Fall 8-29-2019

Effect of a Learned-Threat on Pain Perception and Behaviors

Eric Kruger
University of New Mexico

Fall 8-29-2019

Effect of a Learned-Threat on Pain Perception and Behaviors

Eric S. Kruger

Eric Kruger

Candidate

Psychology

Department

This thesis is approved, and it is acceptable in quality and form for publication:

Approved by the Thesis Committee:

Jacob Vigil, PhD , Chairperson

Kevin Vowles, PhD

Derek Hamilton, PhD

Eric Ruthruff, PhD

Effect of a Learned-Threat on Pain Perception and Behaviors

BY

Eric Kruger

B.S., Exercise Science, The University of Montana, 2002

D.P.T., Doctor of Physical Therapy, The University of Montana, 2007

Thesis

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Masters of Science

Psychology

The University of New Mexico

Albuquerque, New Mexico

May 2017

Effect of a Learned-Threat on Pain Perception and Behaviors

BY

Eric Kruger

B.S., Exercise Physiology, The University of Montana, 2002

D.P.T., Doctor of Physical Therapy, The University of Montana, 2007

M.S. Psychology, University of New Mexico, 2017

ABSTRACT

Pain is an adverse experience and a motivating force which allows for effective responding to environmental threats. There has been limited research in how pain related consequences can be learned outside of direct experience. The purpose of this study was to investigate if a non-painful threatening stimulus can modulate pain behavior. Forty-three male participants were trained via a computer task to respond to a threatening visual symbol (i.e. learned-threat). Participants also completed a painful task, a cold-pressor task (CPT), prior to and after threat training and were randomly assigned to threat/non-threat conditions during a CPT after the threat training. Repeated measures mixed-effects model compared tolerance time and pain ratings between conditions. The threat condition did not significantly influence CPT tolerance time or pain intensity. Therefore, a recently learned non-painful threatening stimulus does not affect pain intensity or tolerance during a CPT.

Keywords: experimental pain, operant conditioning, risk-taking, computerized task, immersive environment, learning

Table of Contents

LIST OF FIGURES

LIST OF TABLES

Chapter 1

Introduction

Survival throughout evolution has ensured that organisms respond to environment threats and there is a continual requirement for organisms to learn of threats throughout development (Boyer & Bergstrom, 2011). Conceptualizing pain as a component of a larger system for responding to environmental threats has gained considerable attention in recent years (Larson, Aronoff, Sarinopoulos, & Zhu, 2009; Legrain, Iannetti, Plaghki, & Mouraux, 2011). Animals primarily learn of environmental threats via direct experience, but in humans our understanding of threat identification becomes increasingly complex, as humans demonstrate several additional learning mechanisms. In addition to direct experience, humans can learn indirectly of threats via observation and language. It is these additional forms of learning that augment directly acquired knowledge of threats.

Environmental stimuli prompt our emotional regulatory systems to organize our behavior around two-general classes of responses; appetitive and defensive responding (Lang, Bradley, & Cuthbert, 1998). Pain behaviors of withdrawal, escape, and avoidance are characterized as defensive responses produced by unconditioned stimuli (UCS; e.g. noxious mechanical, thermal or electrical stimuli). Within the central nervous system, no single area has been shown to be responsible for the production of these behaviors and considerable overlap exists between these areas and those responsible for non-noxious stimuli. Within the neocortex the insula, anterior cingulate cortex, posterior parietal and the secondary somatosensory cortex have the strongest activation signature of noxious (painful) stimuli and are associated with defensive responding (for a review see: Legrain

et al., 2009, 2011). However, these same areas have also been shown to be multimodal in their activation profile (i.e. they show activation independent of sensory modality; A. Mouraux & Iannetti, 2009; André Mouraux, Diukova, Lee, Wise, & Iannetti, 2011). In the brain stem, neural systems underpinning unconditional fear responses, specifically the basolateral complex of the amygdala, have also been demonstrated to process sensory information independent of sensory modality (Campeau & Davis, 1995). Multimodal processing centers that are responsible for defensive responding to threat stimuli tend to be the norm rather than the exception. Thus, instead of being identified as nociceptive specific, these areas have been labeled as threat or salience detection areas (Legrain et al., 2011). It is based on this general idea that these areas underserve a broader threat detection function that enables dynamic and contextually specific responses to threat stimuli in order modulate defensive behavior. Specifically, based on these multimodal threat detection areas one would expect that combinations of threat stimuli would modulate (potentiate or attenuate) responding in a predictable manner.

Indeed, this is the case, as it has been demonstrated that the presentation of multiple simultaneous unconditioned noxious stimuli modulates defensive responding in a predictable manner. Noxious sound in combination with electrical stimulation has been demonstrated to attenuate pain responding in both humans and rats (Fanselow & Helmstetter, 1988; Helmstetter & Bellgowan, 1994; Rhud & Meagher, 2001; Terman, Shavit, Lewis, Cannon, & Liebeskind, 1984). It has also been observed that when noxious stimuli are paired with the expectation of reinforcement, that defensive responding (the startle reflex) is potentiated. For example, Skolnick and Davidson (2002) measured the startle response in conjunction with a computerized lottery task which

provided subjects with monetary rewards for correct responses and punished them with an aversive noise blast for incorrect responses. The experimenters found that at peak anticipation of reward there is a large induction of a startle (eye blink) response. After learning that one has received a reward the same induction produces a smaller startle response. In the reward condition the response was similar to a startle response that was produced in anticipation of a loud noise blast. These results by Sklonick and Davidson provide support that that reward obtainment can modulate unconditioned defensive responding in humans. In summary, pairings of unconditioned noxious stimuli attenuate defensive responding in animals and humans. While in humans only the anticipation of reward increases defensive responding (at least for the startle reflex). These studies have focused on responding to unconditioned noxious stimuli, while cognitive threats such as beliefs, appraisals and catastrophizing statements, have also been shown to play an important role in responding to painful stimuli.

Human cognition is uniquely complex and presents challenges in the study of pain. These challenges arise primarily by the way in which humans use cognition for the generation of language. Language has been an important evolutionary development because it enables humans to learn of associations between contingencies and actions without direct experience or observational learning. With pain this is especially invaluable, as pain often occurs in conjunction with life-threatening circumstances and an opportunity to learn outside of those direct circumstances has been proposed as a critical adaption to increase survival (Craig, 2009; Finlay and Syal, 2014; Jack and Schyns, 2015).

However, it is these cognitions and their role in maintaining the continued threat of pain and damage that have also been associated with the development and maintenance of disability. Severeijns et al. (2001) found in chronic pain patients that pain catastrophizing predicts pain intensity, disability and psychological distress independent of the level of physical impairment. Beliefs about the perceived threat of physical activity and work (fear avoidance beliefs) also have been shown to account for a significant portion of the variance associated with disability (Crombez, Vlaeyen, Heuts, & Lysens, 1999; Waddell, Newton, Henderson, Somerville, & Main, 1993). Additionally, individuals with chronic pain who reported higher levels of pain vigilance had higher levels of pain intensity, emotional distress, disability and health-care utilization (McCracken, 1997). In addition to these clinical findings, in experimental settings pain catastrophizing, fear of pain and pain hypervigilance have been shown to predict pain intensity (Baum, Huber, Schneider, & Lautenbacher, 2011; Roelofs, Peters, Deutz, Spijker, & Vlaeyen, 2005; Roelofs, Peters, Van Der Zijden, & Vlaeyen, 2004).

Several researchers have sought to understand how cognitive threats are learned by turning to language and investigating how threat appraisals generate fear evoked responses. Responding in the anticipation of pain and/or physical danger is defined as a fear evoked response. Evidence supports a strong influence of verbal information in the acquisition of fear responses to potential pain (Muris & Field, 2010). For example, Field and Schorah (2007) asked children to touch different animals after presenting the children with verbal descriptions of the animals that either implied a potential threat or non-threat (i.e. claws vs no claws). The children who were exposed to the threat appraisal showed an increased delay during the act of approaching the animal compared to the non-threat

condition. Additional support for the role of threatening appraisals is seen in adults where verbal information about the effects of cold water (e.g. potential for frostbite) decreased tolerance time to a hand immersed in cold water (Jackson et al., 2014; Boston & Sharpe, 2005; Damme, Crombez, Wever, & Goubert, 2008; Jackson et al., 2005). In these studies, participants were given differential threat appraisal manipulations that altered the degree of threat associated with the noxious stimuli they were to encounter, in this case cold water. A decrease in pain tolerance and an increase in reported pain intensity was observed after the threat manipulations. Several limitations exist in these studies. First, they assume a prior learning between words likely to cause pain and/or damage (e.g. description of potential for threat such as frostbite or claws), and second they do not directly measure the learning process itself.

Addressing some of these limitations, other studies have sought to explain how pain related fear is learned and generalizes in language. The process by which defensive responses (e.g., pain related fear) can generalize to words is called symbolic generalization. Symbolic generalization allows pain-related fear to spread from a conditioned stimulus to other stimuli that share conceptual or functional similarities with the original conditioned stimulus. Several authors have demonstrated that fear generalizes between stimuli that are perceptually dissimilar (e.g. needles, splinters and thorns are all spelled differently) but share a functional or conceptual similarity (i.e. the capacity to cause pain; Augustson & Dougher, 1997; Dymond et al., 2011; Valverde, Luciano, & Barnes-Holmes, 2009). A particularly illustrative example is provided by Dunsmoor et al. (2012), in this study the authors paired a noxious-UCS with specific objects in a category (tools that have heterogeneous functions and perceptual characteristics) and demonstrated

that fear of pain generalized to other members in that category, but not functionally dissimilar categories (e.g. animals). Additional evidence is provided by Bennett et al. (2015) as they were able to show that participants developed a fear of pain in the same conceptual category when the symbolic stimulus was paired with a threatening word (e.g. injury, terrible, danger, pain, hurt). This last study provides evidence that threatening words can be used in lieu of nociceptive stimuli for further generalization of defensive responses (i.e. pain related fear) to new stimuli.

In summary, current evidence supports the hypothesis that threats can be acquired via direct association and indirect association with noxious stimuli. Direct association occurs when a noxious UCS is paired with another neutral stimuli and now becomes the conditioned stimulus and subsequently modulates conditioned responses. Indirect association is described as the process by which symbolic generalization occurs in language, in that originally one word becomes directly associated with a noxious stimulus (e.g. “pain”) however after multiple associative parings of the original word with other words (e.g. “needle”, “knife”, “thorn”, etc.) these new words acquire the same defensive response (fear related pain) as the original word.

The conventional assumption is that that the detection of a perceived threat in combination with noxious stimuli modulates pain behaviors. This assumption is supported by shared multimodal processing centers for threat detection within the central nervous system and how combinations of stimuli modulate pain behaviors. Even though the behavioral evidence is divergent and somewhat counterintuitive. Additionally, language adds to the complexity of understanding how these processes interact in humans, but experimental findings continue to support the hypothesis that a cognitive

threat in addition to noxious stimuli potentiates responding. A key limitation in assessing the role of cognitive threats in all human participants is their prior history with language. This prior history contains specific associations of words with prior injuries, physical damage, and environmental threats. To test the question if a perceived threat that is learned (i.e. is not unconditionally aversive) can modulate pain behaviors it would have to be learned in the experimental setting and have no prior associations with one's natural language (i.e. no direct or indirect associations). Simply stated, it remains impossible to tease apart the influences of symbolic threats if those threats have a direct or indirect association with a noxious stimulus.

