THE ROLE OF THE INFRALIMBIC CORTEX IN A PAIN INTENSITY-GRADED RODENT MODEL OF DISTRACTION ANALGESIA

by

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Abstract

THE ROLE OF THE INFRALIMBIC CORTEX IN A PAIN INTENSITY-GRADED RODENT MODEL OF DISTRACTION ANALGESIA

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The misdirection of attention has been used to reduce experimental and clinical pain- a technique known as distraction analgesia (DA). A growing understanding of the important interrelationship between cognition and pain has prompted the need for a deeper understanding of its neural substrates. There is evidence implicating the infralimbic cortex of the rat brain as a putative mediator of DA, but this has never been directly tested. Therefore, this study investigated the role of the infralimbic cortex (IL) in a rat model of DA at high- and low-intensity pain. One hundred fifty two Sprague Dawley rats underwent stereotaxic surgery to receive either a bilateral electrolytic lesion to the IL or a sham procedure. Following recovery, rats underwent a week of daily habituation to a test chamber. Following habituation, rats received a subcutaneous injection of either .5% or 1% formalin into the plantar surface of the left hindpaw and underwent a formalin test in the same chamber to which they had been habituated. Each chamber was either left empty or outfitted with an inverted falcon tube to serve as a distractor. Behavior during habituation and formalin testing was recorded with tracking software. The presence of the distractor was associated with significantly decreased formalin pain scores in sham animals ($p < .05$), indicating successful DA. However, distractor presence did not decrease formalin pain scores in IL-lesioned animals, indicating that the IL lesion

attenuated DA. The IL lesion was also found to reduce formalin pain scores relative to sham controls when tested in the empty chamber ($p < .01$), implicating a direct role for the IL in the processing of pain. Additionally, patterns in the data suggest that high-intensity pain may be more susceptible to DA than low-intensity pain. This study presents the first published evidence of the IL's role in pain processing and in distraction analgesia. Implications and future directions for studying the interplay between cognition and pain are discussed.

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Chapter 1

Introduction

1.1 Prevalence and Importance

Pain costs the United States up to $635 billion annually (Institute of Medicine, 2011) and afflicts roughly 100 million American adults, which is greater than the number of people with cancer, diabetes, and heart disease combined (Tsang et al., 2008). The Centers for Disease Control and Prevention have reported that 1 in 20 Americans misused opioids, a popular class of analgesic drugs, for a non-medical purpose in 2010 (CDC, 2011). The fact that so many Americans live in pain and that certain analgesic drugs are so often abused suggest that modern pharmaceuticals may be an inadequate solution to the problems posed by pain. In order to more effectively reduce the financial and emotional burden of pain, additional treatment strategies, including non-pharmaceutical strategies, must be developed.

1.2 Historical and Theoretical Framework of the Cognitive Modulation of Pain

Ron Melzack and Ken Casey authored the modern theory of the pain experience, which compartmentalized it into three dimensions: sensory/discriminative, motivational/affective, and cognitive/evaluative (Melzack & Casey, 1968). In this framework, the cognitive dimension was described as the component that allowed for the evaluation of pain in the context of past and present experiences. This notion is corroborated by Henry Beecher's oft-cited finding that severely wounded soldiers who were brought off the battlefield and into a clinic reported little pain and requested small amounts of morphine or no morphine at all (Beecher, 1946). Since identical injuries in a civilian would probably result in requests for high analgesic dosing, Beecher speculated that the cause of the requests for low dosing in soldiers might be the cognitive evaluation of the injury. For example, the injury removes the soldier from a dangerous warzone,

puts him in a safe environment, and may result in being sent home. One the other hand, an identical injury in a civilian would be evaluated as the beginning of disastrous consequences for that person, including financial and familial hardships (Beecher, 1946). Since the soldier's contextual evaluation of the pain condition is the primary distinguishing factor relative to the civilian's experience, Beecher reasoned that cognitive/evaluative processes may be capable of modulating the overall pain experience.

The process of pain evaluation can be broken down into at least three constituent components: attention, which directs the focus of the organism; learning, which establishes experience; and memory, which allows the organism to recall what has been learned. Of these cognitive subcomponents, attention is the most foundational because it must be present before more complex cognitive processing, like learning and memory, can occur.

1.3 Pain Demands Attention

A review of the experimental evidence regarding the relationship between pain and attention concluded that the two are inherently linked because pain necessarily demands attention (Eccleston & Crombez, 1999). This idea is based on the classic notion that pain is a warning signal that indicates threat to an organism (Ohman, 1979; Price, 1988). The influence of threat, in the form of pain, on the attentional system is depicted in Figures 1-1 & 1-2 where the thickness of the arrows represents the strength of influence.

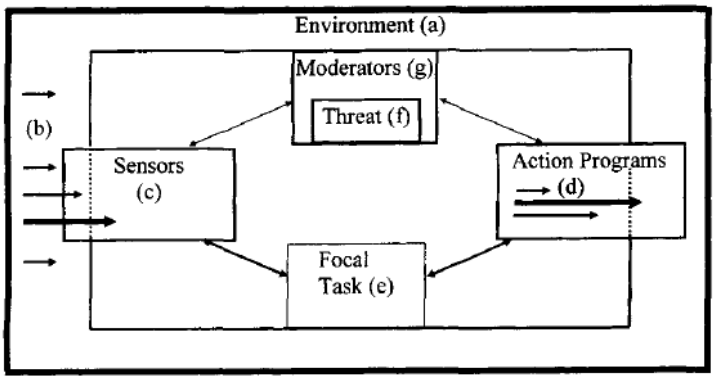

Figure 1-1 The attentional system before the interruption by pain. (Reproduced from Eccleston & Crombez, 1999. No permission required.)

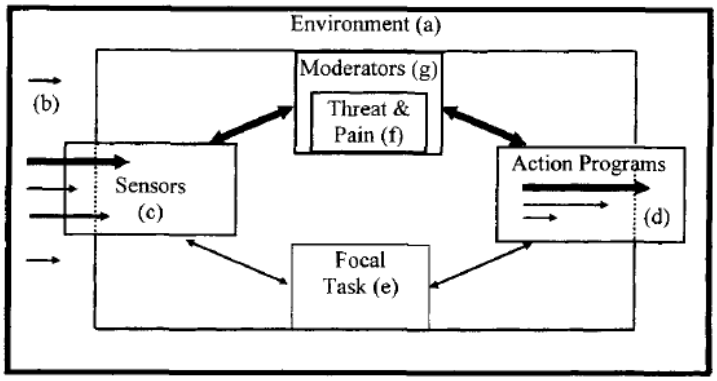

Figure 1-2 The attentional system during pain. (Reproduced from Eccleston & Crombez, 1999. No permission required.)

Note that, when pain is present, the moderating influence of pain on sensory input and on action programs strengthens. The figures show that noxious stimulation alters sensation and resultant behavior by strengthening certain action programs. In Figure 1-2, the action programs strengthened by pain would most likely take the form of escape/avoidance behavior, which would draw attention away from the focal task, thereby disrupting task performance. Thus, pain interrupts attention to focal task performance, which is evident in action programs or behavioral outcomes.

Experimentally, this can be demonstrated with paradigms that compare task performance during pain and non-pain states. If the pain condition is associated with interrupted or impaired task performance, then it can be inferred that the impairment is the result of an attentional shift away from the task and towards the pain. In a representative study (Boyette-Davis, Thompson, & Fuchs, 2008), rats were trained to complete a 5-choice serial reaction time task (5CSRTT), which required the rat to attend to a line of signal lights in an operant box. When a signal light was illuminated, the rat was trained to place its nose in a corresponding hole. Successful nosepokes result in appetitive reward, and unsuccessful trials, which are defined by either failure to nosepoke (omission) or nosepoking the wrong hole (mistake), resulted in no reward. Following training on this task, some rats were administered a noxious formalin injection into the plantar surface of the hindpaw while others received a saline injection as a control. Formalin animals demonstrated a sharp increase in the percentage of omitted trials relative to saline controls, and during those omissions, attended to the noxious stimulus, as measured by pain-related behavior (Boyette-Davis et al., 2008). This shows that noxious stimulation commanded attention and disrupted the focal task, exactly as predicted by Eccleston & Crombez (1999).

Another study showed that human subjects in a high amount of chronic pain perform worse than subjects in a lesser amount of chronic pain on a difficult Stroop-like card sorting task (Eccleston, 1995). Subjects first rated their pain on a 0-100 visual analog scale and were then grouped into the high-pain condition if their score was above the median or grouped into the low-pain condition if their score was below the median. Subjects were shown two cards per trial, each displaying multiple Arabic numerals, and were asked to identify either the card with the higher-value numeral or the card with the greater number of numerals. In Figure 1-3, if the subject was asked which card displayed

the higher-value Arabic numberal, the correct answer would be the card on the right. However, if the subject was asked which card displayed the greater number of numerals, the correct answer would be the card on the left. Results showed an interaction effect between pain group and task type such that high-pain, but not low-pain, subjects displayed increased latency relative to non-pain controls to name the card with the higher number of digits. This suggests that high-intensity pain interrupts attention-demanding tasks more strongly than low-intensity pain. It also suggests that difficult behavioral tasks may be more sensitive to the attention-demanding influence of pain, perhaps because difficult tasks require more attention and, therefore, are more easily disrupted by other attentional demands.

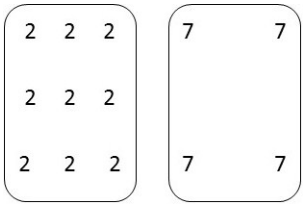

Figure 1-3 An example of the paired cards used as stimuli in Eccleston, 1995.

Additionally, there is evidence that the attention-demanding nature of pain is influenced by pain catastrophization. Catastrophization is “an exaggerated negative orientation toward actual and anticipated pain experiences” (Crombez, Eccleston, Van den Broeck, Van Houdenhove, & Goubert, 2002; Sullivan, Bishop, & Pivik, 1995). In one study (Crombez et al., 2002), pain-free students as well as chronic low back pain patients were assessed with the Dutch version of the Pain Catastrophizing Scale (PCS), which has been validated as a measure of exaggerated negative affect toward pain (Sullivan et al., 1995). Those that scored above the median were analyzed as pain catastrophizers and those that scored below the median were analyzed as non-catastrophizers. Subjects were administered an electrocutaneous 50 Hz AC stimulus to the left forearm for 1500

ms, which was determined in a pilot study to be mildly aversive and tolerable. During stimulation, subjects completed an auditory pitch discrimination task in which either a high 1000 Hz tone or a low 250 Hz tone was presented either 250 ms or 750 ms after electrical stimulus onset. Pain-free and chronic pain catastrophizers took significantly longer than matched non-catastrophizers to identify the tone as high or low in the 250 ms condition ($p < .01$ and $p < .05$, respectively), but not in the 750 ms condition (see Figure 1-4). Interestingly, the chronic pain catastrophizers displayed extremely low response latencies in the 750 ms condition, suggesting a reversal of catastrophization's influence on the attention-demanding task. These results provide corroborating evidence that pain demands attention by interfering with performance on attention-demanding tasks.

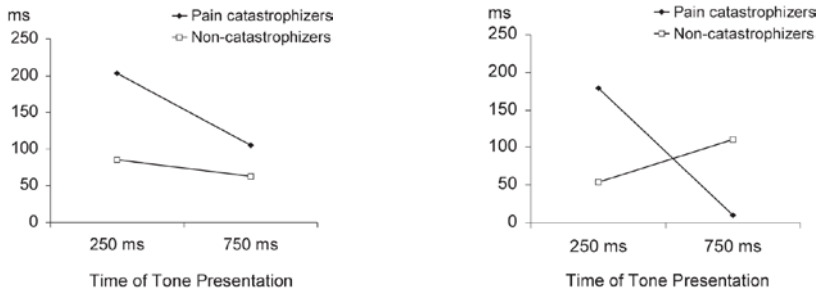

Figure 1-4 Mean interference scores in healthy pain-free subjects (left) and chronic low back pain patients (right). (Reproduced with permission from Crombez et al. 2002. This figure was originally published in Pain Res Manage 2002;7(1):31-39.)

Each of the above experiments regarding the attention-demanding nature of pain viewed pain as an experimental independent variable and viewed behavioral (i.e. operant) or cognitive (i.e. Stroop-like and auditory discrimination task) outcomes as the primary dependent variables. Other research has viewed the relationship between pain and cognition from the opposite perspective where cognitive tasks serve as the independent variables and pain serves as the dependent variable. In order to frame this perspective in the original theoretical construct proposed by Eccleston & Crombez, the

threat/pain would switch roles with the action programs (see Figure 1-5). In such experiments, the cognitive tasks demand attention away from the sensory and affective experience of pain, resulting in a decrease in the amount of pain reported.

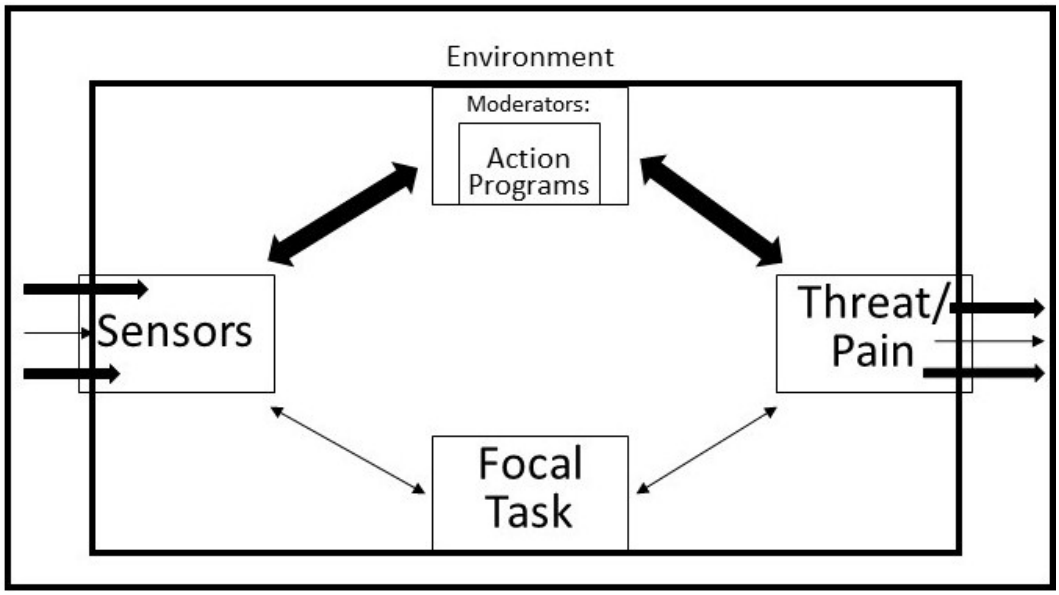

Figure 1-5 Modified attentional system in which action programs or cognitive tasks requiring attention influence pain

1.4 Distraction Analgesia in Humans

Diverting attention away from a default attentional target is called distraction, and a reduction in pain is called analgesia. Therefore, the diversion of attention to reduce the experience of pain is known as distraction analgesia (DA). The following DA experiments provide empirical support for the theoretical construct in Figure 1-5 where a distracting moderator (i.e. behavior or thought process) influences pain.

Distraction has been used in laboratory settings to modulate acute experimental pain in humans. One classic study reported that healthy subjects showed a diminished ability to detect noxious heat pain when attention was diverted away from the stimulus (Bushnell, Duncan, Dubner, Jones, & Maixner, 1985). In this experimental setup (Figure

1-6), the subject was seated in front of a board that had a response button and two signal lights. When the response button was depressed and held, two thermodes attached to each side of the subject's upper lip quickly ramped up to a certain temperature (T1). After a randomly determined amount of time, one of the thermodes increased in temperature to a variable amount (T2), and, if the subject detected the increase within 2 seconds, they were instructed to release the response button. The number of "no response" trials was measured in three experimental conditions: Neutral, in which the procedure was the same as described above; Correct, in which a left- or right-side signal light was illuminated during T1 and correctly indicated which thermode would increase at T2; and Incorrect, in which a signal light would illuminate during T1 and incorrectly indicate which thermode would increase at T2. This paradigm was conducted at two temperature levels in which T1 was a non-noxious 39° C and when T1 was a noxious 45° C. The final temperature of T2 was individualized to each subject such that T2 was detectable on 75% of trials, and the value of the increase fell between 1° and .2° C.

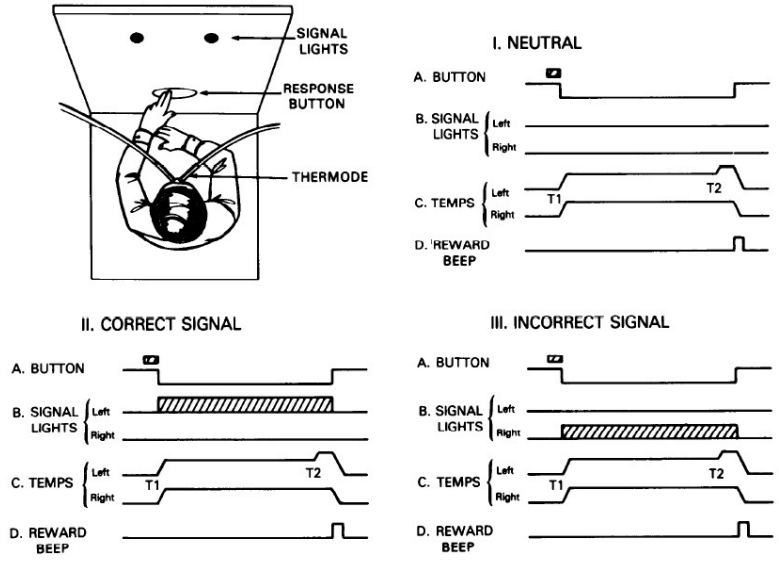

Figure 1-6 The human attention task. (Reproduced with permission from Bushnell, Duncan, Dubner, Jones, & Maixner, 1985).

In the noxious 45° condition, a paired *t*-test revealed that the number of "no response" trials was significantly increased by an incorrect signal relative to the neutral condition, $p < .01$, and that the correct condition was associated with significantly fewer "no response" trials than the neutral condition, $p < .05$ (Figure 1-7). However, in the non-noxious 39° condition, there were no significant differences between signal conditions. The results indicate that the direction of attention toward and away from the correct thermode significantly altered detection of noxious stimulation, but not non-noxious stimulation. This study provided foundational evidence that attention can alter nociception and that it is less effective at influencing non-noxious somatosensation (Bushnell et al., 1985).

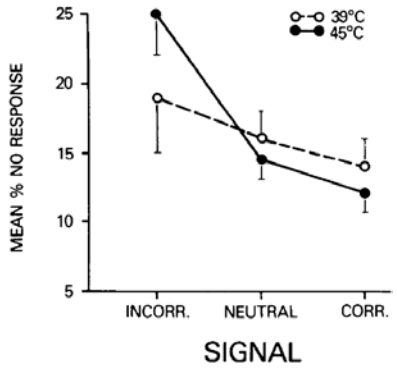

Figure 1-7 Mean percentage of trials in which the subject did not respond to T2 within 2 seconds. (Reproduced with permission from Bushnell, Duncan, Dubner, Jones, & Maixner, 1985).

In a follow-up study with a similar procedure (Miron, Duncan, & Bushnell, 1989), subjects were asked to indicate whether the increase in stimulus intensity occurred in either a single thermode attached to the upper lip or in a visual light stimulus. Subjects were again correctly, incorrectly, or neutrally signaled to attend to either the thermode or the light to detect the increase. This study replicated the previous findings by showing

that the percent of successful trials was marginally higher in the correctly signaled condition relative to the neutral condition, $p = .059$, and significantly lower in the incorrectly signaled condition relative to the neutral condition, $p = .03$. However, this study expanded upon prior research by also quantifying pain intensity and pain unpleasantness on a scale from 0-100 (Figure 1-8). Correctly cueing subjects to the increase in stimulus intensity had no effect on either subjective pain rating. However, incorrect cues significantly reduced both pain intensity ($p = .002$) and pain unpleasantness ($p = .002$) relative to the neutral signal condition. These results suggested that modulating the direction of attention can directly affect the subjective experience of pain and that it is more effective for decreasing pain than for increasing pain. They also demonstrate that the distracting stimulus need not target the same sensory modality as the pain stimulus in order to produce an analgesic effect (Miron et al., 1989).

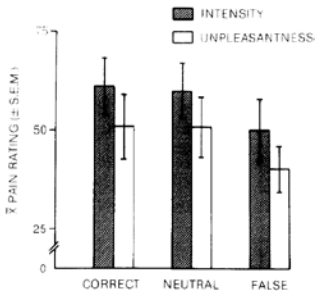

Figure 1-8 Pain Intensity and Pain Unpleasantness of noxious thermal stimulation in each signal condition. (Reproduced with permission from Miron, Duncan, & Bushnell, 1989).

Counter-stimulation is sensory input administered for the purpose of interfering with the perception of another simultaneous sensory stimulus. Vibration has been used as a counter stimulus to pain, and some evidence suggests that it is more effective than placebo at reducing pain (Lundeberg, Nordemar, & Ottoson, 1984). Originally, the pain-reducing effect was explained exclusively in terms of the ascending mechanisms of Gate

Control Theory (Ronald Melzack & Wall, 1965) as well as the finding that large diameter primary afferent fibers interact with pain transmission pathways (Handwerker, Iggo, & Zimmermann, 1975). Building upon that explanation, a more recent study found evidence that attention to a vibratory stimulus and away from pain may contribute to vibration-induced analgesia (Longe et al., 2001), which implicates the engagement of top-down modulatory mechanisms rather than bottom-up Gate Control mechanisms. In this study, a 200 Hz vibrating stimulator was attached to the middle finger of the left hand in the dermatome adjacent to a fast-ramping thermal resistor attached to the volar surface of the left forearm. Subjects were instructed to attend to the pain, the vibration, or an image of a vertical line. When attending to the line, subjects were further instructed to determine whether the image was oscillating slightly, although no such oscillation occurred. Both distraction conditions (i.e. attending to vibration or to the image of the line) were associated with decreased ratings of pain intensity, and more subjects reported that attending to the neutral visual image of the vertical line was more effective at reducing pain than attending to the vibration. This suggests that the analgesic effect of vibration may be partly attributable to the misdirection of attention away from the painful stimulus (Longe et al., 2001).

1.5 Distraction Analgesia is Independent of Affect and of Other Cognitive Factors

There has been some dispute regarding whether DA is an independent phenomenon that can occur without the engagement of affect. In contrast to the evidence above, some early researchers argued passionately that distraction alone could not modulate pain and that, in order for distraction to successfully reduce pain, the relationship between those two factors must be mediated by positive affect, which could be unintentionally introduced into an experiment as an inherent consequence of the distractor (Leventhal, 1992; McCaul, Monson, & Maki, 1992). While affect may play a

significant role in certain forms of DA (Villemure, Slotnick, & Bushnell, 2003), the studies described above provide convincing evidence that emotionally neutral distractor stimuli are sufficient to elicit effective pain reduction (Bushnell et al., 1985; Longe et al., 2001; Miron et al., 1989). Some studies directly addressing the role of affect found that the neural pathways through which distraction decreases pain are partially, but not completely, independent of affect (Roy, Lebuis, Peretz, & Rainville, 2011; Villemure & Bushnell, 2009). It can be concluded that distraction interventions can directly decrease pain in experimental settings without the engagement of affect, although some overlap between mechanisms may exist.

There is additional evidence that the pain modulating influence of attention & distraction is an independent phenomenon that does not rely on other cognitive factors. In one study, distraction was teased apart from placebo treatment, which is more complex because it often involves the additional cognitive factors of learning, memory, and/or expectation. The authors showed evidence that, when used simultaneously, distraction and placebo have an additive pain-reducing effect, which suggests that they can influence pain independently of one another (Buhle, Stevens, Friedman, & Wager, 2012). Another study compared the analgesic effects of conditioned pain modulation, which is used to test the phenomenon known as Diffuse Noxious Inhibitory Control (DNIC), and distraction (Moont, Pud, Sprecher, Sharvit, & Yarnitsky, 2010). This study produced similar results in that, when conditioned pain modulation and distraction were used simultaneously, there was an additive pain-reducing effect on noxious thermal stimulation. These studies provide further evidence that the attentional modulation of pain is largely independent of pain modulation that occurs from learning, memory, and/or expectation mechanisms.

1.6 Clinical Applications of Distraction Analgesia

Most evidence of successful DA has been published using short-term experimental pain manipulations delivered to otherwise healthy subjects, and those studies have been discussed above. DA has also been explored in clinical subjects who had a naturally occurring pain condition before and during their participation in research studies. There is mixed evidence regarding the efficacy of attention/distraction manipulations in the alleviation of clinical pain.

