot on the scale to show how much pain they were feeling four different times throughout the study.

The participant was then randomly assigned to either the "Placebo Group" or "Control Group" and was read the designated script to describe the study. The prompt for the Placebo Group read: "You will be receiving separators that are frequently used in orthodontics to create small spaces between the teeth and are thought to decrease pain", and the prompt to the control group read "You will be receiving separators that are frequently used in orthodontics to create small spaces between the teeth."

At this point a sample of 2-4 milliliters of saliva was collected using the Oragen-DNA collection Kit. Following the study, these samples were shipped to the University of Kentucky to complete the genetic analysis on the participants by extracting genomic DNA by ethanol precipitation.

The first rating on the Visual Analog Scale was taken at this time before the separators were placed. Subjects reported the amount of pain they were in at that moment and the amount of pain they expected the separators to cause them. Eight standard orthodontic separators were then placed between the patients teeth. One separator was placed mesial and distal to all four first molars around the interproximal contact between molar and adjacent teeth. Directions were given for the participant to complete the pain rating on the Visual Analog Scale at 4 hours, 24 hours, and 48 hours after placement. They also recorded how strong their desire was for pain relief and if any medications were taken. Their medication usage was logged at each time point as a "yes or no" response. If they answered "yes", then the amount, drug, and dosage was recorded.

At 48 hours, the participant was scheduled back to the orthodontic clinic and the separators were removed. This completed their physical participation in the study and final data was collected.

A total of 20 subjects were enrolled in the study after initial screening. Three participants did not complete the entire study and said they had to remove the separators due to the large amount of pain they were in. One of these subjects was in the control group and two were in the placebo group.

At the end of data collection, the participants were give a $25 gift card to Publix grocery store for participating.

## Collection of Data

### Pain Intensity Scale

The intensity of pain felt by the subjects before and during separator placement was recorded in a log using the Visual Analog Scale. The left side of the Visual Analog Scale says “no pain sensation at all” and the right end of the scale says “the most intense pain you could possibly imagine”. They recorded their level of pain at four different points throughout the study: before the separators were placed, 4 hours after placement, 24 hours after placement, and 48 hours after placement.

### Oragene-DNA Collection Kit

Each participant gave a sample of 2-4 mL of saliva that was sent to the University of Kentucky for DNA analysis. The DNA will be extracted from the Oragene-DNA stabilized saliva mixture by ethanol precipitation and then will be suspended in Tris-HCl and EDTA with a pH of 8.0. All DNA concentrations will then be measured using a spectrophotometer and analyzed.

## Statistical Considerations

T-tests were completed to compare the placebo and control groups to see if there was any significant differences in pain expectation or pain at the 4 hour, 24 hour, or 48 hour intervals. Age and gender of the subjects were also evaluated. As expected, the placebo group did have a lower pain expectation than the control group, but this was not statistically significant. There was no statistical difference in the amount of pain reported between the placebo or control groups at the 4 hour, 24 hour, or 48 hour time periods. This was a pilot study with a small number of participants and it is suggested that more subjects be evaluated in future studies.

Table 2-1. Outline of inclusion and exclusion criteria.

| | | |
|---|---|---|
| Inclusion criteria | 1. | Males or females in good general health (ASA 1 or 2) between the ages of 18 and 40 years old |
| | 2. | Subjects may have had bonded braces in the past |
| | 3. | Subjects must have all first molars present with two adjacent teeth in each quadrant |
| | 4. | Subjects must be Class I molar, Class I canine, with ideal overbite and overjet |
| | 5. | Willingness and ability to comply with study procedures, attend study visits, and complete the study |
| Exclusion Criteria | 1. | Poor health (ASA 3, 4, or 5) |
| | 2. | Female subjects that are currently pregnant |
| | 3. | Use of any pain medication within the last 48 hours |
| | 4. | Presence of dental or chronic diseases (caries, periodontal disease, heart disease, liver or kidney disease) |
| | 5. | Subjects who have memory of having orthodontic separators or bands placed previously |
| | 6. | No exclusions will be made based on sex or religion |

# CHAPTER 3
# RESULTS

Table 3-1 and Table 3-2 display an outline of the demographic information of the participating subjects. The number of females greatly outnumbered the males in this study (17 females and 3 males) and the subjects had an average age of 22.70 (± 2.31) years.