The purpose of this study is to test if there is behavioral evidence for assumption that a learned-threat, that is learned without a direct or indirect association with bodily harm or pain, can modulate responding to a noxious stimulus. Individuals can develop highly intricate networks of direct and indirect associations between symbols, words, statements, phrases, and pain, therefore to remove any confounding effects of unconditionally defensive behaviors (i.e. the stimulus is an unconditionally aversive, e.g. a loud noise) and conditioned aversive associations (e.g. prior effects of language i.e. indirect associations with words such as pain, injury, harm etc.) an arbitrary visual stimulus was conditioned as the learned-threat. To elicit defensive responding, an operant learning procedure was used to shape responding to the arbitrary visual stimulus (learned-threat). The threat to the research participant, was a symbol that indicated risk of monetary loss. Therefore, participants, learned to shift responding from appetitive to defensive behaviors when this visual stimulus was displayed. Following this learning procedure, a cold-pressor task (CPT) was administered to induce pain during presentation

of either the learned-threat or a non-threat stimulus. To further characterize individual differences in threat responding participants were also measured on several instruments designed to assess pain related fear, pain catastrophizing and pain hypervigilance.

The learned-threat visual stimulus was arbitrary and was expected to not have a prior history with noxious stimuli and thus would be neutral in valence at the start of the learning task and as learning proceeded would generate increased aversive valence. It was hypothesized that an aversive valence would modulate subsequent pain responding during the CPT task. Stated more specifically, the presentation of the learned-threat would result in potentiated pain behaviors (decreased tolerance time and increased pain ratings). However, if either potentiated or attenuated behaviors were observed, then this would provide behavioral evidence that learned-threats can modulate defensive responding to noxious stimuli *a priori* of any association between stimuli. Instead, if pain behaviors remain unchanged in the presence of the learned-threat, then this would support the opposite hypothesis that threats must have a prior history with noxious stimulation, be it acquired via direct or indirect association, to modulate pain responding. In other words, for pain behaviors to be modulated a threat stimulus is either another unconditioned stimulus, a conditioned stimulus learned by direct association (previously paired with a noxious stimulus) or an indirect association (i.e. a stimulus that shares a conceptual/functional similarity with another conditioned stimulus). Finally, based on the findings that the constructs of fear of pain, pain catastrophizing and pain hypervigilance predict experimental pain intensity it was predicted that changes in pain responses to the learned-threat would also be predicted by instruments measuring these constructs.

Chapter 2

Methodology

A total of 97 male participants participated in this study. Participants were recruited from an undergraduate psychology pool at the University of New Mexico (UNM) using the online experimental management system (SONA, The University of New Mexico). All participants signed an informed consent detailing the study objectives, general details of the experimental protocol and reimbursement. In order to remove any possibility of demand characteristics, the consent did not contain any language related to learning or threatening stimuli.

Participants were excluded if they had a history of acute or chronic pain, arthritis, psychiatric disorders or neurological conditions like epilepsy, complex regional pain syndrome, stroke, and or upper extremity pain. In addition, specific contraindications to the CPT include any illness related to a cardiovascular disorder (e.g., high blood pressure, heart disease or dysrhythmia); history of fainting or seizures; history of frostbite; having an open cut, sore or bone fracture on the limb to be immersed in water; or a history of Reynaud's phenomenon.

Male participants were recruited for participation in this study to decrease heterogeneity of intra- and interpersonal sex differences of pain responding. Consistent differences in pain behaviors related to sex have been shown in clinical and non-clinical pain (Fillingim, King, Ribeiro-Dasilva, Rahim-Williams, & Riley, 2009; Wiesenfeld-Hallin, 2005). Females have also demonstrated changes in pain reporting depending on their phase of menstrual cycle (Vigil et al., 2015) and gender interactions between the sex of the experimenter and research participant (Vigil & Coulombe, 2011).

Participants were reimbursed for participation in the experiment with research credits (exchanged for course credit) and a monetary reward that was based on task performance during the experiment (*M* = $5.94, *SD* = $2.01). If students did not wish to participate in this research study, they were given alternative means to obtain course credits.

Experimental Design

The study was divided into two parts, a recruitment phase and experimental phase, see Figure 1 for a flow chart of the experimental protocol. During the recruitment phase participants completed online surveys. A total of 97 participants completed the recruitment phase. Of these, 79 participants participated in the experimental phase. Eighteen participants completed the recruitment phase but did not sign up for the experimental phase.

The experimental phase consisted of two in-person sessions conducted on different days. Experimental sessions were conducted in a sound-attenuated room using a desktop PC (24" monitor; 1920 x 1080 pixels). During each experimental session the participant underwent a baseline CPT, the learning procedure and a post CPT. Participants participated in both experimental conditions (threatening / non-threatening visual stimuli) in a counter-balanced order. With the exception of the manipulation (exposure to the threat stimuli during the second CPT task) the two sessions were identical. Not all participants completed all sessions of the experimental phase because the experimental phase required participants to meet a performance criterion for the operant learning procedure (described below) and for the CPT task: termination of the cold-pressor task before 10 minutes. Twenty-three participants were excluded from full

participation because they did not meet either of these criteria (n = 3 due to learning and n = 20 CPT criteria). One participant was eliminated from final analysis because they did not attend a second session and one participant had self-reported high blood pressure and thus did not continue with the experimental phase.

Operant Learning Procedure

The learning procedure was a behavioral task that the participant completed on a computer in a virtual environment. The experimental task, a virtual environment that reinforces learning of the arbitrary learned-threat stimulus, was developed by the author using Unreal Engine (Epic Games, version 4.12.5). The participants operated the computer using both their dominant and non-dominant hands. Figure 3 shows screen shots of the learning task. Participants were oriented to the virtual environment prior to beginning the learning task. The task consisted of a virtual room that contained 49 blue boxes and an exit. Figure 2 shows a top down map of the virtual environment. At the start of the task, participants were given a minimal instructional set which oriented participants to the goals of the task.

The participant was instructed to "pick-up" as many of the boxes as possible and then exit the room. Boxes were picked-up by moving a virtual character (first-person perspective) over the box and they could exit the room by entering a door-way labeled "exit". Participants navigated the simulated three-dimensional environment via a mouse (direction) and the forward and backward keys on a standard keyboard (movement). With each box that the participant picked-up they earned incrementally more money ($0.02 per box). During a given trial, the more boxes the participant collected resulted in an increased probability that the trial would suddenly end without the participant reaching

the exit. Participants only accrued money across trials by exiting the room. In other words, money earned during a trial was only saved and added to the accrued total if the participant exited the room. Participants received feedback of the total amount they had accrued across all previous trials after every other trial.

Trial failure was determined by picking up boxes that increased the probability of trial end without exit. These boxes are henceforth described as damage-boxes. All other boxes are described as safe-boxes. Visually, damage-boxes and safe-boxes appeared identical to the participant. During each trial the participant was assigned 100 points and this variable was hidden from the participant. Each time the participant picked up a damage-box points were decreased by a set amount, when the participant's points reached zero then the trial ended and a trial-end feedback screen was displayed, see Figure 3 (Image D). Participants completed a total of 30 trials of the task during each session (60 trials total during the experiment). In each session, trials were divided into two blocks of 15 and each block was separated by a two-minute interval.

The distribution of boxes was determined prior to the start of the trial. The distribution of boxes was based on 60 different templates that specified the position of each safe/damage-box and the amount of damage accrued. The order of templates was randomized across participants and each templated was presented one-time during the experiment. There was no visual way to distinguish different templates (i.e. the distribution of damage or safe boxes during each trial). In addition to specifying the location of each box the template also specified the decrement in points of each damage box on that trial. This led to trial-to-trial variation in the probability of trial end for each

template (and hence each trial). Across all trials the probability that any given box was a damage-box or a safe-box was 50%.

During each trial an arbitrary symbol was displayed on the center bottom half of the screen. The abstract figures used for the arbitrary symbol can be seen in Figure 3 (Images A through C). The figures were based on abstract figures used in previous fear acquisition and extinction research (Vervoort, Vervliet, Bennett, & Baeyens, 2014). Each symbol was displayed when the participant's points fell within the specified ranges for each symbol. Figure 3 (Image A) was displayed between 100 and 75 points, Figure 3 (Image B) was displayed between 74 and 40 points, and Figure 3 (Image C) was displayed between 39 and 0 points. The learned-threat was the third visual symbol displayed, Figure 3 (Image C). The symbol is a threat because it was displayed only when the probability of a trial ending was high (i.e. the participant's points were between 39 and zero). Trial end always occurred when the third symbol was displayed and not any other symbol. For the participant to learn to maximize monetary gains they collected boxes until the learned-threat symbol appeared on the screen and then successfully negotiate a path between any remaining boxes and the exit.

In order to shape behavior towards picking up more boxes at the end of each trial participants were given feedback based on their performance (Figure 3, Images D, E & F). If participants exited the room with a symbol other than the learned-threat symbol displayed, they were shown a feedback screen that prompted them to collect more boxes (Figure 3, Image E). If they exited the room with the learned-threat symbol they were given feedback that they performed well on the trial (Figure 3, Image F). If they failed to exit the room before the trial ended then they were given feedback that they failed the

task (Figure 3, Image D). In order to further describe the expected behavior of participants during the experiment the following example provides a trial-by-trial description of one hypothetical participant.

> At the start of the first trial the participant initially enters the room and starts picking up boxes as instructed. At some point they pick-up too many and the trial immediately ends. At this point they are shown the trial failure screen Figure 3 (Image D). During this second trial, they pick-up a few boxes and then exit the room while a non-threat symbol is displayed, Figure 3 (Image A), and thus they are shown a feedback screen that prompts them to collect more boxes on their next trial, Figure 3 (Image E). During the third trial, they collect more boxes than before and this time exit while the threat symbol is on the screen, Figure 3 (Image C). The participant is then shown a feedback screen affirming that they performed well, Figure 3 (Image D). Every other trial they are shown a feedback screen that displays how much they have accumulated in terms of dollars, Figure 3 (Image G)—this is the amount to be awarded at the end of the study.

Participants experienced several failures and successes before they learned to modify their behavior consistent with successfully learning to maximize their gains and avoid the premature trial end.

The goal of this learning procedure was to train individuals to respond to the learned-threat symbol. In order to eliminate the possibility that participants would use alternative strategies to generate the appearance of successful responding several steps were taken. First, location of the damage boxes was pseudo-random across trials and thus could not be predicted by the participant. Second, in order to reduce the chance that

participants could use a counting strategy (i.e. count the number of boxes picked up) the parameters chosen for the number of points that correspond to the differential display symbols, the varied number of damage-boxes on a given trial and the varied decrement in damage points per pickup were pre-selected in order to minimize the correlation between the number of boxes that could potentially be picked up on each trial. In other words, these pre-selected parameters were chosen so that the total number of boxes that could be picked up on each trial varied substantially and the correlation of number of boxes that could be picked up with each symbol on the screen was small. The pre-selected values for these parameters were determined by simulations (N=10,000) of experiment using R version 3.3.1 (R Core Team, 2015). Table 1 displays results of these simulations in terms of number of boxes picked up with each symbol on the screen and their correlations.

Participants had to meet a performance criterion of the task in order to move forward with the experiment. This performance criterion was exiting the room with the learned-threat symbol on the screen in at least 8 of the final 15 trials (53.3% success of the second block) for a given session. It was determined based on simulation (N=10,000) of the experiment conducted in R version 3.31 that any score less than 8 of 15 could be generated by random chance, and thus it could not be determined whether the participant was responding to the on screen stimuli (learned-threat). For example, one such strategy that would not be consistent with responding to the on screen stimuli would be simply walking across the room.