1.6.1 Mixed Efficacy of Distraction on Chronic Clinical Pain

Redirection of attention is often a goal of cognitive-behavioral therapy (CBT), which is usually a component of larger pain management programs that also include pharmacotherapy, functional restoration, and other medical management (Gatchel, Peng, Peters, Fuchs, & Turk, 2007). A meta-analysis of randomized controlled trials concluded that principles of CBT are effective at reducing pain in clinical chronic patients relative to waiting-list controls (Morley, Eccleston, & Williams, 1999). However, since CBT is a loosely defined term that refers to many different techniques, of which attentional manipulation is only one, the meta-analysis cannot reveal the exclusive impact of attentional manipulation on pain. In order to directly determine the impact of attention on clinical pain, more selective manipulations of attention are necessary.

Mindfulness meditation (MM) has been used to more selectively target attentional systems. MM is a technique that aims, in part, to increase control over the direction of attention, and it has been studied for its potential utility in reducing pain by directing attention away from it (Zeidan, Grant, Brown, McHaffie, & Coghill, 2012). Studies using MM as an attentional manipulation have reported that it did not directly reduce clinical pain, but that it successfully improved other metrics related to the experience of pain. The classic example of this finding studied patients with a chronic pain condition lasting

longer than 6 months who participated in a 10-week MM program (Kabat-Zinn, Lipworth, & Burney, 1985). A battery of tests was performed before and after the program, including the Pain Rating Index (Melzack, 1975), Body Parts Problem Assessment (Kabat-Zinn, 1983), number of symptoms on a Medical Symptom Checklist (Kabat-Zinn, 1982), Total Mood Disturbance (McNair, Lorr, & Droppleman, 1971), and General Severity Index on the revised Hopkins Symptom Checklist (SCL-90-R) (Derogatis, 1977). Immediately following the 10-week program, PRI scores were significantly lower compared to baseline, but that difference was no longer apparent in follow-up assessments at 2.5, 5, or 12.5 months. Conversely, many of the other metrics achieved and retained significance in all of the follow-up assessments. Therefore, MM significantly decreased pain in the short-term, but not in the long-term. However, MM significantly improved other pain-related metrics in the long-term, but not in the short-term (Kabat-Zinn et al., 1985).

Similar results were found in a randomized controlled study of chronic low back pain patients in which pain was compared between cohorts that underwent an 8-week mindfulness meditation program or were put on a waiting list control (Morone, Greco, & Weiner, 2008). At the 8-week assessment, the meditation program was associated with non-significant reductions in the short form of the McGill Pain Questionnaire (Melzack, 1987) and the SF-36 Pain Scale (Hays, Prince-Embury, & Chen, 1998). However, the meditation group improved markedly on the Chronic Pain Acceptance Questionnaire (CPAQ) (McCracken, Carson, Eccleston, & Keefe, 2004). Therefore, this literature suggests that attentional redirection (i.e. distraction) via mindfulness meditation may have beneficial effects that are related to the experience of pain, but that direct effects on pain may be weak and brief (Morone et al., 2008).

Additional studies of the influence of distraction on chronic clinical pain are rare. In order to address this gap in the literature, one study used an experimental approximation of chronic clinical pain, capsaicin-induced heat hyperalgesia, to study the influence of distraction (Wiech et al., 2005). When compared to noxious thermal heat pain on untreated skin, the capsaicin model has been associated with increased activity in the medial thalamus, orbitofrontal region, medial prefrontal cortex, perigenual cingulate, anterior insula, and dorsolateral prefrontal cortices (Lorenz et al., 2002). Many of these regions are components of the medial pain system, which is closely associated with the experience of pain affect and chronic pain (Price, 2000). The Wiech et al. study compared thermal stimulation of capsaicin-induced heat hyperalgesic skin to thermal stimulation of untreated skin during an easy distraction task and a difficult distraction task. In the hyperalgesic condition, the difficult distractor task was associated with lower pain ratings than the easy distractor task; however, in the untreated condition, there was no difference in pain between the two distractor tasks (Wiech et al., 2005). In other words, distraction reduced capsaicin-induced heat hyperalgesia, but not noxious thermal pain. This study suggests that distraction may be effective for reducing pain from naturally occurring chronic conditions, and it also corroborates the evidence that higher intensity pain may be more susceptible to DA.

1.6.2 Distraction in Acutely Painful Medical Procedures

Most of the evidence for clinical DA has been reported during acutely painful medical procedures rather than during tonic pain from chronic conditions. For instance, one study reported successful use of distraction to reduce the pain associated with venipuncture for dialysis (Alhani, 2010). Before the venipuncture, patients were presented with two nearly identical cartoon images that contained minor differences. During venipuncture, they were asked to identify all of the differences between the

images and then afterward, report those differences to an experimenter as well as rate the pain of the procedure using a Wong-Baker face pain measurement (Wong & Baker, 1988). This was performed 2-3 times per week until 9 trials had been completed. The distraction task did not reduce pain scores relative to baseline on days 1-5, but the reduction achieved significance on Days 6-9 (Alhani, 2010). Similarly, a head-mounted video display of a beach with corresponding audio significantly reduced pain discomfort during endoscopy relative to no-distraction and audio-only conditions (Lembo et al., 1998).

More complex head-mounted systems have been used to immerse patients in a virtual reality for the purpose of distracting patients from the acute pain of medical procedures (Malloy & Milling, 2010). Virtual reality has decreased pain associated with the cleaning of severe burns (Hoffman et al., 2004, 2008, 2011; Morris, Louw, & Grimmer-Somers, 2009), urological endoscopies (Wright, Hoffman, & Sweet, 2005), physical therapy (Hoffman et al., 2009), and dental pain (Keefe et al., 2012). The use of virtual reality in clinical settings is an interesting example of how distraction can result in improved treatment scenarios for patients. However, there are two major caveats to the virtual reality literature. First, nearly all the studies have been conducted by the same research group, which warrants replication studies by other groups. Second, virtual reality almost certainly engages affect and is therefore not a selective manipulation of attention.

Another study explored the attentional influences on post-operative dental pain, which was slightly longer-term than the acutely painful procedures discussed above, but acute nevertheless. Patients had either 2 or 4 teeth extracted and were then asked to rate their post-operative pain either once or four times. Since rating pain necessitates attention to pain, manipulating the number of pain ratings also manipulates attention to

pain. In patients with two extractions, pain ratings were not different between the 1x and 4x rating conditions; however, in patients with four extractions, pain ratings were significantly higher in the 4x condition than in the 1x condition (Levine, Gordon, Smith, & Fields, 1982). This shows that more frequent attention to pain increased pain ratings. These results suggest again that attentional manipulation may be more effective at higher levels of pain.

1.6.3 Clinical Utility of Distraction Analgesia

The fact that clinical research is less prevalent than experimental research in the field of DA suggests that distraction might be less effective at reducing clinical pain. The available evidence indicates that this publication disparity may be due to the enhanced efficacy of distraction in acute pain compared to chronic pain. Therefore, the primary value of DA in clinical settings appears to be the attenuation of acute increases in pain from medical procedures.

1.7 Chronic Pain May Impair the Ability of Distraction to Produce Analgesia

Chronic pain patients often show enhanced neural responding to experimental pain stimuli relative to healthy controls (Derbyshire et al., 2002; Gracely, Petzke, Wolf, & Clauw, 2002). This hyperalgesic effect is due in part to peripheral and central sensitization (Gwilym et al., 2009), but evidence is amassing that alterations in top-down cognitive modulatory mechanisms, such as attention, may also contribute (Ossipov, Dussor, & Porreca, 2010; Porreca, Ossipov, & Gebhart, 2002). For instance, in a study that presented subjects with colored pain descriptors from the McGill Pain Questionnaire, chronic pain patients took significantly more time than healthy controls to name the color of the words (Pearce & Morley, 1989), suggesting the existence of an attentional bias towards pain-related information. It is intuitive that someone with chronic pain ought to maintain a heightened awareness of pain-related stimuli because it is adaptive for that

individual to avoid threats that may worsen pain. This finding was originally interpreted as a hypervigilance towards pain, but more recent research in rheumatoid arthritis patients suggests that the attentional bias may be better characterized as an inability to disengage attention from pain (Sharpe, Dear, & Schrieber, 2009). This is a critical distinction because it suggests that chronic pain patients may have an impaired ability to direct attention away from pain. This provides a putative explanation for the fact that successful DA is reported more frequently in healthy subjects than in patients with naturally occurring chronic pain conditions. It may be that the chronic patients are less able to successfully direct attention away from their pain and therefore are less likely to show a reduction of pain from distraction. Evidence for the neural substrates of this phenomenon are discussed below.

1.8 Rat Model of Distraction Analgesia

A model of DA has been developed in rats and appears to be an ideal tool to use for the investigation of DA in animals (Ford, Moriarty, McGuire, & Finn, 2008). In the study, rats were habituated to a 30x30x40 testing chamber for 10 minutes per day for 7 days. This was for the purpose of preventing the novelty of the testing chamber from confounding pain behavior later in the study. On the 8th day, rats underwent a formalin test in the same chamber.

The formalin test is a classic preclinical method of assessing pain behavior in which formalin is subcutaneously injected into one hindpaw of the rat and the resulting behavior is quantified over the course of at least 30 minutes (Dubuisson & Dennis, 1977). Formalin testing produces a characteristic tri-phasic pattern of pain behavior which includes 1) an acute phase lasting for about 5 minutes, 2) a latent phase, in which little or no pain behavior is observed, and lastly, 3) a tonic phase, in which sustained pain-related behavior is observed for the remainder of the test.

In the Ford, et al. (2008) study, the formalin tests were conducted in chambers that were either left empty, as in the habituation trials, or that contained a distractor object, which was a falcon tube filled with sand. The study found that animals tested in the presence of the distractor showed significantly lower composite formalin pain scores than controls between 30-45 minutes after formalin injection (Figure 1-9). This finding mimics the distraction analgesic effects found in humans (Longe et al., 2001; Miron et al., 1989). To the author's knowledge, the Ford et al. (2008) study is the only experiment that has directly explored DA in rats.

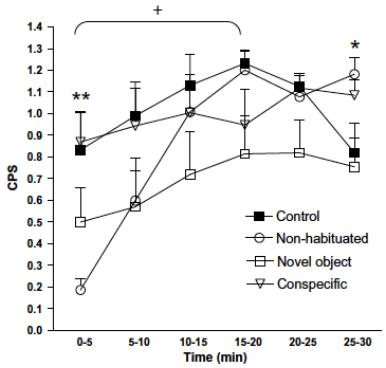

Figure 1-9 Composite Pain Scores (CPS) during the tonic phase of the formalin test. Time 0 corresponds to 30 minutes after the injection of formalin. (Reproduced with permission from Ford et al., 2008).

Critically, the original publication also included supplementary information to validate the model. First, the study aimed to determine whether the rat would attend to the distracting object and therefore measured "directed attention," which was defined as the rat orienting its head within a 2cm annulus of a stimulus. During the formalin test, rats were found to direct significantly more attention toward the falcon tube than to a conspecific that was visible, but not touchable in an adjacent arena, $p < .05$. This suggested that the falcon tube was of more interest to the rat than a conspecific and that the rat was indeed directing attention to the falcon tube.

Because of the potential for overlap between cognitive and affective modulation of pain, it was also critical for the authors to rule out the alternative hypotheses that stress or fear caused the analgesic response. To this end, the authors measured freezing behavior and ultrasonic vocalization (USV) during testing as well as plasma corticosterone, a blood-borne hormone associated with stress-induced activation of the HPA axis, immediately upon conclusion of the test. The study found that no freezing behavior or 22kHz USV's were detected from any rats in any of the experimental conditions. The study also found that none of the distractors used in the study produced significant alterations in plasma corticosterone. This information demonstrates that the effects of the falcon tube on formalin pain scores were not due to stress or fear (affect), and in light of the elevated attention directed to the objects, it can be reasonably concluded that the effect of the falcon tube on the rat was primarily attentional in nature.

1.9 Neural Substrates

One of the major aims in the study of cognitive factors, such as attention/distraction, and pain is to determine which brain regions and circuits mediate their interplay. The identification of those neural underpinnings may lead to the development of non-pharmaceutical treatment strategies that reduce the burden of chronic pain.

1.9.1 Neural Substrates of Attention & Distraction Analgesia in Humans

Corbetta and Shulman proposed a neural system that regulates the 'top-down orienting of attention' (Corbetta & Shulman, 2002). There are two major circuits in this system, the first of which is responsible for goal-directed recognition of stimuli and response selection, and it includes areas of the intraparietal cortex and superior frontal cortex. The second circuit, which includes the temporoparietal cortex and inferior frontal cortex, can modulate the first by orienting attention to behaviorally-relevant stimuli

especially when they are salient or unexpected. The second circuit is more relevant to the experimental study of DA and to the present research study.

The neural substrates of DA have been explored in humans using imaging techniques. In an fMRI study, noxious thermal pain that was consistently rated as 8 or higher on a visual analog scale was delivered to the dorsal surface of the left hand during a distracting cognitive task and during a neutral control task. Both tasks required the subject to identify, using a four-button response pad, the number of words that were presented on a screen; however, the neutral task included non-numerical words and the distracting task included numerical words (Figure 1-10). The distracting task was termed the "interference" task because the numerical words cognitively interfered with the process of counting the number of words present. Pain–related brain activation was significantly decreased in the interference task relative to the neutral task in the contralateral insula, bilaterally in the thalamus, and in the midcingulate (Bantick et al., 2002). All of these regions are consistently activated in experimental pain states relative to resting controls and are components of what has been referred to as the pain matrix (Wager, 2005). Mean pain intensity ratings were also significantly lower in the interference task than in the neutral task (p = .006, Student's t-test). This study demonstrates that DA is associated with significant changes in the activity of specific brain areas critical to the processing of pain.

Interference	Neutral
One	Cat
One	Cat
One	Cat
One	Cat

Figure 1-10 Example word lists from the distracting Interference Task and the Neutral Task used in Bantick et al., 2002.

Another fMRI study instructed subjects to either pay full attention to noxious thermal heat pain or to try to decrease the pain by not attending to it by, for example, thinking of something else (Tracey et al., 2002). The intentional misdirection of attention was associated with lower ratings of pain intensity and aversiveness as well as increased activity in an area of the brain known as the periaqueductal grey (PAG). This is significant because the PAG has a well established role in top-down pain inhibition mechanisms (Fields & Basbaum, 1978; Millan, 2002; Reynolds, 1969). The connection between DA and PAG activation suggests that distraction may reduce pain through classic descending pain modulatory mechanisms. It could be argued that the Tracey el al. (2002) study may be confounded by the fact that subjects were explicitly told to reduce pain by distraction; however, it could also be argued that the critical finding, PAG activation during DA, retains its importance despite the confound. The latter argument is more reasonable because the subjects could not have had conscious knowledge of how to selectively activate their PAG, and it is even less probable that the subjects would have known whether the PAG ought to have been activated during the "attending" or the "not attending" condition. Therefore, the potential confound should not impact the main findings of the study.

A similar fMRI study that was not confounded with the expectation of distraction-induced pain reduction used a cold pressor test (CPT) in tandem with a verbal attention task (VAT) to investigate activity of the perigenual anterior cingulate gyrus (ACG) (Frankenstein, Richter, McIntyre, & Rémy, 2001). Subjects were scanned during the CPT, then during the VAT, then with both CPT and VAT administered simultaneously, which was termed the distraction task (DT). Two ACG subregions of interest, BA24 and BA32, were analyzed based on a prior evidence of their involvement in the processing of pain and cognitive demands, respectively. Data analysis revealed that DA reduced

activity in the pain-processing ACG subregion BA24, but increased activity in the cognition-processing ACG subregion BA32, suggesting that DA differentially affects neural activity in the ACG according to whether the subregion processes primarily pain or cognition.

In order to determine whether the distraction-induced alterations of cingulate pain processing (Bantick et al., 2002; Frankenstein et al., 2001) are functionally related to increases in midbrain activity (Tracey et al., 2002), a similar fMRI study was conducted using experimental heat pain during a visually incongruent color-word Stroop task (Valet et al., 2004). Subjects were scanned during innocuous and noxious heat with and without distraction. Results replicated previous reports of significant reductions in the intensity and unpleasantness of pain during distraction as well as significant increases in activity of the perigenual ACC, orbitofrontal cortex (OFC), posterior thalamus, and PAG. A further analysis revealed that activity in the ACC covaried with activity in the posterior thalamus as well as the PAG only in the condition where noxious heat was presented simultaneously with distraction, but this pattern of covariation was not evident during noxious heat without distraction. This pattern of activity across conditions was interpreted as evidence that the ACC may act via the posterior thalamus and PAG to gate pain during distraction (Valet et al., 2004). Together, these studies correlate subjective reports of DA with a specific pattern of altered brain activity in regions known for their role in pain processing. This provides a basis for understanding DA at the neural level.

1.9.2 Neural Correlates of Impaired Pain Inhibition in Chronic Pain Patients

As discussed in section 1.7, chronic pain may impair cognitive pain-inhibition mechanisms. There is evidence that this impairment is mediated by altered functioning in the brain. An fMRI study showed that, in healthy control subjects, the insula and amygdala were deactivated during anticipation of uncomfortable rectal distention.

However, chronic sufferers of irritable bowel syndrome (IBS) showed less anticipation-induced deactivation of these areas, suggesting an impaired ability of cognitive factors, attention and expectation in this case, to deactivate pain-related neural processing (Berman et al., 2008). This provides direct evidence for a neural mechanism of the impaired inhibition of pain-processing in IBS chronic pain patients.

Additionally, a recent meta-analysis concluded that conditioned pain modulation, which aims to condition the endogenous inhibition of pain, is impaired across a wide variety of chronic pain conditions (Lewis, Rice, & McNair, 2012). This suggests that the neural mechanisms of the impaired inhibition of pain found in the IBS study by Berman et al. (2008) may generalize to other chronic pain conditions. It is important to study these impairments so that clinicians may prevent, delay, or correct them, which may preserve pain-inhibiting cognitive mechanisms thereby improving treatment outcomes for chronic pain patients (Bushnell, Ceko, & Low, 2013).

1.9.3 Role of the Human Orbitofrontal Cortex (OFC) in DA

The orbitofrontal cortex (OFC) is anatomically located within the secondary attention-modulating circuit in Corbetta and Shulman's model, and therefore, it is likely to play a role in attention to unexpected stimuli, which may modulate behavioral responses (Corbetta & Shulman, 2002). A review of the cognitive modulation of pain concluded that the OFC is involved in modulating limbic structures that interact with pain processing, including possibly the amygdala (Petrovic & Ingvar, 2002). Therefore, the human OFC may be a critical brain region in the attentional modulation of pain and the pain-reducing anticipatory inhibition of the amygdala observed by Berman et al. (2008).

Further investigation of the OFC revealed that it is active during the processing of competing attentional stimuli, as opposed to attention itself or pain stimuli (Bantick et al., 2002). For example, the OFC became more active in subjects that performed a

distracting maze task during a cold pressor test than in subjects undergoing the cold pressor test without the distracting task (Petrovic, Petersson, Ghatan, Stone-Elander, & Ingvar, 2000). The study also found evidence of DA in that the intensity and unpleasantness of pain during the cold pressor test were rated significantly lower during the attention-demanding task. These findings were interpreted as evidence that the OFC processes competing attentional stimuli (i.e. pain and maze task) and that it may be involved in the inhibition of pain perception via distraction (Petrovic et al., 2000).

The function of the OFC in humans suggests that it might be an area worthy of investigation in animal research. However, evidence suggests that the attentional functions of the human OFC are performed by other areas in the rat brain.

1.9.4 Neural Substrates of Attentional Set-Shifting in Rats

The neural substrates of two different forms of attentional shifts have been investigated in rats (Ng, Noblejas, Rodefer, Smith, & Poremba, 2007). Attentional shifts can be intradimensional, within the same stimulus modality (i.e. from one scent to a different scent), or extradimensional, between different stimulus modalities (i.e. from a scent to a tactile cue). These can be tested in rats using a rodent version of the Wisconsin card sorting task. In this operant paradigm, rats were presented with two small flower pots filled with a digging medium (i.e. shredded manila folders, aquarium gravel, etc.) and scented around the rim with essential oil (i.e. jasmine, vanilla, etc.). In the first phase, rats were trained to dig for a food reward hidden within a pot associated with a particular dimension (i.e. scent), while another dimension (i.e. medium) was irrelevant to the reward. The criterion for advancement to the next phase was 6 consecutive successful retrievals of the reward. In the next phase, the the scent cue was changed (i.e. from jasmine to vanilla), but the reward continued to be paired with scent. This requires an intradimensional shift (IDS) because the rat must still attend to scent, but

to a different kind of scent. In a subsequent phase, extradimensional attentional shifts (EDS) were tested by pairing the reward with a medium rather than a scent. The primary outcome measure during each phase was the number of trials required to reach criterion. The study tested IDS and EDS in the presence of various electrolytic lesions and found that lesions to the anterior and posterior cingulate cortices (ACC and PCC, respectively) significantly delayed criterion acquisition in the IDS phase, while lesions of the medial prefrontal cortex (mPFC) significantly delayed criterion acquisition in the EDS phase. In other words, rats with a mPFC lesion were less successful at shifting their attentional toward a stimulus that had been irrelevant during training. This is known as perseveration because the rats persevere in accordance with previously learned information while failing to integrate new information. Perseveration is a hallmark of medial prefrontal cortex damage in rats (Birrell & Brown, 2000), monkeys (Dias, Robbins, & Roberts, 1996), and humans (Pantelis et al., 2004). The perseverative effects of mPFC damage will be a critical point later in the discussion of the present research study.

In attentional set-shifting studies like the one discussed above, the EDS is more closely analogous to the DA tasks used on humans in that the new, distracting information most often occurs in a different stimulus modality (Bantick et al., 2002; Longe et al., 2001). Since the mPFC of the rat brain appears to be important for successful EDS, the mPFC of the rat may perform functions similar to those of the human OFC. Experiments are still needed to directly test this hypothesis.

1.9.5 Medial Prefrontal Cortex (mPFC) in the Rat Brain

The mPFC comprises at least three functionally discrete regions: anterior cingulate, prelimbic area (PL), and infralimbic cortex (IL). Early research of the mPFC often explored the region as a whole, failing to tease apart the independent functions of

the subregions, which led some researchers to call for a functional analysis of these areas (Heidbreder & Groenewegen, 2003).

Most animal research of the mPFC has explored the area's role in learning, decision-making, working memory, and inhibitory response control with comparatively little focus on its role in attention and especially pain (Dalley, Cardinal, & Robbins, 2004). Nevertheless, some early research revealed critical information about the functions of the mPFC. In a classic study (Santos-Anderson & Routtenberg, 1976), electrodes were implanted into the mPFC area of rats, which were then placed into a test chamber. The test chamber had an electrified mesh floor and an elevated escape area with a non-electrified floor. When rats were placed into the chamber, they were placed in the escape area. The weight of the animal on the escape platform triggered the delivery of current through the implanted electrode into the mPFC. Thus, the animal could choose to stay on the escape platform and receive mPFC stimulation or descend to the mesh to receive noxious electrical stimulation of the paws. The frequency of descents to the electrified floor were recorded over time. Multiple trials in the test chamber allowed the rats to learn to avoid noxious electrical shock of their paws by decreasing the frequency of descent. Twenty-four hours following the acquisition of this avoidance behavior, rats were again placed in the chamber on the escape platform with no electrical stimulation of the brain and no electrification of the floor. Animals that had received mPFC stimulation during training descended to the floor significantly more often than animals that did not receive stimulation (Santos-Anderson & Routtenberg, 1976). This suggested a role for the mPFC in learning/memory and specifically the integration of past information with present circumstances.

In another classic study from 1962, Olds reported that stimulation of the medial forebrain bundle (MFB) produced marked self-stimulation in rats (Olds, 1962). A decade

later, Routtenberg found that self-stimulation was also possible with electrodes implanted into areas of the mPFC (Routtenberg & Sloan, 1972). Routtenberg alleged that the MFB self-stimulation phenomenon may be due in part to the stimulation of axons which have cell bodies located in the mPFC. In other words, neurons with cell bodies in the mPFC may be the origin of some of the self-stimulation phenomenon seen in other brain regions that receive mPFC afferentation.

More recently, some researchers have presented evidence that lesions to the mPFC disrupt the development of conditioned place preference (CPP) (Tzschentke, 2000). Since CPP is commonly used in experiments seeking to assess reward and/or addiction, this led to the conclusion that the mPFC is involved in reward. While this conclusion is justifiable, the evidence from self-stimulation and learning/memory studies suggest strongly that the functions of the mPFC extend well beyond reward.

With respect to pain specifically, some evidence suggests that the mPFC does not have a significant direct influence on all types of pain. Lesions to the medial frontal cortex selectively attenuated hot plate responses, but had no influence on the formalin test nor on the tail-flick test (Pastoriza, Morrow, & Casey, 1996). Although the lesions in this study were placed quite dorsally and were very large, allowing for the possibility that a more localized lesion may produce different results, it seems clear that the mPFC does not necessarily have a direct influence on all types of pain.

