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Title of Dissertation: Conditioned Pain Modulation in Children: The Effects of Painful and Non-painful Conditioning Stimuli

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ABSTRACT

Title of Document: CONDITIONED PAIN MODULATION IN CHILDREN: THE EFFECTS OF PAINFUL AND NONPAINFUL CONDITIONING STIMULI

Jessica L. Hoehn, Doctor of Philosophy, 2016

Directed By: Lynnda M. Dahlquist, Ph.D., Professor, Director, HSP Graduate Program, Human Services Psychology

Objective: To experimentally test endogenous pain modulation in children in a laboratory setting via a conditioned pain modulation paradigm.

Introduction: Conditioned pain modulation (CPM), a psychophysical measure in which one pain stimulus (conditioning stimulus) is used to inhibit another pain stimulus (test stimulus), is an important indicator of endogenous pain inhibition in adults. The CPM paradigm has interesting clinical relevance in adults, but is understudied in children. There is some preliminary evidence that CPM effects are present in healthy children, and that CPM effects are more robust in older children. However, it is still not fully known if CPM reliably occurs in children or whether CPM responses vary based on child age, and no published study of CPM effects in children has included a nonpainful conditioning stimulus to control for the effects of distraction and other nonspecific effects. Thus, the current study utilized a mixed experimental design, with experimental condition (baseline, painful conditioning stimulus, and nonpainful conditioning stimulus) as the within-subjects factor, child age as the between-subjects factor, and test stimulus (pressure pain threshold) as the dependent variable. It was predicted that children would display a "CPM effect;" i.e., pressure pain thresholds would be inhibited relative to

baseline during the painful conditioning stimulus trial. It was also predicted that older children would display a more robust CPM effect.

Method: Participants were 54 healthy school-aged children, age range 6-12 years, recruited from the local community. Participants were exposed to multiple pressure pain threshold tests, in which pressure was applied to their right hand until the stimulus first began to feel painful. After a baseline assessment, participants underwent two cold pressor trials in which they placed their left hand in a water bath at either 12°C (painful conditioning stimulus) or 22°C (nonpainful conditioning stimulus), and pressure pain threshold was assessed during each cold pressor exposure.

Results: Data were analyzed using a repeated measures ANOVA and multilevel mixed regression modeling, with experimental condition as a within-subjects factor for both analyses, child age (measured as a continuous variable) and duration of cold water exposure included as continuous predictors in the mixed regression models, and pressure pain threshold scores as the dependent variable. Results indicated that participants displayed a significant "CPM effect," such that their pressure pain thresholds were significantly higher during the painful conditioning stimulus trial when compared to baseline and the nonpainful conditioning stimulus trial. There were no significant differences in magnitude of the CPM effect based on age.

Conclusions: The current study replicates and expands on findings regarding CPM in children. It utilized a refined methodology for testing CPM in children, and provides information about the development of descending pain inhibitory pathways. Future studies could utilize pediatric CPM paradigms to examine predictors of chronic pain in children or to test their responses to pain intervention.

CONDITIONED PAIN MODULATION IN CHILDREN: THE EFFECTS OF PAINFUL AND NONPAINFUL CONDITIONING STIMULI

By

Jessica L. Hoehn

Dissertation submitted to the Faculty of the Graduate School of the
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of the requirements for the degree of
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Dedication

To Debra Hoehn, Frederick “John” Hoehn, Jr.,

and Nicole “Nikki” Hoehn

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Conditioned Pain Modulation in Children: The Effects of Painful and Nonpainful Conditioning Stimuli

Conditioned pain modulation is a psychophysical measure of endogenous pain inhibition, in which one pain stimulus (conditioning stimulus) is used to inhibit perception of a second pain stimulus (test stimulus). Conditioned pain modulation paradigms have been frequently tested in adults, and responses to this paradigm have been used as an indicator of endogenous pain inhibitory pathway functioning in the central nervous system in healthy adults. Responses have been found to be impaired in individuals with chronic pain conditions, and they have been used to predict development of chronic pain conditions. Despite the clinical relevance of conditioned pain modulation in adults, this paradigm has rarely been implemented in children. Existing adult paradigms have also rarely utilized control group designs via inclusion of a nonpainful conditioning stimulus, in order to control for nonspecific effects.

The current study tested a conditioned pain modulation paradigm in children. Specifically, the current study focused on age and painful vs. nonpainful conditioning stimuli in conditioned pain modulation in pre- and peri-adolescent aged (ages 6-12 years) children.

Background: **Neurophysiology of Pain**

"Pain" can be defined as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage" (International Association for the Study of Pain, 2012, "Pain," para. 1). Two types of pain can be identified: "nociceptive" pain, i.e., the pain from brief, immediate exposure to noxious stimuli or tissue damage, and "neuropathic" pain, which occurs when sensory

neurons become damaged and produce "hyperalgesic" responses (responses where pain intensity is exaggerated for innocuous and mildly painful stimuli) (Dahlquist & Switkin-Nagel, 2009; Calvino & Grilo, 2006). Acute or "nociceptive" pain can be defined as pain that occurs when tissue damage in the body results in activation of pain-specific neurons (called "nociceptors") which then send "nociceptive" signals to the spinal cord and then the brain. Chronic pain conditions are hypothesized to result from repeated exposure to nociceptive pain, which leads to increased sensitization, or from neuropathic pain conditions (Calvino & Grilo, 2006).

Human pain perception and control can be understood as a multifaceted process and has been described as having sensory, emotional, cognitive, and behavioral dimensions (Calvino & Grilo, 2006). There is ample evidence that pain perception can be amplified/sensitized or inhibited based on several psychological and physiological influences; including stress, coping strategies, gender, age, and social/cultural characteristics (Dahlquist & Switkin-Nagel, 2009). Pain perception is also influenced by a number of complex, often-interacting processes and factors. Recent advances in neurophysiological research and techniques in the past 30-40 years have greatly improved our understanding of the specific biological mechanisms in the brain, spinal cord, and central nervous system that are involved in these processes/pathways.

As outlined by Calvino & Grilo, (2006), tissue damage, or inflammation surrounding tissue damage, leads to stimulation of peripheral nociceptors ("pain sensing" sensory neurons) that are scattered throughout the body. There are two types of peripheral nociceptors: Aδ fibers, which produce "sharp, prickling" (Dahlquist & Switkin-Nagel, 2009) pain sensations, and C fibers, which produce burning or dull

sensations (Calvino & Grilo, 2006). These fibers pass along "nociceptive" signals to the spinal cord. The nociceptors terminate in the "grey matter" in the dorsal horn of the spinal cord. They then transmit the nociceptive signal to neurons located in the dorsal horn. These neurons are either nociceptive-specific neurons, which receive only "high intensity" signals from Aδ and C nociceptors only (i.e. "pain"/nociceptive" signals), or non-nociceptive specific neurons. These neurons then transmit the nociceptive signals to various supraspinal (above the spinal cord) brain structures, including the ventroposterolateral thalamus, the medulla oblongata and mesencephalon (which then transmits nociceptive information to the non-specific medial thalamus), the hypothalamus, and the amygdaloid complex/limbic structure (Calvino & Grilo, 2006). Nociceptive information is then integrated and transmitted from the ventroposterolateral thalamus to the parietal cortex, where pain perception is generated (i.e., the quality of pain perception – intensity, location, sensation, duration), and from the medial thalamus to the frontal, insular, and anterior cingular cortex, where more complex emotional and cognitive responses to pain are generated.

Pain perception can be modulated (i.e., facilitated or inhibited) via various spinal and supraspinal pathways. The above process of pain perception is described as an "ascending" pathway – i.e., nociceptive information is relayed from peripheral sensory neurons up to various supraspinal structures. Melzack and Wall (1965, 1982) posited that both ascending and descending pathways are involved in the perception of pain. Complex physiological "gating" mechanisms located in the spinal cord can modulate nociceptive signals ascending to the brain and other supraspinal structures. Pain perception can also be modulated by "descending" pathways – i.e., the brainstem and

other supraspinal structures can either enhance or inhibit nociceptive processing in the spine, leading to either hyperalgesia or analgesia (Gebhart, 2004). Pathways that can inhibit pain are sometimes described as "endogenous inhibitory pathways," as they represent the body's endogenous ability to inhibit pain. In descending inhibitory pathways, the brainstem and other supraspinal structures are involved in blocking of transmission of nociceptive signals in the spinal cord (Calvino & Grilo, 2006). It is thought that influences from cognitive and emotional factors (i.e., stress, fear, attention, etc.) are mediated via these pathways.

Pediatric Pain

Pain is generally a universal experience in children. In the U.S., painful pediatric medical procedures are nearly universal, due to the widespread use of vaccinations and other routine medical procedures (Blount et al., 2009). There is also a high prevalence of pediatric medical conditions, with an estimate of 20-35% of children currently diagnosed with a painful medical condition in the U.S. (King et al., 2011; Stanford et al., 2008). Several pediatric medical conditions include recurrent bouts of pain and are thus considered to be chronic pain conditions, such as headaches, abdominal pain, back pain, limb pain, and sickle cell disease (Dahlquist & Switkin-Nagel, 2009; King et al., 2011; Stanford et al., 2008). Despite this high prevalence, pain is still relatively understudied in children and adolescents, especially when compared to adults (Blount et al., 2009; Dahlquist & Switkin-Nagel, 2009).

Pediatric pain is different from adult pain, due to developmental differences in pain processing. Though there is ample evidence that children experience and remember painful experiences to the same degree as adults (Schecter, Berde, & Yaster, 2003),

young children lack the cognitive capacities to interpret and report pain in the same manner as adults (Gaffney, McGrath, & Dick, 2003). There is evidence that, while pain facilitative pathways are fully developed in infants, inhibitory pathways are slower to mature, and may not be fully developed in young children (Fitzgerald & Howard, 2003); thus, younger children may be more sensitive to pain than older children and adults. Age plays an important part in how pain is experienced in children, though child age is also understudied. There is evidence that younger children (between ages of 5 and 7) display higher pain intensity than older children (Lander & Fowler-Kerry, 1991; Fanurik, Koh, & Schmitz, 2000; Kleiber et al., 2007). Younger children are also more likely to display behavioral distress and physical and emotional outbursts (Cheng, Foster, & Hester, 2003; Lander & Fowler-Kerry, 1991; Kleiber et al., 2007). It has been suggested that younger children experience and display more distress due to more limited coping strategies and lack of experience with pain (Fanurik et al., 2000). The differences, however, could also be due to differences in neurodevelopment, as there is evidence that some pain processes and pathways develop late in humans and are not fully developed at birth (Fitzgerald & Howard, 2003). Neurodevelopmentally, pain processes that are mature for adults are not fully developed in children.

Laboratory-based experimental pain studies are an effective and clinically relevant way of studying pain in children (Birnie, Caes, Wilson, Williams, & Chambers, 2014). Though experimentally inducing pain in children raises some concerns, pediatric experimental pain research is largely considered to be ethical, and pediatric researchers have taken care to protect child participants from overly burdensome and coercive procedures in pediatric studies (Birnie et al., 2014; Birnie, Noel, Chambers, von Baeyer,

& Fernandez, 2011; McGrath & Brown, 2006). Furthermore, pediatric experimental pain studies have been used to better understand several aspects of pediatric pain. Pediatric experimental pain testing findings have been found to relate to clinical pediatric pain (Birnie et al., 2014), parental influences (Caes, Vervoort, Trost, & Goubert, 2012; Chambers, Craig, & Bennett, 2002; Walker et al., 2006), and influences of psychological and biological variables, such as gender and anxiety (Dufton et al., 2008; Lu, Tsao, Myers, Kim, & Zeltzer, 2007; Myers, Tsao, Glover, Kim, Turk, & Zeltzer, 2006). Pediatric experimental pain has been also used to test the effectiveness of pain interventions. Distraction-based intervention techniques have been found to be particularly effective at reducing pediatric pain, both in experimental and clinical pain studies (Blount et al., 2009; Dahlquist et al., 2010; Dahlquist, Pendley, Landtrip, Jones, & Steuber, 2002; Weiss, Dahlquist, & Wohlheiter, 2011). However, experimental pain is still understudied in children compared to adults. This deficiency is particularly true in studies of endogenous pain inhibition. Endogenous pain inhibitory pathways have been widely examined in adults, but studies are limited in children.

Conditioned Pain Modulation

One measure of endogenous pain inhibitory processes that has promising clinical implications is conditioned pain modulation (CPM), also formerly referred to in the literature as diffuse noxious inhibitory controls (DNIC). Conditioned pain modulation (CPM) refers to the general psychophysical paradigm whereby the perception of a painful stimulus is modulated by the subsequent introduction of a different painful stimulus (Yarnitsky et al., 2010). This phenomenon has been hypothesized to occur due to a specific brainstem-mediated descending inhibitory pathway, labeled as “diffuse noxious

inhibitory controls" (DNIC) in early animal research; ascending nociceptive signals are posited to evoke descending inhibitory pathways, which then exert inhibitory/modulatory effects on any other incoming nociceptive signals (Nir, Granovsky, Yarnitsky, Sprecher, & Granot, 2011). Early studies of CPM utilized the "DNIC" or "DNIC-like" term, as well as other terms such as "pain-evoked hypoalgesia" or "counter-irritation induced analgesia" (Goffaux et al., 2008; Quiton & Greenspan, 2007). CPM is a robust, growing area of research in adults, and has important clinical implications. However, to date it is mostly unexplored in children.

Conditioned Pain Modulation: Definition, Early Research, Terminology

The concept of using pain to inhibit pain, or "counterirritation," is not a new one. Counterirritation methods have been used historically for centuries as a treatment for chronic pain conditions across multiple cultures (Wand-Tetley, 1956). Blisters, electrical stimulation, cauterization/burns (of the skin), and acupuncture were historically used to treat chronic pain conditions such as gout, arthritis, sciatica, etc. (*see* Wand-Tetley, 1956, *for a detailed description of these methods*).

However, specific neurophysiological mechanisms were not examined empirically until the past few decades. Advances in research methodology and understanding of neurophysiology over the past 30-40 years have allowed for considerable advancement in the study of this topic. Early studies of "counterirritation" – i.e., the specific phenomenon of a painful or noxious stimulus inhibiting pain – were first conducted in animals.

The label "diffuse noxious inhibitory controls" (DNIC) was first used by Le Bars, Dickenson, and Besson (1979) to describe the inhibitory effect found in a specific group

of neurons after painful stimulation, in the spinal cords of rats. These neurons, named "wide dynamic range (WDR) neurons" are located in the dorsal horn of the spinal column (for both rats and humans). They receive both nociceptive and non-nociceptive sensory signals and transmit these signals to the brain (Andrews, 2003). In Le Bars, Dickenson, and Besson's (1980) study, the activity of these neurons (noxious activity, non-noxious activity, random firing, etc.) was severely inhibited by various types of painful noxious stimulation in areas throughout the rats' bodies, including heat, "pinching," and electrical stimulation. In the original study, 67 neurons were inhibited by this effect. Nonpainful stimulation (via innocuous light tactile stimulation, i.e., air-jet, paint brush, or blunt probe) to the same areas of the body did not produce this inhibitory effect.

Subsequent studies in rats and other animals replicated and expanded on these findings (Bouhassira, Villanueva, Bing, & Le Bars, 1992; Cadden, 1993; Dickenson, Le Bars, & Besson, 1980). Researchers then replicated these findings in humans, and found robust "DNIC-like" effects in most healthy human adults (De Broucker, Cesaro, Willer, & Le Bars, 1990; Price & McHaffie, 1988; Roby-Brami, Bussel, Willer, & Le Bars, 1987). As research regarding this phenomena increased, researchers increasingly utilized the term "DNIC" to describe any laboratory-based study of pain inhibition via a second painful stimulus, without focus on specific neurophysiological mechanisms. Researchers also used the term "pain-evoked hypoalgesia," "counter-irritation induced analgesia," and "heterotopic noxious conditioning stimulation" (HNCS) to describe similar psychophysical measurement paradigms, among other terms (Goffaux et al., 2008; Kosek & Ordeberg, 2000; Yarnitsky et al., 2010). Thus, "conditioned pain modulation (CPM)" was proposed in 2010 by Yarnitsky and colleagues to describe the methodological

paradigm that theoretically represented DNIC-like effects, but did not necessarily reflect one specific neurophysiological mechanism. Conditioned pain modulation specifically refers to "the phenomenon through which the conditioning stimulus (stimulus used to induce change) affects the test stimulus" (Yarnitsky et al., 2010, p. 339). It can refer to both facilitation and inhibition of pain in the test stimulus, though it is most commonly used to refer to inhibition.

DNIC was hypothesized to reflect a specific endogenous descending pain inhibitory pathway in which pain signals from a stimulus (the conditioning stimulus) block or reduce activity from WDR neurons in the spinal cord. Thus, these neurons are less able to transmit sensory signals from other parts of the body/other stimuli to the brain. Thus, pain signals from other parts of the body are reduced or blocked entirely, resulting in inhibited pain perception (increased pain threshold and tolerance, decreased perceived pain intensity) for the test stimulus. There is evidence that the process does not work in humans and rats with severed spinal cords (De Broucker et al., 1990; Roby-Brami et al.,1987), indicating that supraspinal involvement is necessary for this pathway to work, and that it is a descending inhibitory pathway. There is also evidence that, while attention plays an important role in this mechanism (Longe et al., 2001; Tracey & Dunckley, 2004), attention and distraction alone do not account for the analgesic/pain inhibitory effect of this process (Reinert, Treede, & Bromm, 2000; Tracey & Dunckley, 2004; Willer, De Broucker, & Le Bars, 1989).

"Conditioned pain modulation" does not refer to one specific supraspinal pathway, but rather the methodological paradigm of using a conditioning pain stimulus to modulate (usually inhibit) a test pain stimulus. It is believed that CPM responses do

reflect activation of this descending inhibitory pathway, and endogenous inhibitory pain mechanisms in general. However, there is evidence that there may be two or more neurophysiological mechanisms involved in "counterirritation" analgesia or CPM in human adults (Piché, Arsenault, & Rainville, 2009). In general, CPM is posited to represent a process whereby ascending nociceptive signals activate descending inhibitory pathways, which then exert modulatory effects on any other incoming nociceptive signals (Nir et al., 2011).

Conditioned Pain Modulation: Painful vs. Nonpainful Conditioning Stimuli

CPM effects are usually produced via the use of *painful* conditioning stimuli (i.e., counterirritation), rather than nonnoxious stimuli (Le Bars et al., 1979; van Wijk & Veldhuijzen, 2010). Stronger, more intense conditioning stimuli (CS) seem to provoke stronger CPM effects (van Wijk & Veldhuijzen, 2010). Multiple studies have found that increasing temperatures for heat pain CS or decreasing temperatures for cold pain CS led to stronger pain inhibition responses (Willer, Roby, & Le Bars, 1984; Lautenbacher, Roscher, & Strian, 2002; Tousignant-Laflamme, Pagé, Goffaux, & Marchand, 2008; Nir, Yarnitsky, Honigman, & Granot, 2012).

Some studies have also found CPM inhibitory effects to be absent when nonpainful stimuli are used (Granot et al., 2008; Kagiki, 1994; Price & McHaffie, 1988). However, others have found inhibitory effects from nonpainful stimuli. For example, Lautenbacher and colleagues (2002) found that prolonged exposure (10 minutes) to a hot but non-painful warm water bath (at 42ºC) elicited decreases in ratings of test stimulus pain intensity. These authors found similar results in other studies (Lautenbacher & Rollman, 1997; Lautenbacher, Kunz, & Burkhardt, 2008). Other investigators have

found CPM effects from nonpainful stimuli even without prolonged exposure (Heyman et al., 2010; Bouhassira et al., 1998).

Heyman and colleagues (2010) examined CPM in patients with irritable bowel syndrome, and included both painful (12 ºC) and nonpainful (32ºC) water bath exposure as the CS. They found that participants exhibited test pain inhibition responses for both the painful and nonpainful control CS, though the response was stronger for the painful condition. Heyman and colleagues labeled these nonpainful findings as "pseudo-DNIC effects" and attributed them to nonspecific effects that are not due to the descending inhibitory pathways involved in counterirritation analgesia. Quiton and Greenspan (2007) also included painful and nonpainful conditioning stimuli; they examined sex differences in different types of conditioning stimuli, including distracting (i.e. nonpainful sensory) and painful conditioning stimuli. They found that distracting (nonpainful) conditioning stimuli led to significant reductions in test stimulus pain intensity ratings for both men and women, but that distraction (nonpain) had significantly larger effects for men than women. Some other studies have controlled for these nonspecific effects by including nonpainful conditioning stimuli as a control condition (Edwards, Ness, Weigent, & Fillingim, 2003; Staud, Robinson, Vierk, & Price, 2003; Treister, Eisenberg, Gershon, Haddad, & Pud, 2010), though protocols typically do not include a control condition to control for nonspecific effects (Evans, Seidman, Lung, Zeltzer, & Tsao, 2013).

These studies provide evidence that distraction from the conditioning stimulus may have contributed to reductions in test stimulus pain and thus CPM effects (Heyman et al., 2010). Distraction from pain is known to have a major influence on the perception

of pain, and has been found to inhibit pain responses in both children and adults (Blount et al., 2009; Dahlquist et al., 2002; Defrin, Tsedek, Lugasi, Moriles, & Urca, 2010; Longe et al., 2001; Moont, Pud, Sprecher, Sharvit, & Yarnitsky, 2010). Attention and hypervigilance may play a role in the neurophysiological pathways activated during CPM procedures; Tracey and Dunckley (2004) suggest that supraspinal regions involved in hypervigilance processes may also be involved in the descending inhibitory pathways involved in CPM, which could thus lead to less robust CPM effects in hypervigilant individuals. Others have suggests that hypervigilance to the test stimulus could interfere with CPM responses (Reinert, Treed, & Bromm, 2000).

Thus, the conditioning stimulus may work to inhibit pain not only via activation of counterirritation-based endogenous pain inhibitory pathways, i.e. "DNIC-like" mechanisms, but also via distraction and other nonspecific effects. However, few CPM studies have controlled for this experimentally.

Conditioned Pain Modulation Methodology in Humans

Perception of a painful test stimulus is usually measured prior to, and during and/or after, the application of a (typically) painful second "conditioning stimulus" (Pud, Granovsky, & Yarnitsky, 2009). There is vast variation in the methodology of how this paradigm is measured in humans. Methods vary widely across research laboratories and studies regarding: the type of pain stimulus (for both the test stimulus and conditioning stimulus), the magnitude of stimulus intensity (i.e., temperatures for thermal stimuli), the type of pain perception measured (threshold, tolerance, and/or intensity), the location on the body that the stimuli are applied, the length of time of stimulus exposure, and the timing of test stimulus measurement (during and/or after CS application). As such,

several authors of reviews of CPM studies have noted the limitations of drawing conclusions regarding findings across studies, based on the large variability in CPM study procedures (Pud et al., 2009; Popescu, LeResche, Truelove, & Drangsholt, 2010).