Operant Learning Check

In addition to the performance criteria that probabilistically ensured that participants were responding contingent to the symbolic stimuli presented on the screen,

the participant was also asked two manipulation questions at the end of the experiment. These questions consisted of showing each of the symbols in the context of the task Figure 3 (Images A, B & C). For each symbolic image participants are asked "How likely is it that you would exit the room now?" Participants indicated their likelihood on a continuous visual analogue scale (0 = "very unlikely"; 100 = "very likely"). Participants were also asked "What on the screen tells you that you should exit the room?" Participants selected from the following answers, "The color of the boxes", "The lighting of the room", "The pattern on the floor" or "The symbol in the bottom center of the screen."

Unconditioned Noxious Stimuli

The unconditioned noxious stimuli used in this experiment was the cold pressor task (CPT) which required participants to immerse their hand in cold-water for as long as they could tolerate. During the cold-pressor task the participant is seated so that their non-dominant arm can be comfortably extended downward at their side and lowered into a circulating water bath to wrist level.

A Thermo Electron Corporation (Newington, NH, USA) Neslab RTE17 refrigerated bath circulator (60.0x28.9x47.9cm) is used as the cold pressor. The water is set to maintain a temperature at 5˚C (within 0.1˚C from 4.9˚C to 5.1˚C).

During each session, participants completed two CPTs (baseline and post). During each CPT the participant was required to maintain their attention on the computer screen in front of them. During the baseline CPT participants attended to a white screen with a 2 cm black dot in the center. At the follow up CPT the screen displayed either the learned-threat symbol Figure 3 (Image C) or a non-threat stimuli Figure 3 (Image A). Both the

symbols were presented in the context of the learning procedure (i.e. a screenshot). Participants were briefed on the CPT procedures by watching a 2:30 minute video that outlined how to perform the task. Afterwards, an experimenter answered any questions and clarified the procedures if needed. Prior to beginning the CPT participants were also given written instructions on the screen.

Immediately upon terminating the CPT task participants were asked to rate their pain during the CPT on a 0 to 100 visual analogue scale. Time was recorded by the participant using their hand opposite of the CPT. The timer was a switch that started recording when the spacebar was pressed down and continued until the spacebar was released--when they removed their hand from the water. A maximum time-limit of 10 minutes was used to limit the maximum duration of the CPT. Time was also recorded by the experimenter using a stopwatch, who observed the participant through a closed-circuit video feed. If participants reached the maximum time-limit during any of the CPTs, then they were removed from the study.

Predictors of Changes in Pain Behavior

Prior to the in-person experimental sessions all participants completed a survey that recorded basic participant demographics and psychological constructs regarding pain. The psychological instruments used were the pain vigilance and awareness questionnaire (PVAQ), the fear of pain questionnaire (FPQ), and the pain catastrophizing scale (PCS). Each of these constructs have been shown to predict experimental pain behaviors (Keeley et al., 2008; Roelofs et al., 2004).

The PVAQ is a self-reported measure of attention and hypervigilance to pain that has been used in both clinical and non-clinical samples (McWilliams & Asmundson,

2001; Monticone et al., 2015; Wong, McCracken, & Fielding, 2011). The range of scores of PVAQ is 0 to 80 (higher indicating more vigilance/attention), has an alpha coefficient of 0.92, and is normally distributed.

The FPQ-III consists of a sum 31 items describing fear of hypothetical painful experiences on a 0 to 5 Likert scale (McNeil & Rainwater, 1998). The FPQ has demonstrated excellent internal consistency ($\alpha = 0.93$), retest reliability and has been validated in both clinical and non-clinical samples (Roelofs et al., 2005).

The PCS measures pain related catastrophizing and contains 13 items that are divided into three subscales: rumination, magnification and helplessness. The PCS has been used and validated in both clinical and non-clinical samples (Van Damme, Crombez, & Eccleston, 2002) and demonstrates good internal consistency ($\alpha = 0.87$).

The degree to which persons responded to the learned-threat stimulus during the learning procedure was also evaluated as a potential predictor of pain behavior. The rationale being that individuals who are more responsive to the learned-threat stimulus during the learning procedure would also be more responsive to the learned-threat stimulus during the CPT. The mean number of boxes collected per trial during the second block (last 15 trials of each session) while the learned-threat symbol was displayed was analyzed as a predictor of pain behaviors during the CPT.

Data Analysis

Power was simulated using previous data on CPT in convenience samples prior to conducting the experiment utilizing a gamma distribution of CPT tolerance times, an estimated mode of 60 seconds (*SD* = 30 s) and a correlation of 0.65 for repeated measurements of CPT tolerance time (Koenig et al., 2014; Treister et al., 2015). In order

to detect a medium effect (Cohen's d = 0.5 which equated to a 15 seconds difference in CPT times) a total of 50 participants was required.

Prior to running the analyses, the data was visually examined. Examination of the CPT tolerance times revealed that there were two apparent groups of participants: one group which behaved fairly reliably on the cold pressor time and another group that displayed considerable heterogeneity. The group that displayed heterogeneity generally was characterized by one CPT that was considerably longer than all other CPTs. Based on this observation, Mahalanobis distance (MD) was calculated for the CPT times (Mahalanobis, 1936). Outliers, all participants that had a MD greater than five, were removed from the analysis (n = 12), thus reducing the sample size in the final analysis (n = 43). This also reduced the max time of the CPT of the final data set to 180 seconds and increased the normality of the distribution of tolerance times. In order to further characterize the intra-participant reliability of CPT tolerance times between the outliers and the participants included in the final analysis ICCs were calculated. The outlier's group had an ICC value of 0.37 with a 95% confidence interval from 0.09 to 0.69. The analysis group had an ICC value of 0.67 with a 95% confidence interval from 0.55 to 0.78. Removal of the outlier's group did not substantively change the results of the main effects of the study but did improve power in order to detect effects by reducing unexplained variance by a factor of 3. Including outliers would have raised the magnitude to detect a medium effect (Cohen's d = 0.5) to 45 seconds. Excluding outliers, reduced this to 15 seconds which was similar to what was estimated in the power analysis.

All analysis were conducted using R version 3.1.1 (R Core Team, 2015) and the nlme package was used to estimate mixed-effects models (Pinheiro, Bates, DebRoy, Sarkar, & R Core Team, 2015).

Pearson's correlations were conducted between all predictors and dependent variables. Random intercepts linear mixed-effects models were used to determine whether the factors of Order (first or second session), Time (baseline or post CPT), Condition (non-threat and learned-threat conditions) and the interaction of Time and Condition were significant predictors of CPT tolerance time and pain intensity. The interaction between Time and Condition tests the question whether pain behaviors are different from baseline and post CPT across the threat and non-threat conditions. Models were estimated via maximum likelihood estimation and all continuous variables were grand mean centered. Furthermore, psychological constructs were tested to determine if they were significant predictors of outcomes. Several models were tested by adding continuous predictors (PVAQ, PCS, FPQ and mean boxes-collected) of pain behaviors. These predictors were tested because of prior research demonstrating significant correlations with experimental pain and by including them would account for unexplained variance that would increase the power of the analysis. Additionally, mean boxes collected during the learned-threat symbol was also included as possible predictor because this variable reflected responsiveness to the learned-threat symbol. In deciding which models to include in final reporting, χ^2 goodness-of-fit tests were used to compare models of predictors and *F*-tests for omnibus tests of effects and t-ratio tests for specific contrasts. Model adequacy was summarized by two approximations to the traditional R^2 common to OLS regression: R^2_{GLMM} and R^2_{GLMC}. These approximations of R^2 measure the

estimated proportion of variance accounted for by the fixed effects and fixed effects plus random effects (respectively). Nakagawa and Schielzeth (2013) have shown that both R^2_{GLMM} and R^2_{GLMC} are valid approximations of traditional R^2 and meet many of the requirements for a measurement of model adequacy outlined by Orelien and Edwards (2008). Cohen's *d* when used as a measure of standardized effect was calculated by the mean differenced divided by the standardized sum of all variance components in the mixed model (Westfall, 2015). This method is most consistent with the original form of Cohen's *d* (Cohen, 1992).

Of the models that included potential predictors, the PVAQ and mean boxes collected did result in a significant improvement in the goodness-of-fit over a more parsimonious model. However, in both cases adding these predictors did not improve the estimate of the fixed factors (Time, Condition, and Time and Condition interaction) or reduce standard errors. Also, these predictors did not significantly predict the dependent variables, therefore it was decided to omit these predictors from the final models reported. Thus, the final models reported only used the fixed factors of Order, Time, Condition and the interaction of Time and Condition as predictors of the dependent variables.

Chapter 3

Results

Descriptives

Descriptive statistics were calculated for all participants included in the final analysis (n = 43). The mean age of participants included in this study was 20.14 years (Mdn = 19, SD = 3.4). Forty-nine percent (n = 21) of participants identified as Hispanic. Participants identified with the following races, American Indian or Alaskan Native (n = 4, 9%), Asian (n = 7, 16%), more than one race (n = 3, 7%), other (n = 9, 21%), and White *(*n = 20, 47%). Table 2 displays correlations between predictors and dependent variables, means and standard deviations. No significant correlations were observed between tolerance times, pain intensity and predictors (PVAQ, FPQ, PCS, Boxes Collected). At each time point, across participants, measurements of tolerance time and pain intensity were significantly correlated with each other. A negative correlation between pain intensity and tolerance time approached significance at baseline $r(42) = -0.21$, $p = 0.06$ but not at post $r(42) = -0.05$, $p = 0.57$.Examining the correlations at baseline more closely, there was a significant negative correlation between baseline tolerance time and pain rating during the threat condition, $r(42) = -0.35$, $p = 0.02$, but not the non-threat condition.

The mean time interval between baseline and post CPT was 16.92 min (SD = 3.7 min). A mean of 47.85 seconds was observed for the CPT tolerance time and 65.7 for pain intensity rating. In examining the order of the CPT, there was a significant trend of decreasing tolerance times from the first (first session, baseline CPT) to the last (second session, post CPT), B = -1.67 sec, $F(1,128) = 4.38$, $p = 0.04$, $R^2_{GLMM} = 0.01$, $R^2_{GLMC} = 0.69$.

The same trend for pain intensity ratings was increasing and approached significance, B = 0.72, $F(1,128) = 3.45$, $p = 0.07$, $R^2_{GLMM} = 0.01$, $R^2_{GLMC} = 0.59$.

Given the diversity of ethnicity and race captured in this sample, mixed effects models were used to determine if race or ethnicity significantly explained differences in tolerance times or intensity ratings. Neither race or ethnicity were significant predictors of differences in tolerance time or pain intensity ratings. Also, race and ethnicity did not explain differences in the predictors of pain (PVAQ, PCS and FPQ).

The measurement of predictors in this study appears to have differed significantly from other reported studies. McWilliams and Asmundson (2001) report a mean PVAQ of 33.54 (SD = 13.18) in pool of healthy participants at a Canadian University (N = 256, 77% female, 86.8% Caucasian). This study also reported non-significant differences between sexes of the PVAQ. Our study found a significant 9.9 difference in comparison to that of McWilliams and Asmundson, $t(42) = 6.23$, $p <= 0.001$. This study's reported mean PVAQ is much closer to what McCracken (1997) found in patients with low back pain (M = 47.5, SD =13.5) but remains statistically different McCracken's findings $t(42) = -2.55$, $p = 0.01$. Using the FPQ-III, George, Dannecker, & Robinson (2006) found that a sample of healthy participants had an mean score of 78.8 (SD = 17.5; 74% Cacuasian and 6% Hispanic). Again, FPQ reported in the current study (M = 86.93) was significantly greater than what was reported by George, Dannecker, & Robinson, $t(42) = 2.75$, $p = 0.001$. Van Damme et al. (2002) reported in a sample of 550 Dutch students a PCS mean of 16.56 (SD = 7.78; 74% Female), this study's mean PCS of 16.93 was not significantly different than what was reported by Van Damme et al.