Another early study showed that a lesion to the entire mPFC significantly increased the number of trials required to successfully extinguish a conditioned freezing response to electric footshock (Morgan, Romanski, & LeDoux, 1993). Although freezing behavior indicates fear and is therefore more affective than cognitive in nature, this study is relevant because it substantiates the evidence that mPFC lesions induce perseverative behavior much like the findings of the Ng et al. (2007) attentional set-shifting study.

Additionally, mPFC function and morphology are altered by the presence of pain in ways that decrease functional efficiency. Electrophysiological evidence suggests that inflammatory pain inhibits activity in the prelimbic (PL) area mPFC of rats (Ji & Neugebauer, 2011). In this study, the activity of single neurons in the PL was significantly lower after the injection of 2% carrageenan, an inflammatory agent, through the patellar ligament into the knee joint cavity, which has been shown previously to induce localized inflammation lasting for weeks that is significantly associated with pain behavior (Neugebauer, Han, Adwanikar, Fu, & Ji, 2007). Another study found that an intraplantar injection of carrageenan was associated with decreased spontaneous as well as mechanically and electrically evoked activity in the prelimbic (PL) and infralimbic (IL) cortices of the rat, with peak inhibition occurring 60-min post-injection (Luongo et al., 2013). This effect appeared to be due to an increased expression of group 1 metabotropic glutamate receptors (mGlurR1) in the PL as well as elevated levels of GABA in the PL and IL. Dendritic morphology of the mPFC is also altered by pain. A significant increase in dendritic spining was observed in the basal dendrites of PL neurons 6-8 days following the induction of pain by spared nerve injury (Metz, Yau, Centeno, Apkarian, & Martina, 2009). These studies demonstrate that inflammatory and neuropathic pain in rats produces functional and morphologial changes in the mPFC region of the rat brain, which is associated with cognitive functions such as attentional focus. These changes imply corresponding functional alterations, which may explain attentional impairments in chronic pain patients.

Pain-induced functional and morphological changes in the rat mPFC are consistent with behavioral studies showing cognitive deficits in rats with experimental pain conditions (Boyette-Davis et al., 2008; Low et al., 2012). It is therefore reasonable

to suspect that pain-induced changes in the rat mPFC may account for some of the cognitive deficits observed in animals in pain.

It seems evident that the mPFC is an important brain region in self-stimulation, learning/memory, pain, and behavioral adaptability. This breadth of function associated with the mPFC may be due to a lack of precision in the experimental manipulations that have been used to study the area. It is possible that more localized manipulation of each mPFC subregion may reveal that each of the above functions is associated with a discrete subregion or subregions. Therefore, it seems that future studies should seek to manipulate discrete mPFC subregions in order to develop a clearer understanding of this region's contributions to so many disparate functions.

Of primary importance to the present study is that the contributions of the mPFC subregions to DA remain unclear. The only available evidence comes from the original publication of the rat model of DA (Ford et al., 2008), which used high-performance liquid chromatography (HPLC) to quantify levels of dopamine, serotonin, the dopamine metabolite 3,4-dihydroxyphenylacetic acid (DOPAC), and the serotonin metabolite 5-hydroxyindoleaceticacid (5-HIAA) in the ACC immediately following the formalin test. Relative to the control group, rats tested in the presence of the falcon tube showed a significant reduction of DOPAC and 5-HIAA in the ACC. No other distraction-induced alterations in monoamine or metabolite concentrations were detected in the ACC. Although this demonstrates that distractor presence altered monoamine metabolism in the ACC, clearly implicating a role for the ACC in DA, the relation of these findings to nociception is unclear and requires further investigation. To the author's knowledge, no other studies have specifically investigated the role of the mPFC subregions in DA.

1.9.6 Infralimbic Cortex (IL)

Of the mPFC subregions, the infralimbic cortex may be the most likely to process the distraction component of DA. This is based largely on the pattern of connectivity between the rat IL and other brain regions, which is similar to the human OFC, suggesting that the rat IL and human OFC may be functionally homologous (Hoover & Vertes, 2007; Vertes, 2004). This was determined by iontophoretically delivering the anterograde tracer *Phaseolus vulgaris*-leucoagglutinin to either the IL or the PL and harvesting brains after a survival time of 7-10 days. Tissue was sliced and processed to reveal the destination of efferent projections from the site of tracer delivery. Figure 1-11 shows the efferent projection sites of the IL. Generally, the efferent projection sites of the IL and PL were very different, suggesting that the functional significance of the two areas may be quite different. The author concluded that the projection pattern of the rat IL was similar to that of the primate OFC and therefore, that the functions should be similar (Vertes, 2004). One notable projection site of the IL was the medial and ventromedial PAG in the brainstem (Figure 1-12), which was discussed earlier for its potential role as a mediator of DA in humans (Tracey et al., 2002). The projection of the IL to the PAG suggests that the IL may be capable of influencing descending pain modulatory circuits during DA.

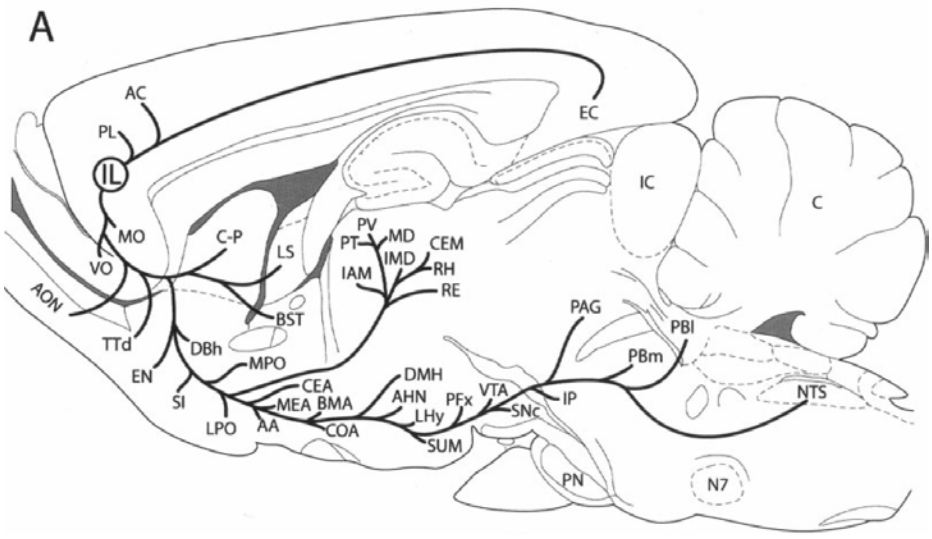

Figure 1-11 Efferent projections sites of the IL cortex in the rat brain. (Reproduced with permission from Vertes, 2004).

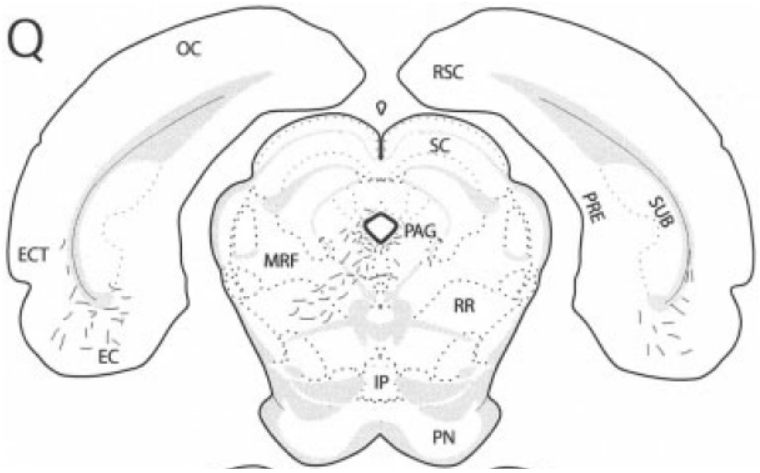

Figure 1-12 Coronal cross-section of the rat brain depicting efferent projections from the IL to the PAG. (Reproduced with permission from Vertes, 2004).

A complementary study used Fluorogold, a retrograde fluorescent tracer, to map the afferent projections that are received by the IL and PL (Hoover & Vertes, 2007). Figure 1-13 shows sources of afferent information to the IL. Notable afferent sources are the dorsal anterior cingulate (AC), hippocampal CA1 and subiculum (SUB), and

basolateral (BLA) and basomedial (BMA) amygdalar nuclei. It was revealed that the hippocampus, specifically the CA1 and subiculum, project "massively" to the IL. Figure 1-14 depicts the afferent sources from the CA1 and subiculum to the IL. The authors interpreted this as potentially indicating a role for the IL in the integration of past and present events to inform future actions. This function is likely to be engaged in the rat model of DA because of its reliance on past information from the habituation phase to inform present responses to the distractor on the test day.

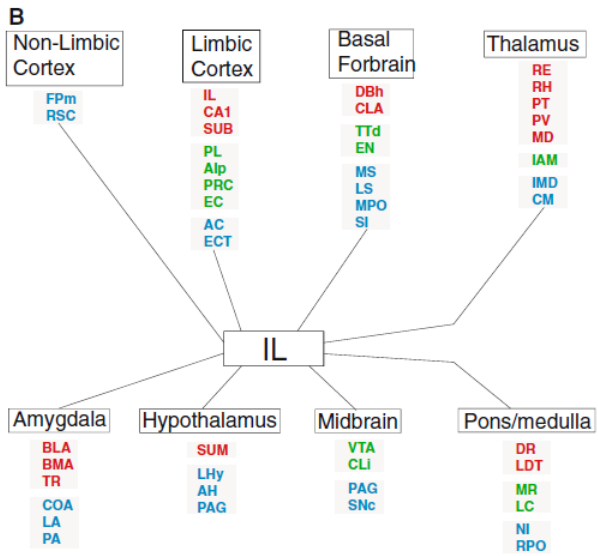

Figure 1-13 Brain regions providing afferent information to the rat IL. Blue = light labeling; Green = moderate labeling; Red = heavy labeling. (Reproduced with permission from Hoover & Vertes, 2007).

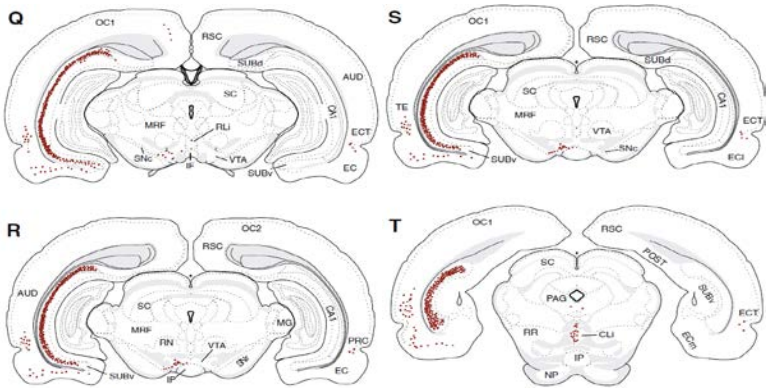

Figure 1-14 Coronal cross-sections of the rat brain depicting sources of afferent projections from the CA1 and subiculum to the IL. (Reproduced with permission from Hoover & Vertes, 2007).

In order to investigate the role of the IL on the integration of past and present information, bilateral excitotoxic quinolinic acid lesions were delivered to the IL of a rat and were then tested in a Pavlovian conditioning experiment (Chudasama, Nathwani, & Robbins, 2005). Rats were trained and tested in a visual discrimination task in which conditioned stimuli presented via an illuminated touch screen indicated whether a lever press would be followed by appetitive reward. The conditioned stimuli (CS) were a white rectangle and a white cross, and each was designated as either the CS^+, indicating that a reward would be delivered following a lever press, or the CS^-, indicating no reward would be delivered. The assignment of shape to CS^+ or CS^- was counterbalanced across animals. Results demonstrated that lesions of the IL did not impair the acquisition of the visual discrimination task, which suggests that IL lesions do not induce perseveration. This is a critical point for any study that induces an IL lesion and then uses a behavioral paradigm reliant on learning or habituation, including the Ford et al. (2008) model of DA, because it shows that IL-lesioned animals can still learn. However, the IL lesion was associated with a non-significant elevation of the time to acquire the reversal task in

which the CS^+ becomes the CS^- and vice versa. The results of Chudasama, et al. (2005) suggest that an IL lesion can be used successfully in a learning/habituation paradigm and that any observed behavioral alterations should not be due to perseveration per se, but rather to an impairment of the ability to incorporate new information into present behavior.

Connectivity of the IL also suggests a role in the regulation of autonomic visceromotor functions (Hoover & Vertes, 2007; Vertes, 2004). Implications of this are discussed in sections 4.2 and 4.12.2 of the Discussion.

Based on the information regarding the mPFC in pain as well as the anatomical connectivity and behavioral significance of the IL, it seems that the IL is the area of the rat brain most likely to be functionally similar to the human OFC and therefore a mediator of the attentional conflict between pain and distractor in experimental models of DA. However, the role of the rat IL in DA has never been directly explored.

1.10 Rationale

It is clear from the information above that researching the neural underpinnings of the interplay between attention/distraction and pain has revealed valuable information that may ultimately be used to decrease clinical pain. It is also evident that nearly all of the research aiming to reveal the brain areas responsible for DA used healthy human subjects in MRI studies (Bantick et al., 2002; Frankenstein et al., 2001; Tracey et al., 2002; Valet et al., 2004). A much deeper understanding could be gained through the increased use of animal research techniques, which include brain lesions, microdialysis, electrophysiological stimulation and recording, immunohistochemistry, etc. These methods would generate a more nuanced understanding of the mechanisms of DA that cannot be gleaned from human studies. However, very little is known about the neural substrates of DA in the rat, and to the author's knowledge, there is only one study that has explored the topic (Ford et al., 2008).

There are a few hints in the literature that higher levels of pain intensity may be more susceptible to the analgesic effect of distraction (Bushnell et al., 1985; Eccleston, 1995; Wiech et al., 2005). To the author's knowledge, this has never been experimentally tested using the same type of pain at varying levels of intensity.

The evidence available in the animal literature suggests that the rat mPFC plays an important role in pain and certain cognitive abilities including attentional focus. Based on connectivity analyses (Hoover & Vertes, 2007; Vertes, 2004), the IL subregion of the mPFC is the area most likely to be functionally homologous to the human OFC, which appears to be critical for processing competing attentional stimuli (Bantick et al., 2002; Vertes, 2004). Furthermore, lesions of the IL do not eliminate the ability to learn or habituate (Chudasama & Robbins, 2003), and therefore may be used to assess behavior in paradigms like the Ford, et al. (2008) model of DA, which require learning through habituation. Despite the evidence regarding anatomical connectivity, functionality, and appropriateness for behavioral research, the subregions of the mPFC, including the IL, have never been explored individually for their role in DA.

Therefore, the present study sought to elucidate the role of the IL in an experimental rat model of DA at high and low levels of pain intensity. This research will reveal valuable information regarding the neural substrates of DA, and that information may be used in the future to inform the development of techniques that preserve cognitive pain-reducing abilities.

1.11 Specific Aims

(1) To demonstrate the analgesic effect of a novel object distractor in the formalin test.

Hypothesis: That rats tested with a novel object in the chamber will display lower formalin pain scores and less licking behavior in the tonic phase than rats tested in an empty chamber.

a. Replicate and further validate the rat model of distraction analgesia.

(2) To measure the magnitude of the distraction analgesia effect at high and low levels of pain intensity.

Hypothesis: That the magnitude of distraction analgesia will be larger at high levels of pain intensity.

a. Determine whether distraction manipulations are more effective for low or high intensity pain.

(3) To elucidate the role of the rat infralimbic cortex (IL) on distraction analgesia.

Hypothesis: That an electrolytic lesion of the IL will attenuate the analgesic effect of the novel object.

a. Determine whether the IL plays a more significant role in distraction analgesia at low or high intensity pain.

Chapter 2

Methods

2.1 Subjects

One hundred fifty two Sprague Dawley rats from the University of Texas at Arlington vivarium between 7-9 months old were used for the study. Animals were housed in groups of 1-3 and maintained on a 12:12 hour light/dark cycle with free access to food and water throughout the study. Prior to investigation, all procedures were approved by the University of Texas at Arlington Institutional Animal Care and Use Committee and were in accordance with the guidelines put forth by the International Association for the Study of Pain (Zimmermann, 1983).

2.2 Surgical Procedures

All animals in the study received a stereotaxic surgical procedure. Each was randomly assigned to receive either a bilateral electrolytic lesion of the infralimbic cortex (IL) or a sham surgical procedure that was identical except for the delivery of electrical current. Animals were anesthetized by intraperitoneal injection of a ketamine (100 mg/mL) and xylazine (100 mg/mL) solution (8.25 mL ketamine + 1.75 mL xylazine) at a volume of .8 ml/kg. The depth of anesthesia was monitored by checking for reflexive behaviors (i.e. eye blink reflex and paw withdrawal reflex) and by visually monitoring the rate and depth of respiration. Following confirmation of the absence of reflexes, the surgical area was shaved with electric clippers to remove surrounding fur, and animals were then secured in a stereotaxic frame with blunt-tipped ear bars. The surgical area was thoroughly cleaned with an antibacterial solution of 10% povidone-iodine solution (Betadine Microbicide).

A midline incision was made in the scalp, and the underlying periosteum tissue was scraped away from the surface of the skull. The location of bregma was determined

in stereotaxic coordinates, and from those coordinates, the location of the burr holes were derived arithmetically (Anterior/Posterior = +3.0; Medial/Lateral = ± .7, Dorsal/Ventral = -5.5) in order to deliver the tip of the electrode to the infralimbic cortex in each hemisphere. For animals in the sham condition, the electrode was inserted into the brain, but no electrical current was passed. For animals in the lesion condition, 1.0 mA was passed for 12 seconds through the electrode.

Following surgery, the wound was cleaned thoroughly with 10% povidone-iodine, closed with surgical staples, and cleaned again with 10% povidone-iodine. Animals were allowed 7 days to recover before further experimentation. During this time, post-surgical signs of infection or overt signs of discomfort were closely monitored.

2.3 Habituation

Following the recovery period, animals began 7 days of daily habituation trials. Prior evidence has suggested that novelty of the test chamber can influence formalin pain scores (Ford et al., 2008). Therefore, the purpose of the habituation procedure was to prevent test chamber novelty from confounding the results. Each habituation trial was designed to precisely mimic every aspect of the upcoming formalin test except for the formalin injection. Rats were first transported to the testing room, removed from their cage, and wrapped in a terry cloth towel. The animal's left hind paw was gently exposed and inverted such that the plantar surface was facing upward. The plantar surface was touched gently by the experimenter for 1-2 seconds, after which the animal was unwrapped and placed into the formalin testing chamber. The formalin testing chambers were constructed of opaque grey Plexiglas (30×30×30 cm). Chambers sat atop an elevated Plexiglas platform that was transparent in order to allow for behavioral observation. Mirrors were angled below the platform to facilitate observation of the hind paws without disturbing the animal.

Each rat was always habituated to and tested in the same chamber in order to eliminate the potential confound of minor structural differences between chambers. If rats were housed two-per-cage, then both rats would be habituated simultaneously in adjacent chambers, and correspondingly, they would also undergo formalin testing simultaneously in adjacent chambers. If a rat was housed individually or was the third rat in a cage of 3, then it was habituated alone, and correspondingly, would undergo a formalin test alone. Chambers were cleaned thoroughly with an antimicrobial soap and water solution before and after each habituation period.

Noldus Ethovision® XT v.7 behavioral tracking software was used to record the locomotion patterns of each rat during each habituation trial and during the Test Day trial. The primary output variables were total distance traveled, time spent in the center zone of the chamber, time spent in the peripheral zone of the chamber, total time spent moving, and heading-to-center. The purpose of recording this information during habituation was primarily to determine whether the lesion induced behavioral effects apart from what could be detected by formalin pain scores. Distance traveled and time spent moving measured total movement. By default, rats prefer to walk adjacent to walls, a behavior called thigmotaxis, and locomotion in the center of a chamber has been used previously as a behavioral indicator of anxiolysis (Prut & Belzung, 2003). Therefore, time spent in the center zone was measured as a behavioral indicator of anxiolysis. The center zone was defined by a circle centered in the middle of the testing arena with a radius half the distance to each chamber wall. Figure 4-2 shows the arena and center zones overlaid on a top-down camera-view schematic image of two testing chambers. Since exploratory locomotion within the chamber, including the center, is expected to decrease over time due to the habituation process, time spent in the periphery may be a better indicator of anxiolysis on later habituation trials. Therefore, time spent in the

periphery was measured in order to provide another indication of exploratory locomotion and habituation to the chamber. Heading-to-center is the average direction that the animal traveled with respect to the center of the chamber and is measured in degrees as per Figure 2-1. Thus, values closer to 0 indicate a heading directed more toward the center and values closer to 180 indicate a heading away from the center. On Test Day, time spent in center zone and heading-to-center were intended to serve as indicators of attention paid to the distractor.

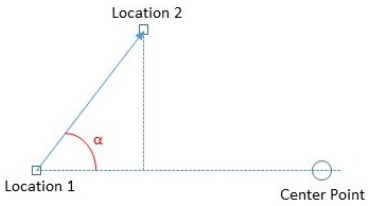

Figure 2-1 Calculation of Heading-to-Center angle α in Noldus Ethovision® software when animal moves from Location 1 to Location 2.

2.4 Formalin Testing

Following 7 days of habituation trials, animals underwent formalin testing. Each animal was transported to the testing room, restrained in the terry cloth towel, and had their left hind paw exposed and inverted exactly as had occurred during each habituation trial. An additional experimenter helped to restrain animals during the injection in order to reduce struggling and maximize the consistency and accuracy of the injections. Each rat was then administered a subcutaneous injection of either .5% or 1% formalin into the plantar surface of the left hind paw. Rats were immediately placed in the same formalin testing chamber to which they had been habituated. Each chamber was either empty, as it had been during habituation, or contained a distractor object. Distractor objects were falcon tubes with orange tops that were inverted and affixed to the center of the formalin

test chamber with Glue Dots® brand removable temporary adhesives. Prior to the start of habituation, all animals were randomly assigned to the formalin concentration condition (.5% or 1%) and to the distractor condition (empty chamber or distractor). The experiment therefore used a 2(IL Lesion/Sham) x 2(.5% Formalin/1% Formalin) x 2(Empty/Distractor) design.

Formalin pain behavior was constantly observed and scored across a 60-minute time period. The number of seconds the animal spent in each of these three behavioral states was quantified: resting the paw on the floor surface (down), elevating the footpad and toes above the floor surface (up), and licking the paw (lick). These behaviors were recorded and quantified utilizing proprietary toggle-key software. The "down" criteria was defined by the animals resting the formalin-injected hind paw on the floor of the chamber with weight applied normally. The "up" criteria was selected during periods in which the animal held the footpad and toes of the left paw above the floor in a guarding position and/or when its weight was placed fully on the contralateral hind paw. "Lick" was selected when the animal was licking, biting, shaking, and/or chewing at the claws of the left hind paw. These three levels were weighted in the following way: Time spent in "down" was multiplied by zero, time spent in "up" was multiplied by 1, and time spent in "licking" was multiplied by 2. These values were then summed and divided by 300 seconds (5-minutes) to produce a weighted composite formalin pain score (CFPS) for each 5-min time bin. The formula used was the following: (Down*0)+(Up*1)+(Lick*2)/300 = CFPS 5-min time bin. This three-level behavioral assessment of the formalin test has been validated and used extensively in previous research (Coderre, Fundytus, McKenna, Dalal, & Melzack, 1993; Donahue, LaGraize, & Fuchs, 2001; P. N. Fuchs, Roza, Sora, Uhl, & Raja, 1999; LaBuda, Donahue, & Fuchs, 2001). Specifically, as the formalin concentration/stimulus intensity increases, paw elevation and licking also increase. At

high levels of stimulus intensity (i.e. 5% formalin solution), more time is spent licking than elevating the paw, which indicates that licking is more closely associated with higher levels of stimulus intensity (Coderre et al., 1993).

2.5 Histology

Following formalin testing, animals were euthanized by overexposure to CO_2 in accordance with AVMA guidelines (Leary et al., 2013), after which their brains were extracted and stored in formaldehyde for at least 48 hours. Once formaldehyde was sufficiently infused into the tissue, brains were then transferred to a 30% sucrose solution where they were stored for at least 48 hours. Using a cryostat, the IL cortex of each brain was sectioned into 80 micron slices, placed on microscope slides, and allowed to air dry for no more than 24 hours.

All slides were then stained with thionine and examined under a microscope to determine the location and extent of lesion damage. The examiner was blind to the experimental conditions of each brain as well as the results of the behavioral testing.

Chapter 3

Results

3.1 Data Screening

3.1.1 Surgical Procedure and Recovery Period

Of the 152 animals in the study, six animals experienced an adverse reaction to the anesthetic drugs and did not survive surgery. One animal was euthanized during the post-surgical recovery period for inflammation under the incision site. Four animals were euthanized for exhibiting dysfunctional motor control upon waking up from the anesthesia.

