AN ANIMAL MODEL OF CONDITIONED PLACEBO ANALGESIA

by

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ABSTRACT

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Research on placebo analgesia (PA) has called for the further elucidation of underlying neural mechanisms. Animal models allow for experimental manipulations that are not possible or feasible in human research such as brain lesions. Therefore, an animal model would be ideally suited to expand upon human PA literature; however, there is currently no animal model of PA. This study used a classical conditioning paradigm in an effort to induce an analgesic response to placebo treatment in rats. Thirty eight female Sprague-Dawley rats underwent an L5 spinal nerve ligation (L5 SNL) to induce a chronic neuropathic pain condition. Animals were conditioned daily for 4 days with subcutaneous injections of saline or active analgesic (gabapentin), then tested for hypersensitivity with a mechanical paw withdrawal threshold test (MPWT). During conditioning, gabapentin effectively attenuated mechanical hypersensitivity as compared with saline controls ($p < .001$). Following the conditioning period, half of the animals switched treatments on the test day. It was expected that animals conditioned with gabapentin would show a modest attenuation of pain threshold when later administered saline as a placebo, but there was no difference between the gabapentin/saline group and the saline/saline

control on test day ($p > .05$). This study revealed crucial information in pursuit of an animal model of conditioned placebo analgesia, most notably the importance of contiguity between the unconditioned stimuli and the unconditioned response.

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CHAPTER 1

INTRODUCTION

1.1 Introduction to the Placebo Effect

In the mid-1950's, the only available explanations of the placebo effect were entirely psychological in nature, relying on methodologies such as the Rorschach and thematic apperception tests, and it was assumed that the effect was merely imagined (Lasagna, Mosteller, von Felsinger, & Beecher, 1954). The emergence of new technology played a considerable role in expanding this view. For instance, it is now understood that placebos elicit measurable effects on the same biological mechanisms as their active drug counterparts, which allows the placebo effect to be viewed as a physiological phenomenon rather than the product of mere imagination. To explain this, a number of ideas have been proposed, most of them belonging to either expectancy theory or conditioning theory. Social factors also seem to play a role in modulating the effects of a placebo treatment. Placebo research has a great deal of clinical relevance especially in the testing of new drugs, but also for the future development of cognitive strategies to enhance drug efficacy. Researchers in the field have called for further elucidation of the neural mechanisms underlying placebo analgesia, but research in humans places certain restrictions on this effort. The purpose of this study is to develop a novel animal model of placebo analgesia in order to allow for basic research of the underlying neural mechanisms.

1.1.1 Physiology of Placebo Analgesia

Early researchers understood that a placebo is capable of inducing behavioral responses similar to an active drug. However, it was unclear whether the placebo was activating physiological mechanisms or having an effect that was merely imagined (Pepper, 1945). A breakthrough came when Levine, Gordon, and Fields published evidence for common

mechanisms mediating both the response to active drug and to placebo. The finding demonstrated that a mere belief in efficacy invokes the same mechanism as the active drug (1978). In the Levine et al. study, data was obtained by antagonizing opioid systems with naloxone, a non-specific opioid receptor antagonist, which almost entirely eliminates a placebo's analgesic effect. The findings suggest that at least part of placebo-induced analgesia depends upon the action of endogenous opioids in the brain, the very same mechanism utilized by active analgesics. It is important to note, however, that the analgesia was not eliminated completely, suggesting that other mechanisms are likely to be involved. Levine's findings have been supported by later studies, one of which addressed the issue from the opposite perspective (Benedetti, 1996; Eippert et al., 2009). Benedetti showed that the potentiation of opioid activity from administration of proglumide, a cholecystokinin antagonist, is followed by a corresponding potentiation of the placebo's analgesic effect. Thus there is evidence that any modulation of endogenous opioid activity, whether up or down, is met with a corresponding change in placebo analgesia, making a clear argument in favor of a contribution of endogenous opioids to the placebo phenomenon. Eippert et al. used fMRI to gather more anatomically specific data and were able to show that naloxone decreased communication between the rostral anterior cingulate cortex (rACC) and periaqueductal gray (PAG), both of which play central roles in pain modulation and contain strong concentrations of μ-opioid receptors.

1.1.1.1 μ-opioid Receptors

Of the three subtypes of opioid receptors, μ-opioid receptors have the highest affinity for opiate drugs such as morphine, heroin, and codeine. It is not surprising then that μ-opioid receptors became the focus of research seeking to identify the mechanisms mediating placebo analgesia in the central nervous system. Efforts to map μ-opioid receptors in the brain and spinal cord have consistently indicated widespread distribution, but stronger concentrations occur in regions that process pain such as the medial thalamus, PAG, median raphe, and clusters within the spinal cord (Mansour & Watson, 1993; Mayberg & Frost, 1990). This finding

is consistent with the powerful pain-relieving effects of opiates (Wilson, Uhelski, & Fuchs, 2008). However, many other brain areas also show high densities of μ-opioid receptors such as the nucleus accumbens, brain stem, thalamus, and striatum (Mansour & Watson, 1993). Although most of these structures are not characterized by a role in pain-processing, their high receptor density is still consistent with opiate functions because of their contributions to positive reinforcement, reward, addiction, and respiratory depression.

1.1.1.2 Dopamine

Other mechanisms are implicated by the fact that opioid antagonism does not completely eliminate PA (Benedetti, 1996). Dopaminergic pathways are a reasonable candidate because of the rewarding nature of opiates, their potential for addiction, and the fact that the mesolimbic dopamine pathway is highly involved in both reward and addiction. PA activates dopaminergic nuclei in the brainstem and forebrain, and when a placebo successfully elicts an effect, the mesolimbic dopamine pathway in particular is activated (Petrovic, Kalso, Petersson, & Ingvar, 2002; Lidstone, Fuente-Fernandez, & Stoessl, 2005). Looking more closely, another study examined the binding potentials of two subtypes of dopamine receptors, D2 and D3, in the striatum, looking for associations between tactile sensitivity and placebo analgesia (Martikainen, et. al., 2005). Oddly, no association was found between binding potentials of striatal D2 or D3 receptors and PA. This finding seems contrary to current understanding, but it does not preclude the possibility that the striatum releases dopamine during PA, nor does it preclude the possibility that dopamine influences PA in other brain structures. Evidence from MRI studies has correlated the magnitude of a placebo's analgesic effect with gray matter density in the ventral striatum, insula, and prefrontal cortex, two of which are major structures in dopaminergic pathways (Schweinhardt, Seminowicz, Jaeger, Duncan, & Bushnell, 2009). The same study also correlated gray matter density in the ventral striatum and the prefrontal cortex to dopamine-related personality traits, which might be used to identify subjects with predispositions to a strong placebo response.