Pud and colleagues conducted a review of "DNIC" methods in 2009, and noted general common practices across studies. The conditioning stimulus is most commonly a painful cold stimulus, usually administered via immersion in a cold pressor; however, thermal heat pain (administered via warm water bath or contact thermode) is also frequently used. Test stimulus pain is induced via a variety of pain stimulation techniques, including but not limited to: thermal heat, electrical, mechanical, and chemical pain stimulation. There does not appear to be one most commonly used test stimulus technique.

Pud and colleagues also concluded that CPM responses tend to fade as time progresses since the application of the CS; thus, designs that administer the test stimulus and conditioning stimulus simultaneously produce the most robust CPM effects. They also noted that there is some evidence that increases in the strength/magnitude of the conditioning stimulus correspond with increases in the strength/magnitude of CPM effects, but some studies failed to find evidence of this relation (Nir et al., 2011).

Most CPM studies apply the conditioning and test stimuli in heterotopic (contralateral) bodily locations, since CPM responses have not always been present in homotopic (ipsilateral adjacent) locations (van Wijk & Veldhuijzen, 2010; Svensson, Hashikawa, & Casey, 1999). However, CPM responses have sometimes been found in homotopic locations (Pud et al., 2009; Pud, Sprecher, & Yarnitsky, 2005). Defrin, Tsedek, Lugasi, Moriles, and Urca (2010) compared different physical distances between

the CS and TS, and found that the magnitude of CPM responses increased as distance between the two stimuli increased. Oono, Nie, Matos, Wang, and Arendt-Nielson (2011) also compared different locations of the test stimulus, (using a CS in the hand across conditions) and found that, when compared to masseter (jaw) and forearm locations, test stimulus pain administered at the leg produced the largest CPM effects.

There is some evidence that thermal cold pain is the most effective conditioning stimulus for inducing CPM effects. Willer et al. (1984) compared cold, muscle, and pressure pain for conditioning stimuli, and found that cold pain produced the largest CPM responses. Oono et al. (2011) compared the impact of multiple types of CS (cold pressor pain, tourniquet pain, and mechanical pressure pain) on pressure pain (including pressure pain threshold, tolerance, and intensity) at multiple body sites. They found that cold pressor pain induced the most robust threshold, tolerance, and intensity ratings of all the CS, and that cold pressor pain produced the least amount of variability across multiple time points and across multiple individuals. Lewis, Heales, Rice, Rome, and McNair (2012) examined the reliability of CPM and compared two conditioning stimuli: ischemic arm pain (administered via inflatable cuff) and tonic cold pain (administered via the cold pressor test). They found that, while both procedures demonstrated excellent within-session reliability, between-session reliability was good for cold pressor test and poor for the ischemic arm tests.

There is some preliminary evidence that differences in heat pain thresholds are not significant when the conditioning stimulus is very mild in intensity. Razavi, Hansson, Johansson, and Leffler (2014) varied the intensity of their conditioning stimuli, and did not find significant differences in heat pain threshold for very mildly painful

conditioning stimuli (i.e., 2 on a pain intensity scale of 1 to 10). They did find differences in threshold for moderate (5/10) and high (7/10) intensity conditioning pain. However, widespread variability between types of pain perception measured for the test stimulus (i.e., threshold, suprathreshold, tolerance, intensity ratings, etc.), as well as body site of assessment and type of pain stimulus (heat, pressure, chemical, electric, etc.), provides inconclusive evidence regarding whether one modality is definitively superior for capturing CPM effects over another.

Conditioned Pain Modulation: Clinical Relevance

The study of conditioned pain modulation is important because, in addition to expanding knowledge and understanding of human pain, CPM also has significant clinical relevance. CPM has been studied extensively in adults in the past several decades, and researchers have found interesting and clinically relevant findings.

Conditioned pain modulation has been found to be highly related to clinical pain experiences. Edwards, Ness, et al. (2003) examined whether multiple laboratory-based pain testing methodologies predicted clinical pain and health-related quality of life in a sample of 77 healthy adults. They measured responses to thermal heat pain threshold and tolerance, temporal summation of thermal pain intensity, and CPM (using cold pressor pain as the conditioned stimulus and thermal temporal summation as the test stimulus), and assessed whether these laboratory pain responses predicted clinical pain and physical health (assessed using the Short-form-36 health survey). They also assessed whether other individual characteristics, such as sex, age, and psychological variables, predicted clinical pain and physical health. They found that, of all the laboratory pain responses analyzed, conditioned pain modulation (DNIC) responses were the only responses to

significantly predict clinical pain symptoms and physical functioning (gender and age were the only other significant predictors). Other studies have replicated and expanded on these findings (Granovsky, 2013; Yarnitsky, 2010).

Impairment in Adult Chronic Pain Populations

CPM has been found to be deficient in some individuals with chronic pain conditions. In general, it has been found that inhibitory CPM effects are reduced or absent in adults with chronic pain conditions, when compared to healthy adults. This absence of CPM is hypothesized to indicate impairment in the body's endogenous pain inhibition pathways, particularly descending inhibition pathways, and could be an indication of further impairment of the central nervous system's ability to reduce pain. It is hypothesized that CPM is impaired in individuals with chronic pain conditions because the CPM effect may "wear out" over time, i.e. that modulation cannot be maintained for a long period of time or after multiple activations (van Wijk & Veldhuijzen, 2010).

Initial studies of CPM and chronic pain focused primarily on fibromyalgia (Van Wijk & Veldhuijzen, 2010; Staud, Robinson, Vierck, & Price, 2003). This impairment has been found in other chronic pain conditions, including: chronic/persistent headache (Pielsticker, Haag, Zaudig, & Lautenbacher, 2005; Cathcart, Winefield, Lushington, & Rolan, 2010; Drummond & Knudsen, 2011; Perrotta et al., 2010), migraine (Sandrini et al., 2006) osteoarthritis (Kosek & Ordeberg, 2000; Arendt-Nelson et al., 2010), irritable bowel syndrome (Wilder-Smith, Schindler, Lovblad, Redmond, & Nirkko, 2004, Wilder-Smith & Robert-Yap, 2007; Piche, Arsenault, Poitras, Rainville, & Bouin, 2010), temporomandibular joint disorder (King et al., 2009; Kashima, Rahman, Sakoda, & Shiba, 1999), and post-operative lumpectomy pain (Edwards et al., 2013). DNIC-like

responses have also been found to be impaired/absent in other chronic medical conditions, including chronic fatigue syndrome (Meeus, Nijs, Van de Wauwer, Toeback, & Truijen, 2008) and insomnia/sleep disturbance (Edwards et al., 2009; Haack et al., 2012; Smith, Edwards, McCann, & Haythornthwaite, 2007).

However, CPM impairment has not been universally found in all chronic pain conditions in adults; for example, studies have found CPM effects to be preserved in samples with chronic lower back pain, rheumatoid arthritis (Leffler, Kosek, Lerndal, Nordmark, & Hansson, 2002), myalgia (Leffler, Hansson, & Kosek, 2002), stroke (Roosink et al., 2011; Tuveson, Leffler, & Hansson, 2009), and Parkinson's disease (Mylius et al, 2009). As van Wijk and Veldhuijzen (2010) note, there does not seem to be a discernible pattern regarding type of chronic pain condition and preservation vs. impairment of CPM; though some researchers have hypothesized that chronic pain condition differences can be explained by the duration (intermittent vs. long-term) of pain in these conditions, or that CPM is reduced only in medically-unexplained pain syndromes.

Due to these conflicting findings, Lewis, Rice, and McNair (2012) recently conducted a systematic review and meta-analysis of studies examining conditioned pain modulation and chronic pain populations in order to more definitively address whether conditioned pain modulation is "dysfunctional" in adult populations with chronic pain. They conducted meta analyses on 30 studies that compared CPM responses between individuals with chronic illnesses and healthy controls. They found that, across these studies, 69% of statistical comparisons between participants with chronic pain and pain-free controls resulted in significant differences – i.e., modulation was significantly

impaired in patients with chronic pain conditions as compared to healthy controls. Lewis and colleagues calculated effect sizes for each comparison and calculated an overall effect size estimate; they found that the estimated overall effect size was large (based on Cohen's (1988) guidelines) and concluded that conditioned pain modulation is impaired in people with chronic pain conditions.

Lewis and colleagues also found moderating effects of gender and age across studies. Effect sizes for CPM differences were larger for women, suggesting that CPM impairment related to chronic pain is greater in women. However, women were overrepresented in the included studies, so this finding should be interpreted with caution. Effect sizes were also larger for younger participants. This second finding reflects that conditioned pain modulation is reduced in older-aged individuals (see section below); thus, effect sizes are smaller in older individuals because the control groups likely have impaired CPM, as well.

CPM as a Predictor of Future Chronic Pain

In their meta-analysis, Lewis, Rice, et al. (2012) noted that their review of previous CPM studies did not provide conclusive evidence regarding the chronology of the relationship between CPM and chronic pain conditions. It is possible that pre-existing CPM impairment is a risk factor for development of chronic pain, or that chronic pain conditions lead to impairment of endogenous pain inhibitory systems in general.

An impaired endogenous pain pathway could put an individual at greater risk for developing chronic pain conditions, or conversely, chronic pain experiences could lead to a "wearing out"/habituation or "maxing out" of endogenous inhibitory pathways. If CPM can predict chronic pain conditions, then it could be used to identify individuals at greater

risk for developing chronic pain conditions, particularly if they are already at risk (i.e., receiving surgery, recently diagnosed with a disorder that can have variable chronic pain trajectories, etc.). Conversely, impairment in CPM caused by chronic pain could be an explanation on how chronic pain works within the body.

There is some preliminary evidence suggesting that conditioned pain modulation dysfunction can be used to predict later development of chronic pain. Yarnitsky et al. (2008) utilized pre-operative assessment of conditioned pain modulation to predict whether individuals undergoing thoracotomies would develop chronic post-thoracotomy pain (CPTP). They found that pre-operative robust conditioned pain modulation responses predicted lowered risk of CPTP. Impaired conditioned pain modulation predicted increased risk of developing post-operative chronic pain. Conditioned pain modulation was assessed prior to surgery, when individuals were in a "pain-free" state. These findings suggest that conditioned pain modulation could be used to identify individuals who are at greater risk of developing chronic pain conditions. Subsequent studies have found evidence that CPM responses can be used to predict development of chronic pain after surgery, and can potentially be used to predict development of neuropathic pain (Granovsky, 2013; Grosen, Vase, Pilegaard, Pfeiffer-Jensen, & Drewes, 2014; Wilder-Smith, Schreyer, Scheffer, & Arendt-Nielson, 2010; Yarnitsky, 2010).

Kosek and Ordeberg **(**2000) studied individuals with painful hip osteoarthritis, both before and after successful hip replacement surgery. They found that, prior to surgery, participants displayed impaired conditioned pain modulation, but that CPM was restored to normal functioning when reassessed 6-14 months after successful surgery (while individuals were in a pain-free state). These findings suggest that endogenous

pain inhibitory pathways can be modified over time, and that improvements in CPM can reflect improvements in chronic pain conditions. This suggests that CPM can be influenced by treatment of chronic pain conditions, and so could be used as a laboratory-based measure of efficacy of pain interventions.

Conditioned Pain Modulation and Individual Differences

CPM also has interesting and relevant clinical implications due to findings regarding individual differences. CPM can vary from individual to individual based on biological and psychological characteristics.

There is a pronounced association between sex and conditioned pain modulation in adults. Women generally display greater pain sensitivity than men in other experimental pain responses (i.e., women report lower pain tolerances and lower pain threshold levels (Edwards, Sarlani, Wesselmann, & Fillingim, 2005; Pud et al., 2009). Chronic pain diagnoses are also vastly more prominent in women when compared to men (Pud et al., 2009). There is also evidence in pediatric chronic pain conditions that girls are more likely to report greater frequency and duration of pain episodes and higher pain intensity in several pediatric pain conditions (Unruh & Campbell, 1999; Tsao et al., 2013; Vierhaus, Lohaus, & Schmitz, 2011; Fillingim et al., 2009). Researchers have hypothesized that these sex differences in pain perception may be due to sex differences in endogenous pain inhibition. Thus, it was hypothesized that sex differences in conditioned pain modulation may explain why chronic pain conditions are more prominent in women. France and Suchowiecki (1999) first studied sex differences in conditioned pain modulation in healthy adults; however, they found no differences between sexes. Some other studies also failed to find differences (Baad-Hansen, Poulsen,

Jensen, & Svensson, 2005; Lautenbacher et al., 2008; Pud, Sprecher, & Yarnitsky, 2005; van Wijk & Veldhuijzen, 2010). However, subsequent studies provided evidence that modulation is more robust in men/less efficient in women (Edwards, Ness et al., 2003; Goodin et al., 2009; Pud et al., 2009; Staud et al., 2003; Serrao et al., 2004; van Wijk & Veldhuijzen, 2010). Popescu et al. (2010) conducted a systematic review of conditioned pain modulation and gender differences, and found that, while several studies found that conditioned pain modulation is more efficient in males and sometimes absent in females, presence and magnitude of gender differences appeared to differ based the methodology and measurement of CPM (i.e., methods are too varied to definitively draw conclusions). It has been theorized that sex differences in pain perception and processing arise either due to biological differences, or socio-cultural differences (Popescu et al., 2010).

Van Wijk and Veldhuijzen (2010) also reviewed sex differences in CPM, and reported that significant sex differences were present in conditioned pain modulation in about 50% of studies. Studies have also found that sex differences may be partially or fully mediated by psychological variables, such as pain catastrophizing **(**Weissman-Fogel, Sprecher, & Pud, 2008). Sex has also been found to be a moderator of relationships between psychological variables (such as catastrophizing) and conditioned pain modulation (Goodin et al., 2009). Sex differences have also been found in relation to conditioned pain modulation and chronic pain; the relation between CPM impairment and chronic pain has sometimes been found to be moderated by sex. In Lewis, Rice, et al.'s (2012) meta-analysis of CPM in chronic pain, gender was found to be a significant moderator of the relation between impaired CPM and chronic pain conditions. Lewis and colleagues found that studies of women-only participants show larger effect sizes for

differences between healthy and chronically ill women then studies that include men, suggesting that women's CPM responses may be more impaired than men in the context of chronic illness. Sex seems to be more relevant for some particular pain conditions (Lewis, Rice, et al., 2012).

There is also evidence for differences in conditioned pain modulation due to other variables, such as ethnicity/race, and age (see section below). There is much evidence that efficiency/magnitude of modulation differs based on ethnicity (Campbell et al., 2008; Goodin et al., 2013). There is also evidence that modulation is impacted by several psychological variables, including catastrophizing, stress, anxiety, dispositional optimism, etc. (Edwards et al., 2013; Goodin et al., 2009; Goodin et al., 2013; King et al., 2013; Quiton & Greenspan, 2007). The impact of these variables on CPM provides further evidence for supraspinal involvement in endogenous inhibitory pain pathways.

Developmental Changes in Conditioned Pain Modulation

There is substantial evidence that age is an important moderator in adult studies of conditioned pain modulation. Studies of conditioned pain modulation have consistently shown that older adults display impaired/dysfunctional responses. For example, Lewis, Rice, et al. (2012) found a small but significant moderating effect of age on the relation between conditioned pain modulation responses and chronic illness. For younger adults, there was a large discrepancy in conditioned pain modulation between individuals with and without chronic pain conditions. However, in older adults, this difference was much smaller, largely because CPM responses were also impaired in older adults even without chronic pain conditions.

Edwards, Fillingim, and Ness (2003) compared conditioned pain modulation responses for healthy younger and older adults, and found impaired CPM in the older adults (the sample of older adults actually displayed facilitation of test stimulus pain rather than inhibition). This finding suggests impairments in endogenous pain inhibition in older adults, which may also explain the higher prevalence and severity of chronic pain conditions in older adults. Other studies have replicated this decrement for older adults (Lariviére, Goffaux, Marchand, & Julien, 2007; Washington, Gibson, & Helm, 2000). These findings indicate that pain processing pathways (specifically descending inhibitory pain pathways) are not static, but rather change across the lifespan.

There is also some evidence that descending modulatory pathways in general are slow to mature. Though many questions regarding developmental neurophysiology of pain still need to be answered, there is some evidence that certain pain processes are not fully developed in younger organisms; specifically, descending inhibitory pathways are not fully developed in young/newborn organisms (Walker, 2014). Research on this topic is necessarily limited due to ethical concerns, but animal research provides more information. There is evidence that general descending inhibitory controls are not fully developed at birth in both animals and human infants (Fitzgerald & Howard, 2003; Fitzgerald & Walker, 2009; Walker, 2014) and it seems that descending pathways from the brainstem to the dorsal horn are particularly slow to mature (Fitzgerald & Howard, 2003). Descending pain modulatory pathways appear to be predominantly facilitating, rather than inhibiting, pain in neonates (Fitzgerald & Walker, 2009; Walker, 2014).

There is ample evidence that early exposure to painful experiences (such as multiple medical procedures from NICU stays) is associated with greater sensitization to

pain in middle childhood (Hermann, Hohmeister, Demirakca, Zohsel, & Flor, 2006; Hohmeister et al., 2010; Lax et al., 2013). These findings could imply that early pain exposure activates and alters pain modulatory pathways in young children, though it is possible that other factors (such as increased exposure to stress, increased likelihood of developmental delays, etc.) could lead to future increased sensitization.

There is also evidence that, while facilitative pathways are more fully developed at birth, descending inhibitory pathways do not fully develop until pre-adolescence or adolescence (Fitzgerald & Walker, 2009; Walker, 2014). Studies with young rats have found evidence that rats do not produce analgesia from brainstem stimulation until they reach a pre-maturation period (which the authors labeled as a "preadolescent" period) (Van Praag & Frenk, 1991). Hathway and colleagues (2012) studied brainstem-mediated pain control (i.e., descending pain inhibitory pathways) in young rats. They found that for very young rats, brainstem-mediated pain control was mostly facilitative of pain signals from the spine, but that it underwent a "switch" to inhibiting pain signals from the spine prior to the rats' maturation. Based on these results, the authors hypothesized that preadolescence or "peri-adolescence" may be a critical period in development of supraspinal (i.e., descending) pain inhibition.

Studies of neurodevelopment in general have also identified late childhood/early adolescence as an important period of brain development (Salum, Polanczyk, Miguel, & Rohde, 2010). Though structural brain developmental trajectories are highly individualized from person to person, based on neuroimaging studies, there does seem to be a generalized pattern of early increases in grey matter in the brain through early childhood, which is then followed by a phase of decrease in grey matter during late

childhood/early adolescence (Salum et al., 2010; Shaw, Gogtay, & Rapaport, 2010; Sowell, Thompson, & Toga, 2004). This "thinning" in grey matter has been attributed to synaptic pruning and increased myelination, and is believed to enhance speed of cognitive processing and/or improve communication between neurons in general (Gogtay & Thompson, 2010; Salum et al., 2010; Sowell et al., 2004).

Based on the animal studies of descending pain inhibitory pathways, it has been hypothesized that endogenous inhibitory pathways are less developed and therefore less effective in human children, which could contribute to pediatric chronic pain conditions (such as juvenile rheumatoid arthritis) (de Lalouviére, Iannou, & Fitzgerald, 2014). If these descending inhibitory processes are not fully matured in younger organisms, they could contribute to greater intensity of pediatric pain – and could also contribute to pediatric chronic pain conditions. Because conditioned pain modulation is thought to reflect one (or more) endogenous inhibitory pathways, it is possible that endogenous inhibitory processes in CPM paradigms would also be delayed/slow to mature in children.

Thus, it cannot be assumed that conditioned pain modulation works in the same way for children as adults. Evidence suggests that CPM responses should be present in younger children, but it is also possible that this type of modulation is reduced in younger children–or it is possible that current CPM methodology may not effectively produce CPM responses in children. This must be verified. There have been a limited number of studies of conditioned pain modulation: to date, investigators have published articles from three separate studies of conditioned pain modulation in children.

Goffaux et al. (2008) was, to my knowledge, the first study of CPM in children (also indicated as the first study by Tsao et al., 2013). The investigators were specifically interested in the relation between pre-term birth, neonatal pain, and conditioned pain modulation. They predicted that high exposure to invasive/painful medical procedures as a neonate would relate to impaired inhibitory pain processes (i.e. impaired conditioned pain modulation). A total of 26 children (ages 7-11 years, $M = 9.3$, $SD = 1.3$) underwent conditioned pain modulation procedures; 13 children were born at full-term (and were presumed pain-free at birth) and 13 were born pre-term, with 6 classified as "low-pain preterm at birth" and 7 classified as "high-pain preterm at birth." Length of time in the NICU was used as a proxy measure for number of invasive/painful procedures as the exact number of procedures was not known. Children who spent a longer number of days in the NICU were classified as "high-pain" preterm, as it was hypothesized that they would have experienced a higher number of invasive painful procedures. Investigators utilized cold pressor pain as the conditioned stimulus and thermal heat pain threshold and thermal heat pain intensity as the test stimuli. They found that the conditioned stimulus significantly reduced thermal heat pain intensity in full-term and "low-pain" pre-term children, thus showing that these children displayed CPM. However, the "high-pain" children did not show a CPM response. These results suggest that children ages 7-11 can display CPM, and, if replicated, implies that children who experience early painful medical procedures may have impairments in endogenous pain inhibition.

Tsao et al. (2013) were the first to study conditioned pain modulation in a sample consisting only of healthy children. Tsao and colleagues were interested in replicating sex difference findings from adults in younger children, and examining age differences in

CPM between adolescents and elementary school-aged children. They conducted CPM procedures with 133 healthy children, (mean age 13 years; younger children ranged in age from 8 to 11 years; older children 12 to 17 years). The sample was 52.6% female; ethnic/racial makeup was 45.5% White/Caucasian, 31.5% Latino, 8% African American, and 27.3% Multiracial.

The test stimulus was operationalized as pain intensity ratings for a pressure stimulus applied to the participant's left thumbnail using a 1x1 cm rubber probe that produced a "moderate" amount of pain. Each participant underwent a "tailoring" procedure to determine the individualized amount of applied pressure that would produce a moderate pain intensity rating. Tailoring involved the multiple random staircase (MRS) pressure pain sensitivity method – a validated method of determining the stimulus level needed for specific pain/sensory thresholds whereby participants are exposed to multiple pressure stimuli at varying intensities and asked to verbally rate each on a numeral pain rating scale from 0 to 10. Based on participants' responses to this task, each participant was assigned a pressure stimulus that would produce a moderate amount of pain (a rating of 6 on a 0-10 numerical pain rating scale). Participants were then exposed to this pressure stimulus at baseline, and then during and after the conditioning, and asked to rate the intensity of their pain from the stimulus on a 0-10 scale.

The conditioning stimulus was provided via cold pressor exposure, in which participants were asked to immerse their right hands in a cold water bath at a temperature of 5℃ for approximately 30 seconds. The investigators found that, across age and sex groups, children displayed robust CPM responses (i.e., their pressure pain intensity was reduced from baseline during and immediately after cold water exposure). CPM was

significantly larger in magnitude for older children compared to younger children. There were no significant sex differences.