3.1.2 Formalin Injection

Two animals were excluded from the analysis of formalin pain scores due to a failed injection of formalin. A formalin injection was considered to have failed if it was delivered into the wrong area of the footpad or if the paw was leeching formalin during the test. One of the excluded rats kicked during plunger depression, causing formalin to be injected into the lateral edge of the footpad. The other excluded rat also resisted restraint, kicked the needle multiple times, and, consequently, leeched a considerable amount of blood and formalin from the footpad onto the surface of the testing chamber during the test. To ensure conservatism, all other rats that experienced anomalies during the injection process due to struggling, including multiple needle insertions, were left in the final analysis provided that the formalin was delivered to the center area of the footpad and the formalin remained in the paw during testing.

3.1.3 Histological Analysis

Overall, 139 animals underwent stereotaxic surgery and successfully completed the protocol. Twenty eight of these animals were not included in the final analysis due to lesion size (less than 75% bilateral damage to the IL) and/or location. Therefore, the final

analysis of formalin pain scores included 111 animals. Histological analysis of lesion location (Figure 3-1) indicated that the average anterior-posterior extent of damage was 1.33 (±0.03) mm. The maximal extent of the damage for the largest number of animals was located at 3.20 mm relative to bregma. All lesions involved at least 75% bilateral damage to the area of the IL. At the maximum extent of the lesion, the structures that were damaged included the IL and the dorsal peduncular cortex (DP). In almost all lesions, there was some damage to the ventral/medial portion of the forceps minor corpus callosum (fmi). More anterior, there was damage to the medial ventral region of the ventral orbital cortex (VO) in 44 of the animals (bregma 4.20 mm) and slightly more posterior, all lesions included the medial orbital cortex (MO) and most ventral region of the prelimbic cortex (PrL). At the middle extent of the lesion, 9 of the animals had some damage to the PrL region which usually involved the most ventral portion of the PrL. A smaller number of animals had lesions that extended into the medial region of the ventral orbital cortex (VO) (n=6) and dorsally encroaching near the region of the anterior commissure (n=3). Posteriorly, a couple lesions (n=2) involved the most anterior region of the septum (bregma 1.7 mm). A one-way ANOVA of lesion size and location revealed no systematic difference among the experimental conditions $F(3, 54) = .5$, $p = .69$, ns.

3.1.4 Behavioral Tracking Analysis

Four additional animals were excluded from only the locomotion analyses because their data was lost due to a software malfunction on Test Day. Therefore, the final analyses of locomotion included 107 animals. The malfunction was caused by the computer entering hibernation mode during the 60-min Test Day trial. This problem was prevented on subsequent trials by playing a full-screen video during behavioral tracking.

3.2 Behavioral Tracking Data During Habituation

The variables distance traveled, time spent moving, time spent in the center zone, time spent in the periphery, and heading-to-center on Days 1-7 were each analyzed with a repeated measures ANOVA in which lesion (IL or Sham), concentration (.5% or 1%), and distractor presence (Distractor or Empty) served as the between-subjects independent variables. The multiple days allowed for within-subjects comparisons over time. Test Day data was not included in these analyses because the primary purpose was to test for differences in locomotion patterns following the surgical procedure, but prior to the administration of formalin and distractors. The assumption of sphericity was violated for all repeated measures ANOVA's conducted in this study, and therefore, the multivariate analysis with a Wilks' Lambda correction is reported for all within-subjects main effects and interactions.

The analysis of distance traveled (Figure 3-2) revealed a main effect for time, $F(6, 94) = 2.93$, $p < .05$, which was due to a general trend of decreasing distance traveled over time. However, there was not a significant interaction between time*lesion, $p = .33$, ns, indicating that the trend did not differ between sham and IL animals during the habituation phase.

The analysis of time spent moving (Figure 3-3) showed a main effect for time, $F(6, 94) = 14.69$, $p < .001$, but no time*lesion interaction, $p = .79$, ns. This analysis also showed a general trend of decreasing movement over time during the habituation phase that did not differ between sham and IL animals.

The analysis of time spent in the center zone (Figure 3-4) also showed a main effect of time, $F(6, 94) = 9.51$, $p < .001$, but no time*lesion interaction, $p = .33$, ns. There was a trend in both groups to spend a large amount of time in the center zone on the first day and to spend a much lower amount of time in the center on subsequent days.

Time spent in the periphery (Figure 3-5) also showed a main effect of time, $F(6, 94) = 9.49$, $p < .001$, but no time*lesion interaction, $p = .36$, ns. Corresponding to the time in center analysis, there was a trend in both groups to spend a small amount of time in the periphery on the first day and to spend a greater amount of time in the periphery on subsequent days.

The heading-to-center variable quantified the orientation of the animal with respect to the center point of the chamber (Figure 3-6). This analysis did not reveal a main effect of time, $p = .21$, ns, nor a time*lesion interaction, $p = .94$, ns.

3.3 Behavioral Tracking Data on Test Day

The tracking data on Test Day was analyzed separately from habituation days because the Test Day session was 6 times longer, influenced by formalin and distractor presence, and therefore, not comparable to habituation trials. The primary purpose of these analyses was to quantify attention to the distractor object with the variables time in center and heading-to-center, but also to determine the effect of lesion, concentration, and distractor presence on general locomotion. The variables total distance traveled, time spent moving, time spent in center zone, and heading-to-center on Test Day were each quantified in 5-minute time bins across the 60-minute formalin test. Each variable was analyzed with a repeated measures ANOVA in which lesion, concentration, and distractor were the independent between-subjects variables, and the multiple time bins allowed for within-subjects comparisons.

In the analysis of total distance traveled, there was a significant main effect of time, $F(11, 89) = 6.23$, $p < .001$, which was due to a large amount of movement during the first 5-minutes followed by comparatively less movement for the remainder of the test. No main effects were detected for any between-subjects variables and there were no significant interactions for time*lesion, time*concentration, or time*distractor. These

findings suggest that none of the independent variables meaningfully influenced distance traveled on Test Day. There was, however, a significant time*concentration*distractor interaction, $F(11, 89) = 2.24$, $p < .05$ (Figure 3-7). Posthoc analysis revealed a significant difference between .5%/Distractor and .5%/Empty at the 35-minute time bin, $p < .05$. There were also significant differences between 1%/Distractor and 1%/Empty at the 45 and 60-minute time bins, $p < .05$.

In order to assess differences between all experimental conditions in distance traveled on Test Day, another repeated measures ANOVA was run with a composite independent variable, henceforth referred to as "condition," that comprised lesion type, formalin concentration, and distractor presence. Therefore, "condition" had 8 levels: Sham/.5/Distractor, Sham/.5/Empty, Sham/1/Distractor, Sham/1/Empty, IL/.5/Distractor, IL/.5/Empty, IL/1/Distractor, and IL/1/Empty. This analysis did not reveal a main effect of condition, $p = .29$, ns. Consequently, no further investigation of distance traveled was conducted.

Analysis of time spent moving also revealed a significant main effect of time, $F(11, 89) = 42.49$, $p < .001$, which was also due to a large amount of movement during the first 5-minutes and comparatively little movement afterward. No main effects were detected for any of the between-subjects independent variables. However, there was a marginally significant time*concentration interaction, $F(11, 89) = 1.89$, $p = .052$, ms (Figure 3-8). The high formalin concentration spent significantly more time moving than the low concentration at the 30, 35, and 40 minute time bins. This indicates that the high concentration of formalin increased locomotion at the beginning of the tonic phase of the test. There was also a significant concentration*distractor interaction, $F(1, 99) = 5.26$, $p < .05$ (Figure 3-9). Posthoc pairwise comparisons showed that the distractor significantly increased movement in the 1% concentration, $p < .05$, but not in the .5% concentration.

Another repeated measures ANOVA was run with condition as the independent variable and time spent moving on Test Day as the dependent variable. No main effect of condition was detected, $p = .16$, ns, and therefore, no further investigation of time spent moving was conducted.

In the analysis of time spent in the center zone, there was a significant main effect of time, $F(11, 89) = 3.26$, $p < .001$ with a tendency to spend more time in the center during the first 5-minutes and less during subsequent time bins. No main effects were detected for any between-subjects independent variables. However, there was a significant time*distractor interaction, $F(11, 89) = 2.70$, $p < .01$ (Figure 3-10). Posthoc analysis of this interaction revealed that the empty condition spent more time in the center zone during the first 5 minutes, $p < .001$, but that the distractor condition spent more time in the center zone during the 35-minute time bin, $p < .05$. Another repeated measures ANOVA was run with condition as the independent variable and time spent in the center zone as the dependent variable. No main effect of condition was detected, $p = .55$, ns, and therefore, no further investigation of time spent in the center zone was conducted.

The analysis of heading-to-center found no significant main effects or interactions. An additional repeated measures ANOVA with condition as the independent variable also did not show a main effect of condition, $p = 71$, ns. Overall, the results of the locomotion analyses on Test Day indicate that the independent variables lesion, concentration, and distractor had only a minor impact on distance traveled and time spent moving. However, these results also demonstrated that the presence of the distractor did not alter heading-to-center and only slightly increased time in the center zone. These findings are addressed in the Discussion section.

3.4 Composite Formalin Pain Scores (CFPS) Across Entire Formalin Test

CFPS was analyzed using a repeated measures ANOVA with lesion type (IL or Sham), formalin concentration (.5% or 1%), and distractor presence (Distractor or Empty) as the between-subjects independent variables. The dependent variables were the composite formalin pain scores for each 5-minute time bin across the 60-minute test. The multiple time bins allowed for within-subjects comparisons. Figure 3-11 shows CFPS in sham groups, and Figure 3-12 shows CFPS in IL groups.

Main effects were detected for lesion type, $F(1, 103) = 5.27$, $p < .05$, formalin concentration, $F(1, 103) = 43.71$, $p < .001$, and time, $F(11, 93) = 30.29$, $p < .001$. Significant interactions were detected between time*concentration, $F(11, 93) = 5.18$, $p < .001$, time*distractor, $F(11, 93) = 2.55$, $p < .01$, and time*lesion*distractor, $F(11, 93) = 2.24$, $p < .05$. Although there was no main effect for the presence of a distractor ($p = .3$, ns), there was a significant interaction between lesion*distractor, $F(1, 103) = 4.39$, $p < .05$.

The time*concentration, time*distractor, and lesion*distractor interactions were further probed with posthoc analyses. Pairwise comparisons revealed that the two formalin concentrations displayed significantly different CFPS in all time bins of the acute and tonic phases of the formalin test, $p < .001$. Interphase bins at 15-min, $p = .3$, ns, and 20-min, $p = .29$, ns, were not significant, but the 25-min bin showed a marginally significant difference between formalin concentrations, $p = .053$, ns (Figure 3-13).

The time*distractor posthoc analysis showed that the distractor presence significantly reduced CFPS at 5, 15, and 40-min time bins, $p < .05$ (Figure 3-14).

Posthoc analysis of the lesion*distractor interaction revealed critical information. There was a significant difference between the empty and distractor conditions in sham animals, $p < .05$, but not in the IL animals, $p = .45$, ns. Also, IL animals showed

significantly lower CFPS than sham animals in the empty chamber, $p < .01$, but not in the presence of the distractor, $p = .89$, ns. These findings unexpectedly demonstrated that the IL lesion was associated with lower CFPS than the sham condition in the empty chamber. Also, the distractor decreased CFPS in the sham animals, but not in the IL animals (Figure 3-15).

In order to explore differences in CFPS among experimental conditions, another repeated measures ANOVA was run with condition as the independent variable. Figure 3-11 shows CFPS in all sham conditions, and Figure 3-12 shows CFPS in all IL conditions. This analysis revealed main effects for time, $F(11, 93) = 30.29$, $p < .001$ and condition, $F(7, 103) = 7.51$, $p < .001$ and a time*condition interaction, $F(77, 564.74) = 1.80$, $p < .001$. Posthoc pairwise comparisons between distractor conditions in each lesion group were probed at each time bin. The Sham/1/Distractor group displayed a significantly lower CFPS than Sham/1/Empty at the 45-minute time bin, $p < .05$, as well as a marginally significant difference at 40, $p = .057$, and 55 minutes, $p = .072$. The Sham/.5/Distractor condition was significantly lower than Sham/.5/Empty at the 5-minute time bin, $p < .05$, but not at any other time bin. These posthoc comparisons suggest that the magnitude of the distraction effect may have been larger in the high 1% concentration of formalin than in the low .5% concentration. In the IL groups (Figure 3-12), posthoc analysis revealed no significant differences in CFPS between Distractor conditions and respective Empty conditions at any time bin of the formalin test. This finding, relative to the many significant distraction effects in the Sham animals, suggests that the IL lesion attenuated the influence of the distractor on CFPS.

3.5 Licking Behavior Across Entire Formalin Test

A repeated measures ANOVA was run with lesion type (IL or Sham), formalin concentration (.5% or 1%), and distractor presence (Distractor or Empty) as the between-

subjects independent variables. The dependent variable was time spent engaged in licking behavior, which was organized into 5-minute time bins across the 60-minute test. The multiple time bins allowed for within-subjects comparisons. Figure 3-16 shows licking behavior over time in sham animals, and Figure 3-17 shows licking behavior over time in IL animals.

Main effects were detected for formalin concentration, $F(1, 103) = 54.87$, $p < .001$, distractor presence, $F(1, 103) = 7.43$, $p < .01$, and time $F(11, 93) = 30.68$, $p < .001$. There were also significant interactions between time*concentration, $F(11, 93) = 8.16$, $p < .001$, time*distractor $F(11, 93) = 2.52$, $p < .01$, as well as lesion*distractor, $F(1, 103) = 4.15$, $p < .05$. These findings are very similar to the analyses of CFPS. The most notable differences in the licking analysis are that there was a main effect of distractor and there was not a main effect of lesion. These both suggest that licking is a better indicator of the distraction manipulation than CFPS.

The three significant interactions were further probed with posthoc analyses. Pairwise comparisons revealed that the two formalin concentrations displayed significantly different amounts of licking behavior at the 5-min time bin of the formalin test as well as time bins 25-55, $p < .05$ (Figure 3-18).

Posthoc analysis of the time*distractor interaction (Figure 3-19) revealed significant differences between distractor conditions at the 5-min, $p < .001$, and 35-min time bins, $p < .05$. The 30 and 40-min time bins were also marginally significant, $p = .06$, ms.

Pairwise comparisons in the lesion*distraction interaction for licking (Figure 3-20) were very similar to those in the CFPS analysis. There was a significant difference between the empty and distractor conditions in sham animals, $p < .001$, but not in the IL animals, $p = .62$, ns. Also, IL animals showed significantly lower licking than sham

animals in the empty chamber, $p < .05$, but not in the presence of the distractor, $p = .74$, ns. These findings mirror those of the CFPS analysis by showing that the IL lesion was associated with less licking than shams in the empty chamber and that the distractor decreased licking in the sham animals, but not in the IL animals.

Another repeated measures ANOVA was run with the composite independent variable and licking behavior as the dependent variable. This analysis revealed main effects of condition, $F(7, 103) = 9.7$, $p < .001$, and time, $F(11, 93) = 30.68$, $p < .001$, as well as a time*condition interaction, $F(77, 564.74) = 1.86$, $p < .001$. Posthoc pairwise comparisons between distractor conditions in each lesion group were probed at each time bin. Figure 3-16 shows the licking behavior over time in sham conditions, and Figure 3-17 shows the licking behavior over time in IL conditions. The Sham/.5/Distractor condition displayed significantly less licking than Sham/.5/Empty in the 5-min and 25-min time bins, $p < .05$. Sham/1/Distractor was marginally lower than Sham/1/Empty at the 5-min timepoint, $p = .06$, ms. Licking behavior in the Sham/1/Empty group began to spike at the beginning of the tonic phase, resulting in a significant difference from Sham/1/Distractor at the 35-min time bin, $p < .001$, and the 40-min time bin, $p < .05$. Consistent with the CFPS analysis, these results suggest that the distraction effect was larger in the 1% condition than in the .5% condition. Given that the distractor caused a dramatic reduction of licking in the tonic phase of the Sham/1/Distractor group and that licking, but not CFPS, showed an acute-phase distraction effect in the IL groups, licking may be a more sensitive measure of DA.

3.6 Mean CFPS in Acute Phase of Formalin Test

Since differences between groups are muted in the interphase of the formalin test, analyzing the acute and tonic phases separately often reveals information that

cannot be gleaned from an analysis of the full test. For analysis of the acute phase, group means were generated across the 5 and 10-minute time bins.

A one-way ANOVA was run with lesion, concentration, and distractor as the independent variables and mean acute-phase CFPS as the dependent variable. Results are shown in Figure 3-21. There were significant main effects of lesion, $F(1, 103) = 4.76$, $p < .05$, and concentration, $F(1, 103) = 38.94$, $p < .001$. There was also a marginally significant lesion*distractor interaction, $F(1, 103) = 3.72$, $p = .056$, ms (Figure 3-22). Posthoc probing of this interaction revealed that the sham animals had significantly higher acute-phase CFPS than IL animals in the empty chamber, $p < .05$, but not in the presence of the distractor. Also, the distractor significantly reduced acute-phase CFPS in sham animals, $p < .05$, but not in IL animals. These results are similar to the lesion*distractor interactions seen in the full-test analyses of CFPS and licking. Specifically, these analyses suggest that the IL lesion reduced acute-phase CFPS and that distraction reduced acute-phase CFPS in sham animals, but not in IL animals.

Another one-way ANOVA was run with condition as the independent variable in order to investigate group difference in acute-phase CFPS (Figure 3-21). This analysis revealed a main effect of condition, $F(7, 103) = 6.83$, $p < .001$. Pairwise comparisons revealed that, in sham lesions, there was a significant difference between Sham/.5/Distractor and Sham/.5/Empty, $p < .05$. However, the difference between Sham/1/Distractor and Sham/1/Empty was not significantly different, $p = .26$, ns. As expected, in the IL lesions, IL/.5/Distractor and IL/.5/Empty were not significantly different, $p = .55$, ns, nor were IL/1/Distractor and IL/1/Empty, $p = .97$, ns. These results demonstrate that distraction significantly reduced low-intensity acute-phase CFPS in sham animals, but did not reduce high-intensity acute-phase CFPS. The results also clearly show that the distraction effect was absent in the IL animals.

3.7 Mean Licking Behavior in Acute Phase of Formalin Test

Licking behavior was also analyzed in the acute phase of the formalin test. For these analyses, acute-phase licking means were generated by averaging licking scores in the 5- and 10-minute time bins.

A one-way ANOVA was run with lesion, concentration, and distractor as the independent variables and mean acute-phase licking as the dependent variable. Results are shown in Figure 3-23. There was a main effect of concentration, $F(1, 103) = 23.89$, $p < .001$, and of distractor, $F(1, 103) = 12.23$, $p < .001$. There was also a lesion*concentration*distractor interaction, $F(1, 103) = 3.98$, $p < .05$. Posthoc probing of this interaction revealed that the IL/.5/Empty condition was significantly lower than Sham/.5/Empty, $p < .05$; however, this difference was not apparent in the higher concentration in the empty or distractor condition. This suggests that the lesion decreased acute-phase licking in the low-intensity condition, but not in the high-intensity condition. Also, in sham animals, the distractor significantly decreased acute-phase licking in the .5% condition, $p < .01$, but not in the 1% condition, $p = .17$, ns. Conversely, in IL animals, the distractor significantly decreased acute-phase licking in the 1% condition, $p < .01$, but not in the .5% condition, $p = .97$, ns. In other words, for the sham animals, distraction reduced acute-phase licking in the low-intensity condition, but for the IL animals, distraction reduced acute-phase licking in the high-intensity condition.

Another one-way ANOVA was run with condition as the independent variable and mean acute-phase licking as the dependent variable. This analysis revealed a main effect of condition, $F(7, 103) = 6.09$, $p < .001$. Pairwise comparisons confirmed the same significant differences in the 3-way lesion*concentration*distractor interaction described above.

3.8 Mean CFPS in Tonic Phase of Formalin Test

Since the stratification of group differences was most apparent and sustained during the tonic phase (see Figure 3-11), additional analyses were run on data from only the tonic phase. For these analyses, group means were generated across the 30-60 minute time bins.

A one-way ANOVA was run with lesion, concentration, and distractor as the between-subjects independent variables and with mean tonic-phase CFPS as the dependent variable. Similar to the full 60-min analysis of CFPS, this analysis revealed main effects for lesion, $F(1, 103) = 5.24$, $p < .05$, and concentration, $F(1, 103) = 50.26$, $p < .001$, as well as a lesion*distractor interaction, $F(1, 103) = 4.79$, $p < .05$. Posthoc analysis of this interaction revealed that the distractor reduced CFPS in Shams, $p < .05$, but not in IL animals, $p = .51$, ns. Also, CFPS was significantly lower in IL animals relative to shams in the empty chamber, $p < .01$ (Figure 3-24).

Another one-way ANOVA was run with the composite independent variable in order to investigate group differences in tonic phase CFPS. Figure 3-25 depicts the results. This analysis revealed a main effect of condition, $F(7, 103) = 8.52$, $p < .001$, justifying further exploration of group differences. In the sham lesions, the difference in mean tonic-phase CFPS between Sham/1/Distractor and Sham/1/Empty was marginally significant, $p = .054$. The difference between Sham/.5/Distractor and Sham/.5/Empty was not significant, $p = .16$, ns. As expected, in the IL lesions, IL/1/Distractor and IL/1/Empty were not significantly different, $p = .66$, ns, nor were IL/.5/Distractor and IL/.5/Empty, $p = .62$, ns. The pattern of these results suggests that the distraction effect is nearing significance and slightly underpowered in the Sham/1% conditions. It is also clear that there is no effect of the distractor on the IL conditions at either concentration of formalin.

3.9 Mean Licking Behavior in Tonic Phase of Formalin Test

A one-way ANOVA was run with lesion, concentration, and distractor as the independent variables and with mean tonic-phase licking behavior as the dependent variable. Main effects were found for concentration, $F(1, 103) = 51.94$, $p < .001$, and distractor, $F(1, 103) = 4.14$, $p < .05$, but there was no main effect for lesion, $p = .16$, ns. There was also a lesion*distractor interaction, $F(1, 103) = 5.14$, $p < .05$ (Figure 3-26). Posthoc analysis of this interaction showed, like the tonic-phase CFPS analysis, that the distractor reduced licking in the sham animals, $p < .01$, but not in the IL animals, $p = .87$, ns. Also, licking was significantly lower in IL animals relative to shams in the empty chamber, $p < .05$ (Figure 3-27).

Another one-way ANOVA was run with the composite independent variable in order to investigate group differences in tonic phase licking behavior. Figure 3-27 depicts the results. In this analysis, there was a main effect of condition, $F(7, 103) = 8.92$, $p < .001$, which justified further exploration of group differences. As expected, Sham/1/Distractor displayed significantly less licking behavior than Sham/1/Empty, $p < .01$. Although Sham/.5/Distractor was lower than Sham/.5/Empty, the difference did not reach significance, $p = .18$, ns. Also as expected, the distractor did not reduce licking in any of the IL conditions. IL/1/Distractor was not significantly different from IL/1/Empty, $p = .96$, ns, and IL/.5/Distractor was not significantly different from IL/.5/Empty, $p = .78$, ns. These results support the notion that licking behavior is more sensitive than CFPS to the effect of the distractor. They also support the evidence that the effect of the distractor is larger in the 1% formalin concentration.

Chapter 4

Discussion

4.1 Overview of Results

This study used a rat model of distraction analgesia (DA) to investigate the role of the infralimbic cortex (IL) in DA during high- and low-intensity formalin-induced nociception. Results provide the first known evidence that the IL plays a major role in the ability to experience a reduction of composite formalin pain scores (CFPS) via attentional diversion. The study also found the first known evidence for reduced CFPS following a bilateral electrolytic IL lesion, which suggests a role for the IL in pain-related behavioral outcomes irrespective of distraction. Below, nuances of the data are discussed, broader implications for the interplay between pain and attention are presented, and future directions are suggested.

4.2 IL Lesion Had No Impact on Locomotion or Anxiety-like Behavior

As expected, locomotion patterns during the habituation portion of the protocol were not different between the IL lesion and sham groups in distance traveled, time spent in the center zone, time spent in the periphery, time spent moving, or heading-to-center (Figures 3-2, 3-3, 3-4, 3-5, & 3-6). Each variable except heading-to-center showed a main effect of time, which was due to a trend of decreasing locomotion and exploratory behavior over time as the protocol progressed. However, the lack of time*lesion interactions in each analysis indicated that, when present, the downward trends did not differ between IL and sham animals. Therefore, the IL lesion had no impact on general locomotion, behavior indicative of anxiety, or orientation ability during habituation. Importantly, this means that the analyses of pain scores were not confounded by any lesion-induced alterations to locomotion or anxiety. This strengthens the veracity of

conclusions that can be drawn with respect to the lesion's impact on pain scores and distraction.

Because the behavioral tracking software measures locomotion patterns of the whole animals, the findings do not rule out the possibility that the IL is involved in visceromotor control, which was suggested by the authors of the IL connectivity analyses (Hoover & Vertes, 2007; Vertes, 2004). It is unlikely that visceromotor control impacted the present study, however the limitations of this research in revealing the full role of the IL are acknowledged below.