1.1.2 Theoretical Understanding of Placebo Analgesia

It is clear that the study of the placebo response, and placebo analgesia in particular, has adopted an increasingly physiological perspective, especially in the last twenty years. Nevertheless, a theoretical explanation of the effect was not immediately implicated by these data. To develop adequate theories, the contributions of expectation, experience, and conditioning, all of which are traditionally behavioral or cognitive topics, must also be considered. Indeed, a comprehensive explanation of PA demands the recognition of physiological as well as behavioral and cognitive data. Theorists generally tended to divide into two camps: expectancy theory and conditioning theory.

1.1.2.1 Expectancy Theory

It has been demonstrated that placebo analgesia is a function of the expectation of drug efficacy (De Pascalis, Chiaradia, & Carotenuto, 2002). However, expectancy alone cannot account for all of a placebo's effect, as evidenced by the fact that even patients who were told they would be given an inactive substance still reported significant decreases, up to fifty percent, in pain ratings (Zubieta, Yau, Scott, & Stohler, 2006). One of the ways to induce expectancy is through experience with the active drug. The contribution of experience to PA has been explored in studies that asked whether a placebo could elicit an effect without prior experience with active drug (Benedetti, et al., 1998; Colloca & Benedetti, 2006). The semi-synthetic opiate buprenorphine was given to lung lobectomy patients after surgery, and on the following day, buprenorphine was replaced with a placebo. Results showed that positive prior experience with an opiate analgesic is critical for future placebo effectiveness, suggesting that PA is not innate and depends upon a critical learning process. This is a critical finding because it demonstrates that experience with active drug is necessary for a placebo to have an effect. Altogether, evidence suggests that a placebo effect can be induced by expectancy and is dependent upon positive prior experience. The role of experience and expectancy may not be

mutually exclusive though since an expectation can be learned or conditioned through experience.

1.1.2.2 Conditioning Theory

Consistent with this idea is yet another theory which argues that the placebo effect is the result of conditioning, and there is a considerable amount of research to support this perspective (Voudouris, Peck, & Coleman, 1989; Wickramasekera, 1980; Wolf, 1950). Since conditioning, expectancy, and experience are intertwined, some theorists recommend that these factors not be dissociated because each theory may be describing the same phenomenon in different ways (De Pascalis, et. al., 2002; Wall, 1992). In the debate between expectancy theory and conditioning theory, there is evidence that conditioning plays the more substantial role in eliciting the placebo effect (Voudouris, Peck, & Coleman, 1990). In one study, human subjects were conditioned to believe in the efficacy of a treatment by repeatedly pairing an inert treatment with an electrical shock that had been surreptitiously lowered from the subject's baseline threshold (De Pascalis, et. al., 2002). The procedure induced expectation of analgesia with verbal instructions and allowed for experience with what appeared to be successful analgesia. Results showed decreased pain ratings after placebo treatment in high and medium suggestibility groups, indicating that a placebo effect can be conditioned. The role of conditioning in PA was further explored in a recent *f*MRI study which identified brain structures active specifically during the experience of placebo analgesia (Lui, Colloca, Duzzi, Anchisi, Benedetti, & Porro, 2010). In this experiment, subjects were cued with a colored light to indicate whether they would receive an analgesic treatment (red: no analgesia; green: placebo analgesia) following a painful laser stimulation to the dorsal surface of one foot. Results showed that the dorsolateral prefrontal cortex (DLPFC), especially in the right hemisphere, as well as the medial prefrontal cortex (MPFC) showed greater activity during green light (placebo analgesic) trials than during red light (non-analgesic) trials. This study provides further evidence that PA can be conditioned. It also suggests that the combination of data from

different techniques could provide further insight and calls for more research exploring underlying neural mechanisms.

1.1.3 Psychosocial Factors in Placebo Analgesia

The impact of psychosocial factors on the conditioning process has also been demonstrated. In 1997, Johnson and Din found that Asian participants had a greater response to placebo treatment of experimental pain than White participants. Cheing speculated that the effect might be due to higher cultural pressure in Asian countries to submit to authority figures like doctors and researchers who conduct psychological studies (Cheing & Cheung, 2002). Another study was able to show that social observational learning was much more effective at inducing a placebo response than verbal instructions, which shows that the quality of the experience with the drug effects can influence the magnitude of placebo (Colloca & Benedetti, 2009). Due to the differential magnitude of placebo effect between subjects and unpredictable fluctuations of the effect within individuals, other researchers have focused their efforts on identifying predictors of placebo response. For example, high dispositional optimism and low state anxiety were identified as significant predictors (Morton, Watson, El-Deredy, & Jones, 2009).

1.1.4 The Practical Utility of the Placebo Effect

While the statistical significance of placebo analgesia is well established, it has been criticized for its limited clinical significance. For example, deceiving chronic pain patients into using a placebo to treat their symptoms would clearly be unethical (Hróbjartsson & Gøtzsche, 2006). Attempts to justify placebo as a legitimate treatment method are specious, however, the placebo is still clinically relevant, especially to the cognitive modulation of pain and to the explanation of individual differences in response to pain and analgesic medications. Placebo research may also lead to cognitive strategies for use in conjunction with more conventional pain relief treatments. Conditioning has already been shown to effectively train the brain to modulate peripheral immune system reactivity, a consequence of which is enhanced organ

functioning (Pacheco-Lopez, Engler, Niemi, & Schedlowski, 2006). Still, more research is needed before practical clinical strategies can be developed and implemented effectively.