Evans, Seidman, Lung, et al. (2013) and Evans, Seidman, Tsao, et al. (2013) conducted follow-up analyses using data from Tsao et al. Evans, Seidman, Lung, et al. (2013) examined child sex differences, mother's anxiety regarding pain, and CPM responses. They found that child sex was a moderating factor; for girls, greater maternal pain anxiety significantly predicted greater anticipatory anxiety and pain-related fear regarding the CPM procedure (but not for boys). For boys, they found that greater maternal pain anxiety predicted less efficient CPM responses. Evans, Seidman, Tsao, et al. (2013) also examined heart rate variability and chronic pain in children. They utilized control subjects from Tsao et al. (2013) but also performed CPM procedures on 48 children with chronic pain; however, they did not present CPM results.

These findings are preliminary and require replication, but, if replicated, could imply that conditioned pain modulation (and endogenous pain inhibition/descending inhibitory control in general) improves as children develop. Evans, Seidman, Lung, and colleagues (2013) also noted in their limitations section that there was a potential confounding factor of distraction. The cold water test (conditioning stimulus) could be acting as a distracter instead of activating inhibitory pain pathways. As in adults, more study with better experimental methodology is needed to distinguish responses due to distraction from responses due to pain-induced inhibitory controls.

Williams, Heitkemper, Self, Czyzewski, and Shulman (2013) examined CPM in girls with irritable bowel syndrome. They studied 43 girls (22 with IBS, and 21 healthy controls), ages 7 to 12 years (mean age 9.8 for IBS group, 9.4 for control). They utilized

tonic cold pressor pain as the conditioning stimulus (CS) and phasic thermal heat threshold as the test stimulus. They found that girls with IBS did not display a significant CPM response in comparison to controls (i.e., thermal heat pain threshold did not significantly change from baseline to post-CS exposure for participants with IBS). However, the groups also differed in psychological variables, including greater symptoms of depression, anxiety, and somatization in girls with IBS. When these psychological variables were controlled for in analyses, there were no significant differences in CPM between groups, suggesting that health status differences in CPM may be mediated by psychological variables.

Thus, there is preliminary evidence that children do exhibit conditioned pain modulation, and preliminary evidence that there are differences in the strength of this response based on age. However, more studies and replication are needed.

Summary

Conditioned pain modulation is an important indicator of endogenous pain inhibition in adults. The CPM paradigm has interesting clinical implications in adults, but is understudied in children. There is some preliminary evidence that CPM effects are present in healthy children, and that CPM effects are more robust in older children. However, it is still not fully known if CPM reliably occurs in children, and findings regarding age also require replication. Furthermore, to my knowledge, no published study of CPM effects in children has included a nonpainful conditioning stimulus to control for the effects of distraction and other nonspecific effects.

Thus, the current study contributes to the pediatric CPM literature in multiple ways. It was designed to replicate findings from adult CPM literature and preliminary

findings from the few previous studies of CPM in children. It was also designed to replicate and expand on findings regarding age differences in CPM in children. The current study utilized a refined methodology for testing CPM in children, via inclusion of a nonpainful conditioning stimulus to control for nonspecific effects.

Study Design Overview

For the current study, we utilized a conditioned pain modulation paradigm with cold pressor exposure as the conditioning stimulus, and pressure pain as the test stimulus. The design of the study was a mixed between-subjects and repeated measures (age by experimental condition) experimental design, with age (measured as a continuous predictor) as the between-subjects factor, experimental condition (baseline, painful conditioning stimulus, and nonpainful conditioning stimulus) as the within-subjects factor, and test stimulus (pressure pain threshold) as the dependent variable. All participants were exposed to a painful conditioning stimulus and nonpainful conditioning stimulus in counter-balanced order. Children were stratified by age and sex and randomized to one of the following experimental condition presentations: Order 1: baseline, painful conditioning stimulus, and then nonpainful conditioning stimulus; or Order 2: baseline, nonpainful conditioning stimulus, and then painful conditioning stimulus. Pressure pain thresholds were assessed at three time points: baseline, during the painful conditioning stimulus presentation, and during the nonpainful conditioning stimulus presentation.

Conditioned Pain Modulation Paradigm

Conditioning stimulus: cold pressor test (CPT). We used the cold pressor test (in which participants are asked to submerge their hand in painfully cold or nonpainful

ambient temperature water) to provide the painful conditioning stimulus and the nonpainful conditioning stimulus for this CPM paradigm. The cold pressor test has been demonstrated to show superior reliability when compared to other conditioning stimuli in the adult literature (Lewis, Heales et al., 2012; van Wijk & Veldhuijzen, 2010). There is also evidence that it produces superior CPM responses when compared to other conditioning stimuli (Lewis, Heales, et al., 2012; van Wijk & Veldhuijzen, 2010), and all existing published pediatric CPM studies have utilized the cold pressor as the conditioning stimulus. The cold pressor test has been noted to be an ethical pain testing modality in children (Birnie et al., 2011; Birnie et al., 2014; von Baeyer, Piira, Chambers, Trapanotto, & Zeltzer, 2005).

Test stimulus: Pressure pain threshold. We used pressure pain threshold (PPT) (measured by applying a steady rate of pressure to the child's thumbnail until the child indicates that the procedure first feels painful) as the test stimulus for this paradigm. PPT was measured at baseline and during both the painful and nonpainful conditioning trials. Pressure pain threshold was chosen as an appropriate test stimulus for this study for a number of reasons, including prior use in adult CPM studies and pediatric experimental pain studies, evidence of reliability, and level of burden/harm it places on participants.

Pressure pain threshold has been frequently used in adult CPM studies and has been found to demonstrate good intrasession and intersession reliability (Lewis, Heales, et al., 2012). Pressure pain modalities have also been widely used in experimental pain studies in children and adolescents, for both healthy children and children with chronic pain conditions (Birnie et al., 2014). An advantage of using pressure pain as the test stimulus is that it may have clinical relevance–i.e., it can simulate the experience of

musculoskeletal pain, and has been used in clinical settings as a diagnostic tool for musculoskeletal pain disorders (Birnie et al., 2014; Ylinen, 2007). Pressure pain also was chosen because in comparison to some other quantitative sensory testing methodologies, it is relatively non-invasive. Furthermore, because *threshold* (rather than tolerance or intensity) was used, the harmful impact was minimized in child participants. As Birnie and colleagues (2014) note in their discussion of different experimental pain modalities in children and adolescents, pressure thresholds are preferable to tolerance or intensity when using pressure pain stimuli in children, as pressure pain threshold (PPT) minimizes any risk of tissue damage.

Specific Aims of the Current Study

The primary aim of the current study was to expand the basic understanding of endogenous pain inhibitory processes in children by conducting a methodologically rigorous examination of conditioned pain modulation in children. To do so, the current study examined the following research questions:

Research question 1: Do healthy children display an inhibitory conditioned pain modulation response, above and beyond nonspecific/sensory distraction effects? Are conditioned pain modulation effects more robust (larger) in children when a painful conditioning stimulus rather than a nonpainful conditioning stimulus is used?

Based on the few published existing pediatric conditioned pain modulation studies (Goffaux et al., 2008; Tsao et al., 2013; Williams et al., 2013), it could be expected that healthy children will show a CPM effect. Children's perceptions of a painful stimulus should be inhibited by the subsequent introduction of a painful conditioning stimulus.

However, all of these studies have tested CPM effects using only a painful conditioning stimulus.

As described previously, some adult studies have only found a conditioned pain modulation response when a painful (as opposed to nonpainful) conditioning stimulus is used (Granot et al., 2008; Kagiki, 1994; Price & McHaffie, 1988). However, some adult studies have produced CPM-like effects with a nonpainful conditioning stimulus (Heyman et al., 2010; Quiton & Greenspan, 2007; Lautenbacher et al., 2002; Bouhassira et al., 1998). Heyman and colleagues (2010) included both painful and nonpainful conditioning stimuli and found that inclusion of a nonpainful conditioning stimulus produced CPM-like effects, but that the strength of these effects were not as high as effects produced from a painful conditioning stimulus. They concluded that any effects from nonpainful conditioning stimuli represent effects from distraction and other nonspecific effects.

These findings suggest that nonpainful conditioning stimuli should be included in research designs of conditioned pain modulation as a control group (to compare the effect of painful conditioning stimulus with the effect of sensory distraction and other nonspecific effects). To date, no published pediatric conditioned pain modulation study has included such a control group. Based on Heyman et al. (2010), Quiton and Greenspan (2007), and other previous studies, it could be expected that a nonpainful conditioning stimulus would significantly inhibit test pain responses, as compared to baseline. It also could be expected that the inhibitory effect of a nonpainful conditioning stimulus would not be as strong in magnitude as the inhibitory effect of a painful

conditioning stimulus, because the painful conditioning stimulus would be evoking the specific endogenous inhibitory pathways involved in counterirritation.

***Hypothesis 1*:** It was predicted that painful conditioning stimuli would lead to significantly more inhibition than nonpainful conditioning stimuli, indicating that healthy children display CPM responses above and beyond sensory distraction or other nonspecific effects.

Research question 2: Do conditioned pain modulation responses vary based on child's age? Will the difference in effect between painful and nonpainful conditioning stimuli vary based on the child's age?

For this research question, we compared CPM effects across child age. The targeted age range (6-12) for this study was selected because it specifically targets children at the upper and lower limits in the "middle childhood" developmental age group, a time of important advances in cognitive and CNS development (Eccles, 1999; Wenar & Kerig, 2000). The age range will capture at its lower limit children at the start of the "middle childhood" age range described by developmental psychologists; at its upper limit, it will also capture preadolescent children.

As described previously, there is evidence that descending inhibitory pathways are slow to mature (Walker, 2014). There is some evidence that preadolescence may be a crucial time for development of descending inhibitory pathways (Hathway et al., 2012; Walker, 2014); descending inhibitory processes are present in rats in this age group, but not in younger age groups. There is also some evidence for general neurodevelopmental changes during late childhood/early adolescence (Salum et al., 2010). There is also already some preliminary evidence for age differences in CPM; Tsao et al. (2013) found

differences between children in late middle childhood (ages 8-11 years) and adolescents (ages 12-17 years). If the above-cited animal studies can be interpreted as valid models of neurodevelopment in humans (as they often are), then, based on the above findings regarding inhibitory pathways in preadolescence in rats, we would also expect to find differences across child age from younger to pre-adolescent children in descending pain inhibition.

Thus, the current study used a slightly younger, more restricted age range than Tsao et al. in order to examine differences across age from younger to pre-adolescent children. Comparison of children in middle childhood with older, pre-adolescent children should provide a sufficient age range to provide information about the developmental changes in conditioned pain modulation processes in early middle childhood through pre-/early adolescence. Based on neurodevelopmental findings, findings from one previous study of age differences in CPM and children (Tsao et al., 2013), and age differences in pediatric experimental pain in general (Lander & Fowler-Kerry, 1991; Fanurik et al., 2000), it is expected that there will be significant differences based on age. Age differences (more robust CPM effects in older children) could have implications about the development of endogenous inhibitory pain pathways in humans. Age differences would imply that these processes are still developing in children in middle childhood through preadolescence, rather than being fully matured at a younger age.

It is also possible that the magnitude of difference in effect between painful and nonpainful conditioning stimuli may vary based on a child's age. Distraction has been shown to be an effective strategy to reduce child pain responses, both during medical

procedures and during experimental pain procedures (Dahlquist et al., 2010). There is also some evidence that endogenous pain pathways develop late in humans and are not fully developed at birth (Andrews, 2003; Fitzgerald & Walker, 2009; Walker, 2014). Neurodevelopmental differences in endogenous inhibitory pain pathways could explain in part why younger children report greater pain intensity and behavioral distress than older children. Thus, it may be expected that endogenous inhibitory pathways involved in conditioned pain modulation may not be as developed for younger children. It could also be hypothesized that, when younger children experience inhibitory effects during a conditioned pain modulation paradigm, their inhibitory effects may have more to do with distraction and other nonspecific effects than specific counterirritation-related endogenous pain inhibitory pathways, especially as compared to older children and adults.

Hypothesis 2: It was expected that, when compared to younger children, older children would display more robust conditioned pain modulation. Although painful conditioning stimuli were expected to lead to significantly more inhibition than nonpainful conditioning stimuli for both younger and older children, the magnitude of the difference in inhibition between painful and nonpainful conditioning was expected to be greater for older children compared to younger children.

Method

Participants

Recruitment and exclusion criteria. Fifty eight child participants were initially recruited and completed at least some study procedures, and a final sample of 54 participants was used for data analyses. Participants were healthy children ranging in age

from 6 – 12 years. The target N for the study was 48 participants based on *a priori* power analyses. Recruitment was stratified based on age, in order to ensure a varied distribution in the sample age range of 6 to 12 years. Because the target sample size for the study was 48 participants, recruitment efforts were targeted to recruit at least 16 children who were 6 or 7 years old, 16 children who were 8 or 9 years old, and 16 children who were 10, 11, or 12 years old. Once the study sample reached the target of 48 participants, we over-recruited in each age range to allow for potential exclusion during data analyses. See Figure 1 for a breakdown of participant stratification and randomization.

In order to participate, it was stipulated that children must not have an open wound on either hand, or a diagnosis that is contraindicated with cold water or pressure pain exposure, such as Reynaud's disease, sickle cell disease, musculoskeletal conditions, etc. Children also could not participate if they had a diagnosis that would interfere with their ability to follow instructions or use the equipment (such as severe intellectual disabilities, severe vision, hearing, or motor problems), or if they had a major medical condition (since a major medical condition could lead to impairment in endogenous inhibitory processes). No children were excluded prior to participation based on these exclusionary criteria.

Participants were recruited from the local suburban community using flyers, and from a summer day camp at UMBC. Flyers were posted at community locations, including: libraries, restaurants, shops, parks, around campus, etc. Flyers were also posted electronically on the UMBC website and through Facebook and other social media sites. Flyers included contact information for our research laboratory; parents who were interested in having their child participate contacted our lab, and an appointment was

scheduled for sensory testing procedures to be conducted in the laboratory.

Families who participated were given flyers that they could distribute to other families who may be interested. Participants were also given a "finder's fee" for referring other participants to the study; they were provided with $5 if they referred a friend who completed all study procedures. Participants were also compensated for their time: child participants were given $10 in cash after they completed procedures. Parents who brought their child to UMBC for the sole purpose of participating were given $10 as compensation for transportation, parking, and inconvenience.

Fifty eight child participants were initially recruited and completed at least some study procedures. One participant's data was excluded because he fell outside the study's targeted age range (he was 13 years old), while another participant's data were excluded due to equipment malfunctions and missing data (the algometer failed to correctly save and display his mean PPT scores during both CPM trials). Two additional participants were excluded because they demonstrated difficulty understanding PPT instructions (i.e., required multiple explanations of the PPT procedure, appeared confused about when to say "stop") and because their baseline mean PPT scores were very high (greater than 50 N) and close to the PPT ceiling of 60 N. These two participants were also revealed to be outliers when examining baseline mean PPT scores (using PP and QQ plots). Thus, a final sample of 54 participants was used for data analyses in this study.

Demographic information. Participants were 54 healthy school-aged children, ranging in age from 6 – 12 (M = 9.05, SD = 1.84) years. Thirty-four (63.0%) participants were male, and 20 (37.0%) were female. Participants were predominantly identified as Caucasian/White (79.6%); see Table 1 for a breakdown of participant race, gender, and

education. The majority of parents rated their child's pubertal development as about the same as other children (75.9%); 4 parents rated their child's development as somewhat earlier (7.4%), 6 rated it as "somewhat later" (11.1%) and 1 rated as "much later" (1.9%).

Setting

All CPM procedures were conducted in the same 4.88 x 3.65 m room located at our research laboratory at UMBC. The set-up of the sensory testing room is diagrammed in Figure 2. The room temperature was maintained between 21℃ and 23℃. There were two adjacent cold pressors in the room. One cold pressor was set to 12℃ (painful temperature), while the other was set at an ambient temperature (22℃). Participants sat in a chair with the cold pressors to their left side.

Equipment

Algometer. Pressure pain threshold (test stimulus) trials were conducted with an algometer, a handheld commercial device available to medical professionals and pain researchers. The device is used to deliver pressure mechanically at a steady rate via a blunt round probe at 0.5 cm^2 in diameter. The device is held perpendicular to the skin surface area and pressure is applied a constant, steady rate until the subject reports pain (or a targeted sensory intensity level), at which time the researcher stops the action of pressure (Ylinen, 2007). The maximum applied pressure is displayed in a digital display in units of N (Newton = 100 kPa (kilopascal)). Pressure algometers have been used clinically for diagnoses of neuromuscular conditions, and are also commonly used in quantitative sensory testing research (Ylinen, 2007). Pressure pain threshold has been tested in children as young as 4 years of age (Han et al., 2012; Nikolajsen et al., 2011),

and researchers have found that the procedure is well-tolerated by child participants (Chaves, Nagamine, de Sousa, de Oliveira, & Grossi, 2010; Birnie et al., 2014).

Metronome. A personal clip-on-ear metronome was used to assist researchers in providing a steady rate of pressure to participants' thumbnails while using an algometer. Two brands of metronome were used throughout the study: a Korg MM-2 Personal Clip-On-Ear Metronome, and a Korg MM-1 MetroGnome In Ear Metronome. Metronomes were clipped into the experimenter's ear and set to make a small noise/beep once per second. Researchers were trained to apply algometer pressure at a rate of approximately 2 N per second. This method of delivering a steady rate of pressure has been used with success in previous pediatric studies utilizing the algometer (Chaves, Nagamine, de Sousa, de Oliveira, & Grossi, 2007; Ferracini, Stuginsk-Barbosa, Dach, & Speciali, 2014; Ylinen, 2007).

Cold pressor. Cold pressor (conditioning stimulus) trials were conducted with the use of a Neslab RTE17 refrigerated bath circulator (60.0 x 28.9 x 47.9 cm) manufactured by Thermo Electron Corporation (Newington, NH). The cold pressor maintained the water temperature at 12°C (±0.1 °C), a temperature used in previous studies of conditioned pain modulation in adults to elicit moderate pain intensity without inducing pain tolerance (i.e., maximum tolerable pain) (Heyman et al., 2010; Lewis et al., 2012). This temperature has also been used in one previous pediatric CPM study (Williams et al., 2013) and has been found to elicit moderately painful responses. Most participants in the above study were able to tolerate prolonged exposure (1 minute) without reaching pain tolerance. For the nonpainful cold pressor trials, the same equipment and procedure was used; however, the temperature was set at ambient (room)

temperature (approximately 22°C), a temperature used in previous adult studies which included a nonpainful conditioning stimulus (Heyman et al., 2010).

Warm water bath. Pre- and post-trial warm water exposures were conducted using an Igloo commercial insulated plastic container measuring 23.5 x 24.0 x 23.5 cm. The container was filled with water to approximately 21 cm in depth. Water was heated to 35°C via a commercial aquatic water heater; safety mechanisms prohibit the heater from increasing water temperature above 35°C. A waterproof thermometer was placed within the water bath to monitor the water temperature.

Thermal feedback system. A Thermal Feedback System (Model DT-100; Power ID-91) manufactured by Bio-feedback Systems, Inc. (Boulder, CO, USA) was used to measure participants' finger surface temperature.

Measures

Family demographics and medical history. Parents completed a brief questionnaire assessing child's age, gender, ethnic identity, grade in school, and questions used to determine socioeconomic status (SES). SES was determined based on Hollingshead's (1975) index of socioeconomic status, which utilizes parents' occupations and highest year of school completed. Questionnaires also included questions regarding the child's medical history and diagnoses, past pain experiences, and parents' medical history.

Pubertal development. Parents also completed a brief 4-to-5-item questionnaire assessing their child's pubertal development, in order to detect signs of early-onset puberty. The questionnaire was based on a modified version of the Pubertal Development Scale (Carskadon & Acebo, 1993; Petersen, Crockett, Richards, & Boxer,

1988) which has been previously modified for parent-report (Mensah et al., 2013). This measure has been found to be highly reliable (Carskadon & Acebo, 1993; Petersen et al., 1988) and to have satisfactory predictive validity (Robertson et al., 1992). Parents were asked about external puberty indicators: skin changes, changes in height/growth spurt, body hair, breast growth (girls only), voice deepening (boys only), and facial hair (boys only). For each item, the parent rated their child's development on a four-point Likert type scale, as either "has not started yet," "has barely started," "has definitely started," or "seems complete." Scores for each item are then averaged to obtain a mean Pubertal Development Scale (PDS) score. Any children who are rated as "seems complete" on any indicator were considered for exclusion from analyses.

The measure also includes an item regarding the parent's perception of the child's pubertal timing: "does your son's/daughter's physical development seem to be earlier or later than most of the other boys/girls his/her age?" Parents will select one of five response options: 1) much earlier, 2) somewhat earlier, 3) about the same, 4) somewhat later, and 5) much later. This question has been used in previous studies as a measure of early pubertal development (for example, Coakley, Holmbeck, Friedman, Greenley, & Thill, 2002; Wasserman, Holmbeck, Lennon, & Amaro, 2012), and was found to predict high levels of family conflict/low family cohesion in healthy children (thus demonstrating predictive validity). Any children who were rated as "much earlier" on this item were considered for exclusion from analyses.

VAS anxiety and pain intensity ratings. Participants rated their anxiety regarding both the pressure and cold water procedures using a visual analog scale (VAS) rating. VAS ratings were 10cm lines with anchors of "not at all worried or nervous" at

the left end and "very worried or nervous" at the right end; participants were asked to make a mark somewhere on the line to show how worried or nervous they were about the pressure test and cold water test. Participants also completed VAS ratings to rate their pain intensity after each CPM trial, with anchors of "no pain" on the left end and "worst pain imaginable" on the right end. This pain intensity measure was included as a manipulation check to ensure that participants reported more pain during the painfully cold water trial as compared to the room temperature water trial. Prior research has found VAS ratings to be valid and reliable measures of pediatric pain (McGrath, 1990).

Procedure

Pilot testing. The study procedures were first piloted with 9 adult participants, and later with 2 child participants. Based on pilot testing, timing of procedures was altered. Prior to pilot testing, the total duration of cold pressor exposure was set at 120 seconds; this was chosen because it matched with adult studies that have used the same cold pressor temperature (12°C) as a conditioning stimulus and pressure pain threshold as test stimulus (i.e., Lewis, Heales, et al., 2012). Other adult studies have used even longer cold water exposure; for example, Zheng, Wang, Yao, Xue, & Arendt-Nelson (2014) used a 5-minute cold pressor exposure at 4°C. However, pilot testing revealed that a prolonged exposure time would likely not be tolerated by child participants. Of the nine adult pilot participants, four (44.4%) could not tolerate the originally proposed cold water conditioning stimulus of a 120-second exposure to 12°C degree water. One of the two child pilot participants also could not tolerate the original 120 second cold water exposure (she removed her hand after approximately 60 seconds).