Table 3-3 shows a comparison of the average Visual Analog Scores between the control and placebo groups. The subjects first gave their VAS for the amount of pain they expected to have with the separators. The sliding scale ranged from 0-10. Because of the suggestive prompts, we would have expected to see the control group's expected pain to be higher than the placebo group. On average, the placebo group expected pain of 1.1 (±0.5) and the control group expected to feel a pain of 2.0 (±1.5). Although this did follow the trend we expected, it was not clinically significant (p=0.1128).

At the 4 hour time point, the placebo group rated their pain intensity to be 1.6 (±0.5) and the control group rated theirs to be 2.0 (±3.1). At 24 hours, the control group's average pain intensity increased more than the placebo group, reaching 3.6 (±2.7) while the placebo group peaked at 2.8 (±3.3). Finally at 48 hours, both groups had a decrease in pain intensity and the controls ended at 2.8 (±1.6) and the placebo group ended at 1.8 (±2.0).

Although the placebo group did in fact have lower average pain expectation and lower recorded pain intensities at all three time points, the differences between the groups were not statistically significant at any of these four points. Figure 3-1 is a visual

chart showing the comparison of VAS scores between the control and placebo groups at 4, 24, and 48 hours.

Figure 3-2 is another depiction of the data over the 48 hour period showing that there was a pain response at 4 hours, the pain peaked at 24 hours, and then decreased between 24-48 hours. This trend is agreeable with separator studies on pain completed in the past [2].

Table 3-1. Gender of subjects

| Variable | N | Control | Placebo |
|---|---|---|---|
| Total | 20 | 10 | 10 |
| Females | 17 | 7 | 10 |
| Males | 3 | 3 | 0 |

Table 3-2. Age of subjects

| | N | Mean (yrs) | SD | Min (yrs) | Max (yrs) |
|---|---|---|---|---|---|
| Total | 20 | 22.7 | 2.64 | 18.00 | 28.00 |
| Control | 10 | 22.90 | 2.73 | 19.00 | 28.00 |
| Placebo | 10 | 22.50 | 2.68 | 18.00 | 28.00 |

Table 3-3. Comparison of Average Visual Analog Scores

| Study Group | Pain Expected | 4 hours | 24 hours | 48 hours |
|---|---|---|---|---|
| Placebo Avg. VAS | 1.1 | 1.6 | 2.8 | 1.8 |
| SD | 0.5 | 1.5 | 3.3 | 2.0 |
| SEM | 0.2 | 0.5 | 1.0 | 0.6 |
| Control Avg. VAS | 2.0 | 2.0 | 3.6 | 2.8 |
| SD | 1.5 | 3.1 | 2.7 | 1.6 |
| SEM | 0.5 | 1.0 | 0.9 | 0.5 |
| P Values | P=0.1128 | P=0.7623 | P=0.5587 | P=0.2624 |