1.1.5 The Placebo Effect in Animal Research

The use of animals in research specifically investigating placebos has been limited, but the evidence available suggests that an animal model of the placebo effect is possible and desirable for the advancement of veterinary medicine (McMillan, 1999). The limited use of animals in placebo research reflects, in part, an assumption by theorists that the effect is an inherently human phenomenon, which is understandable given the necessity of deceit in the methodology and the contributions of expectancy, experience, and conditioning. However, none of those factors precludes the use of an animal model, particularly given the important contribution of conditioning to the placebo effect. The only difficulty is that an animal does not comprehend verbal instructions and therefore cannot be verbally deceived, so the mechanism for inducing expectation must be non-verbal. Conditioning paradigms can serve this purpose, so a reliable conditioning procedure is the key to an animal model of placebo analgesia. Pavlov acknowledged that the effects of morphine could be conditioned, and Skinner proposed that the placebo effect was a conditioned response (Pavlov, 1927; Skinner, 1953). In this view, the inert placebo treatment is the conditioned stimulus (CS), which, after repeated pairings with an unconditioned stimulus (UCS) such as an active drug treatment, elicits a conditioned response (CR). In the few animal studies that have been conducted, placebo treatments have reportedly induced learning deficits, hyperactivity, vomiting, defecation, increased sleep, and acute hyperglycemia (Hernnstein, 1962; Pihl and Altman, 1971; Metalnikov and Chorine, 1926; Siegel, 1975). With certain drugs, for example d-amphetamine sulfate, a single dose provided sufficient exposure to allow for placebo-induced hyperactivity (Ross, 1963). These findings, however sparse, provide enough evidence to suggest that conditioning, experience, and expectancy play a large enough role to warrant use of animals in placebo research (Pacheco-Lopez, et. al., 2006; McMillan, 1999). Many researchers have called for further elucidation of neural

mechanisms underlying the placebo effect (Benedetti & Amanzio, 1997; Pacheco-Lopez, et. al., 2006; Cheing & Cheung, 2002; Craggs, Price, Verne, Perlstein, & Robinson, 2007; Lui, et. al., 2010). The practical and ethical restrictions in human studies limit the techniques that can be used in brain research, making an experimental model in animals advantageous because those restrictions would be eased. Specifically, an animal model would make possible lesion studies and a number of histological assays that examine protein expression indicative of brain activity.

1.1.6 Summary

In summary, the study of the placebo effect in general, and placebo analgesia in particular, has evolved substantially in the last two decades and its implications might prove to have clinical utility. The fact that dopaminergic and μ-opioid mechanisms can be modulated by a mere belief in drug efficacy is a key discovery that may allow for the study of other cognitively mediated neurophysiological events. The role of conditioning in the placebo effect and the call for research to elucidate underlying neural mechanisms provides a rationale for the development of an animal model.

1.2 Rationale for An Animal Model of Placebo Analgesia

1.2.1 Purpose and Hypothesis

To the author's knowledge, there is currently no animal research exploring specifically the analgesic effects of placebo treatment. Moreover, there is not yet a standard animal model for studying placebo effects, analgesic or otherwise. The proposed study aimed to develop an animal model for the study of conditioned placebo analgesia. It was expected that, in rats with a chronic neuropathic pain condition, a single inert vehicle treatment occurring after repeated treatments with gabapentin would decrease hypersensitivity in a way that models the characteristics of the human conditioned placebo analgesic response.

1.2.1 Compensatory Response Theory of Tolerance

On the other hand, it was also possible that, after repeated treatments with gabapentin, the vehicle would elicit increased nociception. The compensatory response theory of tolerance

offers one theoretical explanation. This is the theory used to explain the heroin "overdose" phenomenon whereby a user dies from their standard dose in a novel environment (Siegel, Hinson, Krank, & McCully, 1982). Research has shown that, after repeated morphine administration, a dose of saline can appear to increase measures of pain (Siegel, 1975). This finding is in direct opposition to the placebo effect. The compensatory response explanation for this unusual finding is that the administration of certain drugs, including morphine, is countered in the body by a change in homeostasis. This homeostatic change is viewed as a conditioned response (CR) in anticipation of drug administration, and, after conditioning, can be elicited by the associated environmental stimuli alone. In the case of morphine anticipation, it is assumed that the homeostatic change is a hyperalgesic CR, an effect that is not attenuated by saline. When saline is injected, the hyperalgesic CR occurs without the morphine-induced analgesia. This is why the administration of saline appears to result in an increase of pain.

If the compensatory response model were applied to the proposed experiment, the regimen of daily gabapentin followed by a dose of vehicle would produce an increase in hypersensitivity. This outcome was not likely because the compensatory response theory of tolerance is contingent upon the treatment inducing a homeostatic change. Gabapentin was chosen because its mechanism of action is not known to disrupt homeostatic mechanisms and does not induce a high degree of tolerance such as that seen following repeated morphine administration. However, if the vehicle had produced an increase in hypersensitivity following gabapentin administration, the compensatory response theory would provide a compelling explanation.

CHAPTER 2

METHODS

2.1 Subjects

38 female Sprague-Dawley rats from the University of Texas at Arlington vivarium, weighing approximately 200-300g, were used in the study. Animals were housed in groups of 3-5 and maintained on a 12:12 hour light/dark cycle with free access to food and water throughout the study. Prior to investigation, all procedures were approved by the University of Texas at Arlington Institutional Animal Care and Use Committee and were in accordance with the guidelines put forth by the International Association for the Study of Pain (Zimmermann, 1983).

2.2 Induction of the Neuropathic Pain Condition

On Day 1, all animals received a unilateral left-side L5 spinal nerve ligation as described previously (Kim & Chung, 1992). Animals were anesthetized with 3% isoflurane for induction and 2% for maintenance. Depth of anesthesia was confirmed by the absence of the eye blink reflex as well as the withdrawal reflex to pinch stimulation of the hindpaws. The animal's health was monitored throughout anesthesia with periodic checks of breathing rate and reflexive responses. The incision area was shaved and then cleaned with povidone-iodine. The first incision was 1.5-2 inches in length and was 2-3 millimeters lateral and to the left of the spinal cord. Muscle tissue was removed to expose the overlying transverse process, which was also removed. The L5 spinal nerve was exposed and tightly ligated using 6-0 silk thread. Povidone-iodine was applied once again to the wound before suturing the internal tissue with 4-0 silk thread and closing the overlaying skin with surgical staples. Animals were allowed to recover for three full days (Days 2-4) before any behavioral tests were conducted. Post-operative signs of infection or discomfort were closely monitored during the recovery phase as

well as during behavioral testing. The L5 spinal nerve ligation model of neuropathic pain was chosen for this study because the resulting condition is chronic in nature and is known to be treatable with gabapentin (Kim & Chung, 1992; LaBuda & Little, 2005).