Performance During the Operant Learning Procedure

All participants in the final analysis met the performance criterion during the operant learning procedure (exiting the room with the learned-threat symbol on at least 8 out 15 trials during the final 15 trials). Six participants did not meet the performance criterion at the end of the standard amount of trials and therefore were given another 15 trials. At the end of these 15 trials these six participants met the performance criterion. A considerable range of variability of success was observed across all participants (M = 83.3%, SD = 17%), see Figure 4 for a plot of the distribution of success rate for participants.

Along with this variability of success rate in the final 15 trials there was also considerable variability in how responsive participants were to the learned-threat symbol. This responsivity was measured in the mean number of boxes collected while the learned-threat symbol was displayed (M = 0.92. SD = 0.75), See Figure 5 for plot of this distribution.

Finally, a significant negative correlation was observed between percent success and mean number of boxes collected, $r(86) = -0.85$, $p < 0.001$.

Manipulation Check

A mixed-effects repeated measures model was performed to determine differences in the likelihood of exit for each symbol. A significant omnibus test revealed significant differences in the likelihood of exit for each symbol, $F(2,84) = 59.78$, $p < 0.001$. This was followed up by pairwise comparisons between the different symbols using Tukey's adjustment for multiple comparisons. A significant difference was observed between likelihood of exit of the learned-threat and the second symbol (M_D =

54.73, *SEM* = 6.87), t(84) = 7.95, p < 0.001, *d* = 1.69; the second and first symbol (M_D = 17.29, *SEM* = 6.87), t(84) = 2.51, p < 0.04, *d* = 0.53; and the learned-threat and first symbol (M_D = 72.01, *SEM* = 6.87), t(84) = 10.473, p < 0.001, *d* = 2.25.

When responding to multiple choice questions 100% of participants correctly identified that the symbol on the screen was the stimuli that they used to accurately exit the room.

Learned Threat on Pain Behaviors

For both the dependent variables of interest (CPT tolerance time and pain intensity rating) mixed effects models were used to test if the fixed effect of Order (first or second session), Time (baseline or post CPT), Condition (non-threat and learned-threat conditions) and the Time/Condition interaction were significant.

For CPT tolerance time the model was not significant $\chi^2 = 6.16$, $p = 0.19$, $R^2_{GLMM} = 0.01$, $R^2_{GLMC} = 0.69$, in comparison to the null intercept only model. The main effects of Order, Time, Condition, and the Time/Condition interaction were non-significant. The main effect of Order (first vs. second session) approached significance as a predictor, $F(1,125) = 3.38$, $p = 0.053$. CPT tolerance time decreased by an estimated 4.98 seconds from the first to the second session. Examining the difference in tolerance between post CPTs across conditions of threat a mean difference 4.28 s was observed, $t(125) = 1.93$, $p = 0.23$, $d = 0.15$, 95% CI = [-0.49, 0.72]. Figure 6 displays a plot of the estimates of means of tolerance time for the Threat and Condition factors.

For pain intensity rating the overall model was not significant $\chi^2 = 5.55$, $p = 0.24$, $R^2_{GLMM} = 0.01$, $R^2_{GLMC} = 0.59$, in comparison to the null intercept only model. The main effects of Order, Time, Condition, and the Time/Condition interaction were non-

significant. Examining the difference in pain intensity between post CPTs across conditions of threat, a mean difference 0.81 was observed, $t(125) = 0.44$, $p = 0.64$, $d = 0.07$, 95% CI = [-0.50, 0.64] Figure 7 displays a plot of the estimates of means of pain ratings for the Threat and Condition factors.

Chapter 4

Discussion

This study sought to describe differences in CPT pain tolerance and intensity during exposure to a learned-threat stimulus. The learned-threat, a visual stimulus which indicated potential monetary loss, was trained during an operant learning procedure. The aim was to determine if a learned-threat without a prior direct or indirect association with noxious stimuli could modulate pain tolerance or pain intensity.

To recapitulate, direct associations are acquired when a participant learns of a conditioned association between an unconditionally noxious stimulus and a neutral stimulus—after conditioning the neutral stimulus is called the conditioned stimulus. Indirect associations occur when a conditioned stimulus (previously associated with an unconditionally noxious stimulus is further generalized to another arbitrary stimulus). An example of an indirect association would be if the word “pain” is initially associated with noxious stimulation, and then at later time is associated with color red. Responding, to the color red as if it were painful would be an example of a fear evoked response to pain based on the indirect association with previous noxious stimulation.

The results of this study did not support that a learned-threat representing monetary loss can modulate pain intensity, or tolerance during a CPT. Further, cognitive predictors of pain responding (pain catastrophizing, fear of pain, pain hypervigilance) were not significant predictors of CPT tolerance time or pain intensity. Neither was the measure of task performance (mean boxes picked up while the learned-threat symbol was displayed). In summary, these results do not support a hypothesis that learned-threat can

modulate pain behavior when it has no prior direct or indirect association with noxious stimuli.

The learning procedure used in this study successfully trained participants to switch responding from appetitive to aversive responding when the learned-threat symbol was displayed. This was evidenced in the percentage of successful trials (measured by the ratio of trials in which the participant successfully exited the room with the learned-threat symbol on the screen), the reported likelihood of exit with each symbol on the screen (greatest with the learned-threat stimuli), and participants' identification of the correct stimuli used to guide responding (as measured by a multiple choice question). Several controls were taken to eliminate the possibility of alternative strategies (chance success and counting strategies) and these controls ensured that participants' responses were due to the on-screen stimuli (learned-threat).

In contrast with previous research, which indicates that tolerance time and pain intensity are correlated (Hirsh, George, Bialosky, & Robinson, 2008; Lee, Watson, & Frey Law, 2010), this study found results that approached significance only for the baseline measurements. This finding may have been in part due to only using male participants. Studies that report correlations between intensity and tolerance time often do so with combined male and female samples and thus these correlations are not reported for each sex (Hanssen, Vancleef, Vlaeyen, & Peters, 2014; Koenig et al., 2014; Wang, Jackson, & Cai, 2016). The omission of females in this study may explain why pain intensity and tolerance time were largely uncorrelated. The reduction in the correlation at the post CPT may also reflect habituation to repeated CPTs that resulted in diminished responding. This was supported by the observation that participants did show a

significant decreasing trend of -1.67 seconds for each subsequent CPT they performed and a near significant trend of increasing pain ratings.

In contrast to prior research that shows that measures of pain catastrophizing, fear of pain, and pain vigilance and awareness are predictive of experimental pain behaviors (Roelofs et al., 2004; Sullivan, Bishop, & Pivik, 1995), there were no significant correlations between these variables and our dependent variables of interest (pain intensity and pain tolerance). The reasons for this may be multifactorial. Most striking our sample was characterized by higher scores of fear of pain beliefs and pain vigilance than has been found in other non-clinical university convince samples (McCracken, 1997; McWilliams & Asmundson, 2001). These findings are remarkable as all participants in this study were males and denied a history of acute or chronic pain. Societal and cultural differences may have also contributed to departures from norms reported in other convenience samples for the PVAQ and FPQ. Additionally, because this is a convenience sample it cannot be excluded that a selection bias was not occurring and this could also contributed to the differences reported (Wainer, 1986).

Nearly half our sample identified as Hispanic and whites constituted a majority-minority. However, all predictors and dependent variables showed no differences in scores based on race or ethnicity. Therefore, the departures observed in this study from previously reported norms of fear of pain beliefs and pain vigilance are less likely due to cultural factors as expressed through self-reported race and ethnicity. However, this does not exclude the fact that there may be cultural factors intrinsic to this sample.

Ultimately, it is unclear why the predictors displayed significant departures from norms reported in non-clinical samples. As mentioned above, this could have been

partially due to cultural factors or sex differences in responding. It is less likely that these differences were simply due to inattentive responding by the participants as all significant differences were in the same direction (i.e. increased fear of pain beliefs, and pain vigilance) and sample standard deviations in this study were similar to what has been reported in other convenience samples (McCracken, 1997; McWilliams & Asmundson, 2001; Sullivan et al., 1995). This study may indicate a need for further research validating these measurement instruments in heterogeneous samples that represent membership to different populations in non-clinical university convince samples.

The observed pain rating of 65.7 was lower than what was reported by Treister et al. (2015), who observed a mean pain rating of 79.5 in a combined sex sample of healthy participants (N = 648). More striking, the distribution of pain intensity ratings in the current study's sample was considerably more normally distributed than reported by Treister et al. However, Treister et al. used 3° C bath versus the 5° C bath used in this study and water temperature has been shown to affect pain ratings (Mitchell, MacDonald, & Brodie, 2004). In Treister et al. participants reported intensity verbally to experimenters in the room and in this study were recorded alone via a computer input. Thus, differences in pain intensity between this study and similar study's involving a CPT could have arisen due to the type of measurement (verbal versus computer input), presence of experimenters, and/or temperature of the water bath.

The observed mean tolerance time was 47.85 seconds for participants included in the final analysis. This was consistent with previous reported studies of CPT tolerance time in non-clinical samples (Koenig et al., 2014; Treister et al., 2015). Considerable heterogeneity exits in CPT tolerance time across studies and the reasons for this are

difficult to assess. Part of the difficulty in assessing differences between studies using a CPT is due to heterogeneity in apparatus and parameters used (e.g. varying water temperatures, instructions, contextual cues, circulation and variable stopping times) (Mitchell et al., 2004; Vigil, Rowell, Alcock, & Maestes, 2014; Von Baeyer, Torvi, Hemingson, & Beriault, 2011).

Tolerance time may have also been influenced in our study by the participant's knowledge that they were to perform the task a total of four times (twice each session). This was evidenced by a significant overall trend towards shorter tolerance times with each subsequent CPT and a non-significant trend for pain intensity ratings (with outliers removed). The overall effect size was very small for the slope of pain tolerance and intensity, $R^2_{GLMM} = 0.01$ (for both). In comparison the overall effect size when conditional on participants (random factor) increased to $R^2_{GLMC} = 0.68$ and 0.58 for pain tolerance and intensity, respectively. Thus, in both cases, the majority of variance is explained by the individual and too a much less extent by trends over time.

Also, our selection criteria for the CPT differed compared to most studies using the CPT. Most studies involving a CPT have one or two measurements, this study required four. In addition, we allowed participants to remain in the CPT for up to 10 minutes, as opposed to the frequently used 3 min max time criterion. The aim of the longer CPT criterion was to have a larger window of time in which to capture individual differences in responding. However, it became apparent at the end of the study that participants could be characterized by two types of responding--one group which behaved reliably on all CPTs and another group that did not. This unreliable group's tendency was to have a much longer tolerance time on at least one of the four CPTs.