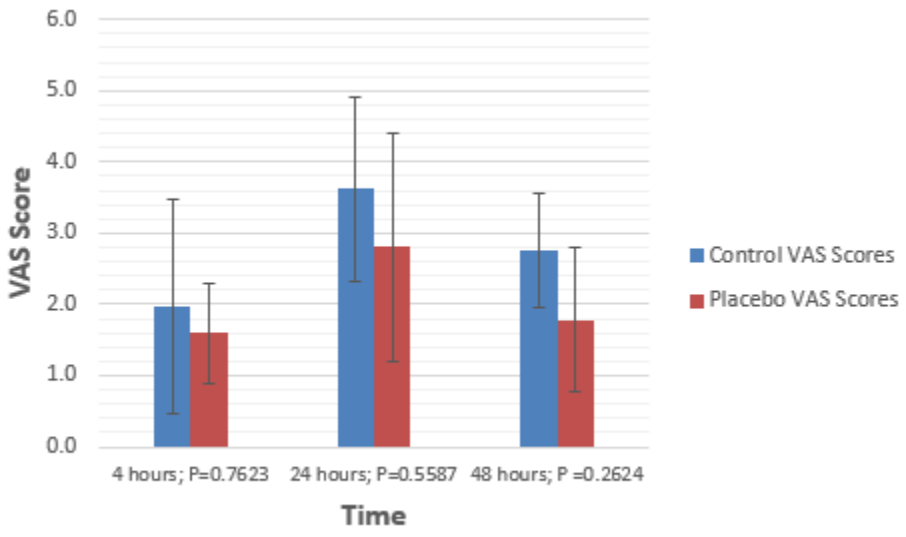


Figure 3-1. Comparison of average VAS Scores over the 48 hour period.

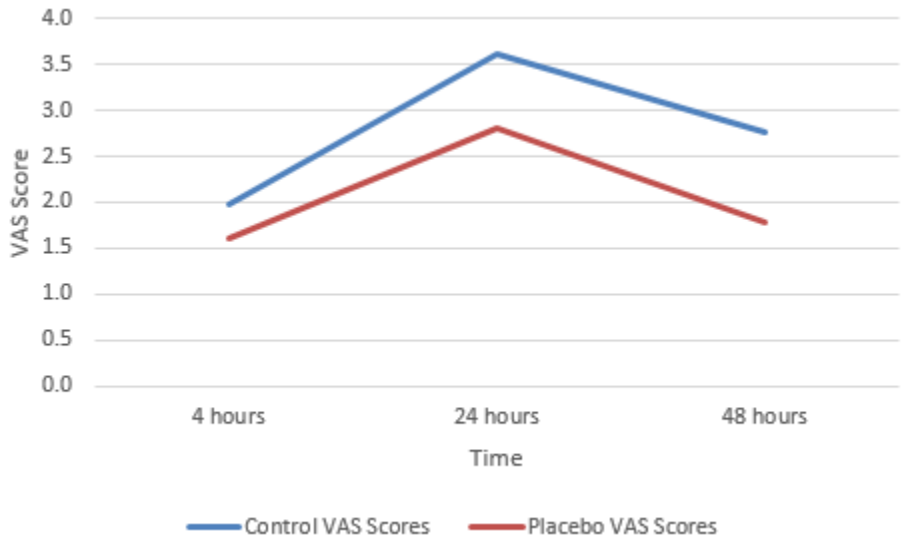


Figure 3-2. Trend of pain intensity over 48 hour period.

# CHAPTER 4
# DISCUSSION

Although there was not a significant difference between the placebo and control group in this study, it is important to recognize that the separator pain model was appropriate in inducing experimental pain and the findings were comparable to those of previous studies [2, 25]. Like the separator studies completed in the past, our results also showed that the amount of pain peaked at 24 hours after separator placement and then decreased between 24-48 hours after placement. This is important to note that we were able to replicate the separator model and the pain experience generated from orthodontic separators can be a valid model for future studies of orthodontic pain.

Patient recruitment was challenging in this study and could be improved in future studies. Our study was a pilot study and had a fairly small sample size (N=20) and we recommend a much larger sample size in follow-up studies. Patients were recruited by posting flyers around the University of Florida main campus and College of Dentistry. This resulted in a large number of participants being dental students. Because the dental school is a small community, we realized that some communication was going on among the students prior to their participation. This could have skewed the data because the subjects were hearing rumors of the separators being painful before they even presented to our clinic. This could have incorporated some bias into their expectations of pain and more than likely diminished the placebo effect. We recommend to avoid using subjects in future studies where this problem could result.