Thus, it was decided that the cold water exposure time be changed to 60 seconds rather than 120 seconds. This duration of exposure mirrored the temperature and duration of one pediatric CPM study, Williams et al (2013). Williams et al (2013) also used a 12℃ cold pressor test as a conditioning stimulus and pressure pain threshold as the test stimulus. They used this timing of the initial cold pressor exposure and pressure pain threshold tests, and found robust, reliable CPM responses. The chosen 60 second exposure time was also longer than in one previous pediatric CPM study; Tsao et al. (2013) used a 30-second exposure at 5℃, with 20 seconds preceding the test stimulus. Goffaux et al. (2008) included at 3 minute exposure at 13℃, but did not describe when test stimuli were measured during this 3-minute period. Thus, the current study included a cold pressor exposure length that mirrored a previous child study with similar paradigms. It should be noted that, despite the variability in cold water exposure, adult pilot participants showed evidence of an overall effect of CPM.

Feedback from pilot participants was also used to determine the appropriateness of the video shown during the study's 10-minute break. During the 10-minute break, participants viewed a neutral, child-appropriate video clip. The video clip was taken from Disney's *Oceans*, a family-themed nature documentary. This clip was included in pilot-testing to ensure appropriateness for children, and to ensure that it was not overly emotionally valenced (i.e., it did not make participants feel particularly strong negative or positive emotions). Adult and child pilot participants provided qualitative feedback that the clip produced generally positive or neutral emotions; all pilot participants indicated that they either liked the video clip or found it be "boring," but none reported that they

disliked the video clip or found it to be upsetting. Pilot testing was also used to clarify study instructions and to determine the appropriateness of the procedures overall.

Experimenter training. Ten undergraduate research assistants were trained to conduct study procedures as experimenters. These experimenters were trained to criteria before they administered procedures with participants. Criteria included successfully completing a rehearsal full protocol with a practice participant, with minimal corrections from the principal investigator. Experimenters also underwent rigorous training for administering pressure tests. They were trained to apply pressure at a steady rate of approximately 2 N per second, using the algometer to apply pressure and the metronome to assist with timing. After multiple practice administrations over the course of at least one week, experimenters were deemed ready to reliably administer pressure pain threshold tests after meeting training criterion. To meet the training criterion, experimenters were observed and timed while completing at least two pressure tests, and were required to complete at least two PPT trials in which they reached approximately 40 N (±1 N) in 20 seconds. Participants from each age group were evenly distributed across experimenters. To minimize experimenter effects, experimenters were kept blind to study hypotheses.

Pre-session. Participants were recruited via community sources and a UMBC summer day camp. Parents completed a family demographics form, medical history form and the pubertal development scale prior to the testing session. Parents also signed consent forms. Researchers scheduled a time for parents to bring their child in to the research laboratory to participate.

Testing session. The timing and structure of study session procedures is outlined in Figure 3. Two researchers were present for every sensory testing session (either the investigator and a research assistant, or two research assistants/graduate students).

Assent procedures and acclimation period. Upon entering the lab, participants spent approximately 10 minutes completing assent procedures and pressure pain threshold practice. This time served as an acclimation period to the lab setting, and paralleled the 10-minute break provided between conditioning stimulus trials. The experimenter timed all procedures using a stopwatch.

The experimenter read an IRB-approved assent form to all participants. Children ages 7 -12 were asked to sign the form to indicate that they have provided assent. For 6-year old participants, parents signed an extra section of their consent form indicating that they waived assent for their child, however, 6-year-old participants were still asked to verbally assent.

In order to avoid coercion, child participants completed a special assent procedure in addition to reading and signing assent forms. The experimenter read a script developed by Dahlquist et al., 2007. It has been used successfully in several studies of laboratory-based pain and distraction featuring elementary school-aged children. The script is as follows:

Before I start, tell me quickly something you don't like to do. [Child should provide an answer like "eat bugs," "do homework," "clean," etc.] *So if I asked you to come to our office and* [insert child's answer], *what would you tell me?* [child should say no; prompt and correct until they do]. *Okay, well, just like you said "no" if I asked you to _____, you are also allowed to say no to doing this study. It is important that you*

understand that I will not be mad at you if you decide to not be in the study, and nobody else, like your parents, will be mad at you if you decide not to be in the study. We do not want any children doing something that they don't want to do. So, do you have to do this study if you don't want to? Will I be mad at you if you don't want to do this study? Will your parents be mad at you if you do not want to do this study?" [Child should say no to all three questions]. *"So do you still want to be in the study?"* [Child should say yes].

Participants were informed that they may ask to stop the procedures at any time, and they would still receive a prize and compensation. Researchers were instructed to terminate procedures if any child seemed confused about assent procedures, but no study session was terminated because the child seemed confused about assent.

Once assent was completed, the experimenter placed the child's hand in a pre-trial warm water bath in order to help standardize the conditioning stimulus experience across participants, and to help control for nonspecific effects (such as outdoor temperature, recent exercise) that could impact the surface temperature of the participant's hand. The participants placed their left hand in the warm water bath for 60 seconds. Finger temperature was also measured before and after the warm water bath and cold pressor exposure, as a manipulation check.

Test stimulus: Pressure Pain Threshold. After assent procedures were completed, children then completed two practice pressure pain threshold ratings in order to acclimate the participants to the pressure procedure, reduce procedural anxiety, and increase accuracy of the subsequent baseline pressure tests. Pressure pain threshold (PPT) was measured by applying a steady rate of pressure (using an algometer) to the

child's right thumbnail. Participants were prompted to face the wall for all pressure tests and CPM trials.

The experimenter first explained the pressure procedure to the participant: they displayed the algometer to the participant. *"This is called an algometer. We use it to measure pressure. It has a soft rubber tip. We're going to use the algometer to apply a steady rate of pressure to your thumbnail. At first, it should just feel like normal pressure – like when you hold or squeeze your own thumbnail."* [Researcher demonstrates by squeezing his/her own thumbnail]. *"Eventually, the pressure will start to hurt. Try squeezing your own thumbnail to see what it feels like"* [participant should squeeze their own thumbnail with their opposite hand]. *"We're going to apply pressure until your thumbnail FIRST starts to hurt. We want you to say 'STOP' as soon as the pressure FIRST starts to hurt. When you say 'STOP,' we will stop the pressure test. It's really important that we stop the test as soon as it FIRST starts to feel painful."*

Researchers then demonstrated the pressure procedure on their own thumbnail, and participants completed a VAS rating regarding their anxiety for the upcoming pressure procedure.

Researchers then completed two "practice" PPT trials on participants' right thumbnails. Researchers applied a steady rate of pressure to the participants' thumbnail using a metronome to ensure that they maintained a rate of pressure at approximately 2 N per second. As soon as the participant stated "stop," the experimenter immediately terminated application of pressure. The experimenter recorded the maximum amount of pressure, as displayed digitally on the device. After each pressure trial, participants were also prompted to verify that they said "stop" as soon as the pressure *first* began to feel

painful. Participants then warmed their left hand in a warm water bath and completed a sleep questionnaire, after which researchers conducted two additional pressure pain threshold tests on the participants' right thumbnail. These two pressure pain threshold scores were averaged into one baseline pressure threshold score.

The site for pressure pain stimulation, the child's thumbnail, was chosen based on prior pediatric experimental pain studies incorporating pressure, including one pediatric CPM study (Tsao et al., 2013). The thumbnail is a relatively non-invasive site for pressure pain testing as compared to other sites used in adult studies, such as the trapezius muscle or masseter muscle in the cheek.

For each test stimulus trial, pressure pain threshold was measured two times, and the two scores were averaged to produce a mean pressure pain threshold rating. Multiple assessments of pressure pain threshold were included for each trial, in order to obtain an accurate estimate of the child's true pressure pain threshold at that time point. Two trials were chosen to obtain an accurate reading but also to minimize the burden on child participants and reduce the possibility of sensitization to repeated pain stimuli. Lewis, Heales, and colleagues (2012) also used two pressure pain threshold measurements in their study of intrasession and intersession CPM reliability, and found that mean PPT showed excellent reliability across intrasession CPM trials.

All PPT tests also had a "ceiling" of 60 N; experimenters were instructed to immediately stop a pressure test if the maximum amount of pressure reached 60 N, regardless of whether participants had stated "stop." Researchers were instructed to review PPT instructions with any participants who reached 60 N, in order to ensure participants understood the procedure. This ceiling was established based on previous

pediatric studies utilizing PPT; previous pediatric studies using pressure pain threshold have found that participants report threshold and elect to stop the procedure well before this maximum value is met. For example, Nikolajsen et al. (2011) studied pressure pain threshold reliability in 50 children ages 4-12 years, and found that all children understood and tolerated the procedure. All participants completed four separate pressure pain threshold tests, and only one reported a single pressure pain threshold above 50.0 N. Thus, a ceiling of 60 N was deemed appropriate for allowing full variability in PPT while also protecting the participant from exposure to unnecessarily high amounts of pressure.

Conditioned Pain Modulation procedure. Researchers then provided instructions to participants regarding the length and sequence of the conditioned pain modulation procedure.

*"In a little while, we're going to do **TWO** different tests at once. You're going to place your hand in one of our water baths,"* [researchers point to the cold pressors] *"and we're going to do the pressure tests just like we did before. You'll be doing the water test with your left hand"* [point to left hand] *"and the pressure tests with your right hand"* [point to right hand]. *"We're going to do both the water test and the pressure tests at the **SAME TIME**."*

"So this is how it's going to work: in a little while, [RA2 NAME] is going to put your left hand in the water. The water is either going to feel cool, or it will feel very cold. If the water is very cold, after a while, your hand may start to feel uncomfortable or hurt. I'm going to time when you put your hand in the water, and I want you to keep your hand in the water for about 1 minute. You can see the timer there." Research assistants then

set up a visual timer for one minute; this timer was prominently displayed and visible to participants throughout the CPM procedures.

"Once the number on the timer gets to zero, I will tell you to take your hand out of the water. I want you to TRY YOUR BEST to keep your hand in the water for the entire minute, until the timer gets to zero and I tell you to take your hand out. If you can't keep your hand in the whole time because your hand hurts way too much or it feels way too uncomfortable, you can take your hand out before I tell you to, but I want you to try to keep your hand in the whole time. So, when should you take your hand out of the water?" Participants were asked when they should take their hand out of the water as a manipulation check; participants should say "*when you tell me to or when it hurts too much*" but not "*when it first starts to hurt.*" Researchers repeated instructions if necessary.

"After your left hand is in the water for a little while, I will do the two pressure tests on your right thumbnail again. Remember, for the pressure tests, you should say "STOP" as soon as your thumbnail first starts to hurt. Try to pay attention to your right thumbnail during these tests. Do you have any questions?"

After reading the instructions and ensuring that the participant was ready, the experimenters then placed the participant's left hand in the cold pressor up their wrist for 60 seconds. The water temperature was set at either 12℃ or 22℃ (ambient temperature), depending on the participant's randomized order. Twenty-five seconds after the start of hand immersion in the water, the first pressure pain threshold test was conducted; it generally lasted approximately 15 seconds. Forty-five seconds after the start of hand immersion in the water, the pressure pain threshold test was conducted

again. Evoking the test stimulus during the conditioning stimulus (rather than after) has been found to lead to robust CPM responses in both adults (Lewis, Heales, et al., 2012) and children (Tsao et al., 2013).

The first pressure test was conducted 25 seconds after initial immersion in the cold pressor for all participants, regardless of when the participant took their hand out of the water; immediately after the two pressure tests are done, participants were told to remove their hand from the water if they had not taken their hand out already. Participants who removed their hand early were told to place their hand back in the water if they could; additionally, participants' hands were not dried or warmed until after both CPM PPT trials were completed. The amount of time that participants kept their hand out of the water (in seconds) was recorded. Participants then rated the intensity of the pain in their hand using a VAS rating. Participants also placed their hand in the warm water bath after cold pressor exposure for 60 seconds, and had their left index finger temperature taken as a manipulation check.

Participants then had a 10 minute break to allow for sufficient time for CPM effects to dissipate. The 10 minute break period was chosen based on previous studies of the duration of CPM effects; most adult studies have found that CPM effects do not persist for more than 5-10 minutes (Van Wijk & Veldhuijzen, 2010). Tsao et al (2013) found that significant CPM effects in children and adolescents dissipated after 1 minute; there were no longer significant differences between subsequent and initial test stimuli. However, because Tsao and colleagues used a briefer cold pressor exposure time (30 seconds) than the planned exposure time for this study (60 seconds), we used a longer (10-minute) break period in order to fully ensure that any CPM effects have dissipated.

During the 10-minute break, participants viewed a neutral, non-emotionally-valenced video clip from Disney's *Oceans*, a family-themed nature documentary.

After the break, the conditioned pain modulation procedure was repeated, with either painful or nonpainful cold pressor temperature as the conditioning stimulus (depending on the participant's randomized order). Procedures were exactly the same, with the only difference being the temperature of the water in the cold pressor. Prior to the start of the CPM procedure, baseline pressure pain threshold ratings were also measured again as a manipulation check to ensure that any inhibitory effects from the previous conditioning stimulus trial had dissipated. Baseline PPT was also measured again in order to ensure that each CPM trial procedure was identical except for the water temperature of the conditioning stimulus.

Once both CPM trials were completed, participants completed a "post-study questionnaire" in which they provided feedback regarding study procedures (i.e., questions regarding whether they could complete the study again, what they thought of the video, etc.).

Compensation. After participants completed the study protocol, participants were allowed to pick a small age-appropriate prize from a prize box (estimated worth $1-$5). Child participants were compensated with $10 as a thank you for participating. Parents of children recruited from the community who were brought to campus solely to participate received $10 to help defer the costs and inconvenience of providing transportation for their child to the UMBC facilities. If parents chose, child participants were entered into a raffle to win an iPad Mini at the end of the study. Children were also informed that they could refer friends, classmates, and other families to the study; they

received $5 for each referred friend who completed the study. We asked all participants who completed the study about how they heard about the study; if they provided the name of a previous participant, we contacted that participant and their parent/guardian and provided previous participants with $5 for each referred person who completed the study. Four participants were referred to the study in this manner.

Results

Preliminary Analyses

Statistical analyses for the current study were completed using SAS version 9.3 and SPSS version 23. The data were first examined for outliers on all variables of interest using PP and QQ plots. As stated above, two participants were revealed to have outlying scores on their baseline PPT scores, and were also reported to display confusion regarding study instructions. Thus, these two participants were excluded from data analyses.

Mean PPT scores were calculated for baseline 1, baseline 2, cold water CPM trial, and room temperature CPM trial. See Table 2 for an overall summary of descriptive statistics for all variables.

To assist with preliminary analyses, change scores were calculated for both the painful and nonpainful CPM trials. To calculate change scores, each of the two mean PPT baseline scores were subtracted from their corresponding mean CPM PPT scores (i.e., the painful cold water CPM PPT mean score was subtracted by the mean of the baseline PPTs that immediately preceded the painful cold water CPM trial, while the nonpainful CPM mean PPT score was subtracted by the baseline mean PPT that immediately preceded that trial). An additional "discrepancy" score was calculated by

subtracting the nonpainful (room temperature) CPM change score from the painful (cold) CPM score. This variable was calculated for preliminary analyses and in order to assist visual comparisons between the painful and nonpainful change scores. For the discrepancy score, positive numbers indicate greater pain inhibition for the painful CPM condition as compared to the nonpainful CPM condition, whereas numbers near zero indicate little difference in change scores between the painful CPM and nonpainful CPM conditions, while negative numbers indicate increased inhibition for the nonpainful CPM condition as compared to the painful CPM condition. See Figure 4 for a plot of all participants' change scores for both the painful and nonpainful CPM condition. Figure 4 also plots participants' discrepancy scores.

Predictors and outcome variables were assessed for significant skew and kurtosis using Tabachnick and Fidell's (2007) guidelines regarding normality and transformations. Z-scores were calculated for both skew and kurtosis. Tabachnick and Fidell (2007) recommend that conservative alpha levels (.01 or .001) should be used to evaluate significant skew and kurtosis z-scores when utilizing small sample sizes. Thus, the current study utilized a relatively conservative alpha level of .01. Based on this criterion, no variables were found to be significantly skewed or kurtotic. Change scores and discrepancy scores were also examined for significant skew and kurtosis, and were not found to be significantly skewed or kurtotic.

Data were then examined for order effects using paired-sample *t* tests and change scores. There was no significant impact of order on cold water CPM change scores or room temperature CPM change scores ($ps \geq .695$). There was also no significant main effect of order when included in a RM ANOVA of condition (defined as baseline 1,

painful conditioning stimulus, and nonpainful conditioning stimulus), $p > .10$. Thus, order was not included as a predictor variable for subsequent analyses.

An independent samples t-test was performed that tested whether white and nonwhite children differed based on age; results showed no significant differences in age between participants who are white and participants who are nonwhite ($t(52) = -0.38$, $p = .704$).

Reliability between the two baseline PPT ratings. Because "baseline" PPT was taken twice immediately before each conditioning stimulus trial, the reliability of the two baselines was examined. The post-break "baseline" (BL2) PPT ratings were compared with the initial baseline (BL1) PPT ratings via paired-sample t-tests and intraclass correlations. T-test results revealed no significant differences between BL1 and BL2, $t(53) = 1.57$, $p = .123$. Additionally, intraclass correlations revealed the two baseline ratings to be highly reliable, Cronbach's alpha = .95.

Because the two baseline PPTs were found to be highly correlated and not significantly different, for subsequent analyses, we decided to calculate the mean of both baselines in order to create a "mean baseline PPT" variable. This "mean baseline PPT" was used in subsequent analyses examining the impact of "condition." For subsequent analyses, condition was defined as: baseline PPT (the mean of both BL1 and BL2 PPT), PPT during the painful conditioning stimulus (cold water CPM), and PPT during the nonpainful conditioning stimulus (room temperature CPM).

Cold water exposure time. Several participants had difficulty tolerating the painful conditioning stimulus (12 degree cold water bath) for the full 60 seconds. Mean time of hand in water across all participants was 42.87 seconds ($SD = 18.77$), with

approximately 28 participants (51.85%) removing their hand at some point before the 60 second timer. An outlier analysis was conducted using PP and QQ plots and scatter plots comparing time in cold water with several variables, including age, race/ethnicity, and CPM change scores, but no clear outliers could be identified. Thus, all participants were included in analyses regardless of the time of their cold water exposure.

Because this variability in exposure time could be a potential confounding variable for making inferences based on this study's results, the child's cold water exposure time was examined as a potential covariate. We first correlated cold water exposure time with both painful (cold) and nonpainful (room temperature) CPM change scores. There was no significant correlation between duration of cold water exposure and either the painful CPM ($r(53) = -.04$, $p = .792$) or nonpainful ($r(53) = -.11$, $p = .415$) change scores. Time in water also did not significantly correlate with VAS ratings of pain intensity for the painful conditioning stimulus, $r(53) = -.15$, $p = .288$.

Notably, duration of cold water exposure did significantly correlate with child age, $r(53) = .43$, $p = .001$, such that younger children were more likely to take their hand out at an earlier time, while older children were more likely to take their hand out at a later time or keep their hand in for the entire 60 seconds. Because of this variable's potential as a confound for drawing conclusions regarding age, it was considered as a covariate for models which included age as a predictor.

Main Analyses

Research question 1: Do healthy children display an inhibitory conditioned pain modulation response, above and beyond nonspecific/sensory distraction effects? Are conditioned pain modulation effects more robust (larger) in children

when a painful conditioning stimulus rather than a nonpainful conditioning stimulus is used?

***Hypothesis 1*:** It was predicted that painful conditioning stimuli would lead to significantly more inhibition than nonpainful conditioning stimuli, indicating that healthy children display CPM responses above and beyond sensory distraction or other nonspecific effects.

This hypothesis was tested via a one-way repeated measures ANOVA, with condition (baseline, painful conditioning stimulus, and nonpainful conditioning stimulus) as the within-subjects factor and PPT as the dependent variable. As predicted, there was a significant main effect of condition, $F(1.63) = 9.06$, $p = .001$, $\eta^2_p = .146$. Post-hoc tests utilizing the Bonferroni correction revealed that painful CPM PPT scores ($M = 22.50$, $SD = 10.71$) significantly differed from both baseline PPT ($M = 19.84$, $SD = 8.91$), $p = .038$, and nonpainful CPM PPT ($M = 19.85$, $SD = 9.34$), $p = .009$. Nonpainful CPM scores did not significantly differ from baseline 1 PPT scores, $p > .900$. See Figure 5 for a graph of the estimated marginal means.

Thus, hypothesis 1 was supported; the painful conditioning stimulus led to significantly more inhibition than the nonpainful conditioning stimulus when examining the overall sample. Additionally, the nonpainful conditioning stimulus did not evoke significant pain inhibition.

Research question 2: Do conditioned pain modulation responses vary based on child's age? Will the difference in effect between painful and nonpainful conditioning stimuli vary based on the child's age?

Hypothesis 2: It was expected that, when compared to younger children, older children would display more robust conditioned pain modulation. Although painful conditioning stimuli were expected to lead to significantly more inhibition than nonpainful conditioning stimuli for both younger and older children, the magnitude of the difference in inhibition between painful and nonpainful conditioning was expected to be greater for older children compared to younger children.

To examine these hypotheses, data were analyzed using multilevel modeling, specifically mixed effects linear models. As described by Cohen, Cohen, West, and Aiken (2003), multi-level modeling is an appropriate analysis technique for longitudinal designs in which there is "clustering" of outcome data (for example, repeated measures data, in which data points are not independent) and/or individual differences in systematic changes over time are expected. Also, unlike the repeated measures ANOVA model, multi-level mixed regression models allow for examination of interactions with scale variables (rather than categorical variables only), and also allow for significance testing of quadratic, cubic, and other non-linear changes over time. Thus, multi-level mixed regression modeling was considered to be an appropriate analysis for these data, because it would allow us to examine individual differences in patterns of change in repeated measures data, and because it would also allow us to test the interaction between a repeated measure (condition) and a continuous variable (age).

First, predictor variables (i.e., age and duration of cold water exposure) were centered along the mean (mean subtracted by score). This was done in order to decrease multicollinearity among predictors, based on recommendations from Cohen et al. (2003). Several regression models were then generated using mixed regression modeling in SAS.

An initial model was created with only random effects as predictors; predictor variables were then added in a step-wise fashion for each additional model. Maximum likelihood comparisons were used to indicate whether predictor models significantly fit the data better than previous, simpler models. Table 3 summarizes the results of these models. As in the RM ANOVA results, when a model including "condition" (baseline PPT, room temperature CPM PPT, and cold CPM PPT) as a linear predictor was compared to a baseline model, fit was significantly improved, χ^2 (1) = 12.30, $p < .001$. This indicates that there is a significant linear effect of condition.