These participants were identified using Mahalanobis distance and were eliminated based on a cut off score of 5. This eliminated participants that had tolerance times greater than 3 minutes. The post-hoc elimination of participants lead to a decrease in the mean CPT tolerance time compared to the mean including all participants (M = 86.6 s versus M = 47.85 s). The mean with outliers excluded was consistent with results from Treister et al. (2015) who examined CPT times in 648 healthy volunteers. Finally, the greater number of CPTs required in this study increased the chance that any one participant would have reached the maximum time criterion, and this could have negatively biased the CPT tolerance times compared to other studies using a fewer number of measurements.

Many studies have demonstrated that a wide array of stimuli are able to modulate pain behavior during the CPT, for example, colors (Helsen, Goubert, Peters, & Vlaeyen, 2011) and threat appraisals (Arntz & Claassens, 2004; Jackson et al., 2005, 2014; Muris & Field, 2010). However, these studies all share a commonality in that the evoking stimulus shares some direct or indirect association with an unconditionally noxious (painful) stimulus.

Previous studies have shown that pairings of unconditioned stimuli can modulate defensive behavior in a predictable manner (Fanselow & Helmstetter, 1988; Helmstetter & Bellgowan, 1994; Rhud & Meagher, 2001; Terman et al., 1984). Unconditioned stimuli are evolutionarily conserved responses that are determined by the organism's phylogenetic history. Thus, an expression of this phylogenetic history would be expected in the nervous system in order to produce reliable responses to stimuli without a learning history. Indeed, there is considerable overlap between neurobiological areas in the central nervous system responsible for threat detection and modulation of pain behaviors. These

areas included the amygdala (basolateral amygdala) and the neocortex (insula, posterior parietal, anterior cingulate, second somatosensory cortex). The overlap in brain processing areas for pain and threat detection amounts to circumstantial evidence in support of a theory that learned-threats not associated with pain can modulate pain responding. Despite this circumstantial evidence, no prior study has examined the effect of a UCS and an operantly derived learned-threat (not associated with noxious stimuli) on pain behaviors.

In the case of the present study, the aim was to determine if pain behaviors can be modulated by an operantly learned-threat that has no prior learning history with pain. This threat was an operant threat because learning occurred in the context of seeking reinforcement (monetary gain). The threat, the potential for monetary loss, was learned across several trials of the operant task. Because the visual stimulus was arbitrary it was expected that it would have no prior history with noxious stimuli and thus would be neutral in valence at the start of the task and as learning proceeded would generate increased aversive valence. It was hypothesized that this aversive valence would moderate subsequent pain responding. The fact that this study did not find behavioral evidence for this hypothesis casts doubt that pain responding can be modulated outside a direct or indirect learning history with noxious stimulation. In other words, for an arbitrary stimulus to modulate pain it must be paired with a noxious stimulus at some point in the individuals learning history.

This study thus provides evidence for a functional interpretation of how an individual learns and responds to threats. In that, responses to operantly learned-threats are functionally related to the consequences they invoke and the contexts that they are

learned. As opposed to a structuralist perspective that threats are processed by a common neurobiological center and that modulation of behavior would occur via any stimulation of that center. In this case, the learned-threat was associated with a specific consequence related to monetary gain and had no relation to noxious stimulation and when presented with noxious stimulation did not modulate responses.

Further evidence of the functional perspective that learned-threats must be paired with noxious stimulation in one's learning history in order modulate behavior, comes from patients with congenital insensitivity to pain. Congenital insensitivity to pain (CIP) is a rare syndrome that results in a dramatic impairment of pain perception from birth. CIP is characterized by nerve pathologies involving the small-fiber nerves responsible for transmitting nociceptive sensory information (Nagasako, Oaklander, & Dworkin, 2003). The pathology specifically effects the peripheral nervous system and has no effects on the central nervous system. These nerve pathologies result in a significant decreased ability of these nerves to transmit nociceptive information. Essentially, individuals with the worst forms of this condition can feel no pain associated with physical stimulation. Thus, individuals afflicted with this condition can form no learning history with noxious stimulation because they cannot acquire the original direct association between unconditioned noxious stimulus and a neutral stimulus. For these individuals to learn of physical threats in their environment (ones that would be typically associated with pain) they must do so by learning through language (i.e. being told not to perform an action because of potential for harm). Despite language related learning, these individuals have extreme difficulty in learning of environmental threats. They susceptible to an increased risk to traumatic injuries and often lose digits and limbs as they age (Axelrod & Simson,

2007). Individuals with CIP suffer from shortened life expectancy as the result of this condition despite being cognitively aware of the risk of damage to their physical body (Kim et al., 2013). If arbitrary stimuli (i.e. language) could modulate defensive responding without a prior association with pain, then one would expect that these individuals would be able learn to avoid threatening (potentially physically harmful) situations. However, that is not the case and therefore the clinical presentation of individuals with this condition suggest that defensive responding cannot be modulated without a prior association with noxious stimulation.

Limitations

This study has several limitations. Other studies have sought to differentiate time to pain threshold, pain intensity at threshold and peak pain intensity (Mitchell et al., 2004; Treister et al., 2015) Including, measures of pain threshold or pain intensity at threshold may have been more sensitive to change than the methods used to assess pain in this study (pain intensity at trial end and tolerance time). Participants were not required to make distinctions between pain threshold and peak pain intensity so that they would maintain their attention on the screen (the threat/non-threat visual stimulus) and not have to shift their attention from screen during the task to other stimuli such as a pain scale for making pain ratings during the CPT.

In this study pain intensity was only measured by a rating immediately after the conclusion of the CPT task. When making this rating the participant was instructed to "rate their pain". This instruction may not have been specific enough to capture peak pain intensity during the CPT, which may be more sensitive to change. However, no matter the language of the pain intensity question, if conducted at the end of the CPT the answer

still requires a retrospective self-report and there may not be a reliable or valid way to differentiate current pain immediately post CPT and any memory of peak intensity during the CPT.

It was inferred, that the change from appetitive to defensive behavior was represented by the cessation of picking-up boxes and exiting the virtual room. This behavior was demonstrated by all participants included in the final analysis. However, when the participant exited the room, they received positive reinforcement in the form of a verbal stimulus praising, "'Good Work! You performed well on this trial", Figure 3 (Image F). Thus, it cannot be excluded that this change in behavior constituted an approach to the positive reinforcement of praise. Due to this the learned-threat stimulus may also have generated positive valance in that it signals to the participant to approach the exit in order to achieve reinforcement.

Regarding the perception of threat, while the participant was asked about their likelihood to exit the room with each symbol displayed, they were not asked about their perception of threat. Therefore, while the learned-threat stimulus successfully resulted in a change from appetitive to defensive behaviors, it remains unknown if the person cognitively recognized this symbol as a threat.

While the threat of monetary reward was enough to modulate responding during the task, it is possible that if the risk for monetary loss was not great enough to cause aversive valance to modulate behavior with the presentation of the noxious stimuli. Studies have shown reward magnitude moderates task performance (Bornovalova et al., 2009; van den Bos, Houx, & Spruijt, 2006). Each trial paid out on average $0.14 this was based on $0.02 reward per box picked-up. It is possible that increasing the reward may

have increased the degree to which the learned-threat stimulus modulated pain responding.

In order to avoid confounds of experimenter and gender characteristics influencing pain responding we only included male participants in this study. Vigil et al. (2014) showed that cold pressor sensitivity was modulated when the experimenter was a biological male compared to a female. All participants and researchers were biological males. However, research indicates robust evidence of sex-differences in risk taking—females are more risk aversive than males (Charness & Gneezy, 2012; Harris, Jenkins, & Glaser, 2006). The operant procedure used in this task is conceptually similar to behavioral measures of risk taking—the Balloon Analogue Risk Task and the Bomb Risk Elicitation Task (Crosetto & Filippin, 2013; Lejuez et al., 2002). Being that females are more risk-aversive, it is possible that they would have been more responsive to the learned-threat stimulus in combination with noxious pain (CPT).

Conclusion

While this study did not find a significant effect, it should be cautioned that this alone is not sufficient evidence that there is no effect. Our study was powered at 80% to detect an effect of 15 seconds in tolerance time, which means that the effect could be appreciably smaller than this and we were unable to find such an effect given the number of participants in this study. Confidence intervals on the effect sizes estimated of pain intensity and tolerance time ranged from a medium to large effect sizes on either side of zero which indicates low precision in this study's ability to reasonably estimate the population effect size. Therefore, the fact that we did not find a significant effect constitutes partial evidence that for threats to modulate pain behavior, the threat must be

acquired via direct or indirect associations with noxious stimulation. The reasons we did not find a significant effect may have also been due to the limitations of this study.

In order to address some of these limitations, future directions would include looking at explicitly measuring the threat appraisal of the participant, including females in the sample, using an alternative unconditionally noxious pain stimulus (thermal, electrical and mechanical), increasing the reward magnitude associated with the threat of monetary loss and measuring pain thresholds in addition to pain intensity and tolerance time.

In summary, this study did not find support for the hypothesis that a learned-threat, without a prior association with pain can modulate pain behavior. These results should be viewed as consistent with the observation that for pain behavior to be modulated the stimulus must be either be an unconditioned stimulus or a conditioned stimulus acquired directly or indirectly. Pain behavior, despite sharing numerous brain centers for threat detection, remains highly functional and contextual and is dependent on a learning history that involves noxious stimulation.

Figure 1 Flow chart depicting experimental design

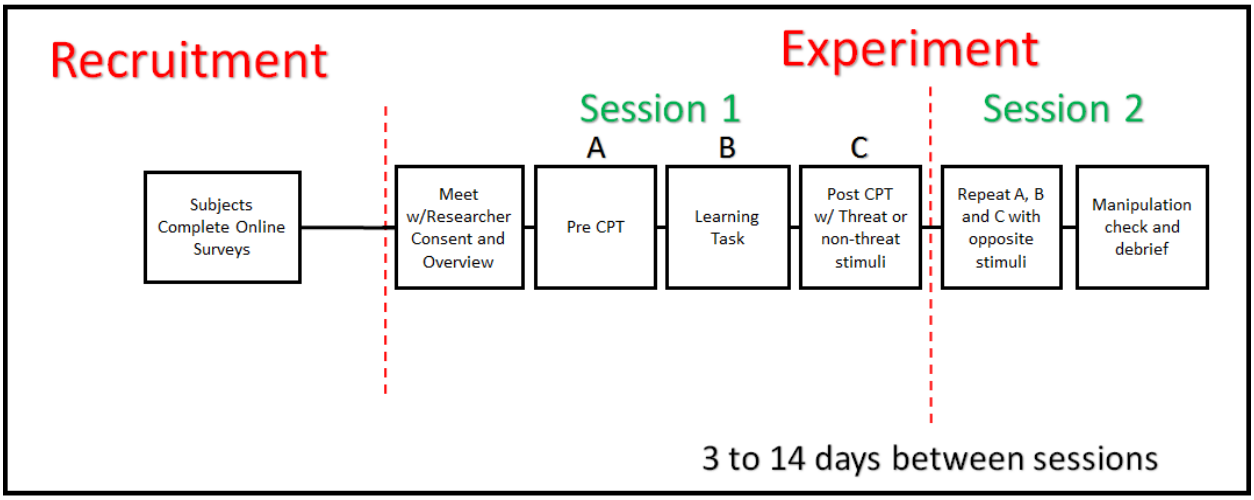
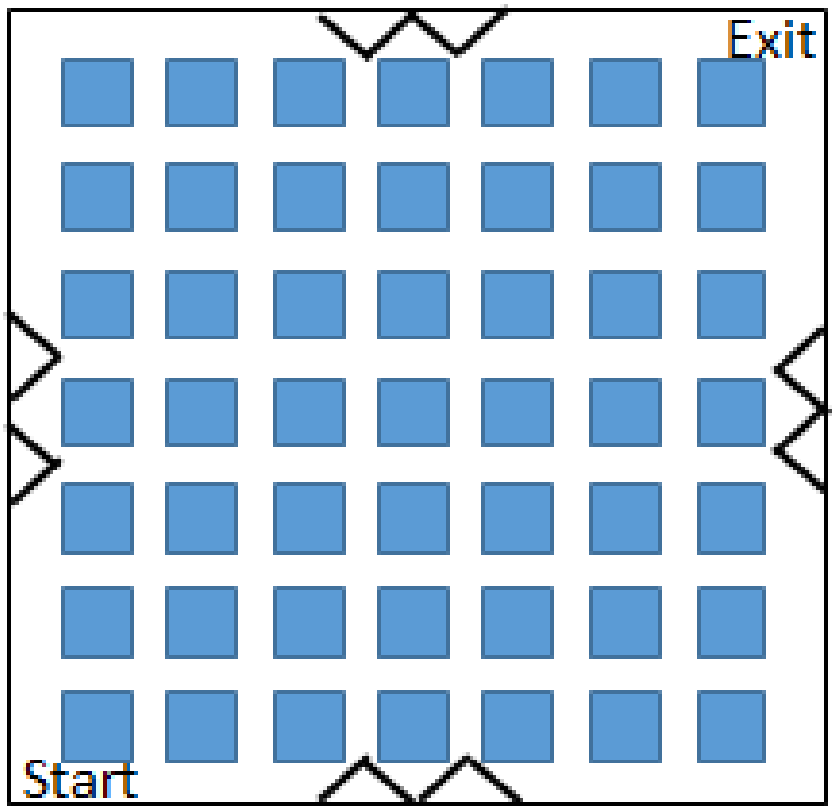