2.3 Drug Preparation

Gabapentin was mixed at a concentration of 90 mg/ml in a .9% normal saline solution and administered subcutaneously (s.c.) at a dosage of 90 mg/kg. This concentration, dosage, and route of administration for gabapentin has been shown to reliably attenuate sensory pain thresholds as measured by the mechanical paw withdrawal threshold test (LaBuda & Fuchs, 2000). The dosage of saline was the same volume as a dose of gabapentin in order to maintain maximal similarity between treatments. Like gabapentin, saline was also administered subcutaneously.

2.4 Measurement of Tactile Allodynia

To assess mechanical hypersensitivity, a Mechanical Paw Withdrawal Threshold (MPWT) test was conducted before surgery, after the surgical recovery period, and each day 60 minutes after the treatment injection. To derive MPWT scores, animals were placed in a Plexiglas chamber (20x10.5x40.5 cm) and habituated for 10 minutes. The chamber was positioned on top of a mesh screen so that tactile stimuli could be administered to the plantar surface of both hindpaws. Tactile sensitivity for each hindpaw was measured using the up/down method (Dixon, 1980) with eight von Frey monofilaments (3.91, 5.91, 9.97, 19.81, 38.82, 78.14, 141.99, and 239.04 mN). Each trial began with a von Frey force of 9.97 mN first delivered to the right hindpaw for approximately 1 s, then the left hindpaw. The next highest force was delivered if there was no withdrawal response, while the next lowest force was delivered if there was a response. This procedure was repeated until no response was made at the highest force (239.04 mN) or until four stimuli were administered following the initial response. The 50% paw withdrawal threshold for each trial was calculated using the following formula: [*X*th]log=[*vFr*]log+k*y* where [*vFr*] is the force of the last von Frey used, k=0.2591 which

is the average interval (in log units) between the von Frey monofilaments, and *y* is a value that depends upon the pattern of withdrawal responses (Dixon, 1980). When an animal did not respond to the highest von Frey monofilament (239.04 mN), then y=1.00 and the 50% mechanical paw withdrawal response for that paw was calculated to be 447.20 mN. This procedure was performed three times and averaged to determine the mean threshold to tactile stimulation for the right and left paws of each animal.

Following the recovery period, animals underwent a pre-treatment MPWT test on Day 5 (Day 5 Pre) in order to confirm the development of a neuropathic pain condition. The pre-test was critical because roughly 15% of animals that undergo the standard L5 SNL procedure do not develop hypersensitivity, and the present study requires all animals to be hypersensitive. Two animals were excluded from further testing and data analysis due to a Day 5 Pre MPWT that failed to fall below 50% of the animal's baseline score. In order to ensure that the pre-treatment MPWT test did not interfere with the conditioning procedure, it was conducted in a separate room without the neutral stimuli of the conditioning procedure.

2.5 Conditioning Procedure

Animals not excluded by the Day 5 Pre MPWT were randomly assigned to one of four injection conditions: gabapentin-throughout, saline-throughout, gabapentin with Day 9 saline, and saline with Day 9 gabapentin. Each animal then began a 4-day conditioning process [Days 5(post)-8] in which they received daily injections according to their treatment condition. Five sensory cues (NS) were incorporated into the conditioning procedure to maximize the association between the injection process and analgesia. Figure 1 depicts how the components of this experiment correspond with classical conditioning terminology. First, all injections took place in a separate room, providing an environmental cue. A temporal cue was incorporated by administering all injections at the same time each day. In order to establish olfactory cues, a Glade® plugIns® scented oil warmer with Glade® plugIns® French Vanilla scented oil was activated in the conditioning room during the injection process and a single experimenter with a

unique olfactory signature administered the daily injections. A visual cue was incorporated by illuminating the injection room with a single General Electric Reveal®, enhanced spectrum, incandescent light bulb in an upward projecting, indirect lamp. Tactile cues were provided by the use of a terry cloth towel, which was used to wrap the animals for injecting while on a hard, flat surface that they had never experienced outside the context of the injection procedure. Animals were also left in the injection room for 60 minutes while the treatment took effect.

On Day 9, the procedure was the same as described above with the exception that two of the groups changed treatments. Day 9 therefore served as a test trial in that, if a placebo effect had been conditioned, this was the first opportunity to test and detect it. The placebo effect or conditioned response (CR) was defined as a significant difference in Day 9 MPWT between the gabapentin/saline and saline/saline groups. A nocebo effect, also considered a CR, was defined as a significant difference in Day 9 MPWT between the saline/gabapentin and gabapentin/gabapentin.

Following Day 9 test day, the daily procedure continued through Day 14 with each group receiving the same treatment administered on Day 9. Days 10-14 therefore served as a follow-up phase in which, if a CR was detected (nocebo or placebo), the dissipation pattern could be tracked. A timeline of the methodology is provided in Figure 2.