For the next model, "condition x condition" was included as an additional predictor. Similar to the "test of within-subjects contrasts" that is sometimes included in SPSS output for repeated measures ANOVA, this predictor tested whether the effect of condition could be better described as quadratic rather than linear (i.e., do PPT scores increase from baseline to nonpainful CPM to cold CPM in a linear fashion? Or is the pattern of scores better described as a curve?). When condition was included as a quadratic predictor, the fit of the model was significantly improved as compared to when condition was included as a linear predictor. This indicates that there was a significant quadratic relationship between condition and PPT scores, which suggests that the pattern of the effect of condition can best be described as curvilinear rather than linear.

Next, the total time participants' hand was in the water was added to the model. This model's fit was not significantly improved compared to previous models and cold water exposure time was not a significant fixed predictor, indicating cold water exposure time was not predictive of PPT scores. Age was then added to the model as a fixed predictor, and fit was significantly improved, indicating that older age significantly

predicted higher PPT scores across time points. Finally, linear and quadratic interactions between condition and age were added. Neither interaction term significantly improved fit of the model. This indicated that, while there was a significant main effect of age, there was no significant interaction between age and condition. Condition remained a significant fixed predictor even when these other predictors were added. See Figure 6 for a graph of the estimated marginal means comparing younger vs. older children (median split) across condition. In addition, participants' age did not significantly correlate with cold or room temperature CPM change scores or participants' discrepancy scores, *p*s > .10. See Figure 7 for a scatter plot of the discrepancy scores and each participants' age. Thus, hypothesis 2 was not supported, as there was no evidence of a significant interaction between age and condition.

Sample size and power analyses. Sample size for the current study was 54 participants total. Based on a priori power analyses, it was determined that a sample size of 42 participants was needed to ensure adequate power to detect moderate effect sizes (i.e., $\eta^2_p \geq .06$) for all analyses. This determination was based on moderate to large effect sizes for main effects present in the previous literature (such as Tsao et al., 2013, $\eta^2_p = .30$ for main effect of CPM and $\eta^2_p = .06$ for main effect of age) and moderate effect sizes for interactions (Tsao et al., 2013, $\eta^2_p = 0.04$). We proposed collecting data from at least 48 participants in total in order to effectively counterbalance age and sex groups across orders.

Post hoc power analyses were also conducted for the main analyses. For the main effect of condition (i.e., the conditioned pain modulation response), effect size was large ($\eta^2_p = .146$) and achieved power for this analyses was .94. Effect size for the main effect

of age was moderate with an achieved power of .86, and effect size for the interaction between age and condition was small with an achieved power of .13.

Exploratory analyses: Exploring responders vs. non-responders to the CPM paradigm. Exploratory analyses were conducted to examine whether participants' response style to the various CPM conditions (i.e. whether or not participants showed an inhibitory effect to the painful CPM condition as compared to the nonpainful condition) was related to other variables of interest. Though there was a significant main "CPM effect" when examining the full sample, as seen in Figure 4, there was variability between participants, and a proportion of the participants did not display a "CPM effect" (i.e., their pain was not inhibited during the painful CPM condition as compared to during the nonpainful CPM condition). These participants were considered to be "non-responders" while participants who did display an inhibitory effect of the painful CS when compared to the nonpainful CS were labeled as "responders."

A new variable was created to examine if there were any significant relations between participants' response style (i.e., whether they were a "responder" or a "non-responder") and other study variables. To create this variable, the discrepancy score variable was recoded so that participants displaying an overall inhibitory effect of the painful CS were coded as "responders" to the CPM paradigm, while participants who did not display an overall inhibitory effect were coded as "non-responders." A "responder" score was defined as having a score greater than or equal to 2 N on the discrepancy score variable (indicating these participants showed at least a 2 N increase in PPT scores during the cold CPM trial as compared to the nonpainful CPM trial and baseline). Twenty-seven participants were labeled as "responders" and 27 were labeled as "non-responders" using

this criterion. See Figure 8 for a plot of all participants' discrepancy scores, with responders and non-responders labeled.

Responders and non-responders were then compared on participant age and duration of hand in cold water using independent samples *t* tests. There was no significant difference between responder and non-responder for both child's age and the duration of the child's hand in the water (ps $\geq .230$). Given the possibility that "responders" PPT scores may show different patterns compared to non-responders, multilevel modeling analyses for Hypotheses 2 were re-run with the subsample of responders only. Results were replicated from the full sample; there was no significant interaction between age and condition, $p > .547$.

Discussion

Summary of Findings and Implications

The primary aim of the current study was to expand the basic understanding of endogenous pain inhibitory processes in children by conducting a methodologically rigorous examination of pediatric conditioned pain modulation. The study was also designed to test whether the strength of conditioned pain modulation responses would vary based on a child's age, which would have implications for the timing of the development of some endogenous pain inhibitory processes. These aims were tested by the following hypotheses:

***Hypothesis 1*:** It was predicted that painful conditioning stimuli would lead to significantly more inhibition than nonpainful conditioning stimuli, indicating that healthy children display CPM responses above and beyond sensory distraction or other nonspecific effects.

This hypothesis was generally supported; pressure pain thresholds measured during the painful cold water conditioning trials were significantly higher than thresholds during both the baseline and the nonpainful (room temperature) conditioning trials. Based on this finding, it can be concluded that the study methodology produced a "conditioned pain modulation (CPM) effect" for some participants. It also suggests that the children in this study displayed a "CPM effect" that was above and beyond sensory distraction or other nonspecific effects.

This finding provides evidence that conditioned pain modulation responses are present in younger children, and replicates and expands on the limited existing literature examining the presence of CPM in children (Goffaux et al., 2008; Tsao et al., 2013; Williams et al., 2013). It suggests that children can understand and tolerate CPM procedures, and that current CPM methodology can effectively and reliably produce CPM responses in children. The finding also suggests that the underlying descending pain inhibitory processes that produce this effect appear to be intact in at least a proportion of the pre- and peri-adolescent children sampled in this study. This is especially significant given the evidence that descending inhibitory pathways may be slower to develop (Fitzgerald & Howard, 2003), and that younger children generally display greater pain sensitivity when compared to older children (Lander & Fowler-Kerry, 1991; Fanurik, Koh, & Schmitz, 2000; Kleiber et al., 2007).

This finding provides important implications for future studies of pediatric pain. Given the wide-ranging use of CPM paradigms in several areas of adult experimental pain research, this study provides evidence that CPM could also be used to test clinically relevant aspects of pain perception with children. Pediatric researchers could utilize the

CPM paradigm to better understand individual differences in pain perception (such as gender, race, level of catastrophizing, etc.) and to examine predictors of chronic pain in children. It could also be used to test the efficacy of pediatric pain interventions.

However, it is important to note that while a significant CPM effect was found when examining group comparisons across all participants, the effect was not universally present at the individual level for all participants. While some participants displayed increased inhibition during the painful conditioning stimulus, a subset of participants did not, based on their change scores (see Figures 4 and 8). Participants' responder status did not significantly relate to their age or the duration of exposure to the painfully cold stimulus. Other CPM studies have found subsets of "non-responders" who fail to demonstrate the inhibitory CPM effect in healthy populations, with some individuals displaying a facilitating CPM effect, even among healthy populations (Hermans et al., 2015). In addition, lack of inhibitory CPM response has been previously associated with several factors, including age, gender, expectations/anticipatory anxiety, chronic pain conditions, early exposure to pain, etc. (Edwards et al., 2003; Hermans et al., 2015; van Wijk & Veldhuijzen, 2010; Williams et al., 2013). It is possible the wide variability in the current study is a reflection of population-based variability in pediatric CPM responses, as found in some adult CPM studies. However, it is also possible that factors unique to this study's design contributed to the proportion of non-responders in this sample. Notably, none of the three published studies of pediatric CPM discussed the proportion of "non-responders" or participants with impaired CPM, so it is difficult to draw conclusions regarding the current study's proportion of non-responders. Further

study is needed to examine factors related to "non-responders" or pain facilitative CPM in a pediatric population.

Interestingly, there was no significant difference between PPT measured during the baselines and during the nonpainful (room temperature) conditioning stimulus, and PPT means between these two conditions were nearly the same value (*M*s = 19.84 and 19.85 N, respectively). This finding suggests that a control condition appeared to have little effect on pain threshold scores in this study. These findings are in contrast with the findings of Heyman et al., (2010), who found some evidence of "nonspecific" or sensory distraction effects when a nonpainful control condition was used in CPM protocols with adults. However, these findings replicate and support findings from other adult literature (Quiton & Greenspan, 2007; Granot et al., 2008; Kagiki, 1994; Price & McHaffie, 1988; Le Bars et al., 1979; van Wijk & Veldhuijzen, 2010) that the conditioning stimulus must be painful to produce significant pain inhibition in the test stimulus. Although the specific sham conditioning stimulus used in this study did not appear to function as a distractor or influence pain responses, , given that some previous studies have found nonspecific effects from nonpainful conditioning stimuli, controlling for these nonspecific effects strengthened this study's design and adds confidence in the study's findings. Moreover, the addition of the sham conditioning control did not appear to adversely affect the feasibility of the study procedures. Therefore, the inclusion of a sham conditioning control should be considered in future research.

While the current study's methodology was successful in producing a significant "CPM effect" for some participants, several participants could not tolerate the full 60-second exposure to the painful conditioning stimulus. Younger children were more likely

to withdraw their hand early in comparison to older children. However, the length of cold water exposure did not appear to have a significant effect on pressure pain threshold ratings, and did not appear to relate to the strength of the "CPM effect." It also did not significantly relate to participants' subjective ratings of pain intensity. This is in contrast to other findings in the adult literature (van Wijk & Veldhuijzen, 2010; Willer, Roby, & Le Bars, 1984; Lautenbacher, Roscher, & Strian, 2002; Tousignant-Laflamme et al., 2008; Nir et al., 2012), which have suggested that stronger CPM effects can be found based on the strength or "dose" of the conditioning stimulus.

There are several possible explanations for this finding. It is possible that, for the children in this study, the effect of the cold water exposure was so powerful that the total length of time did not produce much of a difference in magnitude in inhibitory responses. Exposure of even short duration may have been adequate for producing an inhibitory effect. It could also be that differences based on exposure duration were too small or subtle to detect with this study's power. Given that the study was adequately powered to detect moderate effect sizes but not small effect sizes for an age by condition interaction, this explanation is possible.

Despite the fact that shortened duration of cold water exposure did not appear to interfere with obtaining a significant CPM effect for some participants, the cold water exposure methodology for this study would likely benefit from refinement before replication in order to prevent more participants from removing their hand early. Even though the current study controlled for duration of water exposure statistically, ideally, duration of exposure to the conditioning stimulus should be uniform across all participants, in order to reduce variability between participants. Thus, future studies

could utilize a slightly milder painful conditioning stimulus (either through a warmer temperature or shorter exposure time) to ensure uniform duration of exposure.

Hypothesis 2: It was expected that, when compared to younger children, older children would display more robust conditioned pain modulation. Although painful conditioning stimuli were expected to lead to significantly more inhibition than nonpainful conditioning stimuli for both younger and older children, the magnitude of the difference in inhibition between painful and nonpainful conditioning was expected to be greater for older children compared to younger children.

This hypothesis was not supported. Although overall age effects were consistent with previous findings in the literature that younger children tend to have lower pain tolerance and higher subjective experiences of pain intensity when compared to older children (Lander & Fowler-Kerry, 1991; Fanurik, Koh, & Schmitz, 2000; Kleiber et al., 2007), there were no significant differences in the strength of the "CPM effect" based on age. This finding contrasts Tsao and colleagues' (2013) report of age differences in CPM response in healthy children ages 8-17. There are several possible explanations for the current study's failure to find age moderation effects. One explanation is that it is possible that age differences in the strength of the CPM do exist for this study's age range (6 – 12 years), but that these differences are very subtle. This study had sufficient power to detect moderate effect sizes, but not sufficient power to detect small effect sizes. However, when estimated marginal means are compared between the younger and older children in the study, as seen in Figure 5, there does not appear to be even a small difference in pattern between the two age groups.

It could also be that the design of the CPM paradigm used in the current study was not sensitive enough to detect subtle differences in the strength of CPM responses based on age. Tsao et al (2013) and Goffaux et al. (2008) utilized a test stimulus that tested *suprathreshold* pain responses (i.e., a tailored measure of moderate pressure pain intensity), rather than simply threshold responses. There is some evidence that pain threshold is sensitive to a variety of factors (related to both the stimuli and the participant) during testing compared to other pain testing measures, including participant ethnicity, age, and anxiety (Hansson, Backonja, & Bouhassira, 2007; Komiyama, Kawara, & De Laat, 2007; Nahman-Averbuch, Nir, Sprecher, & Yarnitsky, 2016; Lautenbacher, Kunz, Strate, Nielsen, & Arendt-Nielsen, 2005), which could affect its ability to detect subtle differences. Thus, it is possible that methodology utilizing suprathreshold for the test stimulus would be better suited for detecting subtle differences in CPM magnitude. However, pressure pain threshold was chosen as the test stimulus for multiple reasons, in part due to its ease and lack of expense for implementation, and also because it is considered to be a less invasive method of pain testing in children (Birnie et al., 2014). It has also been shown to be a reliable and efficient method of testing CPM in adults (van Wijk & Veldhuijzen, 2010; Lewis et al., 2012).

It is also possible that the study's age range was not wide enough to capture differences in magnitude of CPM responses. Tsao et al.'s age range was much broader; they included children of ages ranging from 8 to 17 years, and also included much older adolescents in their sample. As stated previously, there is some evidence that descending inhibitory pathways could be slow to develop in children (Fitzgerald & Howard, 2003; Hathway et al., 2014; Walker, 2014), and may not develop until pre- or peri-adolescence.

However, little is known on the exact timeline of development. It is possible that the descending inhibitory pathways that are activated in conditioned pain modulation paradigms are already well-developed in the children in my sample age range (i.e., ages 6-12 years), which would explain why no significant age-based differences in CPM effects were found. It is also possible that these descending pathways are slow to develop until adolescence (rather than peri-adolescence as theorized by Hathway and colleagues (2012) and as I hypothesized), which would then explain why Tsao and colleagues found age-based differences in CPM magnitude but no significant differences were found in this study. It is also possible that these pathways are developed to an extent prior to adolescence, but then develop further in adolescence. Further study and replication is needed to shed light on the developmental trajectory of these descending inhibitory pathways.

Limitations

The current study contains some limitations that could limit the generalizability and interpretation of findings. The current study's relatively small sample size limited power to detect small affects. Although previous literature (Tsao et al., 2013) suggested it was reasonable to expect moderate to large effect sizes for effects of condition and an age by condition interaction, the actual effect size for the condition by age interaction was quite small. It is possible that an increased sample size would improve the ability to detect smaller effect sizes for an interaction. However, as previously addressed, when estimated marginal means are compared between the younger and older children in the study, as seen in Figure 5, there does not appear to be a difference in pattern between the

two ages, which would suggest that a significant interaction could not have been found even with a larger sample size.

Additionally, recruitment was somewhat limited by the feasibility of recruiting a large number of child participants over the data collection period. At least 161 participants would have been needed to detect small effects for interaction analyses with adequate power. Previous studies in our lab have recruited between 30-50 children during summer studies (Law et al., 2011; Sil et al., 2014), and a similar number over the course of one year of recruitment. Without additional funding and incentives for participation, it would have taken approximately 4 years to collect a sample large enough to detect small effect sizes, which would be impractical. The current study recruited 58 participants (though data from 4 participants were later excluded from analyses for various reasons) during a six-month period from June to November 2015. Given previous recruitment rates for similar studies conducted in our lab, and difficulty recruiting participants outside of the summer session, recruiting more participants would have been outside the scope of the current study.

Another limitation was the homogeneity of the sample. As shown in Table 1, participants were nearly 80% White/Caucasian, and participants were mostly male (63%). Given that adult CPM studies have found differences based on race/ethnicity and gender, it is possible that a more heterogeneous sample may have produced greater variability in CPM responses, and may have also displayed CPM differences based on gender and race/ethnicity. However, since the current study was designed largely as a feasibility/pilot study of novel methodologies for studying CPM in children, the study

was limited by the demographics of the population we had access to recruit from. Future studies could explore race/ethnicity and gender issues in more detail.

The most prominent limitation is participants' variability in their duration of cold water exposure. Though this variability did not significantly relate to differences in pressure pain threshold, CPM effects, or participants' responder vs. non-responder status, the variability does represent a certain non-standardization of procedures. It also deviates from nearly all previous studies of CPM in adults and children; typically the duration of CPM exposure is standardized across all participants except when it is deliberately varied as part of the study design.

Based on previous findings that prolonged exposure to the conditioning stimulus produces stronger CPM effects, and hypotheses proposed by some adult CPM researchers such as Zheng et al. (2014), the duration of the conditioning stimulus should be prolonged in order to effectively enact the descending inhibitory pathways that are theorized to be activated during CPM protocols. A two-minute exposure time was initially proposed, and this time was shortened after pilot testing revealed that both adults and children had difficulty tolerating the prolonged exposure. The final design of the current study was modeled in part after the few existing pediatric CPM studies; of those three studies, all utilized either a similar (12℃, Williams et al., 2013; 13℃, Goffaux et al., 2008) or colder (5℃, Tsao et al., 2013) water temperature for the conditioning stimulus. Williams et al. utilized a 12℃ water bath for a 60-second exposure with children ages 7-12 years, while Goffaux utilized a 13°C bath for a 3-minute exposure with children ages 7-11 years old. Tsao et al. (2013) utilized a 5℃ water bath for 30

seconds with children ages 8-17 years. According to these researchers, most children in their studies tolerated these procedures.

However, it is possible that differences in cold pressor equipment may have influenced participants' ability to tolerate the cold water bath. Some studies utilizing the cold pressor task do not utilize a refrigerated bath circulator but rather use portable coolers and ice, which can lead to warming of the water immediately surrounding the hand due to the body's natural projection of body heat (Mitchell, MacDonald, & Brodie, 2004). The current study utilized a Neslab RTE17 refrigerated bath circulator, which continuously maintained the water temperature at 12°C ± 0.3°C and continuously circulated water to avoid localized warming of water around the hand. Williams and colleagues utilized "circulated ice water maintained at a temperature of 12°C ± 1°C" (p. 923), but did not specify the specific type of equipment. Because the margin of error was broader for that study's equipment, it is possible that participants in that study experienced a slightly warmer bath when compared to the current study. For Goffaux and colleagues, it is unknown whether an automated refrigerated bath circulator was used for the conditioning stimulus, as it was used in the current study. Thus, it is possible that children from these previous studies were better able to tolerate a similar temperature and exposure time because different equipment was used.

It is also possible that the current study's variability in cold water exposure duration was influenced by the relatively higher proportion of younger children, particularly children ages 6-7 years old. As duration of cold water exposure was highly related to age, it is likely that the current study cold pressor procedures were less well tolerated because the current study included younger children when compared to the

previous three pediatric CPM studies, all of which did not recruit participants younger than 7 or 8 years old. However, though younger children in the current study were much more likely to remove their hand early as compared to older children, some older children in the current study also removed their hand early (28 children in total removed their hand prior to 60 seconds). This suggests that, for future studies, the current study's methodology should possibly be altered, particularly if future studies plan to produce the conditioning stimulus with an automated refrigerated bath circulator, or if future studies plan to study CPM with younger children (ages 7 or less). Future studies could use a milder temperature (for example, 13°C) for 60 seconds, or could decrease the exposure time.

Another limitation of the current study is the absence of a measure of emotional valence or pain catastrophizing from participants during or immediately after CPM trials. It is possible that the CPM responses found in this study were a result of alternative pathways or mechanisms, rather than the traditional "DNIC-like" counter-irritation pathway found by Le Bars and colleagues in the original studies of CPM/DNIC in the 1970s-80s (Le Bars, Dickenson, & Besson, 1979). Because so many participants removed their hands early, it is possible that these participants were experiencing stress from the conditioning stimuli. This stress could have resulted in an alternative analgesic mechanism, such as stress-induced analgesia, an important pain inhibitory mechanism that has been extensively studied in adult experimental pain (Butler & Finn, 2009). In addition, several adult CPM studies have measured various psychological correlates in relation to CPM responses, including anxiety, depression, in vivo pain catastrophizing, etc., and some have found that these emotional states have correlated with impaired

inhibitory CPM responses (Goodin et al., 2008; Nahman-Averbuch et al., 2016; van Wijk & Veldhuijzen, 2010). Future studies utilizing this methodology could incorporate emotional valence ratings immediately before and after CPM trials, which could then be included as a predictor in order to examine the potential influence of stress-induced analgesia and other affect-related effects on CPM responses.

Conclusion

Despite the variability in cold water exposure duration and other limitations, the current study's design was still successful in producing CPM effects. The counterbalanced design was also successful in minimizing order effects, and the design was effective in producing reliable pressure point threshold baseline measures.

By including a nonpainful conditioning stimulus, the current study provides strong evidence that significant pediatric CPM responses cannot be attributed to nonspecific effects. The findings also lend additional validity to previous studies of pediatric CPM, by showing that the inhibitory effects found in this paradigm could not be solely or even partially attributed to sensory distraction or other nonspecific effects. By building and expanding on previous pediatric CPM studies, this study lays the foundation for future avenues of study. As outlined in previous sections, future studies could utilize this paradigm with similarly-aged children but may consider altering the conditioning stimulus. A slightly milder temperature of 13ºC could be used, or a shorter duration. Altered study procedures could potentially be used with children who are even younger than 6 years of age, provided that care is taken to ensure that younger children fully understand CPM procedures. Future studies could utilize a broader age range in general, since this study did not find age differences in CPM magnitude, in contrast to hypotheses

and theorized timing of descending inhibitory pathways. Future studies could also incorporate a suprathreshold measure for the test stimulus, and include emotional valence ratings immediately prior to and following the CPM trials to test for the influence of stress and other psychological factors on CPM responses.

This study provides further evidence that the CPM response is present and testable in children, particularly in children as young as 6 years of age. This implies that future studies of pediatric pain could utilize CPM paradigms to study various aspects of pediatric pain and pediatric endogenous pain inhibition. One avenue for future study is the testing of the multiple individual differences in CPM that are found in adults; pediatric studies could utilize a CPM paradigm to test for differences in gender, race/ethnicity, catastrophizing, and factors that have found to influence CPM responses and to map onto clinically significant differences in pain perception. Pediatric CPM studies could be used to test the involvement of descending inhibitory pathways in the development of these differences. CPM could also be further tested in children with chronic pain conditions, to determine if their responses are impaired when compared to healthy children. CPM paradigms could be further used as a way of empirically testing children's responses to pain interventions, or to see if CPM responses in children can be used as a predictor of developing pediatric chronic pain conditions, as in adults. In sum, conditioned pain modulation could be used as an important indicator of endogenous pain inhibition pathways in children. The findings of this study support the feasibility of implementing conditioned pain modulation studies in children, so that these endogenous inhibitory pathways can be studies more closely in children.