Figure 2 Top-down view of the virtual environment



Note: Blue boxes indicate location of pick-up boxes within the operant task.

Figure 3 Screen shots during the operant task

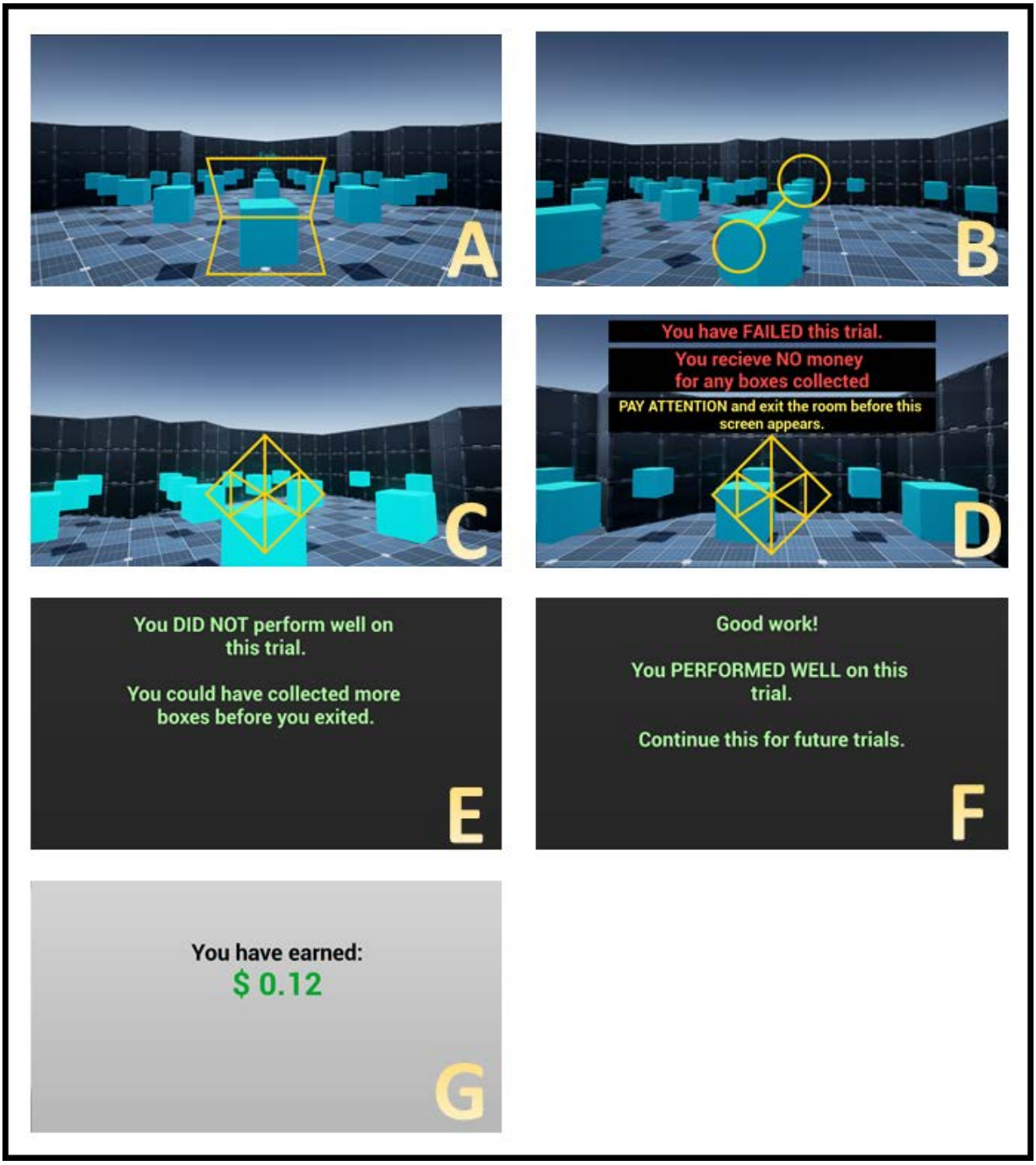


Figure 4 Distribution of % success during the final 15 trials

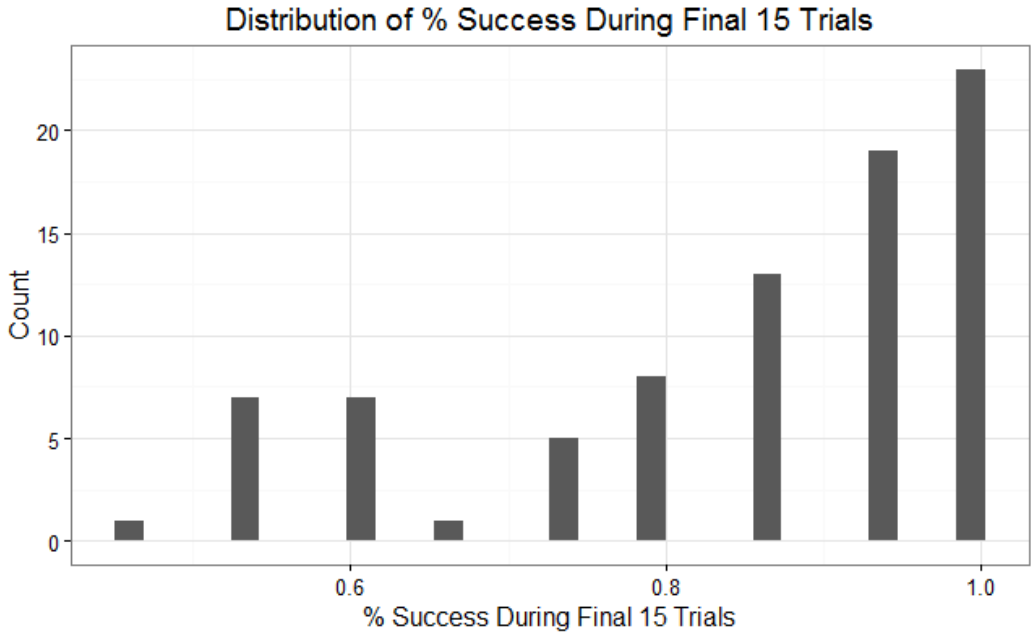


Figure 5 Distribution of mean collected boxes with learned-threat symbol

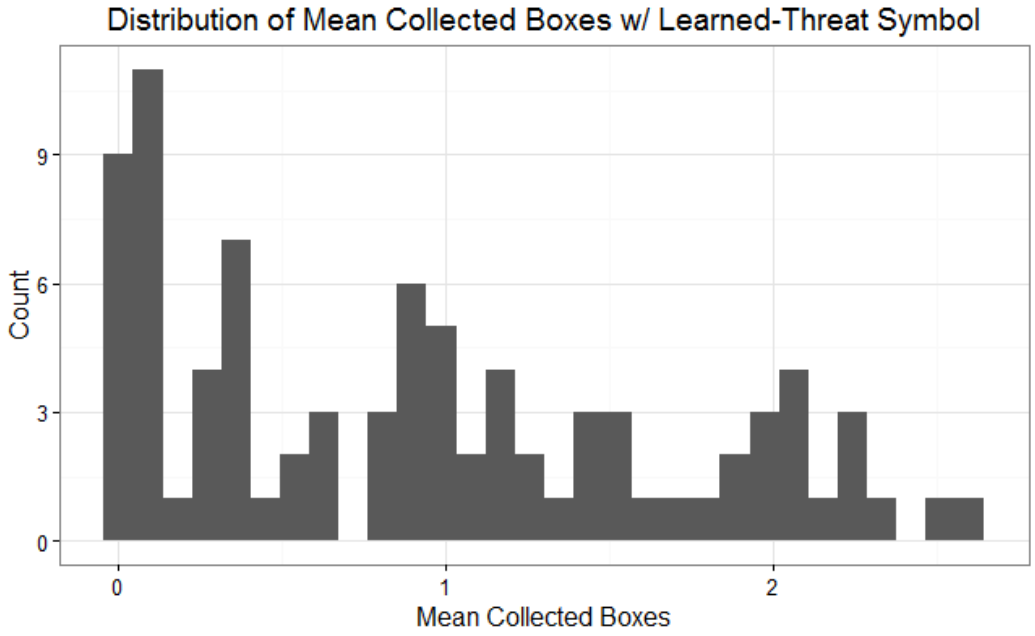


Figure 6 CPT tolerance time by condition and baseline/POST

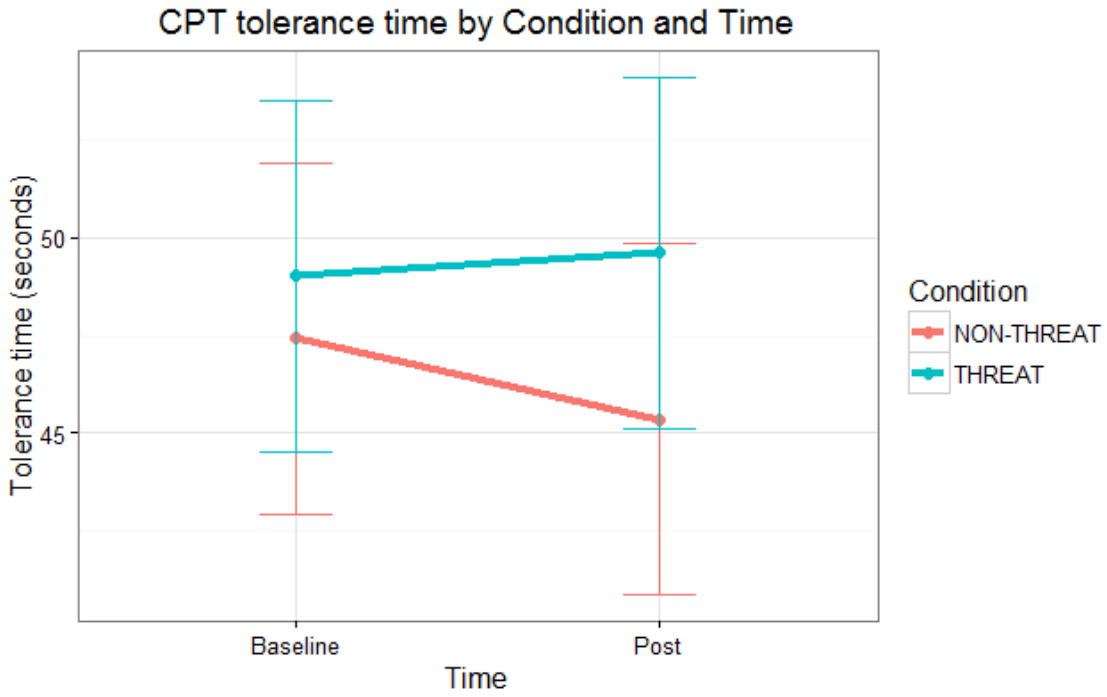


Figure 7 Pain Rating by Condition and Baseline/POST

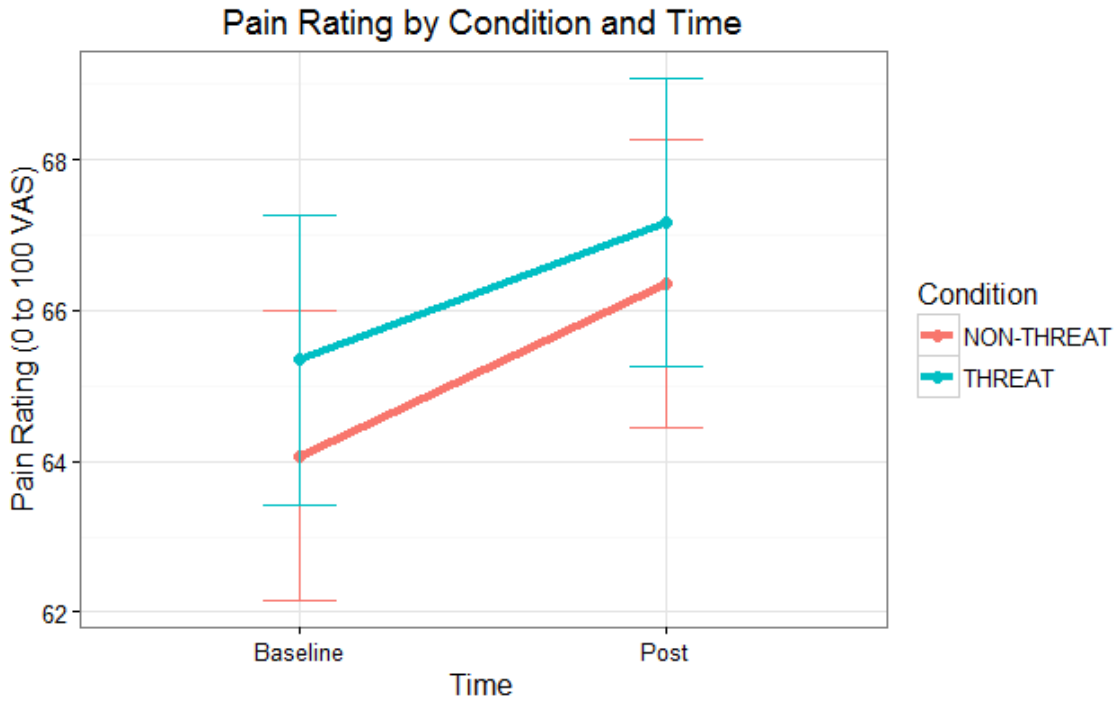


Table 1 Descriptive statistics and correlations of simulated task

	High	Med	Low	Total
High	**4.16**			
Med	0.00	**5.04**		
Low	0.15	0.09	**4.63**	
Mean	3.32	3.00	3.70	10.05
(SD)	2.04	2.24	2.15	4.01

Note. This table describes the number of boxes that would be picked-up during a computer simulation of the task. High = boxes collected between 100 and 75 points, Med = boxes collected between 75 and 40 points, Low = boxes collected between 39 and 0 points

Table 2 Descriptive statistics and correlations of study variables

		Tolerance Time				Pain Rating				Instruments			Boxes Collected w/ Threat Symbol	
		Threat		Non-Threat		Threat		Non-Threat						
		1.Baseline	2.Post	3.Baseline	4.Post	5. Baseline	6. Post	7.Baseline	8.Post	9.PVAQ	10.FPQ	11.PCS	12. Session 1	13. Session 2
1.	Baseline	--	--	--	--	--	--	--	--	--	--	--	--	--
2.	Post	0.75***	--	--	--	--	--	--	--	--	--	--	--	--
3.	Baseline	0.58***	0.63***	--	--	--	--	--	--	--	--	--	--	--
4.	Post	0.56***	0.75***	0.87***	--	--	--	--	--	--	--	--	--	--
5.	Baseline	-0.35*	-0.2	-0.13	-0.11	--	--	--	--	--	--	--	--	--
6.	Post	-0.11	-0.06	-0.06	-0.09	0.63***	--	--	--	--	--	--	--	--
7.	Baseline	-0.17	0.05	-0.11	-0.11	0.56***	0.58***	--	--	--	--	--	--	--
8.	Post	-0.15	-0.17	-0.19	-0.23	0.54***	0.56***	0.70***	--	--	--	--	--	--
9.	PVAQ	-0.11	-0.06	-0.01	0.14	-0.10	-0.02	-0.11	0.02	--	--	--	--	--
10.	FPQ	0.02	0.13	0.07	0.23	0.06	-0.12	-0.04	0.02	0.52***	--	--	--	--
11.	PCS	-0.24	-0.15	0.10	0.07	-0.01	-0.2	-0.12	-0.07	0.28ψ	0.29 ψ	--	--	--
12.	Session 1	-0.14	-0.05	-0.06	-0.04	0.19	-0.03	0.27	0.08	-0.09	0.09	0.1	--	--
13.	Session 2	-0.12	0.02	0.06	0.11	0.26	-0.02	0.21	0.09	-0.10	0.20	0.23	0.62***	--
mean		49.3	49.92	47.14	45.07	65.24	67.06	64.18	66.47	43.44	86.93	16.93	1.00	0.86
SD		30.7	34.5	26.13	25.83	11.11	11.25	12.39	15.18	10.42	19.38	9.54	0.74	0.78

Note. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ψ $p < 0.1$; Box Pick-Ups w/ Threat Symbol is a measure the degree to which participants responded to the threat symbol (with higher numbers the participant goes on to collect more boxes and does not exit the room

References

Arntz, A., & Claassens, L. (2004). The meaning of pain influences its experienced intensity. *Pain*, *109*(1–2), 20–25.

Augustson, E. M., & Dougher, M. J. (1997). The transfer of avoidance evoking functions through stimulus equivalence classes. *Journal of Behavior Therapy and Experimental Psychiatry*, *28*(3), 181–191.

Axelrod, F. B., & Simson, G. G. (2007). Hereditary sensory and autonomic neuropathies: types II, III, and IV. *Orphanet Journal of Rare Diseases*, *2*(1), 39.

Baum, C., Huber, C., Schneider, R., & Lautenbacher, S. (2011). Prediction of experimental pain sensitivity by attention to pain-related stimuli in healthy individuals. *Perceptual and Motor Skills*, *112*(3), 926–946.

Bennett, M., Hermans, D., Dymond, S., Vervoort, E., & Baeyens, F. (2015). From bad to worse: Symbolic equivalence and opposition in fear generalisation. *Cognition & Emotion*, *29*(6), 1137–45.