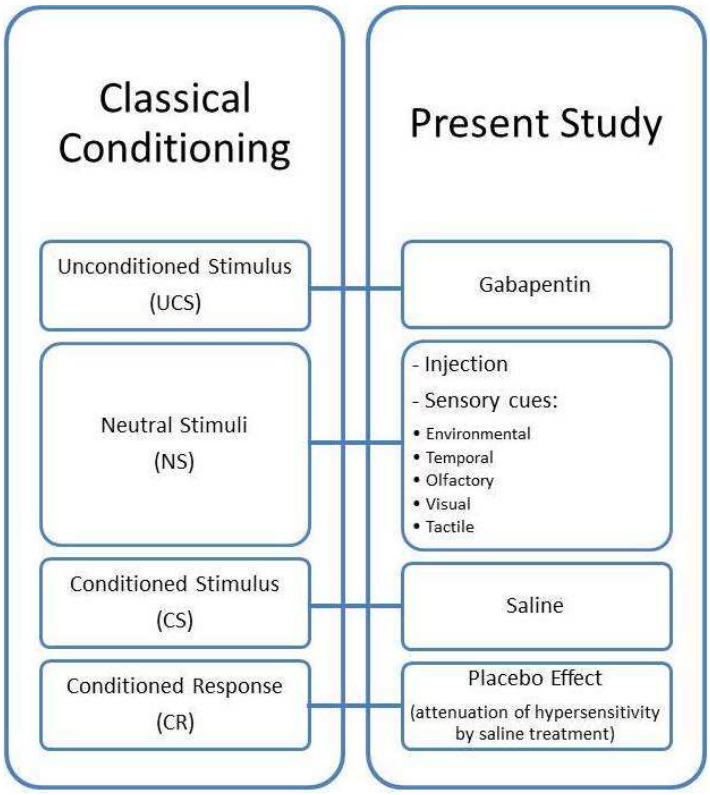

Figure 1 Classical Conditioning vs. Present Study Diagram

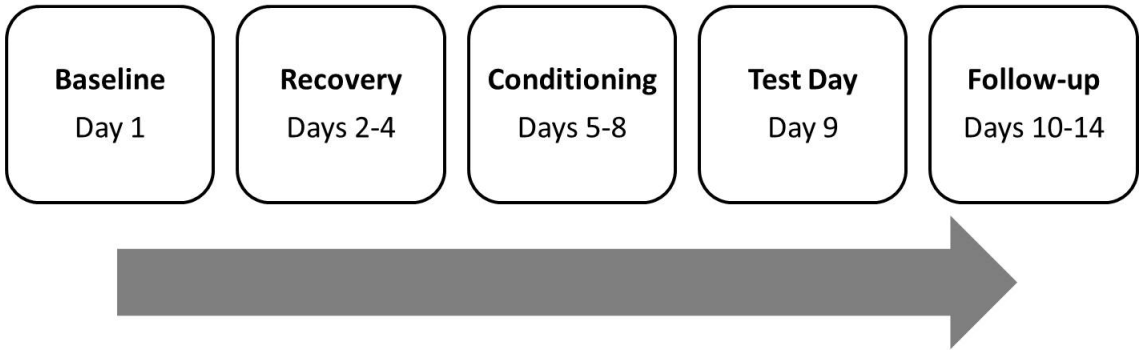

Figure 2 Basic timeline of the study

CHAPTER 3

RESULTS

3.1 Overall Results

A mixed-design, repeated measures ANOVA was used to analyze the left paw MPWT data. As expected, there was a significant main effect for Day [$F(11,374) = 42.161$, $p < .001$] and Treatment [$F(3,34) = 70.193$, $p < .001$], indicating that MPWT's differed depending upon the day of treatment and upon the treatment regimen that was administered. There was also a significant Day by Treatment interaction [$F(33, 374) = 31.09$, $p < .001$].

3.2 Post-hoc Analysis at Baseline and Day 5 Pre

Post-hoc analysis of left paw MPWT (see Figure 3) revealed no differences between any treatment groups at baseline ($p < .05$) nor at the Day 5 pre-treatment test ($p < .05$). The mean threshold score for all treatment groups at baseline was 431.21 mN, which is very near the ceiling value of 447.2 mN. This confirms that, prior to experimentation, no animals were experiencing mechanical hypersensitivity from any naturally occurring conditions. The mean threshold score for all treatment groups at the Day 5 pre-treatment test was 96.32 mN, indicating a significant drop in MPWT from baseline ($p < .001$). This difference between baseline and Day 5 pre-treatment test confirms that the experimental pain condition was effective. Taken together, these two findings provide crucial controls for the experiment: all animals included in the study were not hypersensitive prior to the experiment, the experimental pain condition was effective, and the experimental pain model was the only pain condition influencing the results.

3.3 Post-hoc Analysis during Conditioning Phase

On every day of the conditioning phase (Day 5 post-treatment, Day 6, Day 7, and Day 8), each group receiving gabapentin demonstrated a significantly higher MPWT than each group receiving saline ($p < .001$). This shows that gabapentin induced a powerful and reliable attenuation of mechanical hypersensitivity and that saline does not.

3.4 Post-hoc Analysis on Test Day

Post-hoc analysis of Day 9 test day did not reveal a significant difference between the gabapentin/saline and the saline/saline groups ($p > .05$), demonstrating that the conditioning paradigm with gabapentin was not effective at inducing a conditioned placebo analgesic response. Also of interest on Day 9 was the saline/gabapentin group. There was not a significant difference between saline/gabapentin and gabapentin/gabapentin ($p > .05$), indicating that the conditioning paradigm also failed to induce a nocebo effect.

3.5 Post-hoc Analysis during Follow-up Phase

Results from the follow-up phase (Days 10-14) were similar to the conditioning phase in that gabapentin effectively attenuated hypersensitivity on each day while saline did not. Specifically, on every day of the follow-up phase, each group that was receiving gabapentin demonstrated a significantly higher MPWT than each group receiving saline ($p < .001$). This shows that the effectiveness of gabapentin analgesia is not modulated by the effects of daily, repeated administration within the present conditioning paradigm.