Although the participants in the placebo group did have a lower pain expectancy and recorded average pain level at all three time points, the difference between the

control and placebo groups was not statistically significant. This could have been due to the placebo effect being too weak. In order to induce the placebo response, a suggestive prompt was used saying “You will be receiving separators that are frequently used in orthodontics to create small spaces between the teeth and are thought to decrease pain”. Perhaps this was not enough to induce a strong placebo response. Because most people associate a doctor and all things associated with a doctor’s office to be helpful and to decrease pain, perhaps playing up the doctor role could have initiated more of a response. In the future, if the investigator wears a white coat, a stethoscope, and reiterates more that their pain will be decreased, perhaps the investigator would be more believable. Another way to induce a stronger response could be to show a video instead of reading a prompt where the subject watches a patient having separators placed while smiling and acting like it is a positive situation. Another limitation with our study was that we had two investigators (one male and one female) that alternated reading the prompt and placing the separators on the subjects. This could have had an effect on the participants’ expectations and would be better for future studies for the same investigator to place the orthodontic separators in all subjects.

When looking at the data from the study, it was noted that the standard deviations were very large. This confirmed that regardless of the cognitive suggestions, there is a large variation with pain between individuals and it is very subjective and based on multiple factors. The large standard deviations also confirmed that a larger sample size is needed to detect a statistical difference between study groups.

Although only the averages were mentioned in the results above, it was interesting to look at the ranges of pain among the individual subjects. There were a few subjects in each the placebo and control group that had pain levels from 0-1 throughout the entire study. There were also subjects in each group that reported intense pain over 9 on the VAS scale. This large range in pain must be the result of other factors, such as genetic variations. The saliva samples were sent to the University of Kentucky to complete a genetic analysis and this will also be closely evaluated. Single nucleotide polymorphisms (SNPs) are located within genes and have been associated with moderating pain sensitivity in human beings. SNPs associated with different catechol-O-methyltransferase genotypes have been related to an increase in pain responses [26, 27]. Catechol-O-methyltransferase SNPs have also been linked to the placebo response and should be looked at closely in future studies [27].

# CHAPTER 5
# CONCLUSIONS

The orthodontic separator model to study pain successfully mirrored previous studies. Subjects began to show a pain response at 4 hours, average pain peaked at 24 hours, and then decreased between 24-48 hours. The placebo group had a lower pain expectation than the control group after being read a suggestive prompt, but this was not statistically significant. As expected, the placebo group had lower average reported pain than the control group at 4 hours, 24 hours, and 48 hours, but there was not a significant difference at any of these time points. There was great variability between individual subjects and the amount of pain they reported from the orthodontic separators, and a genetic analysis from their saliva samples will be analyzed in the future. This was a pilot study with only 20 subjects, and a larger sample size is recommended for future research studies.

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# BIOGRAPHICAL SKETCH

Sydney M. Greer was born in Lubbock, Texas to Mr. David Jones and Mrs. Areace Mason. Her father is the Executive Vice President at City Bank Texas in Lubbock and her mother is the Operations Manager at Wells Fargo Advisors in Lubbock. Sydney has one sister, Lauren Schneider, who is a third grade teacher in Hillsboro, Texas. Sydney's family moved to Shallowater, Texas, where her childhood was spent.

After graduation from Shallowater High School, Sydney attended Texas A&M University in College Station and received a BS in biomedical sciences. Sydney was then awarded her DDS degree at Texas A&M Health Science Center Baylor College of Dentisty in Dallas, Texas. Right after dental school, Sydney applied for orthodontics residency and matched at the University of Florida in Gainesville. After her first year of residency, Sydney was married in Dallas, Texas to Preston Greer. In May 2018, Sydney will graduate from the University of Florida with a Master of Science and a certificate in orthodontics. Sydney and her husband will move back to Texas in June 2018 to begin their lives together.

Sydney is a member of the Omicron Kappa Upsilon dental honor fraternity and Xi Psi Phi (ZIPS) dental fraternity. Sydney has many hobbies including travelling, water skiing, camping, Aggie football, and her mini-goldendoodle, Piper.