Bornovalova, M. A., Cashman-Rolls, A., O'Donnell, J. M., Ettinger, K., Richards, J. B., deWit, H., & Lejuez, C. W. (2009). Risk taking differences on a behavioral task as a function of potential reward/loss magnitude and individual differences in impulsivity and sensation seeking. *Pharmacology Biochemistry and Behavior*, *93*(3), 258–262.

Boston, A., & Sharpe, L. (2005). The role of threat-expectancy in acute pain: Effects on attentional bias, coping strategy effectiveness and response to pain. *Pain*, *119*(1–3), 168–75.

Boyer, P., & Bergstrom, B. (2011). Threat-detection in child development: An evolutionary perspective. *Neuroscience & Biobehavioral Reviews*, *35*(4), 1034–1041.

Campeau, S., & Davis, M. (1995). Involvement of the central nucleus and basolateral complex of the amygdala in fear conditioning measured with fear-potentiated startle in rats trained concurrently with auditory and visual conditioned stimuli. *Journal of Neuroscience*, *15*(3), 2301–2311.

Charness, G., & Gneezy, U. (2012). Strong evidence for gender differences in risk taking. *Journal of Economic Behavior & Organization*, *83*(1), 50–58.

Cohen, J. (1992). A power primer. *Psychological Bulletin*, *112*(1), 155.

Crombez, G., Vlaeyen, J. W. S., Heuts, P. H. T. G., & Lysens, R. (1999). Pain-related fear is more disabling than pain itself: Evidence on the role of pain-related fear in chronic back pain disability. *Pain*, *80*(1–2), 329–339.

Crosetto, P., & Filippin, A. (2013). The "bomb" risk elicitation task. *Journal of Risk and Uncertainty*, *47*(1), 31–65.

Damme, S., Crombez, G., Wever, K. N., & Goubert, L. (2008). Is distraction less effective when pain is threatening? An experimental investigation with the cold pressor task. *European Journal of Pain*, *12*(1), 60–67.

Dunsmoor, J. E., Martin, A., & LaBar, K. S. (2012). Role of conceptual knowledge in learning and retention of conditioned fear. *Biological Psychology*, *89*(2), 300–5.

Dymond, S., Schlund, M. W., Roche, B., Whelan, R., Richards, J., & Davies, C. (2011). Inferred threat and safety: Symbolic generalization of human avoidance learning. *Behaviour Research and Therapy*, *49*, 614–621.

Fanselow, M. S., & Helmstetter, F. J. (1988). Conditional analgesia, defensive freezing, and benzodiazepines. *Behavioral Neuroscience*, *102*(2), 233–243.

Field, A. P., & Schorah, H. (2007). The verbal information pathway to fear and heart rate changes in children. *Journal of Child Psychology and Psychiatry*, *48*(11), 1088–1093.

Fillingim, R. B., King, C. D., Ribeiro-Dasilva, M. C., Rahim-Williams, B., & Riley, J. L. (2009). Sex, gender, and pain: A review of recent clinical and experimental findings. *The Journal of Pain: Official Journal of the American Pain Society*, *10*(5), 447–485.

George, S. Z., Dannecker, E. A., & Robinson, M. E. (2006). Fear of pain, not pain catastrophizing, predicts acute pain intensity, but neither factor predicts tolerance or blood pressure reactivity: An experimental investigation in pain-free individuals. *European Journal of Pain*, *10*(5), 457–457.

Hanssen, M. M., Vancleef, L. M., Vlaeyen, J. W., & Peters, M. L. (2014). More optimism, less pain! The influence of generalized and pain-specific expectations on experienced cold-pressor pain. *Journal of Behavioral Medicine*, *37*(1), 47–58.

Harris, C. R., Jenkins, M., & Glaser, D. (2006). Gender differences in risk assessment: Why do women take fewer risks than men? *Judgment and Decision Making*, *1*(1), 48.