4.3 Analysis of Locomotion on Test Day

Also as expected, evidence for differences among experimental conditions in distance traveled or time spent moving on Test Day was sparse. Most notably, there was no main effect of condition detected for these variables on Test Day. Nevertheless, certain significant interactions were found in each analysis. Most notably, Figure 3-7 shows some evidence that the distractor increased distance traveled at certain time points; however, the influence was not consistent over time and did not correspond to the phases of pain-related behavior in the formalin test. Therefore, it seems reasonable to conclude that distance traveled was only minimally affected by the independent variables, including distractor presence, on Test Day.

In the analysis of time spent moving, the 1% groups spent more time moving than the .5% groups at the beginning of the tonic phase (Figure 3-8). Also, the 1% group, but not the .5% group, seemed to increase movement when the distractor was present (Figure 3-9). Since there was no difference between concentrations in the empty chamber, the formalin concentration itself did not alter time spent moving. Therefore, these interactions may reflect increased exploration of the distractor by the high-intensity pain group. This interpretation is supported by the evidence that the distractor had a

larger impact on high-intensity pain during the tonic phase, which is discussed in section 4.9. However, it is somewhat surprising that the corresponding variable, total distance traveled, did not reflect this interaction. The best interpretation of the concentration*distractor interaction seems to be that it is a mere suggestion that the distractor may have attracted more attention from the high-intensity groups than the low-intensity groups. Overall, the evidence demonstrates that general locomotion was only mildly affected by the lesion, formalin concentration, and distractor presence on Test Day, which is critical in order to rule out the notion that locomotion, rather than distraction, was driving the observed changes in formalin pain scores.

Contrary to predictions, the variables intended to quantify attention to the distractor on Test Day, time in center zone and heading-to-center, were only minimally altered by distractor presence on Test Day. Animals tested with distractors spent more time in the center zone at only the 35-minute time bin, and spent less time in the center zone during the first 5-minutes relative to animals tested in the empty chamber (Figure 3-10). The large difference during the first 5-minutes is unlikely to be due to fear or aversion to the distractor, but rather an inability of the rat to enter the center zone with ease. This is discussed in greater detail in section 4.12.3 and is supported by the consistently low time spent in the center zone by the distractor group. These findings provide minimal evidence that the distractor increased time spent in the center zone. Similarly, the analyses also failed to detect an increase in the orientation of movement towards the center of the chamber.

These findings are somewhat surprising given that the original Ford et al. (2008) publication provided convincing evidence that the falcon tube engaged the attention of the rats. There are two reasons for the discrepancy between the original and the present studies. First, the original study measured head orientation while the present study

measured heading and time spent in the center zone. Second, the original experiment compared head orientation between two distractor conditions (falcon tube and conspecific) rather than between the presence and absence of a distractor. It is critical to realize that these results do not indicate a failure of the falcon tube to distract the rat, but rather a failure of the software to quantify the distraction. Based on the prior evidence from Ford et al. (2008), it seems reasonable to conclude that, in the present study, the falcon tube distracted rats, but that Ethovision could not provide behavioral evidence of this. A thorough explanation of why Ethovision did not provide a behavioral correlate of attention to the distractor on Test Day is provided in section 4.12.3 of the Discussion.

4.4 Distractor Presence Reduced CFPS and Licking

Sham animals tested with a distractor had significantly lower mean formalin pain scores (Figure 3-15, 3-22, & 3-24) and mean licking behavior (Figure 3-20, 3-23, & 3-26) than sham animals tested in the empty chamber. Figure 3-11 also shows a clear stratification of CFPS among all sham groups over time. This demonstrates successful DA similar to the effects observed by Ford et al. (2008) and provides evidence that DA can be produced by an affectively neutral distractor stimulus. For clinicians, this is important information because it demonstrates that pain-reducing distraction procedures need not engage affect in order to be effective in the clinic. Also, the magnitude of DA appeared to be larger in the licking scores than in CFPS, and this was particularly true in the high 1% formalin concentration. Implications of this are addressed in section 4.11.

4.5 IL Lesion Significantly Reduced Formalin Pain Scores

One of the most surprising findings of this study was that, relative to sham controls, the IL lesion group was associated with significantly lower formalin pain scores and licking when tested in the empty chamber (Figure 3-15, 3-20, 3-22, 3-24, & 3-26). The effect was similar in magnitude to that of distractor presence, which can be seen in

the listed figures by comparing the Sham/Distractor and the IL/Empty groups. To the author's knowledge, this is the first evidence suggesting that the IL region of the rat mPFC is directly involved in pain processing.

This finding is probably a consequence of the IL inputs from the medial thalamus and limbic structures, including the basolateral and basomedial amygdalar nuclei, because the IL receives very little input from the ACC (labelled AC in Figure 1-13) and virtually no input from other major pain-processing regions such as the primary and secondary somatosensory cortices and the insula. It is of interest that the IL lesion had such a strong impact on pain despite having only secondary connections to the brain regions more commonly cited for their role in processing pain. In this regard, the IL seems to have more of an integrative role in processing pain-related signals from other structures. Moreover, the evidence of pain processing by the IL warrants further investigation by future studies.

4.6 The IL Lesion Attenuated Distraction Analgesia

The primary finding of this study was that the bilateral lesion to the IL sub-region of the mPFC eliminated the rats' ability to experience DA. This is supported by the detection of DA in shams, but not in IL animals. Unlike sham animals, IL animals did not show a distractor-induced reduction of mean CFPS (Figure 3-15, 3-22, & 3-24) or licking (Figure 3-20 & 3-26). The lack of a distraction effect in the IL animals was particularly evident in the analysis of CFPS over time (Figure 3-12) where distractor groups were not significantly different from their respective empty condition at any time bin.

The only evidence of IL-lesioned animals experiencing DA came from the licking analysis in which a significant difference was observed between IL/1/Empty and IL/1/Distractor groups during the first 5 minutes (Figure 3-17). This difference could also be seen in the acute-phase licking analysis (Figure 3-23). Thus, it may be possible for an

IL-lesioned animal to experience DA in the immediate short-term, but not beyond. The fact that the only distraction effect in IL animals was detected in licking behavior suggests again that licking may be a more sensitive measure of DA.

Overall, this study provides direct evidence that distraction meaningfully reduced CFPS and licking in sham animals, but not IL animals. These findings demonstrate that the IL lesion attenuated DA. They also suggest, as predicted, that the function of the rat IL was similar to that of the human OFC in the context of experimental distraction analgesia. To the author's knowledge, this is the first study in rats to reveal one of the brain regions responsible for DA.

The reason that the IL lesion attenuated DA cannot be directly determined from this experiment. However, the evidence available immediately implicates the elimination of IL input to the PAG as a possible cause. As discussed earlier, the PAG is an area of the brain that is known to be involved in descending pain inhibition (Fields & Basbaum, 1978; Millan, 2002; Reynolds, 1969) as well as distraction analgesia (Tracey et al., 2002). In the rat brain, the PAG receives input from the IL (Vertes, 2004). Assuming the PAG is activated during rat DA in the same way that it is activated by human DA, which has not yet been empirically demonstrated, it is possible that the IL lesion eliminated the input to the PAG triggered by the distractor object. This would have resulted in a failure to engage the pain inhibiting effects of PAG activation, which ultimately would produce a lack of distraction analgesia. This is perhaps the most likely explanation given the available evidence; however, it has not been demonstrated empirically. Future research is needed to confirm 1) that the PAG is involved in the phenomenon of DA in the rat and 2) that the lack of IL input to the PAG causes an attenuation of DA.

These results illustrate a role for the IL in the processing of pain that is seemingly self-contradictory in the sense that the lesion reduced pain scores while simultaneously

preventing a form of pain reduction. However, this contradiction is most likely illusory because the evidence from attentional set-shifting (Ng et al., 2007) suggests that the lesion ought to have impacted primarily attentional mechanisms, and it is not contradictory for a lesion to alter attentional mechanisms while also reducing pain scores.

4.7 The Lack of DA in IL Animals is Probably Not Due to IL Lesion-Induced Analgesia

In Figure 3-15, the analgesic effect of the IL lesion may seem to explain the lack of a significant distraction effect in those animals. This argument depends on the existence of a floor effect of the distraction manipulation whereby distraction would have been ineffective at or below the lesion-attenuated CFPS or licking values. However, Figures 3-11, 3-16, 3-21, 3-23, 3-25, & 3-27 show direct evidence that distraction can slightly reduce even low amounts of pain in sham animals. Specifically, in the .5% concentration conditions, which displayed low formalin pain scores in the empty chamber, the distractor further reduced pain scores and licking. Although the effect of the distractor was weaker at the low formalin concentration, the trend clearly indicates that the distractor further reduced already-low levels of formalin pain and licking in sham animals. This evidence suggests that, if there is a floor effect of DA, it is well below the level of the lesion-attenuated CFPS and licking values. Therefore, IL lesion-attenuated analgesia is not a viable explanation for the failure of the IL animals to experience further reduction of their pain scores by distraction. In other words, there is no reason to believe that the presence of IL-induced analgesia precluded further pain reduction by distraction.

4.8 Low-Intensity Pain May Be More Susceptible to DA during Acute Phase

As mentioned above, the foundational literature on DA provided evidence that high-intensity pain may engage more attentional mechanisms (Eccleston, 1995) or that it may be more susceptible to cognitive modulation (Wiech et al., 2005), but these claims were never directly tested between two levels of the same type of noxious stimulus. This

experiment expands upon the prior literature by measuring pain rather than cognition as an outcome and by comparing a single type of noxious stimulation at high- and low-intensity. Therefore, to the author's knowledge, this study represents the first direct comparison of DA on the same type of pain administered at two levels of intensity.

One of the most interest findings in the present study is that, during the acute phase, low-intensity pain appears to be more susceptible to DA (Figures 3-21 & 3-23); however, during the tonic phase, high-intensity pain appears to be more susceptible to DA (Figures 3-25 & 3-27). While this may seem contradictory, it may be explained by the previous literature describing different mechanisms underlying acute and tonic phases of the formalin test (Shibata, Ohkubo, Takahashi, & Inoki, 1989; Tjolsen, Berge, Hunskaar, Rosland, & Hole, 1992). It is conceivable that the distraction manipulation acts more effectively on certain mechanisms depending on the intensity of the pain and on whether it is acute or tonic.

The major caveat to this finding is that licking scores of low-intensity pain in the acute-phase are only susceptible to DA in the sham animals. In the IL animals, acute-phase licking was reduced by distraction only in the high-intensity condition, but not the low-intensity condition (Figure 3-23). It is particularly interesting that the effect of the IL lesion on the acute-phase was only apparent in the licking behavior (Figure 3-23), but not in CFPS (Figure 3-21). In other words, the IL lesion had an effect on acute-phase licking, but not necessarily CFPS. Also, the IL lesion altered the acute response to high and low pain intensity relative to brain-intact controls. The explanation for this is unclear, and therefore, future research is warranted.

4.9 High-Intensity Tonic Pain May Be More Susceptible to Distraction Analgesia Overall

In the tonic phase, the distractor's effect on CFPS and licking in shams was stronger in the 1% groups than in the .5% groups (see Figures 3-11, 3-16, 3-25, & 3-27).

Unlike in the acute-phase, this pattern was not reversed by the IL lesion. Since the tonic phase comprises the longest portion of the formalin test, it may be justifiable to consider the possibility that high-intensity tonic pain is more susceptible to DA.

This finding may seem to pose a challenge to the theoretical assumptions regarding pain as an evolutionarily adaptive and protective function (Ohman, 1979; Price, 1988). For instance, pain has been described as performing the evolutionarily advantageous function of signaling danger to an organism and/or increasing its chances of survival (Institute of Medicine, 2011). In this context, it seems counterintuitive and maladaptive for an organism to be more easily distracted away from high-intensity pain than low-intensity pain. After all, an animal would have a better chance of survival if they paid close attention to high-intensity pain because this would afford them the ability to quickly escape or avoid more survival-threatening stimuli. However, it is not pain itself that is evolutionarily adaptive or protective, but rather the ability to avoid pain. Distraction can be considered a way to avoid experiencing pain and, in that sense, it could perform an extremely protective function. In fact, distraction may be considered an avoidance strategy, albeit a cognitive one, analogous to the behavioral escape/avoidance strategies, which have been used extensively in the preclinical study of pain (Fuchs & McNabb, 2012; Johansen, Fields, & Manning, 2001; LaBuda & Fuchs, 2000; Mauderli, Acosta-Rua, & Vierck, 2000). Discussion of distraction's utility as an avoidance strategy is addressed in the next section. This argument reveals that there is no necessary conflict between DA and the evolutionarily protective function of pain; however, it does not explain why distraction ought to be more effective during high-intensity pain.

The best explanation for the enhanced efficacy of DA at high-intensity pain is provided by studies that compared neural activation patterns between high- and low-intensity pain. Various degrees of subjective pain intensity are associated with

corresponding alterations of peripheral activity in C-fibers (Torebjörk, LaMotte, & Robinson, 1984) as well as central activity in the brain (Coghill, McHaffie, & Yen, 2003). More specifically, Coghill et al. (2003) found that thermal stimulation rated as highly intense was associated with increased activation in areas that are also known to be involved in attentional processing, including, most notably, the perigenual ACC and the ventral prefrontal cortex (see Figure 4-1), which contains the orbitofrontal cortex. Given that those areas are primarily active during high-intensity pain, it should be easier to recruit them for distraction-induced reduction of high-intensity pain. This would explain the increased susceptibility of high-intensity pain to DA as well as the decreased ability of the IL animals to experience high-intensity pain. However, this explanation is contingent upon the notion that pain-evoked "activity" in these regions differs in important ways from distraction-evoked "activity." This seems likely given that the effects of each type of stimulation produce opposite effects on pain. It also seems feasible given that the rat IL is highly interconnected within itself, which may provide a mechanistic explanation for how attention-related activity in the IL could modulate pain-related activity in the IL. To the author's knowledge, this has yet to be demonstrated experimentally. Future research may be warranted to determine the differences between pain- and distraction-evoked activity in these brain regions in humans and animals.

4.10 The Utility of Distraction as a Pain-Avoidance Strategy

The utility of distraction as a pain-avoidance strategy has clear limitations, especially when compared to escape/avoidance. In a situation where escape and distraction are both viable pain-avoidance options, escape from stimulation is clearly ideal. However, if the purpose of pain is to signal the need for escape, then pain becomes utterly purposeless when escape is impossible. In inescapable pain states, diversion of attention may usurp escape as the best option, even for high-intensity pain,

because it would be better than engaging no protective mechanisms at all. Consider, for example, a wartime torture victim in which high-intensity painful tissue damage is imminent and inescapable. For that individual, distraction would retain its protective function despite the high-intensity pain. Therefore, the adaptive and protective value of DA may not be limited by the intensity of the pain, but rather by the availability of escape/avoidance. Since formalin-induced pain is inescapable, this may explain why the present study found evidence that distraction is capable of producing analgesia during the formalin test.

4.11 Licking Behavior Is More Sensitive to the Analgesic Effects of Distraction than CFPS

One of the most interesting findings in this study was that the tonic-phase licking analysis revealed a main effect of distractor while the tonic-phase CFPS analysis did not. Figure 3-16 shows a massive spike in tonic-phase licking in the Sham/1/Empty group that was attenuated by distraction, yet this phenomenon was not proportionally matched by the corresponding .5% groups. This is also evident in Figure 3-27 by the significant distraction effect in the Sham/1% groups, but not the Sham/.5% groups. This suggests that the high sensitivity of licking to DA is due primarily to the massive distraction-induced attenuation of licking behavior in the 1% groups. Also, the distraction effect on tonic-phase licking (Figure 3-27) was greater than the distraction effect on tonic-phase CFPS (Figure 3-25) in Sham/1% groups. Additional evidence is provided by the acute-phase analyses, which showed that licking, but not CFPS, was able to detect a distraction effect in the IL/1% groups. It seems justifiable to interpret these results as an indication that licking behavior is more sensitive to DA than CFPS during higher-intensity pain.

4.12 Limitations and Future Directions

4.12.1 Effects of the Distractor on the Rat

This study clearly shows that the presence of the falcon tube reduced composite formalin pain scores in brain-intact animals, but it does not directly indicate the nature of the object's effect on the rat. Specifically, this study does not provide direct evidence as to whether the distractor's impact is primarily attentional, affective (i.e. fear), or stress-related. However, the original publication of this paradigm showed sufficient and convincing evidence that the most likely impact on the rat is attentional in nature (Ford et al., 2008). Therefore, this limitation should not detract from the veracity of the conclusions that have been drawn.

4.12.2 Function of the Rat Infralimbic Cortex (IL)

The scope of the study was also limited in terms of what it could reveal about the function of the IL region of the rat mPFC. Therefore, more research is needed in order to fully and precisely describe why the IL lesion eliminated DA. For example, it is unknown whether the purported visceromotor functions of the IL influenced behavior in the present study. Also, this study was not designed to determine whether the effects of the IL lesion on DA required past information learned from the habituation period. The literature suggests two possibilities.

Information from the attentional set-shifting literature (Ng et al., 2007) suggests that the lack of DA in the lesioned animals might represent a failure to engage in an extradimensional attentional set-shift (EDS). In other words, the rat may have failed to integrate new information (falcon tube) presented to a different stimulus modality (visual & tactile) that changes a situation to which they were previously habituated (test chamber). If this interpretation is accurate and the primary role of the mPFC, and

perhaps IL specifically, is the mediation of EDS, then the learning which occurred during the habituation period may have been necessary for the lesion to have eliminated DA.

The other possibility suggested by the connectivity literature (Hoover & Vertes, 2007; Vertes, 2004) is that the IL processes competing attentional stimuli, analogous to the function of the human OFC (Bantick et al., 2002), regardless of whether the stimuli have been experienced previously. If this interpretation is accurate, then the learning which occurred during habituation would not be necessary for the lesion to eliminate DA.

These two possibilities could be tested by performing a follow-up study in IL-lesioned and sham rats using chamber novelty, if validated as affectively neutral, as the distractor. Control animals would undergo habituation, then a formalin test in an empty chamber, and would be expected to display normal formalin pain scores; non-habituated animals would be expected to display mildly attenuated pain scores; and the outcome of primary interest would be that of the IL-lesioned non-habituated animals. If the attenuation of DA depends on learning through habituation, then IL-lesioned animals would show full-magnitude pain scores that were not attenuated by chamber novelty. On the other hand, if the attenuation of DA does not depend on learning through habituation, then IL-lesioned animals would show attenuated pain scores. Such a study would indicate whether the role of the IL in DA depends on previously learned information. This would clarify the function of the IL by revealing the type of cognition it processes (i.e. behavioral adaptation or competing attentional stimuli).

4.12.3 Quantifying a Behavioral Correlate of Attention to the Distractor on Test Day

As mentioned previously, this study was unable to detect a behavioral correlate of attention to the distractor. The null result is best explained by three practical factors: 1) limitations of the center-point detection method used in the Ethovision software; 2) the presence of the falcon tube limiting the animal's ability to enter the center zone; 3) the

rat-to-chamber size ratio being too large for the animal to engage in the hypothesized behaviors. These are discussed in the context of the two variables in question: time spent in center zone and heading-to-center.

4.12.3.1 Time Spent in Center Zone

The Ethovision software used center-point detection for this experiment. On this setting, it is possible for a rat to have part of its body in the center zone while its center-point is recorded in the perimeter. Thus, an animal could have been quite close to the distractor without being recorded in the center zone, and this was particularly likely to happen to the large rats used in the study. See Figure 4-2 for a schematic representation of this hypothetical scenario.

Also, the presence of the falcon tube dramatically reduced the likelihood of the rat being detected in the center zone because it forced rats to move around it. In Figure 4-2, consider that the radius of the center zone was 7.5 cm. Subtracting the radius of the falcon tube, 1.5 cm, there were only 6 cm between the distractor and the boundary of the center zone. For a representative rat in this study, the most lateral edge of the body was about 4.5 cm from its center-point as viewed from the camera. This means that the most lateral edge of the rat would need to be within 1.5 cm of the distractor in order for it to have been detected in the center zone. Such positioning would provide the rat with a particularly poor view of the distractor because the rat's head would be oriented away from it. Therefore, since the rat could not have been detected in the center zone while facing the object, time spent in the center zone should not be considered a reliable indication of attention to the distractor in this study.

4.12.3.2 Heading-to-Center

Heading-to-center quantified movement towards the center point of the chamber. This variable was also intended to serve as a behavioral correlate of attention paid to the

distractor. However, the rat-chamber size ratio prevented this measure from being useful. The length of a representative rat used in this study from snout to tail base was 22 cm, which was longer than the distance between the distractor and the corner of the chamber, 21.21 cm. Therefore, it was physically impossible for a rat to position itself such that it was directly facing the distractor. Therefore, if the center-point of the rat moved toward the center of the chamber, the rat's head would be moving past the distractor. Therefore, like time spent in center zone, heading-to-center should not be considered a valid indication of attention to the distractor. Since both of these measures are not valid indicators of attention, the amount of attention that the rat paid to the distractor could not be measured.

It is critical to realize that an animal could have paid great attention to the distractor without spending an elevated amount of time in the center zone and without orienting towards the center of the chamber. Therefore, attention to the distractor cannot be ruled out. Furthermore, evidence from the original Ford, et al. (2008) publication of this model provided sufficient and convincing evidence that the falcon tube distracted the rats. Based on this evidence, it seems valid to assume that the falcon tube distracted the rats in the present study.

4.13 Conclusions

In conclusion, this study presents the first known evidence that the IL is a necessary neural substrate for the induction of DA in a rat. This study also presents the first known evidence that the IL contributes to formalin-induced nociceptive behaviors. The results indicate that the Ford et al. (2008) model is a reproducible method for the investigation of the neural substrates of DA. Future research should clarify the nuances of the cognitive processing that occurs in the IL, the extent to which attentional

processing in the IL is dependent upon previously learned information, and the ability of the IL to engage descending pain modulatory mechanisms.