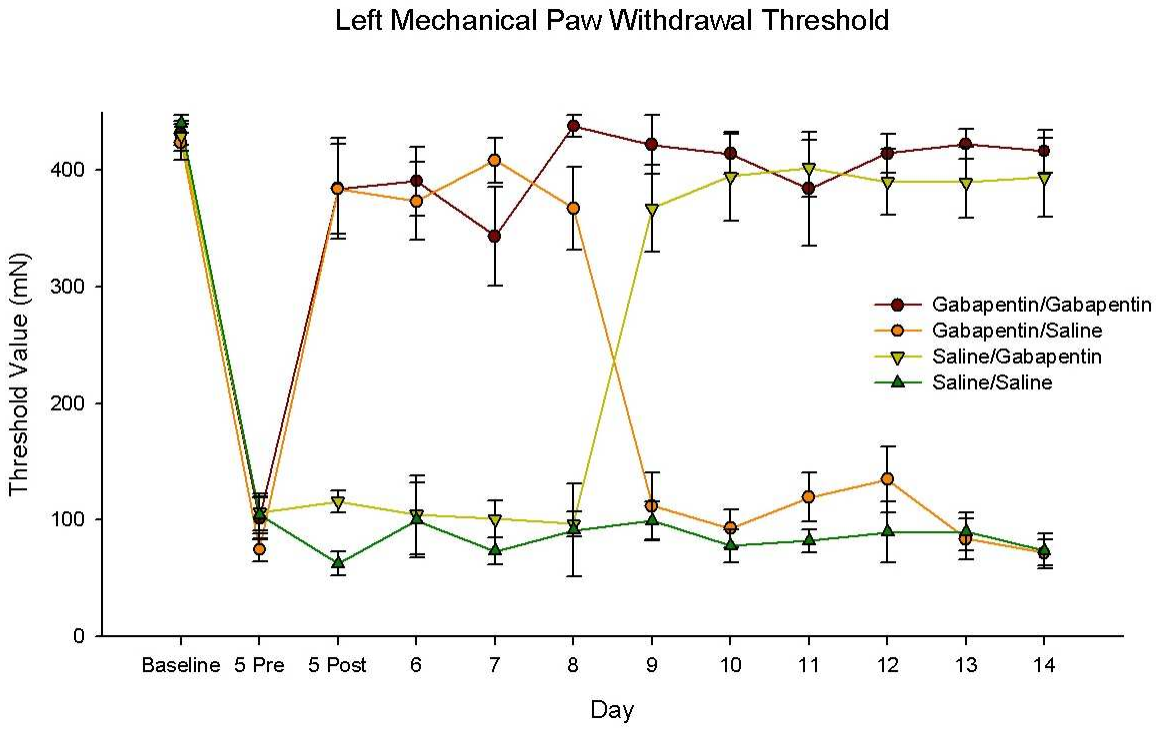

Figure 3 Left Paw Mechanical Paw Withdrawal Threshold across Time

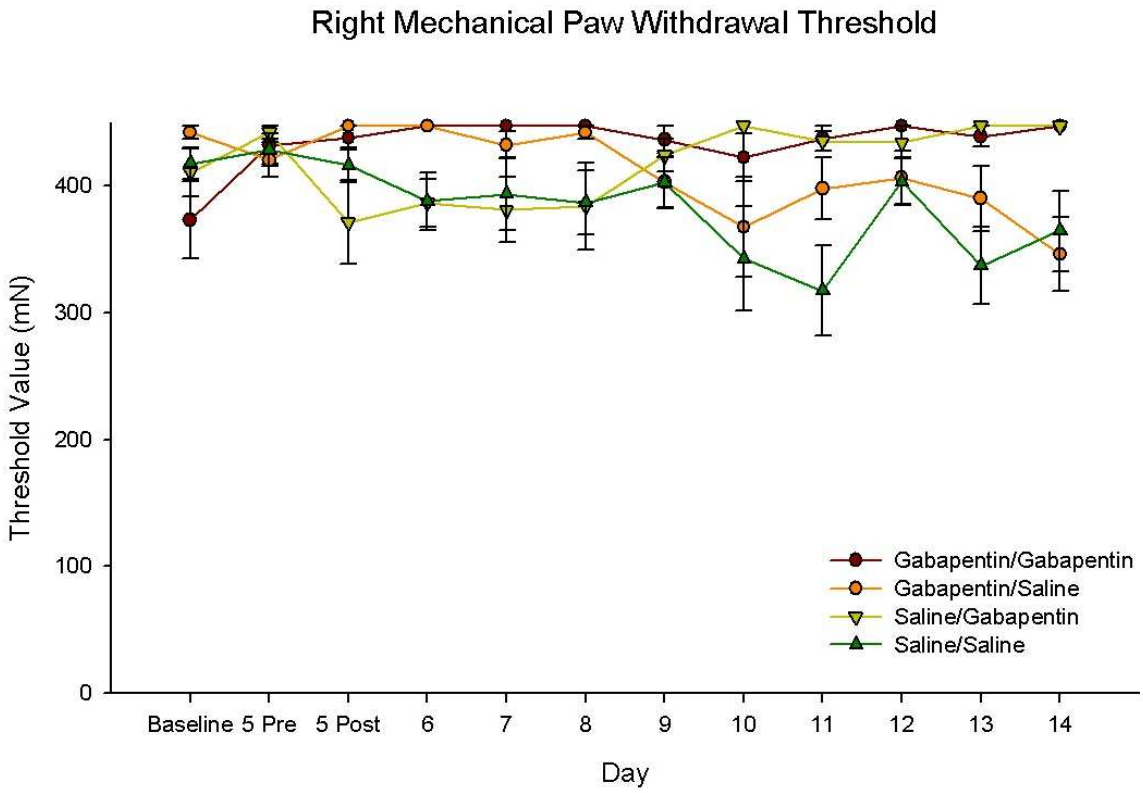

Figure 4 Right Paw Mechanical Paw Withdrawal Threshold across Time

CHAPTER 4

DISCUSSION

4.1 Overview of Results

The purpose of this study was to develop an animal model of conditioned placebo analgesia in order to elucidate underlying neural mechanisms using techniques that are not possible in human research. This experiment revealed a wide array of crucial information toward that end. Firstly, the L5 spinal nerve ligation was very effective at inducing a considerable amount of mechanical hypersensitivity that was dramatically different from the baseline state of the animal. This difference from the baseline state is advantageous because it allows for a wide window in which the treatments can exert their effects. Secondly, gabapentin was very effective at attenuating mechanical hypersensitivity in the neuropathic pain condition, and saline was not. The effectiveness of the treatments created a dramatic difference between the hypersensitive and analgesic states used in the study. These two disparities (between baseline and hypersensitive states and between control and analgesia treatments) are critical components of the paradigm because they increase the likelihood of detecting a placebo effect, and therefore they are elements of the methodology that should be retained in future experiments (Benedetti et. al., 1998).

4.2 Theoretical Explanations for the Results

The findings of this experiment are somewhat surprising given the preponderance of literature citing the influence of conditioning on the analgesic response. The literature provides some clues as to why these findings did not detect a conditioned analgesic effect. Expectancy theory has shown that experience with an active and effective drug is necessary to elicit a placebo response. This criterion is satisfied in our study as demonstrated by the consistent and powerful efficacy of gabapentin during the conditioning days. This suggests that the protocol

should have successfully induced an expectation of analgesia, and therefore, expectancy is unlikely to be an element of the experiment that contributed to the lack of a placebo effect.

Certain elements of conditioning theory reveal a better explanation for the results of this study. Prior studies have repeatedly demonstrated that the learned association between active drug (UCS) and placebo treatment (NS/CS) is critical to elicit a placebo effect. This association depends on and can be modulated by a number of methodological factors, but perhaps the most important factor concerning this experiment is contiguity. Contiguity refers to the temporal distance between the NS and UCS. In classical conditioning paradigms, this relationship is inversely proportional to the strength of the learned association between them (Pavlov, 1927; Jones, 1962). In the present experiment, the injection (UCS) was presented simultaneously with all of the NS sensory cues, satisfying the contiguity principle and increasing the likelihood of a strong CR. This suggests that the contiguous UCS-NS relationship was unlikely to be the reason for the lack of a placebo effect. However, the relationship between the injection (UCS) and analgesia (UCR) was not as contiguous because the injection occurred 60 minutes prior to the analgesic response. Since this is a UCS-UCR relationship, it does not directly violate the contiguity principle, which specifically describes the UCS-NS relationship. However, the 60-minute latency period is quite long for a conditioning paradigm, and it might have diminished the CR by weakening the association between the injection and the other sensory cues. This appears to be a compelling explanation for the lack of a placebo effect in spite of the fact that it does not directly violate the principle of contiguity. It seems reasonable to conclude that the lack of a placebo response was due to inadequacies within the conditioning paradigm, that the latency time between injection and analgesia is too long, and that this weakened the likelihood of developing conditioned placebo analgesia.