Table 1

Demographics

	Frequency	%
Race/Ethnicity		
Black or African American	6	11.1
White/Caucasian	43	79.6
Hispanic/Latino	1	1.9
Biracial	3	5.6
Grade		
1st	8	15.4
2nd	8	15.4
3rd	9	17.3
4th	10	19.2
5th	6	11.5
6th	9	17.3
7th	2	3.8
Gender		
Female	20	37.0
Male	34	63.0

Table 2

Descriptive Statistics of Predictors and Outcome Variables

Variable	*Mean*	*SD*	*Min*	*Max*
Baseline 1 Mean PPT[a]	20.28	9.42	7.00	47.95
Baseline 2 Mean PPT	19.39	8.87	5.70	44.85
Mean of 2 Baseline PPTs	19.84	8.91	6.35	45.60
Painful CPM Mean PPT	22.50	10.71	6.15	61.60
Nonpainful CPM Mean PPT	19.85	9.34	5.25	46.60
Duration of hand in cold water (secs)	42.87	18.77	8.00	60.00
Child age (yrs)	9.05	1.84	6.16	12.39

[a] PPT is measured in Newtons (*N*).

Table 3

	X^2	*Fixed Effects*		
Model		**Estimate**	**SE**	***p***
Condition (linear)	12.30**	18.06	1.24	<.001
Condition (quadratic)	4.5*	1.32	0.62	.035
Duration of hand in cold water	2.2	0.09	0.06	.136
Age	6.5*	1.75	0.67	.011
Age*trial (linear)	0.6	0.16	0.20	.439
Age*trial*trial (quadratic)	0.7	-0.10	0.23	.403

Mixed Regression Model Comparisons

** $p < .01$
* $p < .05$

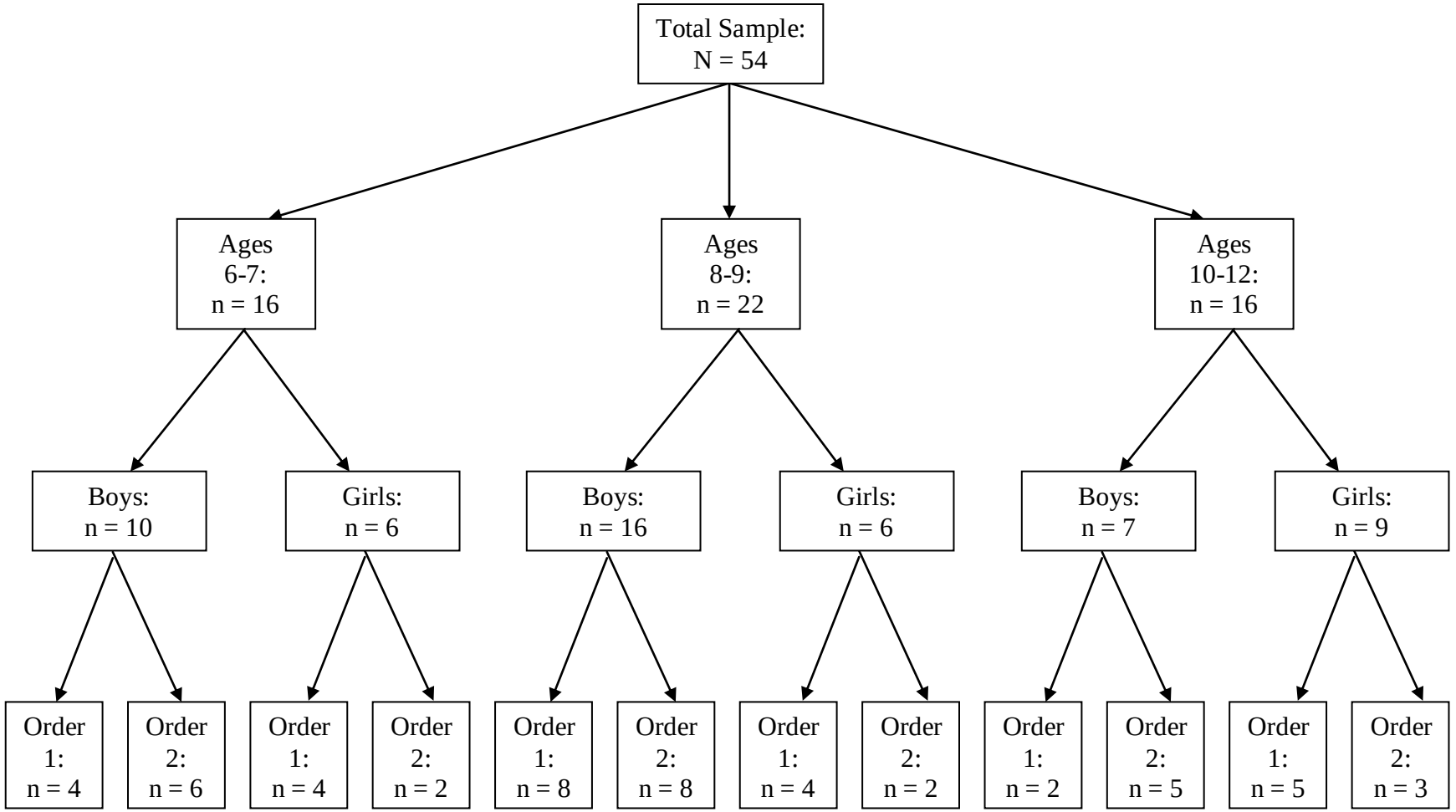


Figure 1. Participant stratification/randomization chart. Participants were stratified based on age and sex, and then randomized to either Order 1 or Order 2.

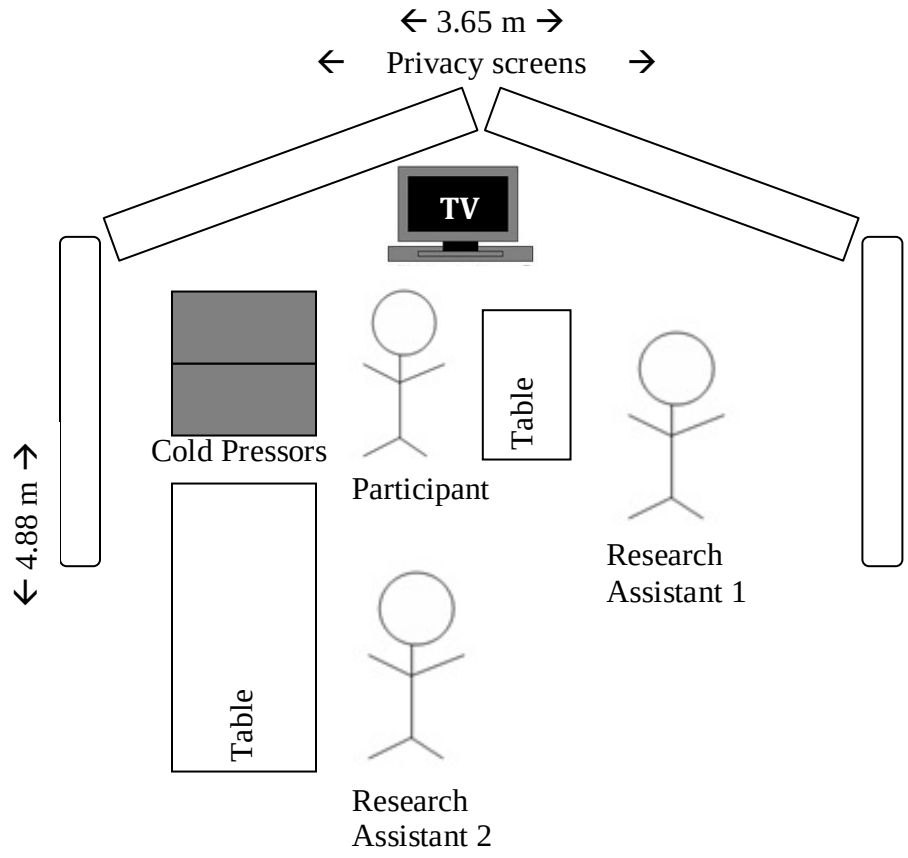


Figure 2. Layout of Study Room

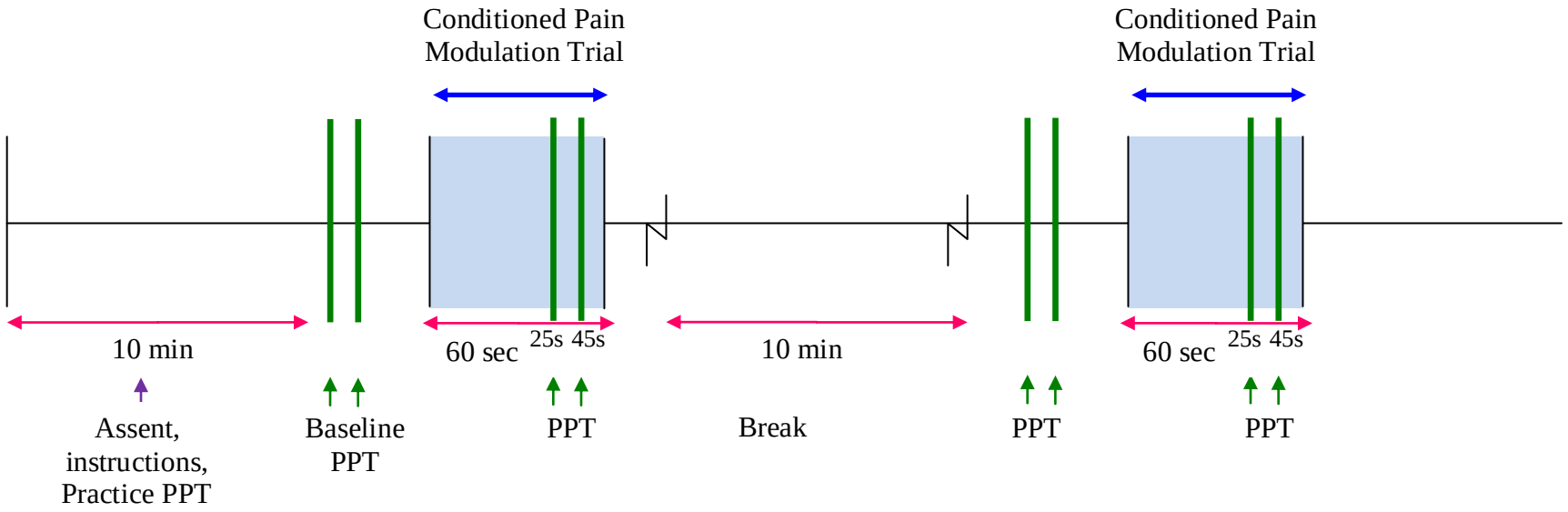


Figure 3. Timeline of Study Procedures

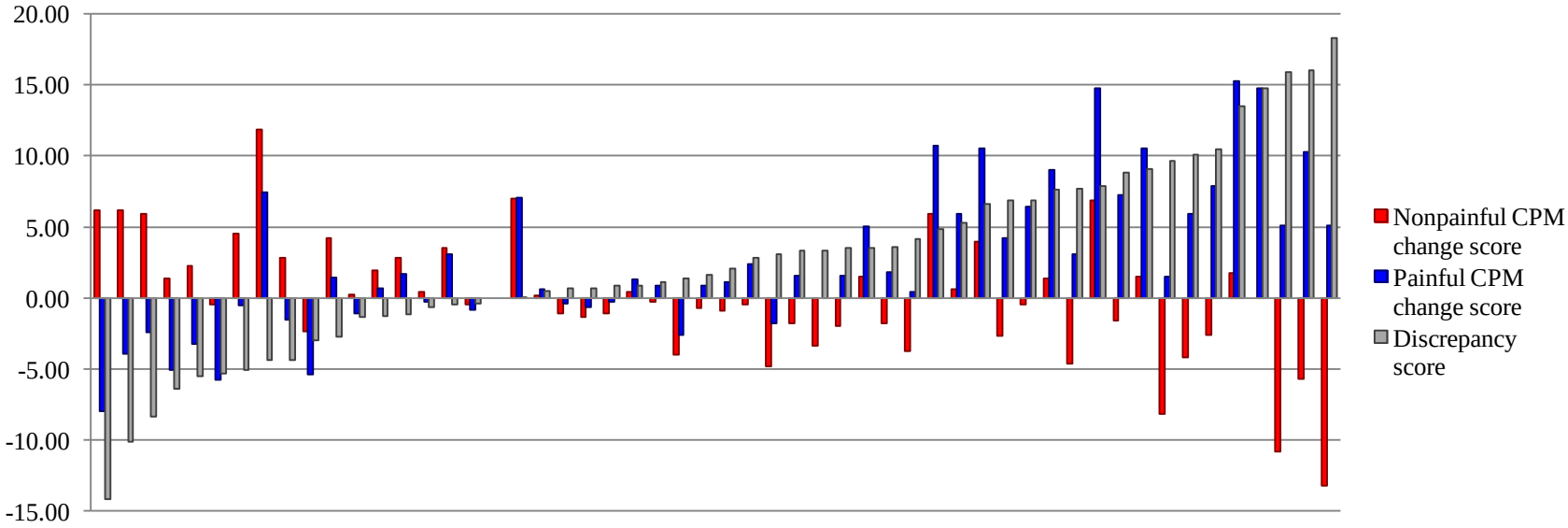


Figure 4. Plot of all participants' change scores for painful (cold) CPM condition, nonpainful (room temperature) CPM condition, and the discrepancy scores (painful [cold] CPM change score – nonpainful [room temperature] CPM change score). Participants' data for this plot were sorted from lowest to highest discrepancy score.

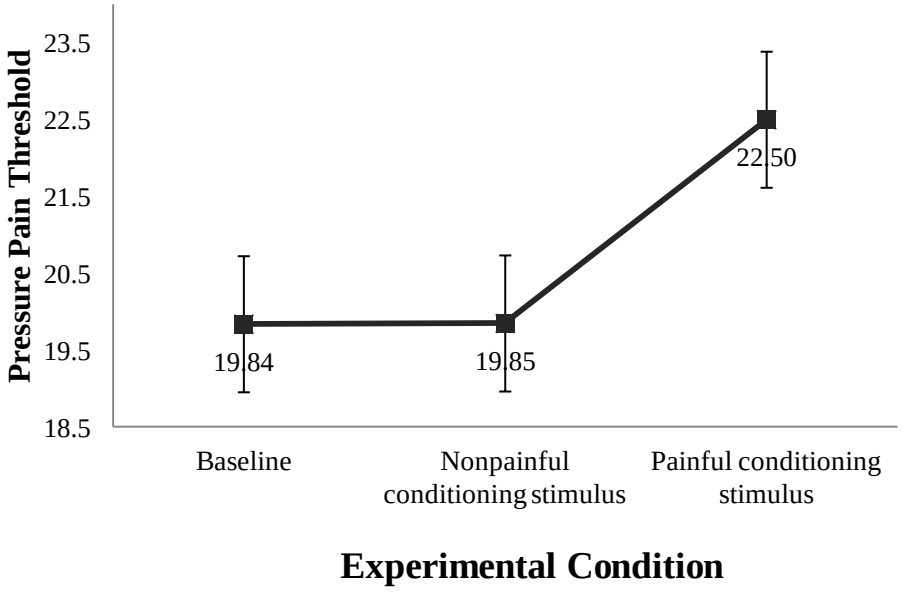


Figure 5. Effect of experimental condition on pressure pain threshold (PPT).

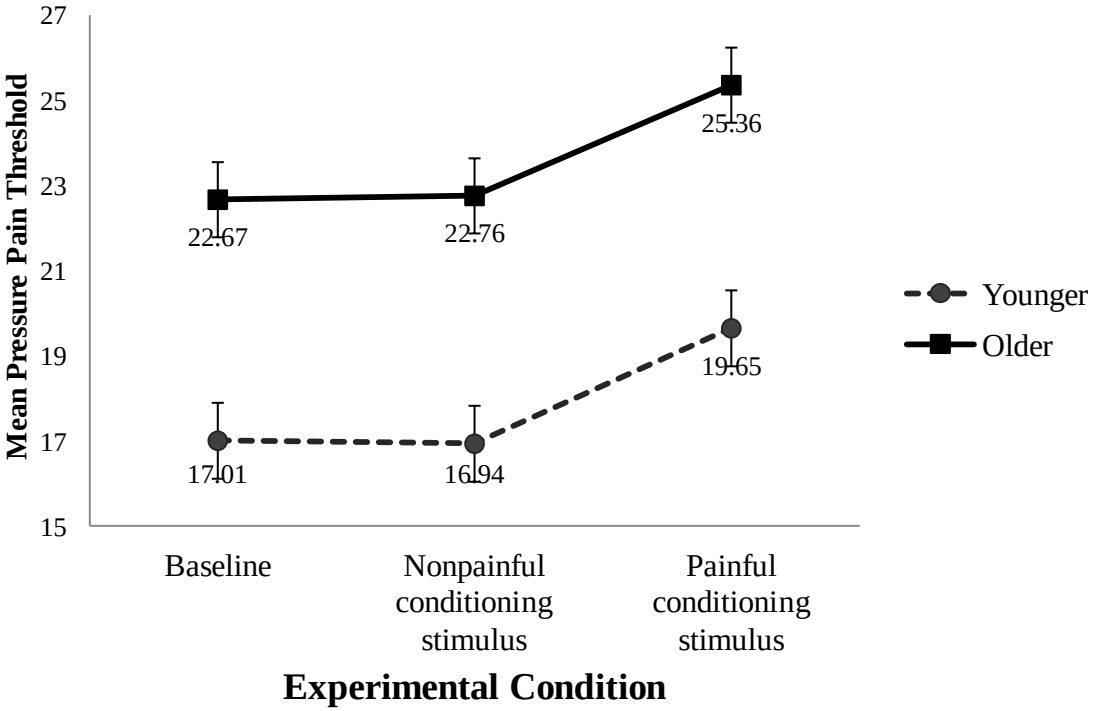


Figure 6. Effect of experimental condition on pressure pain threshold (PPT), split by age.

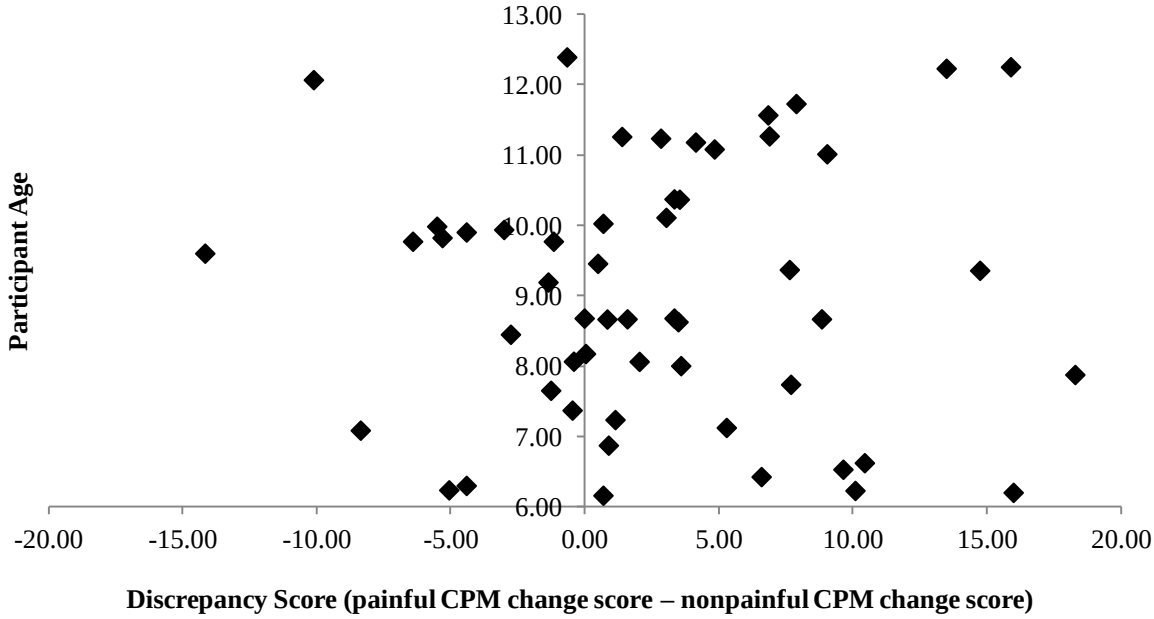


Figure 7. Scatter plot of participants' discrepancy scores (painful [cold] CPM change score – nonpainful [room temperature] CPM change score) plotted according to the participants' age. There was no significant relation between participants' age and their discrepancy scores.

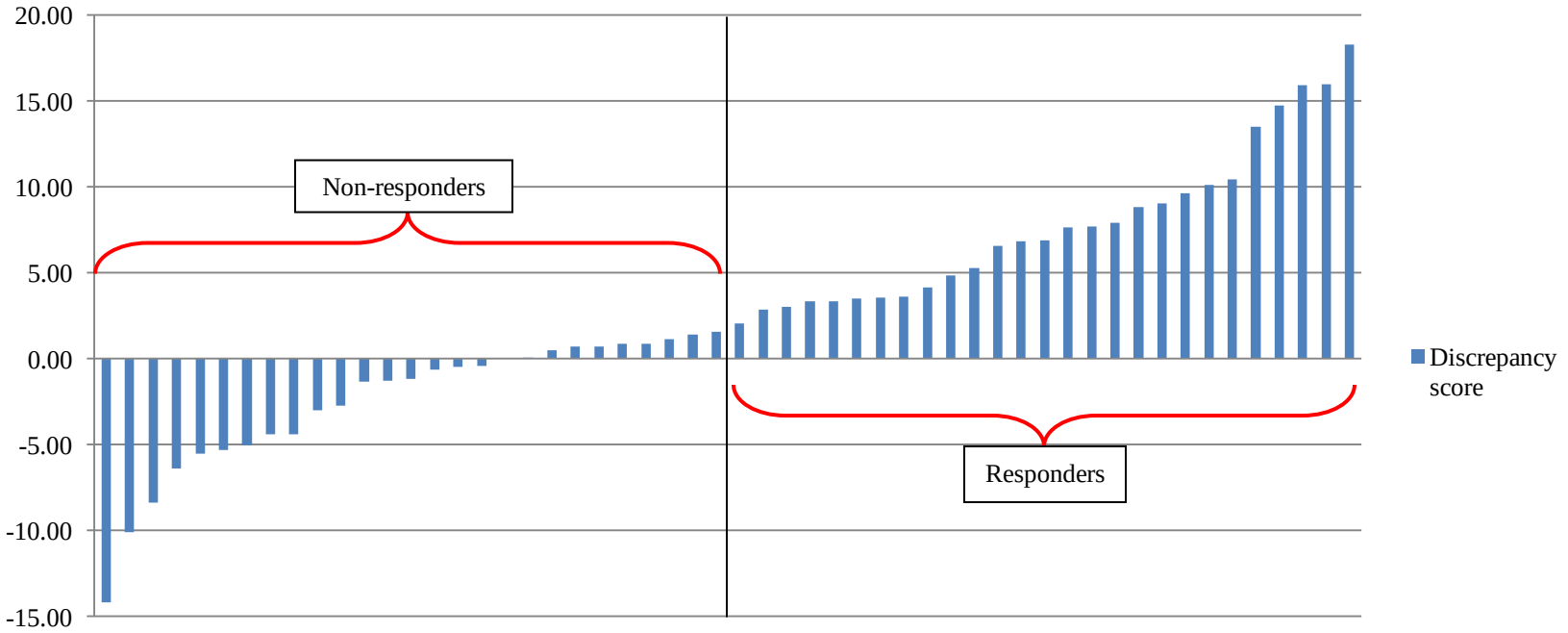


Figure 8. Plot of all participants' discrepancy scores (painful [cold] CPM change score – nonpainful [room temperature] CPM change score). Participants were labeled as "responders" if their discrepancy score was ≥2 N, indicating an inhibitory effect of at least 2 N for the painful (cold) conditioning stimulus.