Appendix A

Graphs and Additional Figures

Figure 3-1

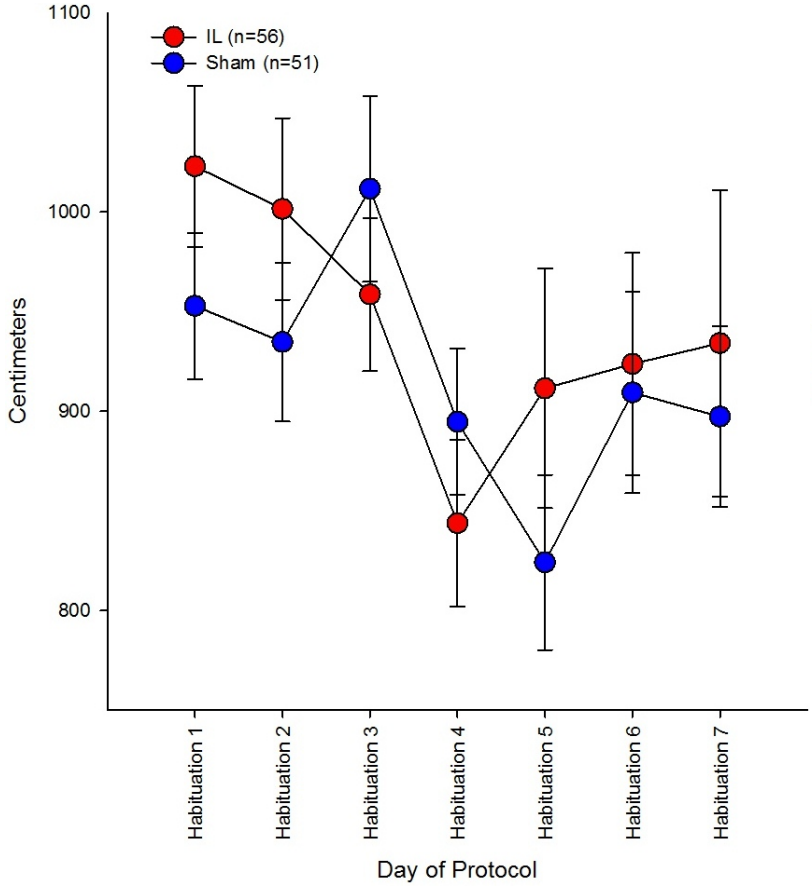

Figure 3-2

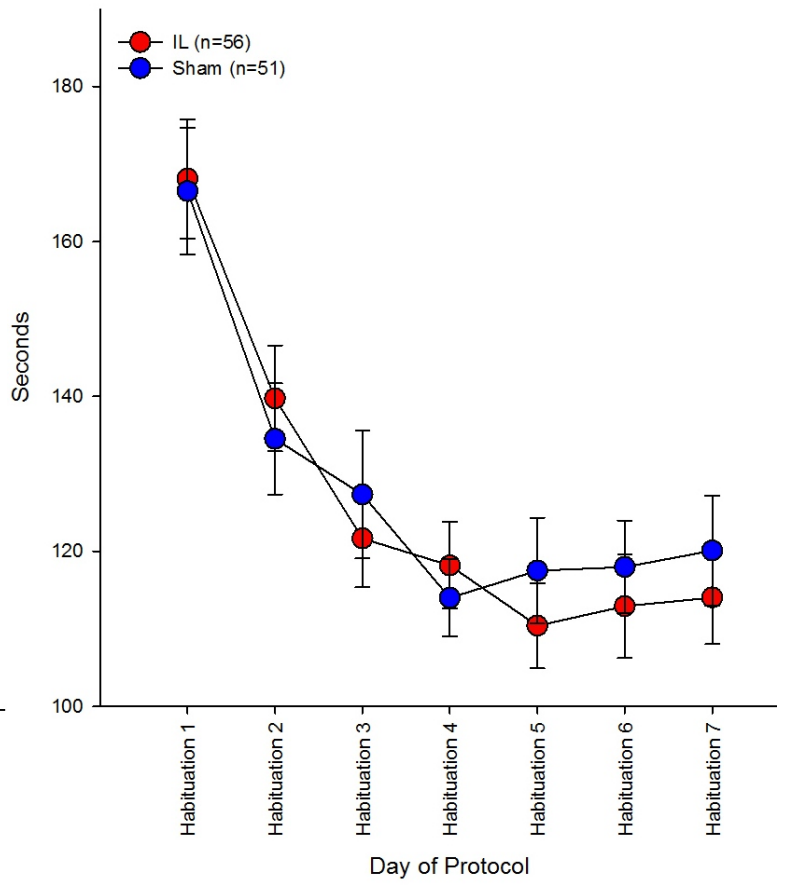

Figure 3-3

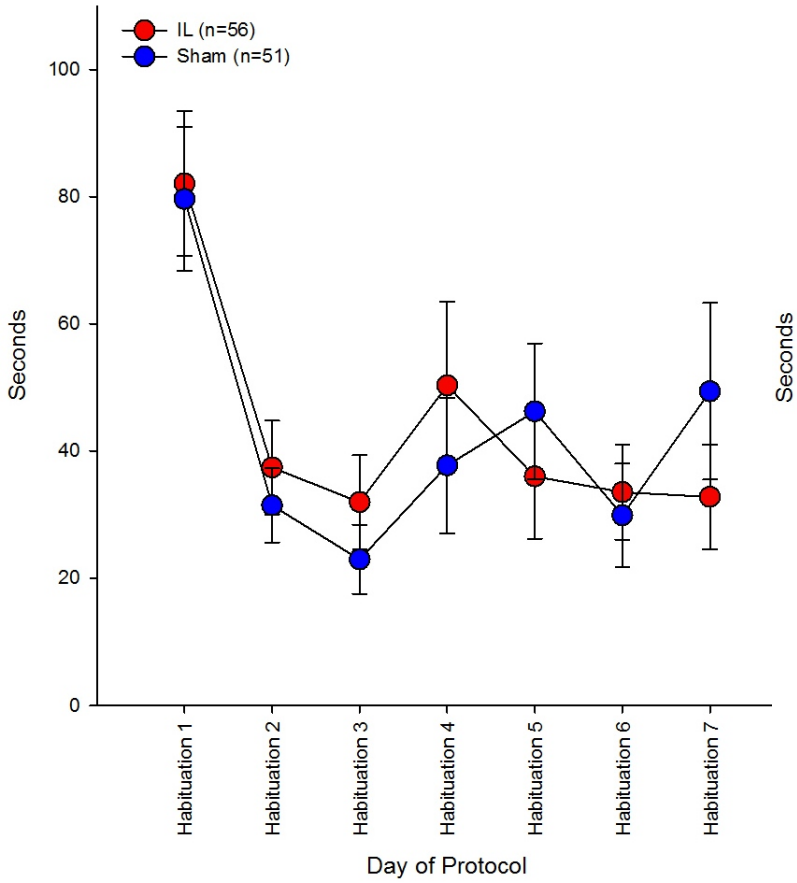

Figure 3-4

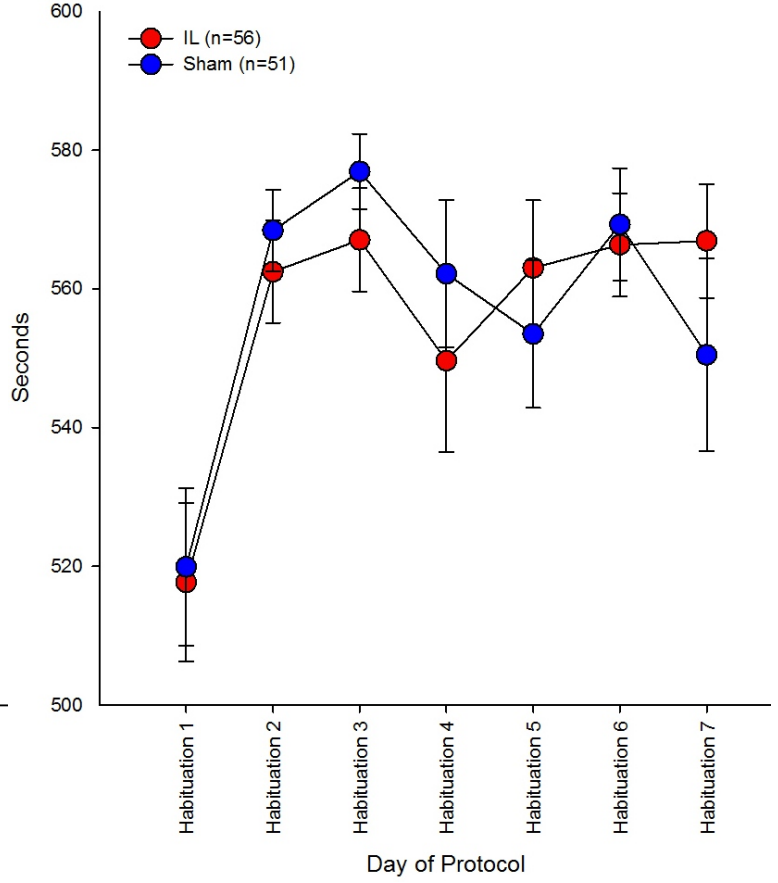

Figure 3-5

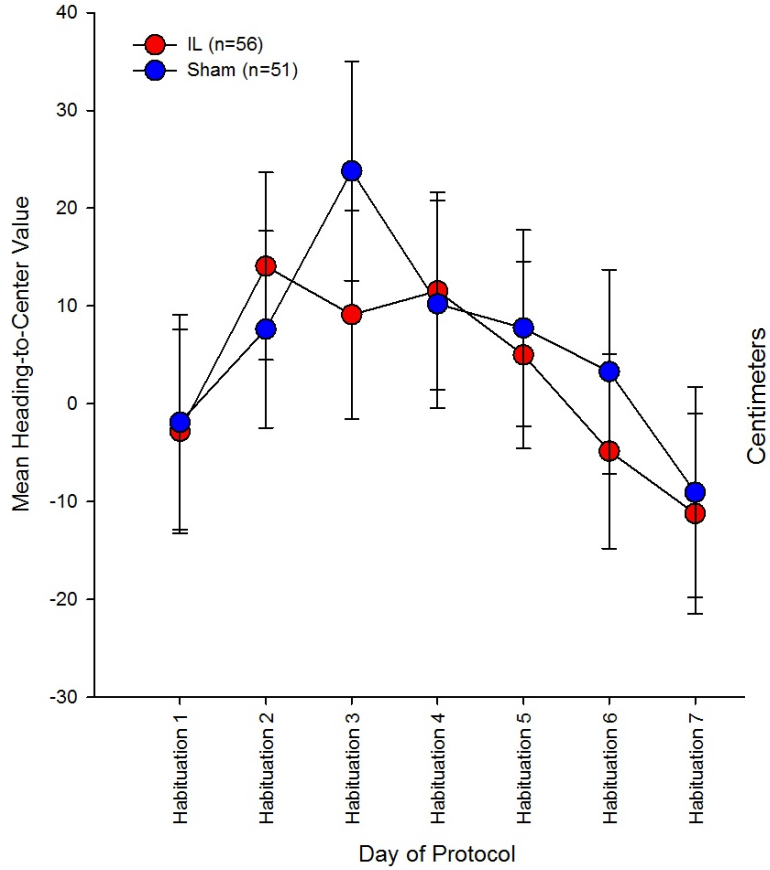

Figure 3-6

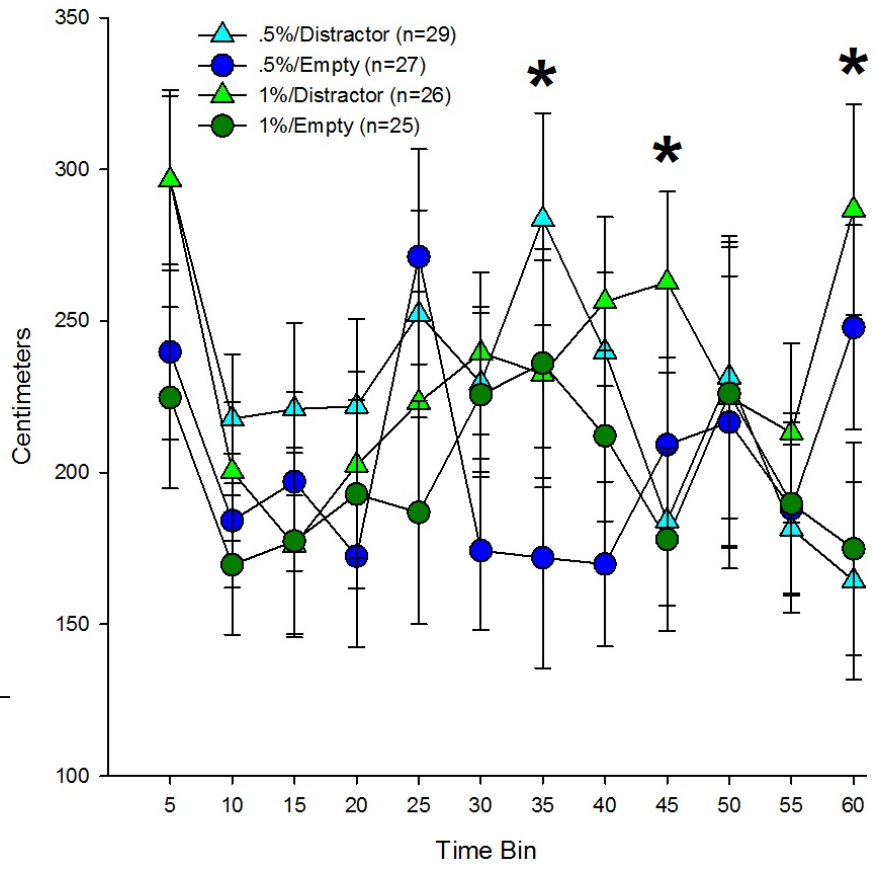

Figure 3-7

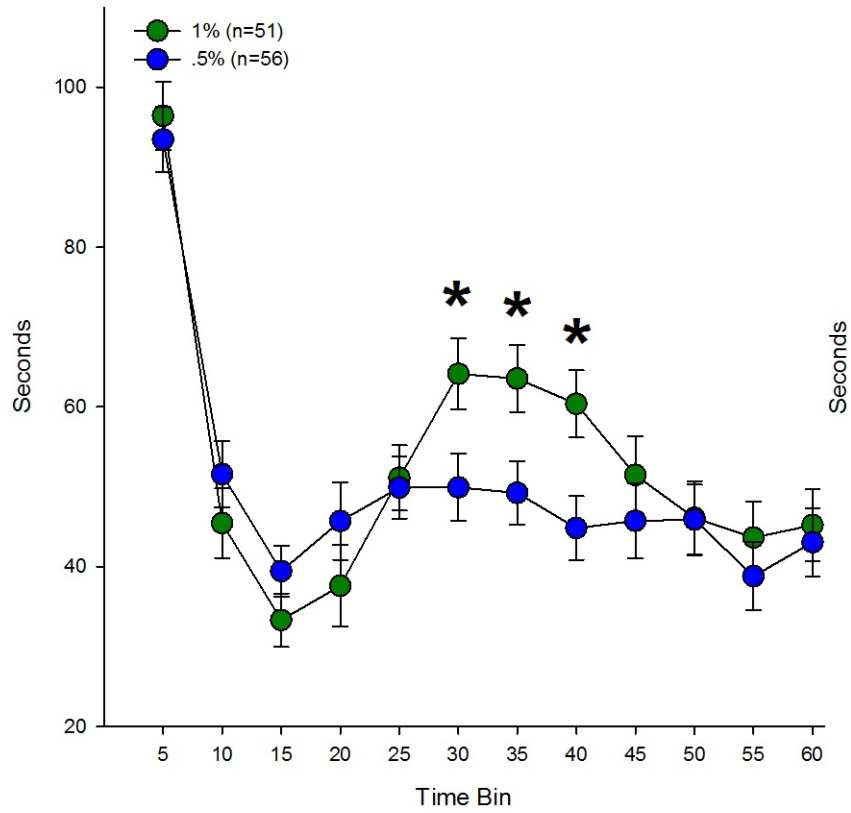

Figure 3-8

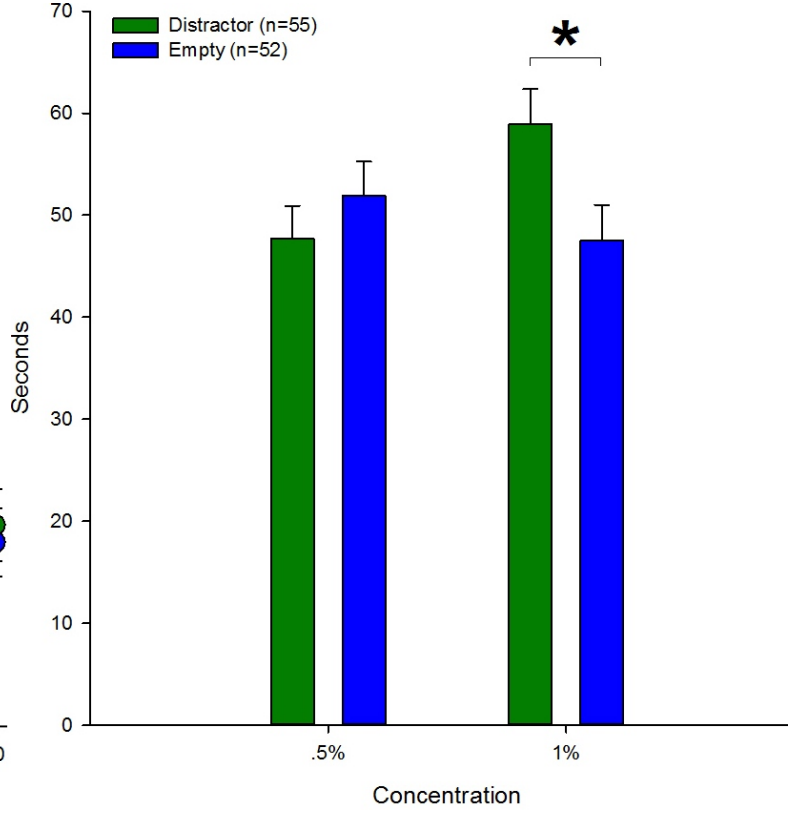

Figure 3-9

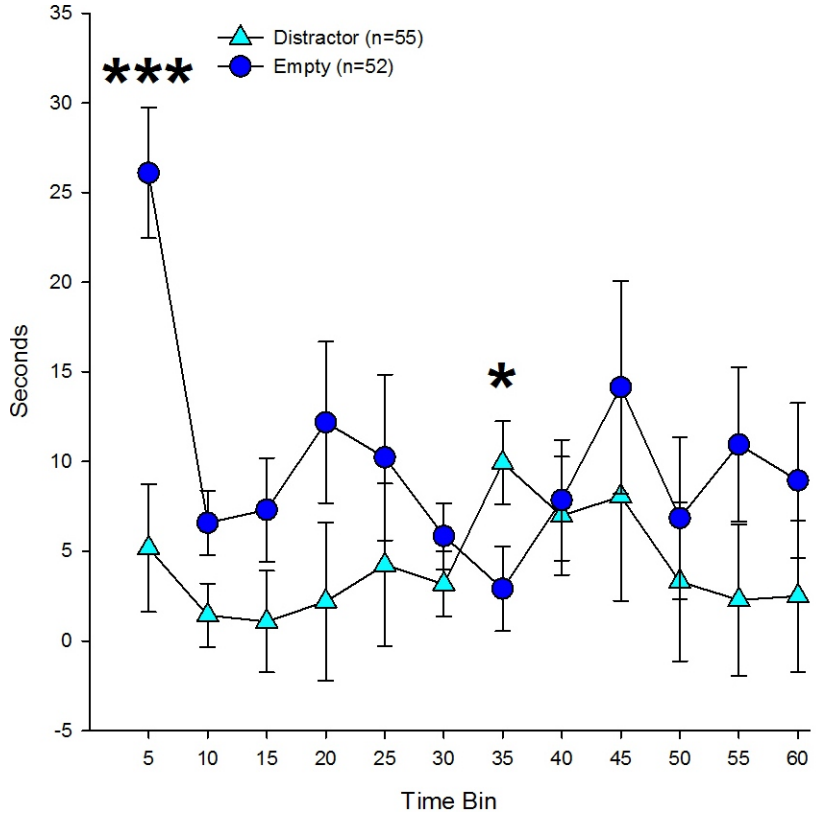
Distractor (n=55)
Empty (n=52)
***
*
Seconds
Time Bin
35
30
25
20
15
10
5
0
-5
5
10
15
20
25
30
35
40
45
50
55
60