4.3 Possible Implications for Classical Conditioning

These findings may hold interesting implications for classical conditioning. If the lack of UCS-UCR contiguity was weakening the CR while the simultaneous UCS-NS exposure was

strengthening the CR, the results imply that the temporal relationship between the UCS-UCR has a stronger influence than that of the UCS-NS on the magnitude of conditioned analgesic responses. Alternatively, it might be that the onset of the NS rather than their presence must be closer in time to the UCR in order to elicit a CR. Future research may be able to parse out these relationships between stimuli and responses in order to determine which explanation is more appropriate.If your work has more than five chapters, repeat the previous chapter text selection and copy operations and chapter paste and rename processes until you have created a template that has as many chapters as your work has.

4.4 Evidence from Odor Aversion Literature

Another possible explanation for the failure to induce conditioned placebo analgesia comes from a serendipitous finding in odor aversion research, which showed that the effect of an olfactory stimulus can be potentiated by the addition of a taste stimulus (Bowman, Batsell, & Best, 1992). It was originally believed that a taste cue would compete with or overshadow the odor cue, but results showed that there was actually a synergistic effect between the two cues such that the reaction to the olfactory cue was potentiated by the presentation of an aversive taste cue. These experiments are considerably d1ifferent from the conditioned placebo analgesia paradigm; however, the introduction of a taste cue might have the same effect and increase the likelihood of eliciting a CR. This provides one future direction for the pursuit of an animal model of conditioned placebo analgesia.

4.5 Methodological Advantages of the Paradigm

It is clear that changes must be made to the conditioning paradigm, but as the development of this model continues in future experiments, it will be critical to note the methodological elements that worked well and should not be changed. Most importantly, the classic format of conditioning, test day, and follow-up appears to be well suited to answering the questions inherent to the pursuit of conditioned placebo analgesia. However, the length of these periods, especially the number of conditioning trials, might be adjusted to suit the needs

of future experiments. The general health of the animal was not a confound within this paradigm, and future adjustments to the protocol should attempt to retain this attribute. Body weight was quite stable throughout all phases of the procedure (data not shown), the injection site did not become hardened, bloody, or unhealthy in any other way, and observations of all characteristic behaviors such as eating, drinking, sleeping, grooming, social interaction, play, and locomotion were all stable within rats and between groups throughout the protocol. While these are merely qualitative observations, they are crucial to the success of the protocol.

4.6 Conclusion

This experiment represents a significant step towards the development of an animal model of conditioned placebo analgesia. Given that placebo effects have been documented in animals and that placebo analgesia is possible in humans, the development of an animal model of conditioned placebo analgesia seems wholly plausible. Future iterations of this paradigm should focus on improving the parameters of the conditioning procedure as these are the most likely reasons for the lack of a placebo effect in this study. Above all, the utility of an animal model of conditioned placebo analgesia should be kept in mind, for its development would make possible a host of new methodologies to placebo research and would advance the study of the cognitive component of pain processing.

REFERENCES

Benedetti, F. (1996). The opposite effects of the opiate antagonist naloxone and the cholecystokinin antagonist proglumide on placebo analgesia. *Pain*, *64*(3), 535-543.

Benedetti, F. & Amanzio, M. (1997). The neurobiology of placebo analgesia: from endogenous opioids to cholecystokinin. *Progress in Neurobiology*, *51*, 109-125.

Benedetti, F., Amanzio, M., Baldi, S., Casadio, C., Cavallo, A., Mancuso, M., et al. (1998). The specific effects of prior opioid exposure on placebo analgesia and placebo respiratory depression. *Pain*, *75*, 313–319.

Bowman, M. T., Batsell, W. R., & Best, M. R. (1992). Evidence that stimulus generalization does not determine taste-mediated odor potentiation. *Bulletin of the Psychonomic Society*, *30*(3), 241-243.

Cheing, G. L. Y. & Cheung, K. S. H. (2002). Placebo analgesia: Clinical considerations. *Physiotherapy*, *88*, 735-743.

Colloca, L. & Benedetti, F. (2006). How prior experience shapes placebo analgesia. *Pain*, *124*, 126–33.

Colloca, L. & Benedetti, F. (2009). Placebo analgesia induced by social observational learning. *Pain*, *144*, 28-34.

Craggs, J. G., Price, D. D., Verne, G. N., Perlstein, W. M., & Robinson, M. M. (2007). Functional brain interactions that serve cognitive–affective processing during pain and placebo analgesia. *NeuroImage*, *38*, 720–729.

De Pascalis, V., Chiaradia, C., & Carotenuto, E. (2002). The contribution of suggestibility and expectation to placebo analgesia phenomenon in an experimental setting. *Pain*, *96*, 393–402.

Dixon, W. J. (1980). Efficient analysis of experimental observations. *Annual Review of Pharmacology and Toxicology, 20*, 441-462.

Eippert, F., Bingel, U., Schoell, E., Yacubian, J., Klinger, R., Lorenz, J., et al. (2009). Activation of the opioidergic descending pain control system underlies placebo analgesia. *Neuron*, *63*, 533–543.

Hernnstein, R. J. (1962). Placebo effect in the rat. *Science, 138*(3541), 677-678.

Hróbjartsson, A. & Gøtzsche, P. C. (2006). Unsubstantiated claims of large effects of placebo on pain: Serious errors in meta-analysis of placebo analgesia mechanism studies. *Journal of Clinical Epidemiology*, *59*, 336–338.

Johnson, M. & Din, A. (1997). Ethnocultural differences in the analgesic effects of placebo transcutaneous electric nerve stimulation on cold-induced pain in healthy subjects: a preliminary study. *Complementary Therapies in Medicine*, *5*, 74-79.

Jones, J. (1962). Contiguity and reinforcement in relation to CS-UCS intervals in classical aversive conditioning. *Psychological Review*, *69*(3), 176-186.