Appendix

Questionnaires

Demographic Questionnaire (Parent) (rev 12-5-14)

Date _____

Child's date of birth (Month/ date/ year) _____

Child's Age _____ Grade __to __ school year _____

Child's race/ethnicity _____ Child's sex (circle): boy girl

Mother's or female caregiver's occupation*
_____

Father's or male caregiver's occupation*
_____

Highest year of school completed—Mother or female caregiver*
_____

Highest year of school completed—Father or male caregiver*
_____
(*If not applicable, please write NA)

Does your child have any of the following health conditions? If yes, please describe.

Health condition	**Yes**	**No**	**If yes, please describe**
Hearing problems			
Vision problems			
Car/motion sickness			
Seizures			
Circulation problems, (e.g., sickle cell anemia, Reynaud's disorder, etc)			
Coordination problem			
Other condition that might affect your child's response to sensory testing (describe)			

Parent Ratings of Past Medical Experiences

For each medical experience listed below, please indicate if your child has ever had this experience (yes/no). **If yes, please indicate approximately how many times and rate your child's reaction.**	**Circle Yes or No**	**If yes, about how many times?**	**Please rate your child's reaction to each experience on a scale of 1 to 7, 1 = very positive and 7 = very negative** [Circle one]								
Admitted to the hospital	Yes No		very pos.	1	2	3	4	5	6	7	very neg.
Emergency Room visit	Yes No		very pos.	1	2	3	4	5	6	7	very neg.
Doctor's Visit	Yes No		very pos.	1	2	3	4	5	6	7	very neg.
Finger stick	Yes No		very pos.	1	2	3	4	5	6	7	very neg.
Venipuncture for a blood test (blood drawn from vein)	Yes No		very pos.	1	2	3	4	5	6	7	very neg.
I.V. Placement	Yes No		very pos.	1	2	3	4	5	6	7	very neg.
Surgery	Yes No		very pos.	1	2	3	4	5	6	7	very neg.
Immunizations	Yes No		very pos.	1	2	3	4	5	6	7	very neg.
Dental Visit	Yes No		very pos.	1	2	3	4	5	6	7	very neg.
Other	Yes No		very pos.	1	2	3	4	5	6	7	very neg.

Pubertal Questionnaire

Instructions for Parents: Because the onset of puberty can impact how children respond to different sensations, we are asking that you complete the questions below. Please complete the following questions about your child's pubertal development:

	Please Circle one response:			
Would you say your child's:	**Has not started yet**	**Has barely started**	**Has definitely started**	**Seems complete**
Growth in height	0	1	2	3
Body hair growth	0	1	2	3
Skin changes (especially pimples)	0	1	2	3
FOR GIRLS: Breast growth	0	1	2	3
FOR BOYS: Voice deepening	0	1	2	3
FOR BOYS: Facial hair growth	0	1	2	3

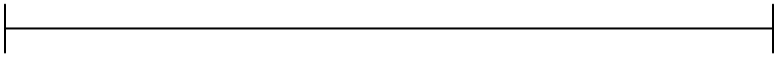
Does your son's/daughter's physical development seem to be earlier or later than most of the other boys/girls his/her age? Please circle one option:

(1) Much earlier (2) Somewhat earlier (3) About the same (4) Somewhat later (5) Much later

Pressure test rating (2/18/15)

Participant ID#_____
Date:_____

Make a mark on the line to show how worried or nervous you are about the pressure test that we are going to do.



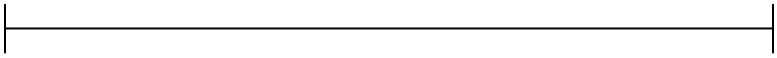
Not at all worried or nervous

Very worried or nervous

Cold water test rating (6/12/14)

Participant ID#_____
Date:_____

Make a mark on the line to show how worried or nervous you are about the cold water test that we are going to do.



Not at all worried or nervous

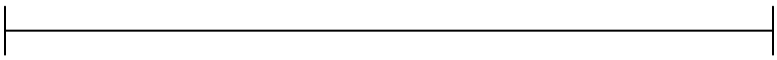
Very worried or nervous

Pain intensity rating (07/08/15)

Participant ID#_____
Date:_____

Circle 1: Frosty Olaf

Make a mark on the line to show how much pain you felt when your hand was in the water bath.



No pain Worst pain I ever felt

References

Andrews, K. A. (2003). The human developmental neurophysiology of pain. In N. L. Schechter, C. B. Berde, & M. Yaster, (Eds.), *Pain in infants, children, and adolescents*. 2nd Ed. (pp. 43-57). Philadelphia: Lippincott Williams & Wilkins.

Arendt-Nielsen, L., Nie, H., Laursen, M., Laursen, B., Madeleine, P., Simonsen, O., & Graven-Nielsen, T. (2010). Sensitization in patients with painful knee osteoarthritis. *Pain*, *149*, 573-581. doi:10.1016/j.pain.2010.04.003

Baad-Hansen, L., Poulsen, H., Jensen, H., & Svensson, P. (2005). Lack of sex differences in modulation of experimental intraoral pain by diffuse noxious inhibitory controls (DNIC). *Pain*, *116*, 359-365.

Birnie, K., Caes, L., Wilson, A., Williams, S., & Chambers, C. (2014). A practical guide and perspectives on the use of experimental pain modalities with children and adolescents. *Pain Management*, *4*, 97-111. doi:10.2217/pmt.13.72

Birnie, K., Noel, M., Chambers, C., von Baeyer, C., & Fernandez, C. (2011). The cold pressor task: Is it an ethically acceptable pain research method in children?. *Journal of Pediatric Psychology*, *36*, 1071-1081. doi:10.1093/jpepsy/jsq092

Blount, R. L., Zempsky, W. T., Jaaniste, T., Evans, S., Cohen, L., Devine, K., & Zeltzer, L. (2009). Management of Pediatric Pain and Distress due to medical disorders. In M. C. Roberts & R. G. Steele, (Eds.), *Handbook of Pediatric Psychology*. 4th Ed. (pp. 171-188). New York, NY: Guilford Press.

Bouhassira, D., Sabaté, J., Coffin, B., Le Bars, D., Willer, J., & Jian, R. (1998). Effects of rectal distensions on nociceptive flexion reflexes in humans. *The American Journal of Physiology, 275*, G410-G417.

Bouhassira, D., Villanueva, L., Bing, Z., & le Bars, D. (1992). Involvement of the subnucleus reticularis dorsalis in diffuse noxious inhibitory controls in the rat. *Brain Research, 595*, 353-357. doi:10.1016/0006-8993(92)91071-L

Butler, R. K., & Finn, D. P. (2009). Stress-induced analgesia. *Progress in Neurobiology, 88*(3), 184-202. doi:10.1016/j.pneurobio.2009.04.003

Cadden, S. W. (1993). The ability of inhibitory controls to 'switch-off' activity in dorsal horn convergent neurones in the rat. *Brain Research, 628*(1-2), 65-71. doi:10.1016/0006-8993(93)90938-J

Caes, L., Vervoort, T., Trost, Z., & Goubert, L. (2012). Impact of parental catastrophizing and contextual threat on parents' emotional and behavioral responses to their child's pain. *Pain 153,* 687–695.

Calvino, B., & Grilo, R. (2006). Central pain control. *Joint, Bone, Spine: Revue Du Rhumatisme, 73*(1), 10-16.

Campbell, C. M., France, C. R., Robinson, M. E., Logan, H. L., Geffken, G. R., & Fillingim, R. B. (2008). Ethnic differences in diffuse noxious inhibitory controls. *The Journal of Pain, 9,* 759-766. doi:10.1016/j.jpain.2008.03.010

Carskadon, M., & Acebo, C. (1993). A self-administered rating scale for pubertal development. *The Journal of Adolescent Health, 14*, 190-195.

Cathcart, S., Winefield, A. H., Lushington, K., & Rolan, P. (2010). Noxious inhibition of temporal summation is impaired in chronic tension-type headache. *Headache:*

The Journal of Head & Face Pain, *50*, 403-412. doi:10.1111/j.1526-4610.2009.01545.x

Chambers, C.T., Craig, K.D., & Bennett, S.M. (2002). The impact of maternal behavior on children's pain experiences: An experimental analysis. *Journal of Pediatric Psychology*, *27*, 293–301.

Chaves, T. C., Nagamine, H. M., de Sousa, L. M., de Oliveira, A. S., & Grossi, D. B. (2007). Intra- and interrater agreement of pressure pain threshold for masticatory structures in children reporting orofacial pain related to temporomandibular disorders and symptom-free children. *Journal Of Orofacial Pain*, *21*(2), 133-142.

Chaves, T. C., Nagamine, H. M., de Sousa, L. M., de Oliveira, A. S., & Grossi, D. B. (2010). Comparison between the reliability levels of manual palpation and pressure pain threshold in children who reported orofacial pain. *Manual Therapy*, *15*(5), 508-512. doi:10.1016/j.math.2010.03.010

Cheng, S., Foster, R. L., & Hester, N. O. (2003). A review of factors predicting children's pain experiences. *Issues in Comprehensive Pediatric Nursing*, *26*, 203-216. doi:10.1080/01460860390246678

Coakley, R., Holmbeck, G., Friedman, D., Greenley, R., & Thill, A. (2002). A longitudinal study of pubertal timing, parent-child conflict, and cohesion in families of young adolescents with spina bifida. *Journal of Pediatric Psychology*, *27*, 461-473.

Cohen, J. (1988). *Statistical power analysis for the behavioral sciences* (2nd ed.). Hillsdale, NJ: Lawrence Earlbaum Associates.

Dahlquist, L. M., McKenna, K. D., Jones, K. K., Dillinger, L., Weiss, K. E., & Ackerman, C. (2007). Active and passive distraction using a head-mounted display helmet: Effects on cold pressor pain in children. *Health Psychology, 26*, 794-801. doi:10.1037/0278-6133.26.6.794

Dahlquist, L. M., Pendley, J., Landtrip, D. S., Jones, C. L., & Steuber, C. (2002). Distraction intervention for preschoolers undergoing intramuscular injections and subcutaneous port access. *Health Psychology*, *21*, 94-99. doi:10.1037/0278-6133.21.1.94

Dahlquist, L. M., Switkin-Nagel, M. (2009). Chronic and recurrent pain. In M. C. Roberts & R. G. Steele, (Eds.), *Handbook of Pediatric Psychology*. 4th Ed. (pp. 153-170). New York, NY: Guilford Press.

Dahlquist, L. M., Weiss, K. E., Law, E. F., Sil, S., Herbert, L., Horn, S., & ... Ackerman, C. (2010). Effects of videogame distraction and a virtual reality type head-mounted display helmet on cold pressor pain in young elementary school-aged children. *Journal of Pediatric Psychology*, *35*, 617-625. doi:10.1093/jpepsy/jsp082

De Broucker, T., Cesaro, P., Willer, J., & Le Bars, D. (1990). Diffuse noxious inhibitory controls in man. Involvement of the spinoreticular tract. *Brain: A Journal of Neurology*, *113*, 1223-1234.

Defrin, R., Tsedek, I., Lugasi, I., Moriles, I., & Urca, G. (2010). The interactions between spatial summation and DNIC: Effect of the distance between two painful stimuli and attentional factors on pain perception. *Pain*, *151*, 489-495. doi:10.1016/j.pain.2010.08.009

de Lalouvière, L., Ioannou, Y., & Fitzgerald, M. (2014). Neural mechanisms underlying the pain of juvenile idiopathic arthritis. *Nature Reviews Rheumatology*, *10*, 205-211. doi:10.1038/nrrheum.2014.4

Dickenson, A., Le Bars, D., & Besson, J. (1980). Diffuse noxious inhibitory controls (DNIC). Effects on trigeminal nucleus caudalis neurones in the rat. *Brain Research*, *200*, 293-305.

Drummond, P. D., & Knudsen, L. (2011). Central pain modulation and scalp tenderness in frequent episodic tension-type headache. *Headache: The Journal of Head & Face Pain*, *51*, 375-383. doi:10.1111/j.1526-4610.2010.01779.x

Dufton, L.M., Konik, B., Colletti, R., Stanger, C., Boyer, M., Morrow, S., & Compas, B. (2008). Effects of stress on pain threshold and tolerance in children with recurrent abdominal pain. *Pain 136*, 38–43.

Eccles, J. S. (1999). The development of children ages 6 to 14. *The Future of Children*, *9*(2), 30-44. doi:10.2307/1602703

Edwards, R. R., Fillingim, R. B., & Ness, T. J. (2003). Age-related differences in endogenous pain modulation: A comparison of diffuse noxious inhibitory controls in healthy older and younger adults. *Pain*, *101*, 155-165. doi:10.1016/S0304-3959(02)00324-X

Edwards, R.R., Grace, E., Peterson, S., Klick, B., Haythornthwaite, J.A., & Smith, M.T. (2009). Sleep continuity and architecture: Associations with pain-inhibitory processes in patients with temporomandibular joint disorder. *European Journal of Pain*, *13*, 1043-1047.

Edwards, R. R., Mensing, G., Cahalan, C., Greenbaum, S., Narang, S., Belfer, I., & … Jamison, R. N. (2013). Alteration in pain modulation in women with persistent pain after lumpectomy: Influence of catastrophizing. *Journal Of Pain & Symptom Management, 46*, 30-42. doi:10.1016/j.jpainsymman.2012.06.016

Edwards, R. R., Ness, T. J., Weigent, D. A., & Fillingim, R. B. (2003). Individual differences in diffuse noxious inhibitory controls (DNIC): Association with clinical variables. *Pain, 106*, 427-437. doi:10.1016/j.pain.2003.09.005

Edwards, R. R., Sarlani, E., Wesselmann, U., & Fillingim, R. B. (2005). Quantitative assessment of experimental pain perception: Multiple domains of clinical relevance. *Pain, 114*, 315-319. doi:10.1016/j.pain.2005.01.007

Evans, S., Seidman, L., Lung, K., Zeltzer, L., & Tsao, J. (2013). Sex differences in the relationship between maternal fear of pain and children's conditioned pain modulation. *Journal of Pain Research, 6*, 231-238. doi:10.2147/JPR.S43172

Evans, S., Seidman, L., Tsao, J., Lung, K., Zeltzer, L., & Naliboff, B. (2013). Heart rate variability as a biomarker for autonomic nervous system response differences between children with chronic pain and healthy control children. *Journal of Pain Research, 6*, 449-457. doi:10.2147/JPR.S43849

Fanurik, D., Koh, J. L., & Schmitz, M. L. (2000). Distraction techniques combined with emla: Effects of IV insertion pain and distress in children. *Children's Health Care*, 29, 87-101.

Ferracini, G. N., Stuginsk-Barbosa, J., Dach, F., & Speciali, J. G. (2014). A comparison pressure pain threshold in pericranial and extracephalic regions in children with migraine. *Pain Medicine*, *15*(4), 702-709. doi:10.1111/pme.12353

Fillingim, R. B., King, C. D., Ribeiro-Dasilva, M. C., Rahim-Williams, B., & Riley III, J. L. (2009). Sex, gender, and pain: A review of recent clinical and experimental findings. *The Journal of Pain*, 10, 447-485.

Fitzgerald, M., & Howard, R. F. (2003). The neurobiologic basis of pediatric pain. In N. L. Schechter, C. B. Berde, & M. Yaster, (Eds.). *Pain in infants, children, and adolescents*. 2nd Ed. (pp. 19-42). Philadelphia: Lippincott Williams & Wilkins.

Fitzgerald, M., & Walker, S. (2009). Infant pain management: a developmental neurobiological approach. *Nature Clinical Practice. Neurology*, *5*(1), 35-50. doi:10.1038/ncpneuro0984

France, C., & Suchowiecki, S. (1999). A comparison of diffuse noxious inhibitory controls in men and women. *Pain*, *81*, 77-84.

Gaffney, A., McGrath, P., & Dick, B. (2003). Measuring pain in children: Developmental and instrument issues. In N. L. Schechter, C. B. Berde, & M. Yaster, (Eds.). *Pain in infants, children, and adolescents*. 2nd Ed. (pp. 129-141). Philadelphia: Lippincott Williams & Wilkins.

Gebhart, G. F. (2004). Descending modulation of pain. *Neuroscience & Biobehavioral Reviews*, *27*, 729. doi:10.1016/j.neubiorev.2003.11.008

Goffaux, P., Lafrenaye, S., Morin, M., Patural, H., Demers, G., & Marchand, S. (2008). Preterm births: Can neonatal pain alter the development of endogenous gating systems?. *European Journal of Pain*, *12*, 945-951. doi:10.1016/j.ejpain.2008.01.003

Gogtay, N., & Thompson, P. M. (2010). Mapping gray matter development: Implications for typical development and vulnerability to psychopathology. *Brain & Cognition, 72,* 6-15. doi:10.1016/j.bandc.2009.08.009

Goodin, B. R., McGuire, L., Allshouse, M., Stapleton, L., Haythornthwaite, J. A., Burns, N., & ... Edwards, R. R. (2009). Associations between catastrophizing and endogenous pain-inhibitory processes: Sex differences. *The Journal of Pain, 10,* 180-190. doi:10.1016/j.jpain.2008.08.012

Goodin, B. R., Kronfli, T., King, C. D., Glover, T. L., Sibille, K., & Fillingim, R. B. (2013). Testing the relation between dispositional optimism and conditioned pain modulation: Does ethnicity matter?. *Journal of Behavioral Medicine, 36,* 165-174. doi:10.1007/s10865-012-9411-7

Granot, M., Weissman-Fogel, I., Crispel, Y., Pud, D., Granovsky, Y., Sprecher, E., & Yarnitsky, D. (2008). Determinants of endogenous analgesia magnitude in a diffuse noxious inhibitory control (DNIC) paradigm: Do conditioning stimulus painfulness, gender and personality variables matter?. *Pain, 136,* 142-149. doi:10.1016/j.pain.2007.06.029

Granovsky, Y. (2013). Conditioned pain modulation: A predictor for development and treatment of neuropathic pain. *Current Pain and Headache Reports, 17,* 361. doi:10.1007/s11916-013-0361-8

Grosen, K., Vase, L., Pilegaard, H. K., Pfeiffer-Jensen, M., & Drewes, A. M. (2014). Conditioned pain modulation and situational pain catastrophizing as preoperative predictors of pain following chest wall Surgery: A prospective observational cohort study. *Plos ONE, 9*(2), 1-15. doi:10.1371/journal.pone.0090185

Haack, M. M., Scott-Sutherland, J. J., Santangelo, G. G., Simpson, N. S., Sethna, N. N., & Mullington, J. M. (2012). Pain sensitivity and modulation in primary insomnia. *European Journal of Pain*, *16*, 522-533. doi:10.1016/j.ejpain.2011.07.007

Han, T., Hong, C., Kuo, F., Hsieh, Y., Chou, L., & Kao, M. (2012). Mechanical pain sensitivity of deep tissues in children--possible development of myofascial trigger points in children. *BMC Musculoskeletal Disorders*, *13*13. doi:10.1186/1471-2474-13-13

Hansson, P., Backonja, M., & Bouhassira, D. (2007). Usefulness and limitations of quantitative sensory testing: Clinical and research application in neuropathic pain states. *Pain*, *129*(3), 256-259. doi:10.1016/j.pain.2007.03.030

Hathway, G. J., Vega-Avelaira, D., & Fitzgerald, M. (2012). A critical period in the supraspinal control of pain: Opioid-dependent changes in brainstem rostroventral medulla function in preadolescence. *Pain*, *153*, 775-783. doi:10.1016/j.pain.2011.11.011

Hermann, C., Hohmeister, J., Demirakça, S., Zohsel, K., & Flor, H. (2006). Long-term alteration of pain sensitivity in school-aged children with early pain experiences. *Pain*, *125*, 278-285.

Hermans, L., Van Oosterwijck, J., Goubert, D., Goudman, L., Crombez, G., Calders, P., & Meeus, M. (2015). Inventory of Personal Factors Influencing Conditioned Pain Modulation in Healthy People: A Systematic Literature Review. *Pain Practice: The Official Journal Of World Institute Of Pain*, doi:10.1111/papr.12305

Heyman, S., Maixner, W., Whitehead, W. E., Klatzkin, R. R., Mechlin, B., & Light, K. C. (2010). Central processing of noxious somatic stimuli in patients with irritable bowel syndrome compared with healthy controls. *The Clinical Journal of Pain*, *26*, 104-109. doi:10.1097/AJP.0b013e3181bff800

Hohmeister, J., Kroll, A., Wollgarten-Hadamek, I., Zohsel, K., Demirakça, S., Flor, H., & Hermann, C. (2010). Cerebral processing of pain in school-aged children with neonatal nociceptive input: An exploratory fMRI study. *Pain*, *150*, 257-267. doi:10.1016/j.pain.2010.04.004

Hollingshead, A. B. (1975). *Four factor index of social status*. New Haven, CT: Yale University.

International Association for the Study of Pain. (2012, May 22). IASP Taxonomy: Pain Terms. Retrieved August 1, 2014, from: http://www.iasp-pain.org/Education/Content.aspx?Item Number=1698&navItemNumber=576#Pain

Kakigi, R. (1994). Diffuse noxious inhibitory control: Reappraisal by pain-related somatosensory evoked potentials following CO2 laser stimulation. *Journal Of The Neurological Sciences*, *125*, 198-205.

Kashima, K., Rahman, O., Sakoda, S., & Shiba, R. (1999). Increased pain sensitivity of the upper extremities of TMD patients with myalgia to experimentally-evoked noxious stimulation: possibility of worsened endogenous opioid systems. *Cranio: The Journal of Craniomandibular Practice*, *17*, 241-246.