Figure 3-10

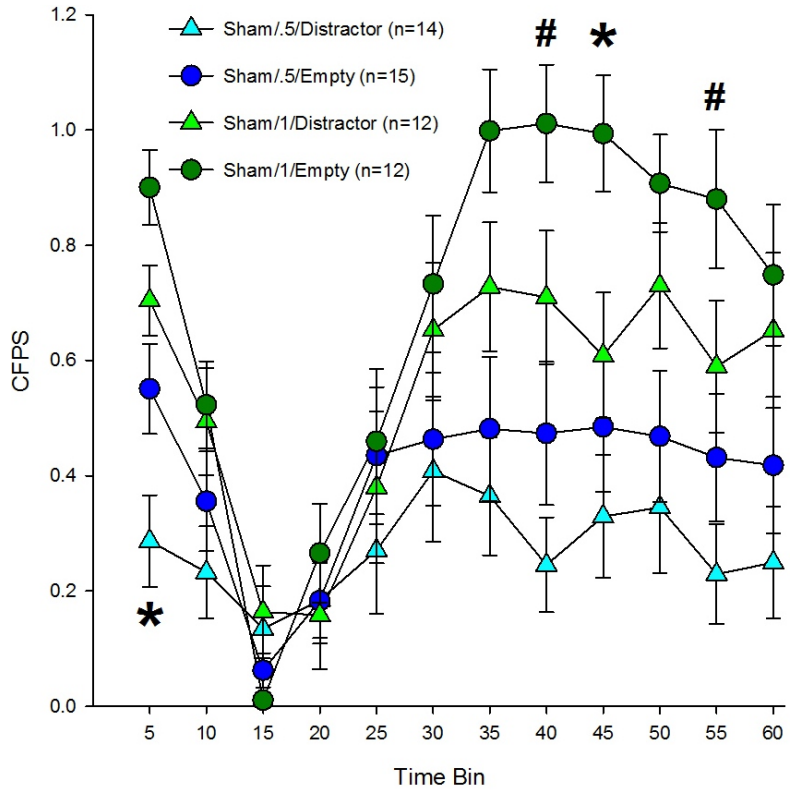

Figure 3-11

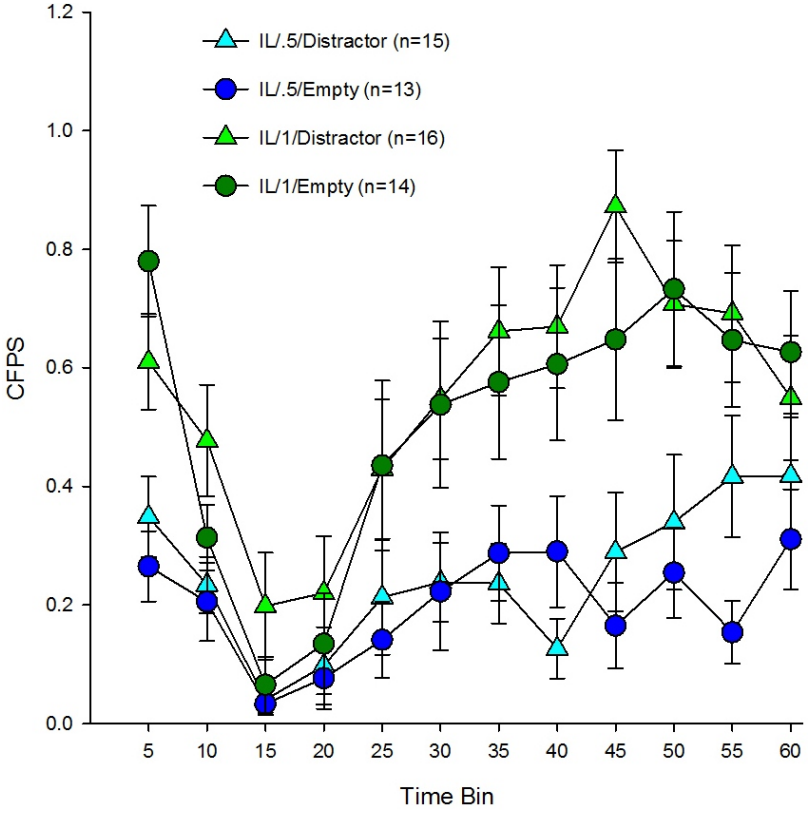

Figure 3-12

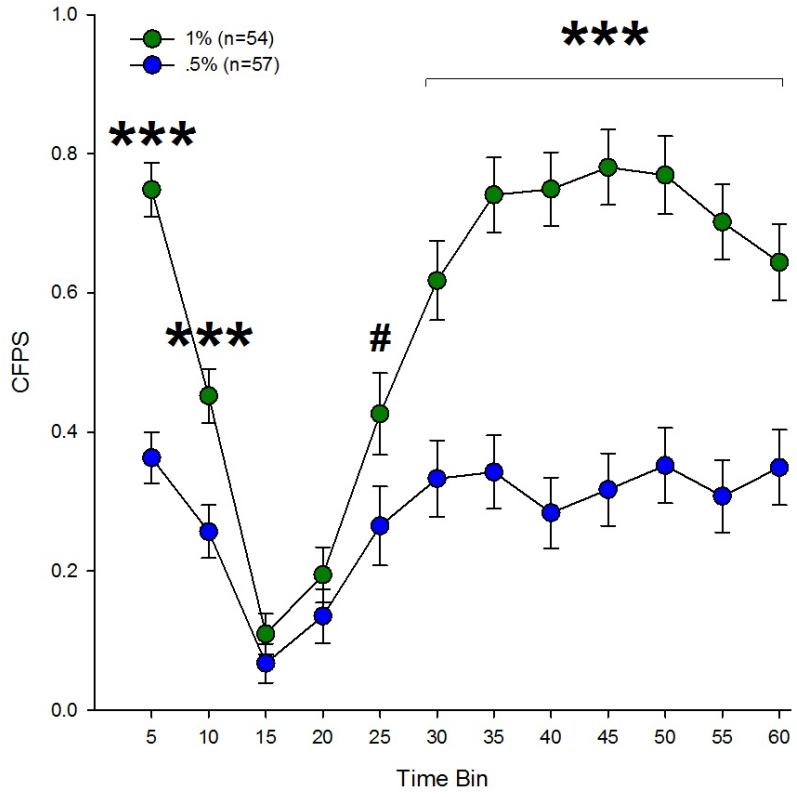

Figure 3-13

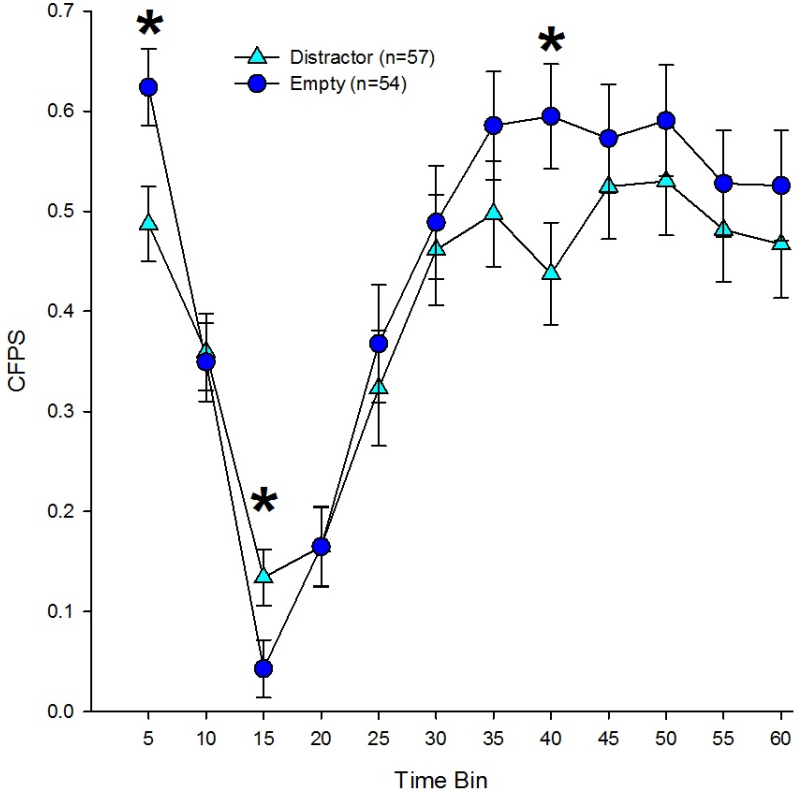

Figure 3-14

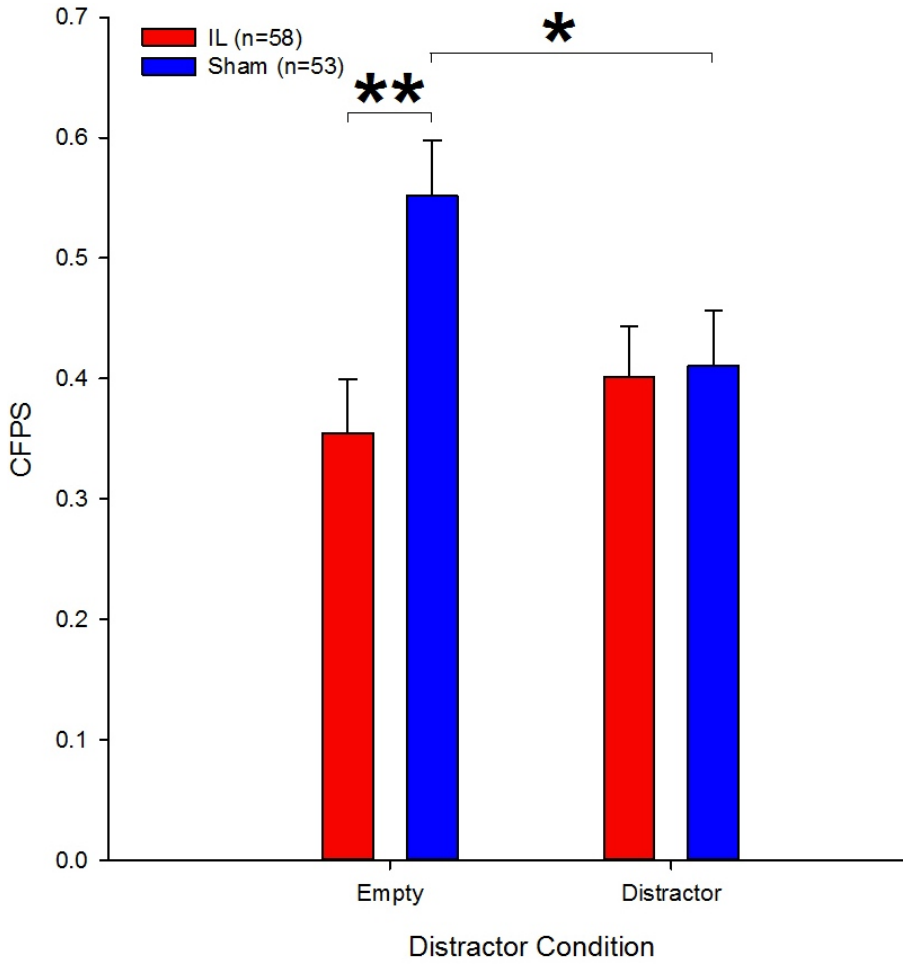
IL (n=58)
Sham (n=53)
**
*
CFPS
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Empty
Distractor
Distractor Condition