Kim, S. H. & Chung, J. M. (1992). An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. *Pain*, *50*, 255-263.

Lasagna, L., Mosteller, F., von Felsinger, J. M., & Beecher, H. K. (1954). A study of the placebo response. *The American Journal of Medicine, 16*(6), 770-779.

Levine, J. D., Gordon, N. C., & Fields, H. L. (1978). The mechanism of placebo analgesia. *The Lancet*, *312*(8091), 654-657.

Lidstone, S. C., de la Fuente-Fernandez, R., & Stoessl, A. J. (2005). The placebo response as a reward mechanism. *Seminars in Pain Medicine*, *3*(1), 37-42.

Lui, F., Colloca, L., Duzzi, D., Anchisi, D., Benedetti, F., & Porro, C. A. (2010). Neural bases of conditioned placebo analgesia. *Pain, 151*, 816-824.

Mansour, A. & Watson, S. J. (1993). Anatomical distribution of opioid receptors in mammalians: An overview. *Opioids I*, Handbook of Experimental Pharmacology, *104* (A. Herz, Ed.), 79-106.

Martikainen, I. K., Hagelberg, N., Mansikka, H., Hietala, J., Nagren, K., Scheinin, H., et al. (2005). Association of striatal dopamine D2/D3 receptor binding potential with pain but not tactile sensitivity or placebo analgesia. *Neuroscience Letters*, *376*, 149–153.

Mayberg, H. S. & Frost, J. J. (1990). Opiate receptors. *Quantitative Imaging: Neuroreceptors, Neurotransmitters, and Enzymes*, 81-95.

McMillan, F. D. (1999). The placebo effect in animals. *Journal of the American Veterinary Medical Association, 215*, 992-999.

Metalnikov, S., Chorine, V. (1926). The role of conditioned reflexes in immunity. *Annales de l'Institut Pasteur, 40*, 893-900.

Morton, D. L., Watson, A., El-Deredy, W., & Jones, A. K. P. (2009). Reproducibility of placebo analgesia: Effect of dispositional optimism. *Pain*, *146*, 194–198.

Pacheco-Lopez, G., Engler, H., Niemi, M., & Schedlowski, M. (2006). Expectations and associations that heal: Immunomodulatory placebo effects and its neurobiology. *Brain, Behavior, and Immunity*, *20*, 430–446.

Pavlov, Ivan. (1927). Conditioned Reflexes: An Investigation of the Physiological Activity of the Cerebral Cortex. ed. Oxford University Press, Cambridge.

Pepper, O. H. (1945). A note on the placebo. *American Journal of Pharmacy*, *117*, 409-412.

Petrovic, P., Kalso, E., Petersson, K. M., & Ingvar, M. (2002). Placebo and opioid analgesia—imaging a shared neuronal network. *Science*, *295*, 1737–1740.

Pihl, R. O. & Altman, J. (1971). An Experimental Analysis of the Placebo Effect. *The Journal of Clinical Pharmacology, 11*(2), 91-95.

Ross, S. S. S. B. (1963). Further support for the placebo effect in the rat. *Psychological Reports. 13*, 461-462.

Schweinhardt, P., Seminowicz, D. A., Jaeger, E., Duncan, G. H., & Bushnell, M. C. (2009). The anatomy of the mesolimbic reward system: A link between personality and the placebo analgesic response. *The Journal of Neuroscience*, *29*(15), 4882-4887.

Siegel, S. (1975). Conditioning insulin effects. *Journal of Comparative Physiological Psychology, 89*, 189-199.

Siegel, S. (1975). Evidence from rats that morphine tolerance is a learned response. *Journal of Comparative Physiological Psychology, 89*(5), 498-506

Siegel, S., Hinson, R. E., Krank, M. D., & McCully, J. (1982). Heroin “overdose” death: Contribution of drug-associated environmental cues. *Science, 216*, 436-437.

Skinner, B. F. (1953). Science and Human Behavior. ed. The McMillan Company, London.

Voudouris, N. J., Peck, C. L., & Coleman, G. (1989). Conditioned response models of placebo phenomena: Further support. *Pain*, *38*, 109-116.

Voudouris, N. J., Peck, C. L., & Coleman, G. (1990). The role of conditioning and verbal expectancy in the placebo response. *Pain*, *43*, 121-128.

Wall, P. D. (1992). The placebo effect: An unpopular topic. *Pain*, *51*(1), 1-3.

Wickramasekera, I. (1980). A conditioned response model of the placebo effect: Predictions from the model. *Biofeedback and Self-regulation, 5*(1), 5-18.

Wilson, H. D., Uhelski, M. L., Fuchs, P. N. (2008). Examining the role of the medial thalamus in modulating the affective dimension of pain. *Brain Research, 1229*, 90-99.

Wolf, S. (1950). Effects of suggestion and conditioning on the action of chemical agents in human subjects- The pharmacology of placebos. *The Journal of Clinical Investigation*, *29*(1), 100-109.

Zimmermann, M. (1983). Ethical guidelines for investigations of experimental pain in conscious animals [editorial]. *Pain, 16*(2), 109-110.

Zubieta, J., Yau, W., Scott, D. J., Stohler, C. S. (2006). Belief or Need? Accounting for individual variations in the neurochemistry of the placebo effect. *Brain, Behavior, and Immunity*, *20*, 15–26.

BIOGRAPHICAL INFORMATION

Christopher T. McNabb has been researching pain processing in the central and peripheral nervous systems as a Graduate Teaching Assistant at the University of Texas at Arlington since 2009. His laboratory experience under the mentorship of Dr. Perry Fuchs has given him extensive exposure to preclinical behavioral methodologies used to assess pain, anxiety, memory, and cognitive abilities in rodents. Christopher's other laboratory experience includes collaborations with faculty at the University of Texas Southwestern Medical School, other neuroscience faculty at UTA, and contract experiments for pharmaceutical companies which assess experimental compounds that may later move on to clinical trials in humans. He also enjoys the rewarding nature of teaching undergraduate courses. Prior to graduate study, Christopher earned a Bachelor's Degree in Music Education with a minor in Psychology from Texas Christian University. His subsequent years as a public school band director primed his interest in the profound effects of sensory neuroscience, which later developed into an interest in the underlying physiological mechanisms, analgesia, and the placebo effect. He hopes to continue research within the field of placebo analgesia and pursue a career in academia.