King, S., Chambers, C. T., Huguet, A., MacNevin, R. C., McGrath, P. J., Parker, L., & MacDonald, A. J. (2011). The epidemiology of chronic pain in children and

adolescents revisited: A systematic review. *Pain, 152*, 2729-2738. doi:10.1016/j.pain.2011.07.016

King, C., Goodin, B., Kindler, L., Caudle, R., Edwards, R., Gravenstein, N., & ... Fillingim, R. (2013). Reduction of conditioned pain modulation in humans by naltrexone: An exploratory study of the effects of pain catastrophizing. *Journal of Behavioral Medicine, 36*, 315-327. doi:10.1007/s10865-012-9424-2

King, C., Wong, F., Currie, T., Mauderli, A., Fillingim, R., & Riley, J. (2009). Deficiency in endogenous modulation of prolonged heat pain in patients with Irritable Bowel Syndrome and Temporomandibular Disorder. *Pain, 143*, 172-178. doi:10.1016/j.pain.2008.12.027

Kleiber, C., Schutte, D. L., McCarthy, A., Floria-Santos, M., Murray, J. C., & Hanrahan, K. (2007). Predictors of topical anesthetic effectiveness in children. *The Journal Of Pain,8*, 168-174. doi:10.1016/j.jpain.2006.08.002

Komiyama, O., Kawara, M., & De Laat, A. (2007). Ethnic differences regarding tactile and pain thresholds in the trigeminal region. *The Journal Of Pain, 8*(4), 363-369. doi:10.1016/j.jpain.2006.12.002

Kosek, E., & Ordeberg, G. (2000). Lack of pressure pain modulation by heterotopic noxious conditioning stimulation in patients with painful osteoarthritis before, but not following, surgical pain relief. *Pain, 88*, 69-78.

Lander, J., & Fowler-Kerry. (1991). Age differences in children's pain. *Perceptual and Motor Skills, 73*, 415-418.

Larivière, M., Goffaux, P., Marchand, S., & Julien, N. (2007). Changes in pain perception and descending inhibitory controls start at middle age in healthy

adults. *The Clinical Journal of Pain, 23,* 506-510. doi:10.1097/AJP.0b013e31806a23e8

Lautenbacher, S., Kunz, M., & Burkhardt, S. (2008). The effects of DNIC-type inhibition on temporal summation compared to single pulse processing: Does sex matter?. *Pain, 140,* 429-435. doi:10.1016/j.pain.2008.09.019

Lautenbacher, S., Kunz, M., Strate, P., Nielsen, J., & Arendt-Nielsen, L. (2005). Age effects on pain thresholds, temporal summation and spatial summation of heat and pressure pain. *Pain, 115*(3), 410-418. doi:10.1016/j.pain.2005.03.025

Lautenbacher, S., & Rollman, G. (1997). Possible deficiencies of pain modulation in fibromyalgia. *The Clinical Journal of Pain, 13,* 189-196.

Lautenbacher, S., Roscher, S., & Strian, F. (2002). Inhibitory effects do not depend on the subjective experience of pain during heterotopic noxious conditioning stimulation (HNCS): A contribution to the psychophysics of pain inhibition. *European Journal Of Pain, 6,* 365-374.

Law, E. F., Dahlquist, L. M., Sil, S., Weiss, K. E., Herbert, L., Wohlheiter, K., & Horn, S. (2011). Videogame distraction using virtual reality technology for children experiencing cold pressor pain: The role of cognitive processing. *Journal of Pediatric Psychology, 36,* 84-94. doi:10.1093/jpepsy/jsq063

Lax, I., Duerden, E., Lin, S., Mallar Chakravarty, M., Donner, E., Lerch, J., & Taylor, M. (2013). Neuroanatomical consequences of very preterm birth in middle childhood. *Brain Structure & Function, 218,* 575-585. doi:10.1007/s00429-012-0417-2

Le Bars, D., Dickenson, A., & Besson, J. (1979). Diffuse noxious inhibitory controls (DNIC). I.: Effects on dorsal horn convergent neurones in the rat. *Pain, 6*, 283-304.

Leffler, A., Hansson, P., & Kosek, E. (2002). Somatosensory perception in a remote pain-free area and function of diffuse noxious inhibitory controls (DNIC) in patients suffering from long-term trapezius myalgia. *European Journal of Pain, 6*, 149-159.

Leffler, A., Kosek, E., Lerndal, T., Nordmark, B., & Hansson, P. (2002). Somatosensory perception and function of diffuse noxious inhibitory controls (DNIC) in patients suffering from rheumatoid arthritis. *European Journal of Pain, 6*, 161-176. doi:10.1053/eujp.2001.0313

Lewis, G., Heales, L., Rice, D., Rome, K., & McNair, P. (2012). Reliability of the conditioned pain modulation paradigm to assess endogenous inhibitory pain pathways. *Pain Research & Management: The Journal of the Canadian Pain Society, 17*(2), 98-102.

Lewis, G., Rice, D., & McNair, P. (2012). Conditioned pain modulation in populations with chronic pain: A systematic review and meta-analysis. *The Journal of Pain, 13*, 936-944. doi:10.1016/j.jpain.2012.07.005

Longe, S., Wise, R., Bantick, S., Lloyd, D., Johansen-Berg, H., McGlone, F., & Tracey, I. (2001). Counter-stimulatory effects on pain perception and processing are significantly altered by attention: An fMRI study. *Neuroreport, 12*, 2021-2025.

Lu, Q., Tsao, J.C., Myers, C.D., Kim, S.C., & Zeltzer, L.K. (2007). Coping predictors of children's laboratory-induced pain tolerance, intensity, and

unpleasantness. *Journal of Pain, 8*,708–717.

McGrath, P. A. (1990). Pain in children: Nature, assessment, and treatment. New York: Guilford Press.

McGrath, P., & Brown, S. (2006). Quantitative sensory testing in children: Practical considerations for research and clinical practice. *Pain, 123*, 1-2.

Meeus, M., Nijs, J., Van de Wauwer, N., Toeback, L., & Truijen, S. (2008). Diffuse noxious inhibitory control is delayed in chronic fatigue syndrome: An experimental study. *Pain*, *139*, 439-448. doi:10.1016/j.pain.2008.05.018

Melzack, R., & Wall, P. D. (1965). Pain mechanisms: A new theory. *Science, 150*, 971-979. doi:10.1126/science.150.3699.971.

Melzack, R., & Wall, P. D. (1982). *The challenge of pain*. New York: Basic Books.

Mensah, F., Bayer, J., Wake, M., Carlin, J., Allen, N., & Patton, G. (2013). Early puberty and childhood social and behavioral adjustment. *The Journal of Adolescent Health,, 53*, 118-124. doi:10.1016/j.jadohealth.2012.12.018

Mitchell, L. A., MacDonald, R. R., & Brodie, E. E. (2004). Temperature and the cold pressor test. *The Journal of Pain, 5*(4), 233-238.

Moont, R., Pud, D., Sprecher, E., Sharvit, G., & Yarnitsky, D. (2010). 'Pain inhibits pain' mechanisms: Is pain modulation simply due to distraction?. *Pain*, *150*, 113-120. doi:10.1016/j.pain.2010.04.009

Myers, C.D., Tsao, J.C., Glover, D.A., Kim, S.C., Turk, N., & Zeltzer, L.K. (2006). Sex, gender, and age: Contributions to laboratory pain responding in children and adolescents. *Journal of Pain, 7*, 556–564.

Mylius, V. V., Engau, I. I., Teepker, M. M., Stiasny-Kolster, K. K., Schepelmann, K. K.,

Oertel, W. H., & ... Möller, J. C. (2009). Pain sensitivity and descending inhibition of pain in Parkinson's disease. *Journal of Neurology, Neurosurgery & Psychiatry*, *80*(1), 24-28. doi:10.1136/jnnp.2008.145995

Nahman-Averbuch, H., Nir, R., Sprecher, E., & Yarnitsky, D. (2016). Psychological factors and conditioned pain modulation: A meta-analysis. *The Clinical Journal Of Pain*, *32*(6), 541-554. doi:10.1097/AJP.0000000000000296

Nikolajsen, L., Kristensen, A. D., Pedersen, L. K., Rahbek, O., Jensen, T. S., & Møller-Madsen, B. (2011). Intra- and interrater agreement of pressure pain thresholds in children with orthopedic disorders. *Journal of Children's Orthopaedics*, *5*(3), 173-178. doi:10.1007/s11832-011-0336-4

Nir, R., Granovsky, Y., Yarnitsky, D., Sprecher, E., & Granot, M. (2011). A psychophysical study of endogenous analgesia: The role of the conditioning pain in the induction and magnitude of conditioned pain modulation. *European Journal of Pain*, *15*, 491-497. doi:10.1016/j.ejpain.2010.10.001

Nir, R., Yarnitsky, D., Honigman, L., & Granot, M. (2012). Cognitive manipulation targeted at decreasing the conditioning pain perception reduces the efficacy of conditioned pain modulation. *Pain*, *153*, 170-176. doi:10.1016/j.pain.2011.10.010

Oono, Y., Nie, H., Matos, R. L., Wang, K., & Arendt-Nelson, L. (2011). The inter- and intra-individual variance in descending pain modulation evoked by different conditioning stimuli in healthy men. *Scandinavian Journal of Pain, 2*, 162-169. doi: 10.1016/j.sjpain.2011.05.006

Perrotta, A., Serrao, M., Sandrini, G., Burstein, R., Sances, G., Rossi, P., & ... Nappi, G. (2010). Sensitisation of spinal cord pain processing in medication overuse

headache involves supraspinal pain control. *Cephalalgia, 30,* 272-284. doi:10.1111/j.1468-2982.2009.01914.x

Petersen, A. C., Crockett, L., Richards, M., & Boxer, A. (1988). A self-report measure of pubertal status: Reliability, validity, and initial norms. *Journal of Youth And Adolescence*, *17*, 117-133. doi:10.1007/BF01537962

Piché, M., Arsenault, M., & Rainville, P. (2009). Cerebral and cerebrospinal processes underlying counterirritation analgesia. *Journal of Neuroscience, 29*, 14236-14246. doi:10.1523/JNEUROSCI.2341-09.2009

Piché, M., Arsenault, M., Poitras, P., Rainville, P., & Bouin, M. (2010). Widespread hypersensitivity is related to altered pain inhibition processes in irritable bowel syndrome. *Pain, 148,* 49-58. doi:10.1016/j.pain.2009.10.005

Pielsticker, A., Haag, G., Zaudig, M., & Lautenbacher, S. (2005). Impairment of pain inhibition in chronic tension-type headache. *Pain, 118*, 215-223. doi:10.1016/j.pain.2005.08.019

Popescu, A., LeResche, L., Truelove, E. L., & Drangsholt, M. T. (2010). Gender differences in pain modulation by diffuse noxious inhibitory controls: A systematic review. *Pain, 150,* 309-318. doi:10.1016/j.pain.2010.05.013

Price, D. D., & McHaffie, J. G. (1988). Effects of heterotopic conditioning stimuli on first and second pain: A psychophysical evaluation in humans. *Pain, 34*, 245-252. doi:10.1016/0304-3959(88)90119-4

Pud, D., Sprecher, E., & Yarnitsky, D. (2005). Homotopic and heterotopic effects of endogenous analgesia in healthy volunteers. *Neuroscience Letters, 380,* 209-213.

Pud, D., Granovsky, Y., & Yarnitsky, D. (2009). The methodology of experimentally induced diffuse noxious inhibitory control (DNIC)-like effect in humans. *Pain, 144*, 16-19. doi:10.1016/j.pain.2009.02.015

Quiton, R. L., & Greenspan, J. D. (2007). Sex differences in endogenous pain modulation by distracting and painful conditioning stimulation. *Pain, 132*, S134-S149. doi:10.1016/j.pain.2007.09.001

Razavi, M., Hansson, P., Johansson, B., & Leffler, A. (2014). The influence of intensity and duration of a painful conditioning stimulation on conditioned pain modulation in volunteers. *European Journal of Pain,, 18*, 853-861. doi:10.1002/j.1532-2149.2013.00435.x

Reinert, A., Treede, R., & Bromm, B. (2000). The pain inhibiting pain effect: An electrophysiological study in humans. *Brain Research, 862*, 103-110.

Robertson, E. B., Skinner, M. L., Love, M. M., Elder, G. H., Conger, R. D., Dubas, J. S., & Petersen, A. C. (1992). The pubertal development scale: A rural and suburban comparison. *The Journal of Early Adolescence, 12*, 174-186. doi:10.1177/0272431692012002003

Roby-Brami, A., Bussel, B., Willer, J., & Le Bars, D. (1987). An electrophysiological investigation into the pain-relieving effects of heterotopic nociceptive stimuli. Probable involvement of a supraspinal loop. *Brain: A Journal of Neurology, 110,* 1497-1508.

Roosink, M., Renzenbrink, G., Buitenweg, J., van Dongen, R., Geurts, A., & Ijzerman, M. (2011). Somatosensory symptoms and signs and conditioned pain modulation

in chronic post-stroke shoulder pain. *The Journal of Pain: Official Journal of The American Pain Society*, *12*, 476-485. doi:10.1016/j.jpain.2010.10.009

Salum, G., Polanczyk, G., Miguel, E., & Rohde, L. (2010). Effects of childhood development on late-life mental disorders. *Current Opinion in Psychiatry*, *23*, 498-503. doi:10.1097/YCO.0b013e32833ead33

Sandrini, G. G., Rossi, P. P., Milanov, I. I., Serrao, M. M., Cecchini, A. P., & Nappi, G. G. (2006). Abnormal modulatory influence of diffuse noxious inhibitory controls in migraine and chronic tension-type headache patients. *Cephalalgia*, *26*, 782-789. doi:10.1111/j.1468-2982.2006.01130.x

Schechter, N. L., Berde, C. B., & Yaster, M. (2003). Pain in infants, children, and adolescents: An overview. In N. L. Schechter, C. B. Berde, & M. Yaster, (Eds.). *Pain in infants, children, and adolescents*. 2nd Ed. (pp. 1-18). Philadelphia: Lippincott Williams & Wilkins.

Serrao, M., Rossi, P., Sandrini, G., Parisi, L., Amabile, G., Nappi, G., & Pierelli, F. (2004). Effects of diffuse noxious inhibitory controls on temporal summation of the RIII reflex in humans. *Pain*, *112*, 353-360. doi:10.1016/j.pain.2004.09.018

Shaw, P., Gogtay, N., & Rapoport, J. (2010). Childhood psychiatric disorders as anomalies in neurodevelopmental trajectories. *Human Brain Mapping*, *31*, 917-925. doi:10.1002/hbm.21028

Sil, S., Dahlquist, L. M., Thompson, C., Hahn, A., Herbert, L., Wohlheiter, K., & Horn, S. (2014). The effects of coping style on virtual reality enhanced videogame distraction in children undergoing cold pressor pain. *Journal of Behavioral Medicine*, *37*, 156-165. doi:10.1007/s10865-012-9479-0

Smith, M., Edwards, R., McCann, U., & Haythornthwaite, J. (2007). The effects of sleep deprivation on pain inhibition and spontaneous pain in women. *Sleep, 30,* 494-505.

Sowell, E. R., Thompson, P. M., & Toga, A. W. (2004). Mapping changes in the human cortex throughout the span of life. *Neuroscientist, 10,* 372-392. doi:10.1177/1073858404263960

Stanford, E. A., Chambers, C. T., Biesanz, J. C., & Chen, E. (2008). The frequency, trajectories and predictors of adolescent recurrent pain: A population-based approach. *Pain, 138,* 11-21. doi:10.1016/j.pain.2007.10.032

Staud, R., Robinson, M. E., Vierck, C. R., & Price, D. D. (2003). Diffuse noxious inhibitory controls (DNIC) attenuate temporal summation of second pain in normal males but not in normal females or fibromyalgia patients. *Pain, 101,* 167-174. doi:10.1016/S0304-3959(02)00325-1

Svensson, P., Hashikawa, C., & Casey, K. (1999). Site- and modality-specific modulation of experimental muscle pain in humans. *Brain Research, 851,* 32-38.

Tabachnick, B. G., & Fidell, L. S. (2007). *Using multivariate statistics (5th ed.)*. Boston, MA: Allyn & Bacon/Pearson Education.

Tousignant-Laflamme, Y., Pagé, S., Goffaux, P., & Marchand, S. (2008). An experimental model to measure excitatory and inhibitory pain mechanisms in humans. *Brain Research, 1230,* 73-79. doi:10.1016/j.brainres.2008.06.120

Tracey, I., & Dunckley, P. (2004). Importance of anti- and pro-nociceptive mechanisms in human disease. *Gut, 53,* 1553-1555.

Treister, R., Eisenberg, E., Gershon, E., Haddad, M., & Pud, D. (2010). Factors affecting—and relationships between—Different modes of endogenous pain modulation in healthy volunteers. *European Journal of Pain*, *14*, 608-614. doi:10.1016/j.ejpain.2009.10.005

Tsao, J., Seidman, L., Evans, S., Lung, K., Zeltzer, L., & Naliboff, B. (2013). Conditioned pain modulation in children and adolescents: effects of sex and age. *The Journal Of Pain*, *14*, 558-567. doi:10.1016/j.jpain.2013.01.010

Tuveson, B., Leffler, A., & Hansson, P. (2009). Influence of heterotopic noxious conditioning stimulation on spontaneous pain and dynamic mechanical allodynia in central post-stroke pain patients. *Pain*, *143*, 84-91. doi:10.1016/j.pain.2009.02.002

Unruh, A. M., & Campbell, M. A. (1999). Gender differences in children's pain experiences. In P. J.McGrath & G. A.Finley (Eds.), *Progress in pain research and management: Vol. 13. Chronic and recurrent pain in children and adolescents* (pp. 199-241). Seattle, WA: IASP Press.

van Praag, H., & Frenk, H. (1991). The development of stimulation-produced analgesia (SPA) in the rat. *Brain Research. Developmental Brain Research*, *64*, 71-76.

van Wijk, G., & Veldhuijzen, D. S. (2010). Perspective on diffuse noxious inhibitory controls as a model of endogenous pain modulation in clinical pain syndromes. *The Journal of Pain*, *11*, 408-419. doi:10.1016/j.jpain.2009.10.009

Vierhaus, M., Lohaus, A., & Schmitz, A. (2011). Sex, gender, coping, and self-efficacy: Mediation of sex differences in pain perception in children and adolescents. *European Journal of Pain*, *15*, 621.e1-621.e8. doi:10.1016/j.ejpain.2010.11.003

von Baeyer, C. L., Piira, T., Chambers, C. T., Trapanotto, M., & Zeltzer, L. K. (2005). Guidelines for the cold pressor task as an experimental pain stimulus for use with children. *The Journal of Pain, 6*, 218-227. doi:10.1016/j.jpain.2005.01.349

Walker, S. (2014). Overview of neurodevelopment and pain research, possible treatment targets. *Best Practice & Research, Clinical Rheumatology*, *28*, 213-228. doi:10.1016/j.berh.2014.03.007

Walker, L., Williams, S., Smith, C., Garber, J., Van Slyke, D., & Lipani T. (2006). Parent attention versus distraction: Impact on symptom complaints by children with and without chronic functional abdominal pain. *Pain 122*, 43–52.

Wand-Tetley, J. (1956). Historical methods of counter-irritation. *Annals of Physical Medicine*, *3*(3), 90-99.

Washington, L., Gibson, S., & Helme, R. (2000). Age-related differences in the endogenous analgesic response to repeated cold water immersion in human volunteers. *Pain, 89*, 89-96.

Wasserman, R. M., Holmbeck, G. N., Lennon, J. M., & Amaro, C. M. (2012). A longitudinal assessment of early pubertal timing as a predictor of psychosocial changes in adolescent girls with and without spina bifida. *Journal of Pediatric Psychology*, *37*, 755-768. doi:10.1093/jpepsy/jsr121

Weiss, K. E., Dahlquist, L. M., & Wohlheiter, K. (2011). The effects of interactive and passive distraction on cold pressor pain in preschool-aged children. *Journal of Pediatric Psychology*, *36*, 816-826. doi:10.1093/jpepsy/jsq125

Weissman-Fogel, I., Sprecher, E., & Pud, D. (2008). Effects of catastrophizing on pain perception and pain modulation. *Experimental Brain Research*, *186*, 79-85. doi:10.1007/s00221-007-1206-7

Wenar, C., & Kerig, P. (2000). *Developmental psychopathology: From infancy through adolescence (4th ed.)*. New York, NY, US: McGraw-Hill.

Wilder-Smith, C., & Robert-Yap, J. (2007). Abnormal endogenous pain modulation and somatic and visceral hypersensitivity in female patients with irritable bowel syndrome. *World Journal of Gastroenterology*, *13*, 3699-3704.

Wilder-Smith, C., Schindler, D., Lovblad, K., Redmond, S., & Nirkko, A. (2004). Brain functional magnetic resonance imaging of rectal pain and activation of endogenous inhibitory mechanisms in irritable bowel syndrome patient subgroups and healthy controls. *Gut*, *53*, 1595-1601.

Wilder-Smith, O., Schreyer, T., Scheffer, G., & Arendt-Nielsen, L. (2010). Patients with chronic pain after abdominal surgery show less preoperative endogenous pain inhibition and more postoperative hyperalgesia: A pilot study. *Journal Of Pain & Palliative Care Pharmacotherapy*, *24*, 119-128. doi:10.3109/15360281003706069

Willer, J., Roby, A., & Le Bars, D. (1984). Psychophysical and electrophysiological approaches to the pain-relieving effects of heterotopic nociceptive stimuli. *Brain: A Journal of Neurology*, *107*, 1095-1112.

Willer, J., De Broucker, T., & Le Bars, D. (1989). Encoding of nociceptive thermal stimuli by diffuse noxious inhibitory controls in humans. *Journal of Neurophysiology*, *62*, 1028-1038.

Williams, A. E., Heitkemper, M., Self, M. M., Czyzewski, D. I., & Shulman, R. J. (2013). Endogenous inhibition of somatic pain is impaired in girls with irritable bowel syndrome compared with healthy girls. *The Journal of Pain*, *14*, 921-930. doi:10.1016/j.jpain.2013.03.003

Wohlheiter, K. A., & Dahlquist, L. M. (2013). Interactive versus passive distraction for acute pain management in young children: The role of selective attention and development. *Journal of Pediatric Psychology*, *38*, 202-212. doi:10.1093/jpepsy/jss108

Yarnitsky, D. (2010). Conditioned pain modulation (the diffuse noxious inhibitory control-like effect): Its relevance for acute and chronic pain states. *Current Opinion in Anaesthesiology*, *23*, 611-615. doi:10.1097/ACO.0b013e32833c348b

Yarnitsky, D., Arendt-Nielsen, L., Bouhassira, D., Edwards, R., Fillingim, R., Granot, M., & ... Wilder-Smith, O. (2010). Recommendations on terminology and practice of psychophysical DNIC testing. *European Journal of Pain 14*, 339. doi:10.1016/j.ejpain.2010.02.004

Yarnitsky, D., Crispel, Y., Eisenberg, E., Granovsky, Y., Ben-Nun, A., Sprecher, E., & ... Granot, M. (2008). Prediction of chronic post-operative pain: Pre-operative DNIC testing identifies patients at risk. *Pain*, *138*, 22-28.

Ylinen, J. (2007). Pressure algometry. *The Australian Journal of Physiotherapy*, *53*, 207.

Zheng, Z., Wang, K., Yao, D., Xue, C. L., & Arendt-Nielsen, L. (2014). Adaptability to pain is associated with potency of local pain inhibition, but not conditioned pain modulation: A healthy human study. *Pain*, *155*, 968-976. doi:10.1016/j.pain.2014.01.024