Helmstetter, F. J., & Bellgowan, P. S. (1994). Hypoalgesia in response to sensitization during acute noise stress. *Behavioral Neuroscience*, *108*(1), 177–185.

Helsen, K., Goubert, L., Peters, M. L., & Vlaeyen, J. W. S. (2011). Observational learning and pain-related fear: An experimental study with colored cold pressor tasks. *Journal of Pain*, *12*(12), 1230–1239.

Hirsh, A. T., George, S. Z., Bialosky, J. E., & Robinson, M. E. (2008). Fear of Pain, Pain Catastrophizing, and Acute Pain Perception: Relative Prediction and Timing of Assessment. *The Journal of Pain*, *9*(9), 806–812.

Jackson, T., Pope, L., Nagasaka, T., Fritch, A., Iezzi, T., & Chen, H. (2005). The impact of threatening information about pain on coping and pain tolerance. *British Journal of Health Psychology*, *10*(3), 441–451.

Jackson, T., Wang, Y., & Fan, H. (2014). Associations between pain appraisals and pain outcomes: Meta-analyses of laboratory pain and chronic pain literatures. *Journal of Pain*, *15*(6), 586–601.

Keeley, P., Creed, F., Tomenson, B., Todd, C., Borglin, G., & Dickens, C. (2008). Psychosocial predictors of health-related quality of life and health service utilization in people with chronic low back pain. *PAIN®*, *135*(1–2), 142–150. https://doi.org/10.1016/j.pain.2007.05.015

Kim, W., Guinot, A., Marleix, S., Chapuis, M., Fraisse, B., & Violas, P. (2013). Hereditary sensory and autonomic neuropathy type IV and orthopaedic complications. *Orthopaedics & Traumatology: Surgery & Research*, *99*(7), 881–885.

Koenig, J., Jarczok, M. N., Ellis, R. J., Bach, C., Thayer, J. F., & Hillecke, T. K. (2014). Two-week test-retest stability of the cold pressor task procedure at two different

temperatures as a measure of pain threshold and tolerance. *Pain Practice*, *14*(3), 126–135.

Lang, P. J., Bradley, M. M., & Cuthbert, B. N. (1998). Emotion, motivation, and anxiety: Brain mechanisms and psychophysiology. *Biological Psychiatry*, *44*(12), 1248–1263.

Larson, C. L., Aronoff, J., Sarinopoulos, I. C., & Zhu, D. C. (2009). Recognizing threat: A simple geometric shape activates neural circuitry for threat detection. *Journal of Cognitive Neuroscience*, *21*(8), 1523–1535.

Lee, J. E., Watson, D., & Frey Law, L. A. (2010). Lower-order pain-related constructs are more predictive of cold pressor pain ratings than higher-order personality traits. *Pain*, *11*(7), 681–691.

Legrain, V., Damme, S. V., Eccleston, C., Davis, K. D., Seminowicz, D. A., & Crombez, G. (2009). A neurocognitive model of attention to pain: Behavioral and neuroimaging evidence. *Pain*, *144*(3), 230–232.

Legrain, V., Iannetti, G. D., Plaghki, L., & Mouraux, A. (2011). The pain matrix reloaded: A salience detection system for the body. *Progress in Neurobiology*, *93*(1), 111–24.

Lejuez, C. W., Read, J. P., Kahler, C. W., Richards, J. B., Ramsey, S. E., Stuart, G. L., … Brown, R. A. (2002). Evaluation of a behavioral measure of risk taking: The Balloon Analogue Risk Task (BART). *Journal of Experimental Psychology: Applied*, *8*(2), 75.

Mahalanobis, P. C. (1936). On the generalized distance in statistics. *Proceedings of the National Institute of Sciences (Calcutta)*, *2*, 49–55.

McCracken, L. M. (1997). “Attention” to pain in persons with chronic pain: A behavioral approach. *Behavior Therapy*, *28*(2), 271–284.

McNeil, D. W., & Rainwater, A. J. (1998). Development of the fear of pain questionnaire-III. *Journal of Behavioral Medicine*, *21*(4), 389–410.

McWilliams, L. A., & Asmundson, G. J. G. (2001). Assessing individual differences in attention to pain: Psychometric properties of the Pain Vigilance and Awareness Questionnaire modified for a non-clinical pain sample. *Personality and Individual Differences*, *31*(2), 239–246.

Mitchell, L. A., MacDonald, R. A. R., & Brodie, E. E. (2004). Temperature and the cold pressor test. *The Journal of Pain*, *5*(4), 233–237.

Monticone, M., Ambrosini, E., Rocca, B., Nava, T., Terragni, E., Cerri, C., & McCracken, L. M. (2015). Development of the Italian Version of the Pain Vigilance and Awareness Questionnaire in subjects with chronic low back pain: cross-cultural adaptation, confirmatory factor analysis, reliability and validity. *International Journal of Behavioral Medicine*, *23*(2), 214–223.

Mouraux, A., & Iannetti, G. D. (2009). Nociceptive laser-evoked brain potentials do not reflect nociceptive-specific neural activity. *Journal of Neurophysiology*, *101*(6), 3258–3269.

Mouraux, André, Diukova, A., Lee, M. C., Wise, R. G., & Iannetti, G. D. (2011). A multisensory investigation of the functional significance of the “pain matrix.” *NeuroImage*, *54*(3), 2237–2249.

Muris, P., & Field, A. P. (2010). The role of verbal threat information in the development of childhood fear. “Beware the jabberwock!” *Clinical Child and Family Psychology Review*, *13*(2), 129–150.

Nagasako, E. M., Oaklander, A. L., & Dworkin, R. H. (2003). Congenital insensitivity to pain: An update. *Pain*, *101*(3), 213–219.

Nakagawa, S., & Schielzeth, H. (2013). A general and simple method for obtaining R2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, *4*(2), 133–142.

Orelien, J. G., & Edwards, L. J. (2008). Fixed-effect variable selection in linear mixed models using statistics. *Computational Statistics & Data Analysis*, *52*(4), 1896–1907.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., & R Core Team. (2015). *nlme: Linear and Nonlinear Mixed Effects Models*. Retrieved from http://CRAN.R-project.org/package=nlme

R Core Team. (2015). *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://www.R-project.org/

Rhud, J. L., & Meagher, M. W. (2001). Noise stress and human pain thresholds: divergent effects in men and women. *The Journal of Pain*, *2*(1), 57–64.

Roelofs, J., Peters, M. L., Deutz, J., Spijker, C., & Vlaeyen, J. W. S. (2005). The Fear of Pain Questionnaire (FPQ): Further psychometric examination in a non-clinical sample. *Pain*, *116*(3), 339–346.

Roelofs, J., Peters, M. L., Van Der Zijden, M., & Vlaeyen, J. W. S. (2004). Does fear of pain moderate the effects of sensory focusing and distraction on cold pressor pain in pain-free individuals? *Journal of Pain*, *5*(5), 250–256.

Severeijns, R., Vlaeyen, J. W., van den Hout, M. A., & Weber, W. E. (2001). Pain catastrophizing predicts pain intensity, disability, and psychological distress independent of the level of physical impairment. *The Clinical Journal of Pain*, *17*(2), 165–172.

Skolnick, A. I., & Davidson, R. I. (2002). Affective modulation of eyeblink startle with reward and threat. *Psychophysiology*, *39*(6), 835–850.

Sullivan, M. J. L., Bishop, S. R., & Pivik, J. (1995). The Pain Catastrophizing Scale: Development and validation. *Psychological Assessment*, *7*(4), 524–532.

Terman, G. W., Shavit, Y., Lewis, J. W., Cannon, J. T., & Liebeskind, J. C. (1984). Intrinsic mechanisms of pain inhibition: Activation by stress. *Science (New York, N.Y.)*, *226*(4680), 1270–1277.

Treister, R., Nielsen, C. S., Stubhaug, A., Farrar, J. T., Pud, D., Sawilowsky, S., & Oaklander, A. L. (2015). Experimental comparison of parametric versus nonparametric analyses of data from the cold pressor test. *The Journal of Pain*, *16*(6), 537–548.

Valverde, M. R., Luciano, C., & Barnes-Holmes, D. (2009). Transfer of aversive respondent elicitation in accordance with equivalence relations. *Journal of the Experimental Analysis of Behavior*, *92*(1), 85–111.

Van Damme, S., Crombez, G., Bijttebier, P., Goubert, L., & Van Houdenhove, B. (2002). A confirmatory factor analysis of the Pain Catastrophizing Scale: Invariant factor structure across clinical and non-clinical populations. *Pain*, *96*(3), 319–324.

Van Damme, S., Crombez, G., & Eccleston, C. (2002). Retarded disengagement from pain cues: The effects of pain catastrophizing and pain expectancy. *Pain*, *100*, 111–118.

van den Bos, R., Houx, B. B., & Spruijt, B. M. (2006). The effect of reward magnitude differences on choosing disadvantageous decks in the Iowa Gambling Task. *Biological Psychology*, *71*(2), 155–161.

Vervoort, E., Vervliet, B., Bennett, M., & Baeyens, F. (2014). Generalization of human fear acquisition and extinction within a novel arbitrary stimulus category. *PloS One*, *9*(5), e96569.

Vigil, J. M., & Coulombe, P. (2011). Biological sex and social setting affects pain intensity and observational coding of other people's pain behaviors. *Pain*, *152*(9), 2125–2130.

Vigil, J. M., DiDomenico, J., Strenth, C., Coulombe, P., Kruger, E., Mueller, A. A., … Adams, I. (2015). Experimenter effects on pain reporting in women vary across the menstrual cycle. *International Journal of Endocrinology*, *2015*, e520719.

Vigil, J. M., Rowell, L. N., Alcock, J., & Maestes, R. (2014). Laboratory personnel gender and cold pressor apparatus affect subjective pain reports. *Pain Research & Management: The Journal of the Canadian Pain Society*, *19*(1), e13-18.

Von Baeyer, C. L., Torvi, D., Hemingson, H., & Beriault, D. (2011). Water circulation and turbulence in the cold pressor task: Unexplored sources of variance among

experimental pain laboratories. Retrieved from http://childpain.org/ppl/issues/v13n1_2011/v13n1_vonbaeyer.pdf

Waddell, G., Newton, M., Henderson, I., Somerville, D., & Main, C. J. (1993). A Fear-Avoidance Beliefs Questionnaire (FABQ) and the role of fear-avoidance beliefs in chronic low back pain and disability. *Pain*, *52*(2), 157–168.

Wainer, H. (1986). The SAT as a social indicator: A pretty bad idea. In *Drawing inferences from self-selected samples* (pp. 7–21). Springer. Retrieved from http://link.springer.com/chapter/10.1007/978-1-4612-4976-4_2

Wang, Y., Jackson, T., & Cai, L. (2016). Causal effects of threat and challenge appraisals on coping and pain perception. *European Journal of Pain*, *20*(7), 1111-1120.

Westfall, J. (2015). PANGEA: Power analysis for general anova designs. *Unpublished Manuscript. Available at Http://Jakewestfall. Org/Publications/Pangea. Pdf.*

Wiesenfeld-Hallin, Z. (2005). Sex differences in pain perception. *Gender Medicine*, *2*(3), 137–145.

Wong, W. S., McCracken, L. M., & Fielding, R. (2011). Factorial validity and reliability of the Chinese version of the Pain Vigilance and Awareness Questionnaire (ChPVAQ) in a sample of Chinese patients with chronic pain. *Pain Medicine*, *12*(7), 1018–1025.