Figure 3-15

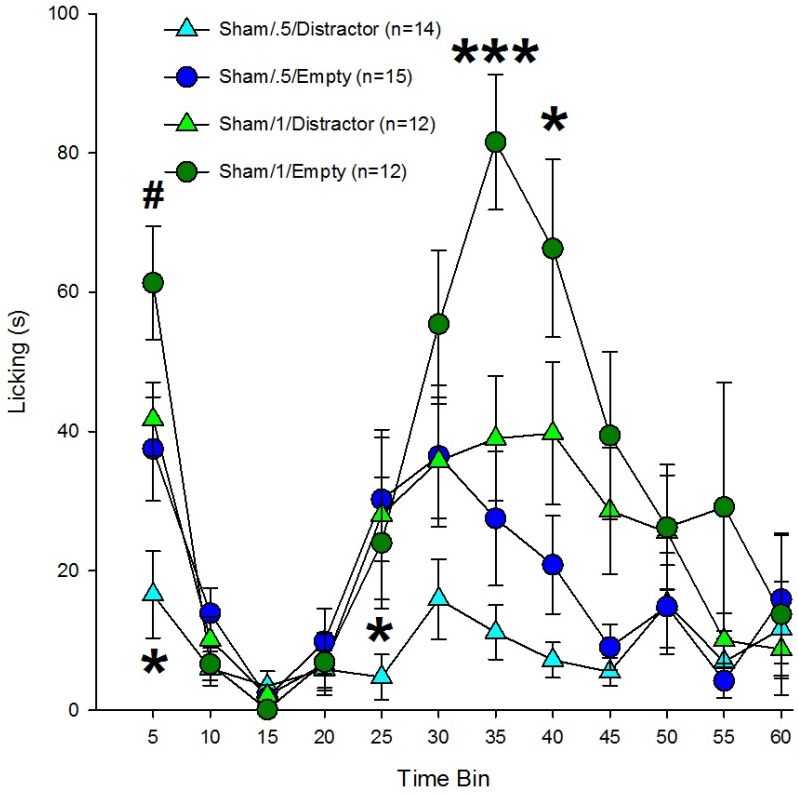

Figure 3-16

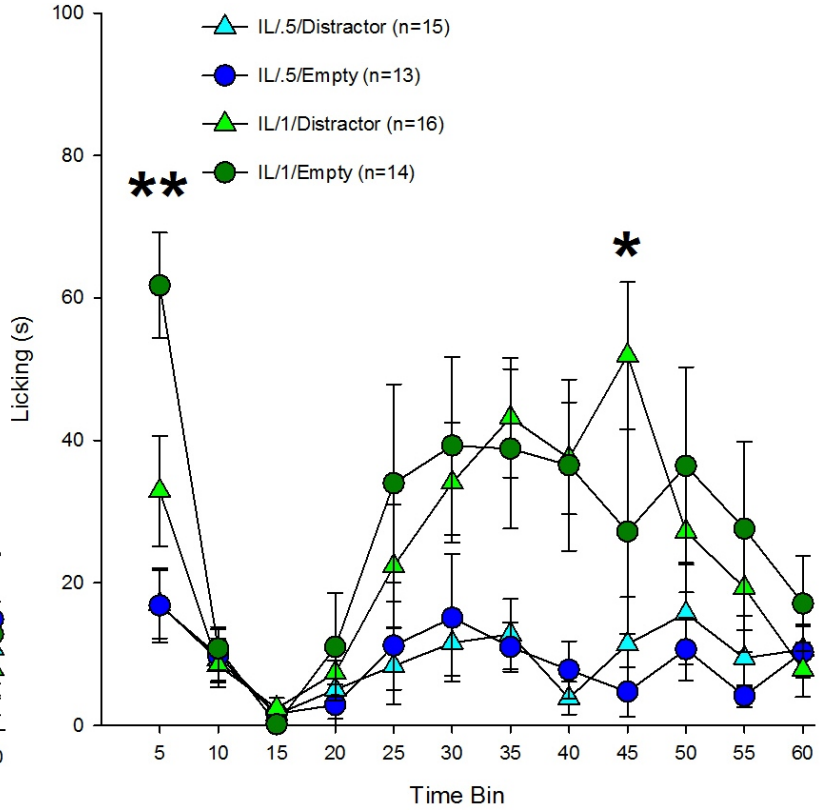

Figure 3-17

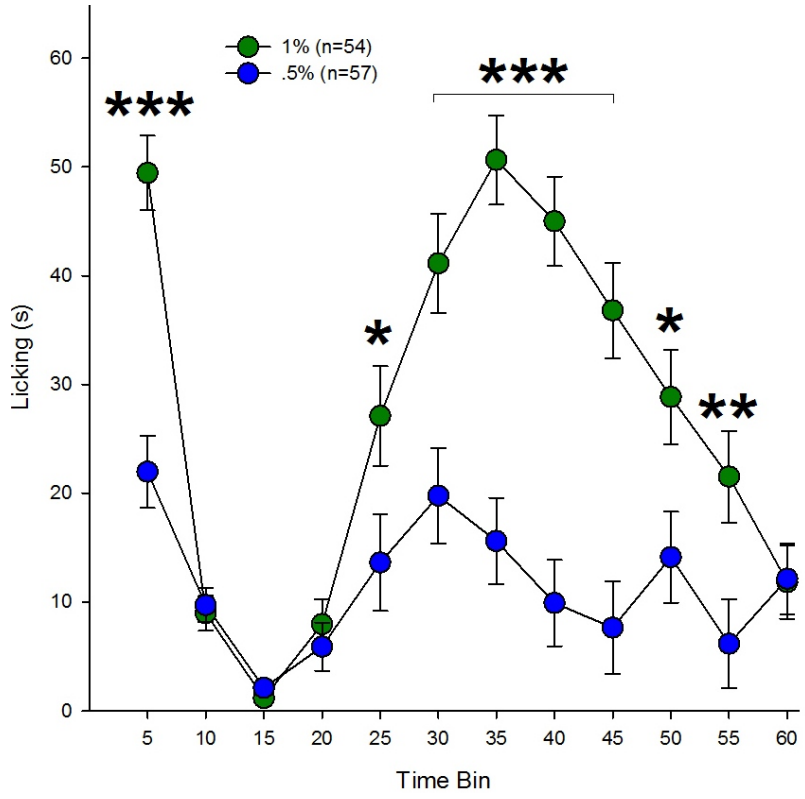

Figure 3-18

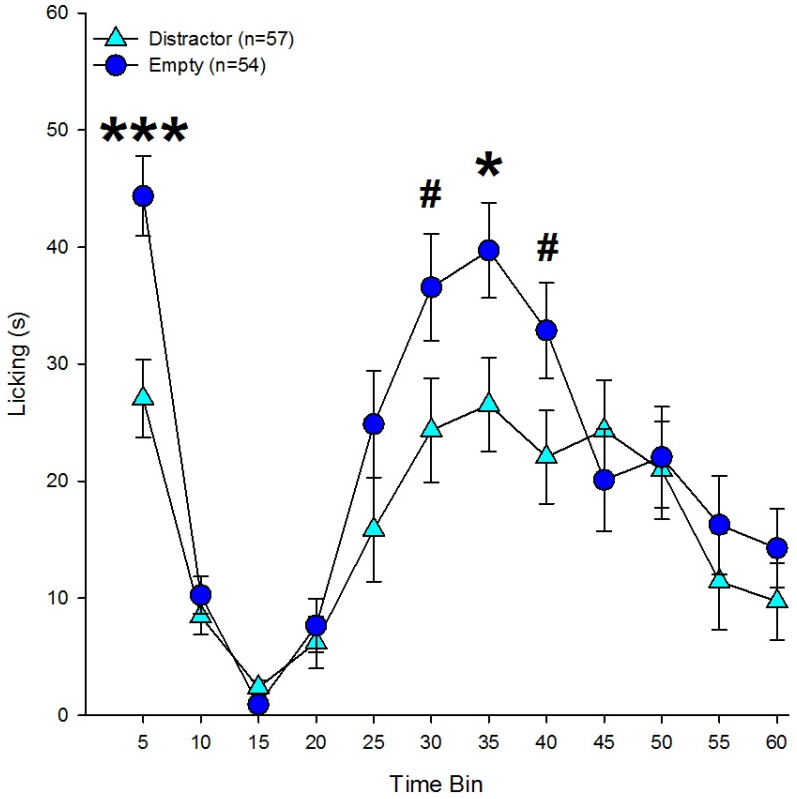

Figure 3-19

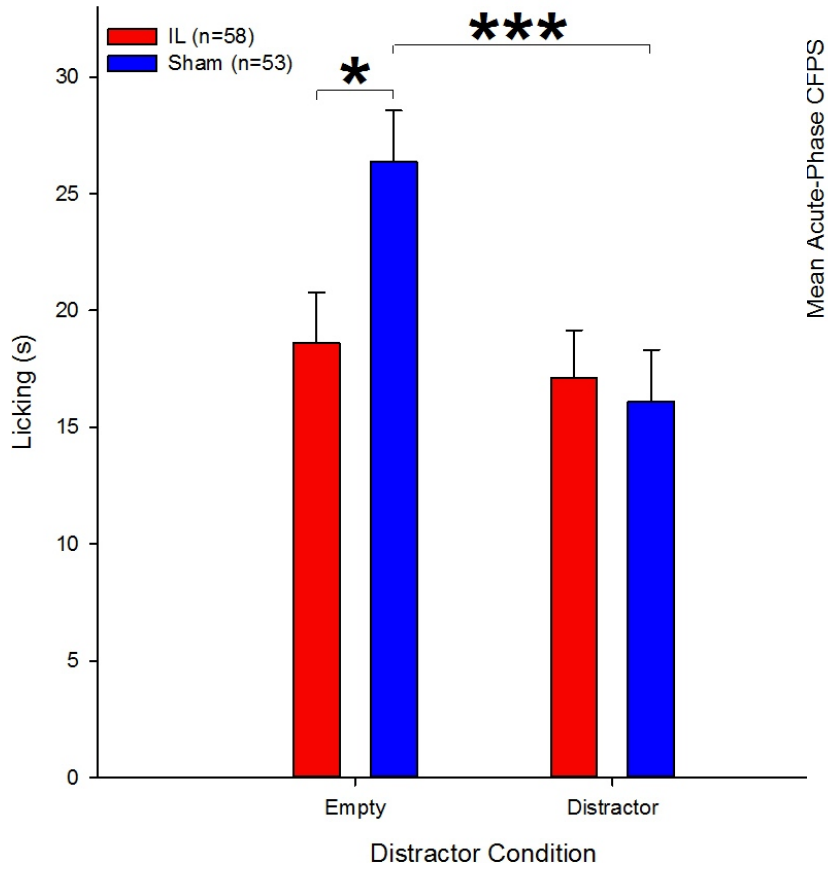

Figure 3-20

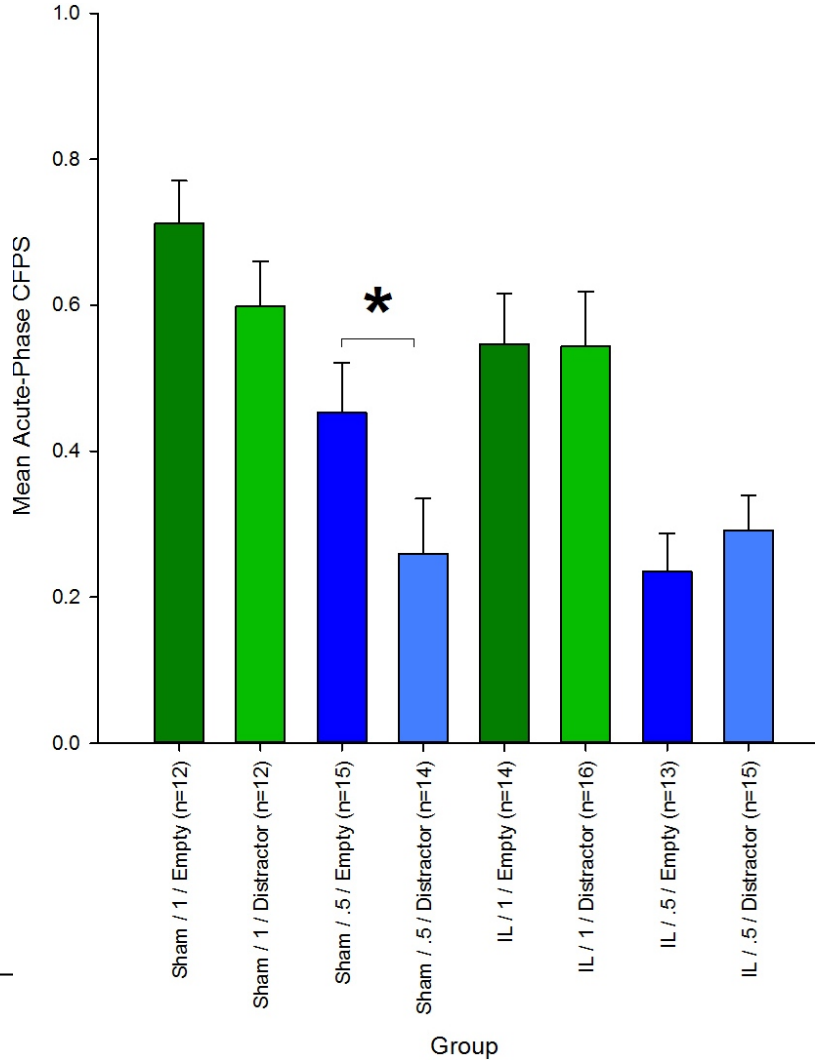

Figure 3-21

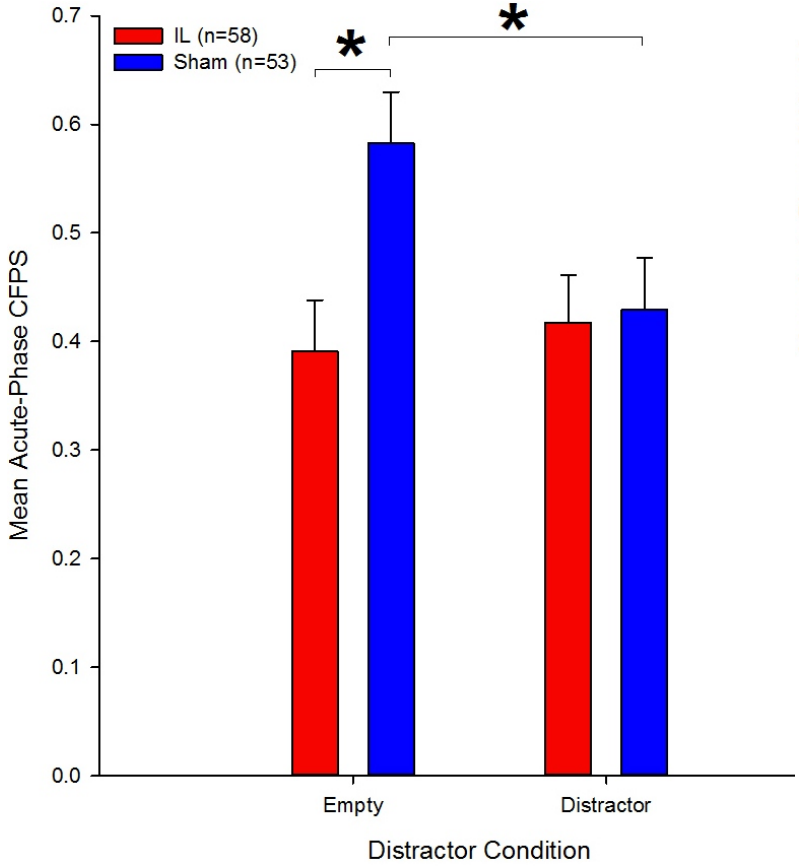

Figure 3-22

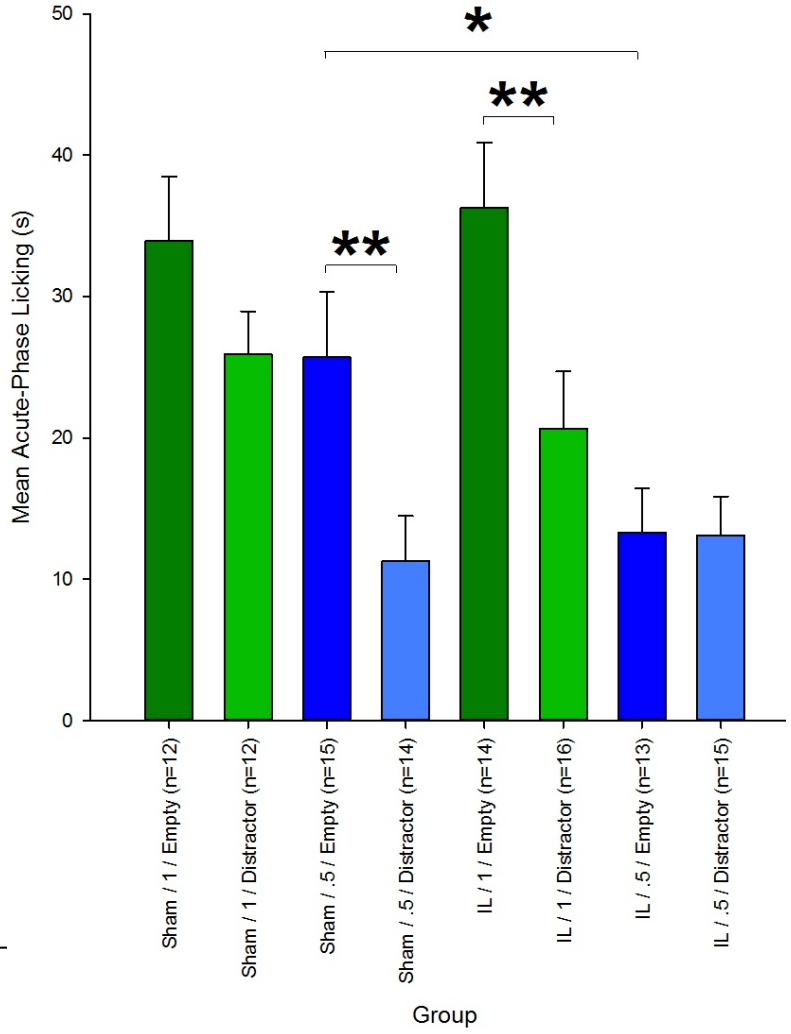

Figure 3-23

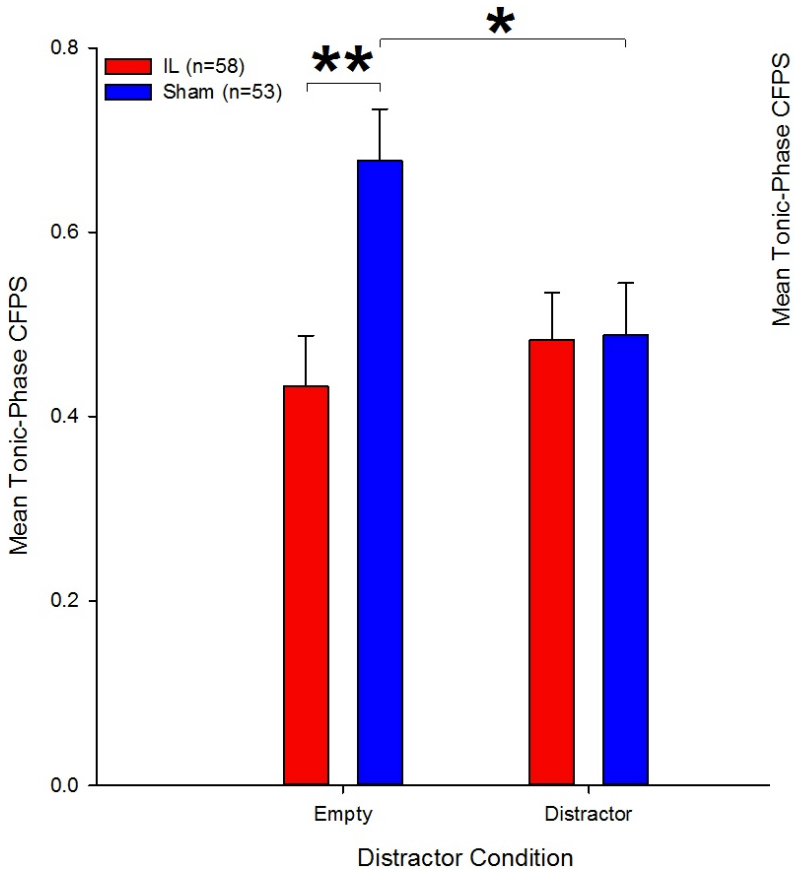

Figure 3-24

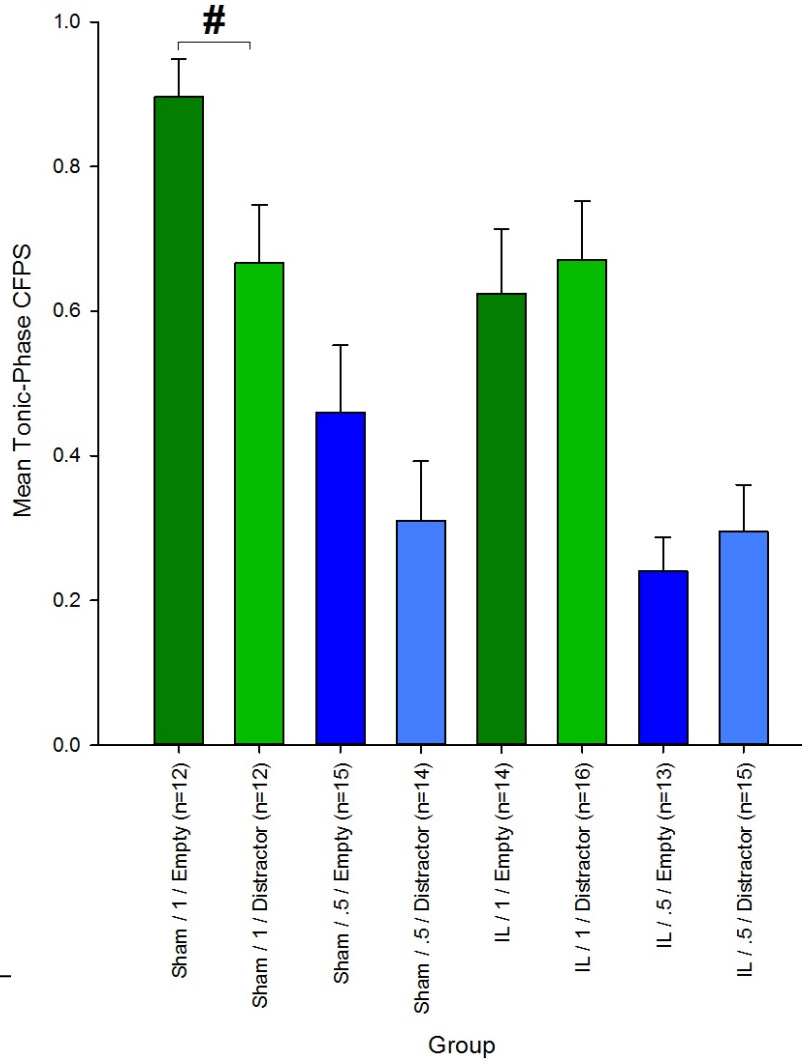

Figure 3-25

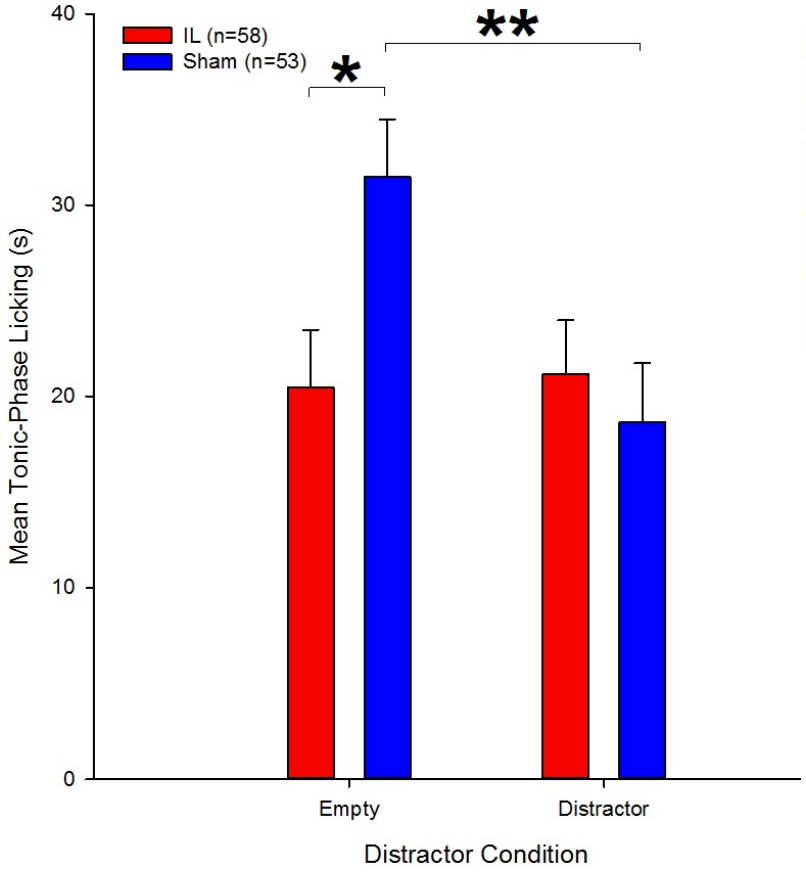

Figure 3-26

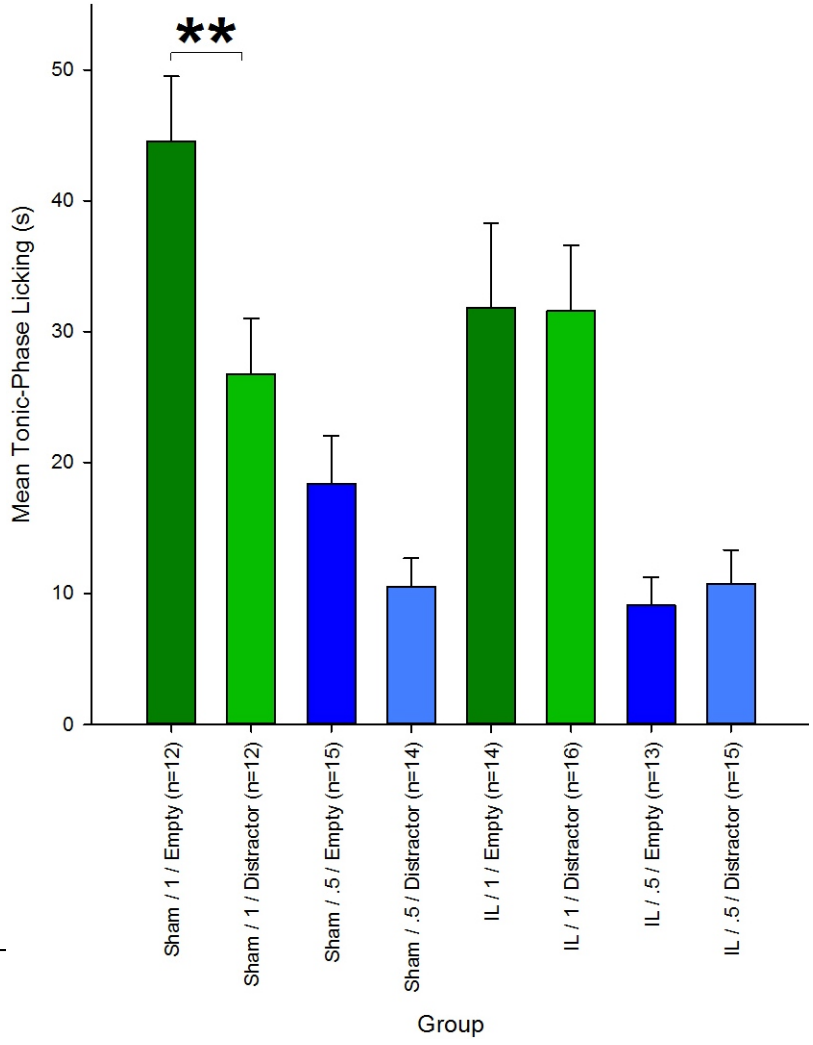

Figure 3-27

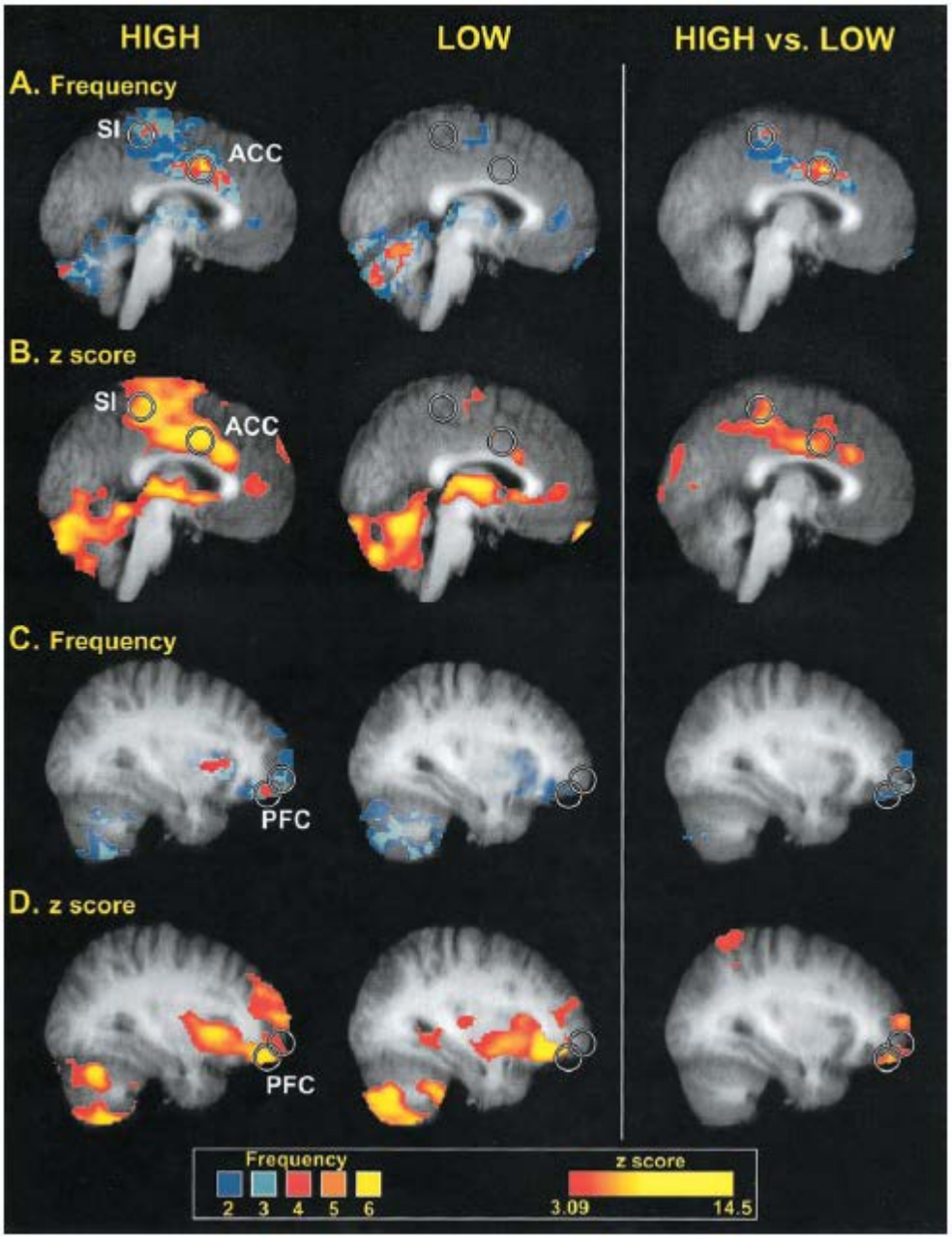
HIGH
LOW
HIGH vs. LOW
A. Frequency
SI
ACC
B. z score
SI
ACC
C. Frequency
PFC
D. z score
PFC
Frequency
2 3 4 5 6
z score
3.09
14.5

Figure 4-1

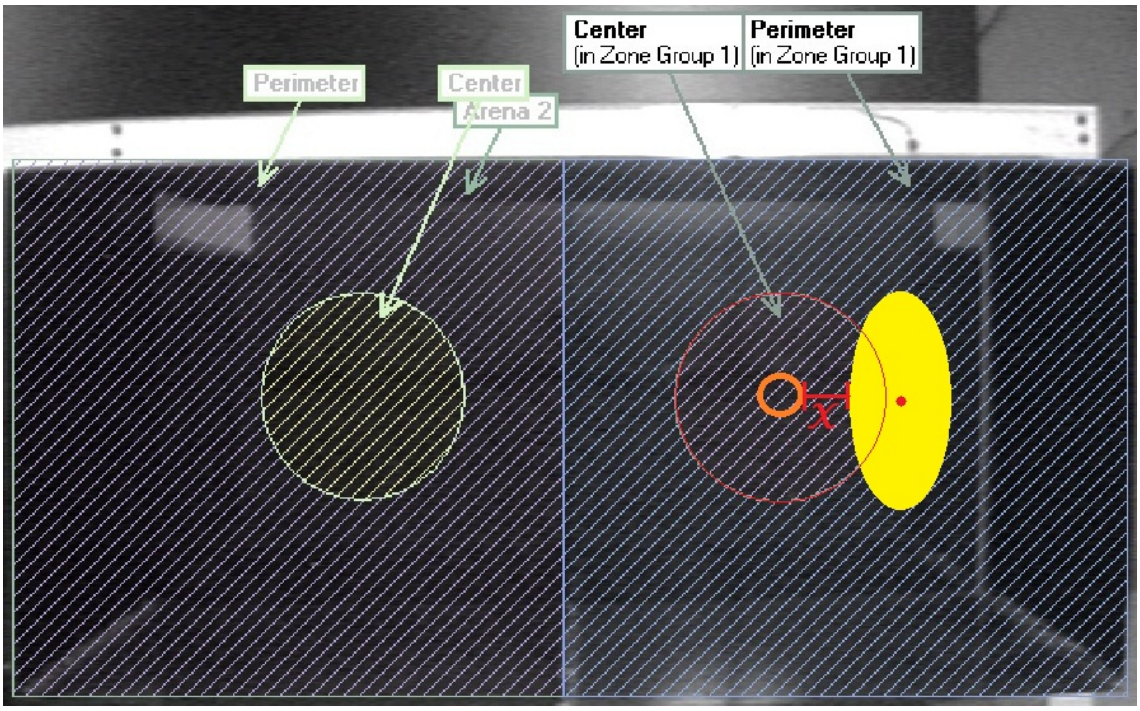
Perimeter
Center
Arena 2
Center
(in Zone Group 1)
Perimeter
(in Zone Group 1)

Figure 4-2

Appendix B

Tables

Table 3-2

Distance Traveled during Habituation by Lesion Groups

	IL Mean	*IL SEM*	*Sham Mean*	*Sham SEM*
Habituation Day 1	1022.94	40.66	952.80	36.70
Habituation Day 2	1001.52	45.55	934.69	39.85
Habituation Day 3	958.56	38.39	1011.66	46.62
Habituation Day 4	843.78	41.86	894.58	36.66
Habituation Day 5	911.47	60.23	824.07	43.95
Habituation Day 6	923.70	55.90	909.39	50.66
Habituation Day 7	934.16	76.96	897.26	45.32

Table 3-3

Time Spent Moving during Habituation by Lesion Groups

	IL Mean	*IL SEM*	*Sham Mean*	*Sham SEM*
Habituation Day 1	168.12	7.69	166.50	8.21
Habituation Day 2	139.74	6.84	134.52	7.19
Habituation Day 3	121.66	6.32	127.33	8.26
Habituation Day 4	118.18	5.60	114.00	4.96
Habituation Day 5	110.39	5.50	117.51	6.84
Habituation Day 6	112.91	6.69	117.98	5.99
Habituation Day 7	114.02	6.01	120.09	7.06

Table 3-4

Time Spent in Center Zone during Habituation by Lesion Groups

	IL Mean	*IL SEM*	*Sham Mean*	*Sham SEM*
Habituation Day 1	82.09	11.43	79.66	11.36
Habituation Day 2	37.41	7.37	31.46	5.87
Habituation Day 3	31.96	7.41	22.96	5.43
Habituation Day 4	50.33	13.20	37.74	10.65
Habituation Day 5	36.01	9.80	46.24	10.68
Habituation Day 6	33.54	7.46	29.88	8.12
Habituation Day 7	32.79	8.23	49.39	13.89

Table 3-5

Time Spent in Periphery during Habituation by Lesion Groups

	IL Mean	*IL SEM*	*Sham Mean*	*Sham SEM*
Habituation Day 1	517.69	11.42	519.92	11.37
Habituation Day 2	562.46	7.37	568.42	5.86
Habituation Day 3	567.07	7.43	576.93	5.42
Habituation Day 4	549.63	13.21	562.18	10.65
Habituation Day 5	563.02	9.79	553.49	10.67
Habituation Day 6	566.36	7.46	569.30	8.10
Habituation Day 7	566.93	8.22	550.46	13.90

Table 3-6

Mean Heading-to-Center Value during Habituation by Lesion Groups

	IL Mean	*IL SEM*	*Sham Mean*	*Sham SEM*
Habituation Day 1	-2.83	10.46	-1.91	11.00
Habituation Day 2	14.07	9.60	7.61	10.08
Habituation Day 3	9.10	10.66	23.81	11.20
Habituation Day 4	11.53	10.09	10.20	10.61
Habituation Day 5	4.97	9.56	7.73	10.05
Habituation Day 6	-4.87	9.95	3.28	10.45
Habituation Day 7	-11.24	10.23	-9.08	10.75

Table 3-7

*Test Day Distance Traveled - Time*Concentration*Distractor*

Time Bin	*.5/Distractor Mean*	*.5/Distractor SEM*	*.5/Empty Mean*	*.5/Empty SEM*	*1/Distractor Mean*	*1/Distractor SEM*	*1/Empty Mean*	*1/Empty SEM*
5	296.46	27.81	239.73	28.83	296.442	29.7116	224.6823	29.9632
10	217.74	21.34	184.13	22.12	200.3644	22.796	169.5542	22.989
15	220.92	28.44	196.99	29.48	176.059	30.3804	177.4345	30.6377
20	221.85	28.82	172.39	29.87	202.4702	30.7897	192.8761	31.0505
25	252.3363	34.2183	271.1755	35.4663	223.1236	36.5523	186.8394	36.8619
30	229.5147	25.0298	174.2233	25.9426	239.4174	26.737	225.6411	26.9634
35	283.5149	35.0639	171.9415	36.3427	232.5628	37.4555	236.0308	37.7727
40	239.8029	26.1489	169.8429	27.1025	256.3942	27.9324	212.0574	28.169
45	184.0798	27.8686	209.1979	28.8849	262.843	29.7694	177.9457	30.0215
50	231.5049	46.5413	216.5531	48.2386	224.8302	49.7157	226.0078	50.1368
55	181.483	27.59	188.0646	28.5962	213.1253	29.4718	189.7162	29.7214
60	164.356	32.5433	247.837	33.7302	286.7178	34.763	174.8359	35.0574

Table 3-8

*Test Day Time Spent Moving - Time*Concentration*

Time Bin	*.5% Mean*	*.5% SEM*	*1% Mean*	*1% SEM*
5	93.47	4.05	96.41	4.27
10	51.56	4.14	45.45	4.36
15	39.43	3.18	33.31	3.35
20	45.70	4.85	37.59	5.11
25	49.8863	3.8922	51.1113	4.0998
30	49.9053	4.2174	64.1282	4.4425
35	49.2234	3.9711	63.5372	4.183
40	44.8308	4.0004	60.374	4.2139
45	45.7292	4.6444	51.4619	4.8922
50	45.8831	4.3854	46.0889	4.6194
55	38.8064	4.3103	43.6499	4.5403
60	43.0538	4.2813	45.1921	4.5098

Table 3-9

*Test Day Time Spent Moving - Concentration*Distractor*

Concentration	*Distractor Mean*	*Distractor SEM*	*Empty Mean*	*Empty SEM*
0.50%	47.68	3.25	51.90	3.37
1%	58.89	3.48	47.50	3.51

Table 3-10

*Test Day Time Spent in Center Zone - Time*Distraction*

Time Bin	*Distractor Mean*	*Distractor SEM*	*Empty Mean*	*Empty SEM*
5	5.17	3.56	26.09	3.64
10	1.43	1.77	6.58	1.80
15	1.08	2.83	7.31	2.90
20	2.19	4.40	12.18	4.49
25	4.2357	4.5278	10.2292	4.6258
30	3.1648	1.8051	5.8442	1.8441
35	9.9263	2.3066	2.9074	2.3565
40	6.9747	3.2944	7.8489	3.3657
45	8.0603	5.8139	14.1393	5.9397
50	3.2963	4.419	6.8414	4.5147
55	2.2819	4.2309	10.9486	4.3225
60	2.5023	4.2228	8.9431	4.3142

Table 3-11

CFPS Across Time in Sham Groups

		Sham/.5/Distractor	Sham/.5/Empty	Sham/1/Distractor	Sham/1/Empty
5	M	0.29	0.55	0.70	0.90
	SEM	0.08	0.08	0.06	0.06
10	M	0.23	0.36	0.49	0.52
	SEM	0.08	0.09	0.09	0.08
15	M	0.13	0.06	0.16	0.01
	SEM	0.07	0.03	0.08	0.00
20	M	0.18	0.18	0.16	0.27
	SEM	0.08	0.07	0.09	0.09
25	M	0.27	0.44	0.38	0.46
	SEM	0.11	0.12	0.13	0.13
30	M	0.41	0.46	0.65	0.73
	SEM	0.12	0.12	0.12	0.12
35	M	0.36	0.48	0.73	1.00
	SEM	0.10	0.12	0.11	0.11
40	M	0.25	0.47	0.71	1.01
	SEM	0.08	0.12	0.12	0.10
45	M	0.33	0.48	0.61	0.99
	SEM	0.11	0.11	0.11	0.10
50	M	0.34	0.47	0.73	0.91
	SEM	0.11	0.11	0.11	0.08
55	M	0.23	0.43	0.59	0.88
	SEM	0.09	0.11	0.11	0.12
60	M	0.25	0.42	0.65	0.75
	SEM	0.10	0.12	0.13	0.12

Table 3-12

CFPS Across Time in IL Groups

		IL/.5/Distractor	IL/.5/Empty	IL/1/Distractor	IL/1/Empty
5	M	0.35	0.27	0.61	0.78
	SEM	0.07	0.06	0.08	0.09
10	M	0.23	0.21	0.48	0.31
	SEM	0.05	0.07	0.09	0.05
15	M	0.04	0.03	0.20	0.07
	SEM	0.03	0.01	0.09	0.05
20	M	0.10	0.08	0.22	0.13
	SEM	0.07	0.05	0.10	0.09
25	M	0.21	0.14	0.43	0.44
	SEM	0.10	0.06	0.12	0.14
30	M	0.24	0.22	0.55	0.54
	SEM	0.07	0.10	0.10	0.14
35	M	0.24	0.29	0.66	0.58
	SEM	0.07	0.08	0.11	0.13
40	M	0.13	0.29	0.67	0.61
	SEM	0.05	0.09	0.10	0.13
45	M	0.29	0.16	0.87	0.65
	SEM	0.10	0.07	0.09	0.14
50	M	0.34	0.25	0.71	0.73
	SEM	0.11	0.08	0.11	0.13
55	M	0.42	0.15	0.69	0.65
	SEM	0.10	0.05	0.12	0.11
60	M	0.42	0.31	0.55	0.63
	SEM	0.10	0.08	0.11	0.10

Table 3-13

*CFPS Time*Concentration*

		1%	0.50%
5	M	0.75	0.36
	SEM	0.04	0.04
10	M	0.45	0.26
	SEM	0.04	0.04
15	M	0.11	0.07
	SEM	0.03	0.03
20	M	0.19	0.14
	SEM	0.04	0.04
25	M	0.43	0.26
	SEM	0.06	0.06
30	M	0.62	0.33
	SEM	0.06	0.06
35	M	0.74	0.34
	SEM	0.05	0.05
40	M	0.75	0.28
	SEM	0.05	0.05
45	M	0.78	0.32
	SEM	0.05	0.05
50	M	0.77	0.35
	SEM	0.06	0.05
55	M	0.70	0.31
	SEM	0.05	0.05
60	M	0.64	0.35
	SEM	0.06	0.05

Table 3-14

*CFPS Time*Distractor*

		Distractor	Empty
5	M	0.49	0.62
	SEM	0.04	0.04
10	M	0.36	0.35
	SEM	0.04	0.04
15	M	0.13	0.04
	SEM	0.03	0.03
20	M	0.17	0.16
	SEM	0.04	0.04
25	M	0.32	0.37
	SEM	0.06	0.06
30	M	0.46	0.49
	SEM	0.06	0.06
35	M	0.50	0.59
	SEM	0.05	0.05
40	M	0.44	0.60
	SEM	0.05	0.05
45	M	0.52	0.57
	SEM	0.05	0.05
50	M	0.53	0.59
	SEM	0.05	0.06
55	M	0.48	0.53
	SEM	0.05	0.05
60	M	0.47	0.53
	SEM	0.05	0.06

Table 3-15

*CFPS Lesion*Distractor*

	M	*SEM*
IL/Empty	0.354	0.045
IL/Distractor	0.401	0.042
Sham/Empty	0.552	0.046
Sham/Distractor	0.410	0.046

Table 3-16

Licking Across Time in Sham Groups

		Sham/.5/Distractor	Sham/.5/Empty	Sham/1/Distractor	Sham/1/Empty
5	M	16.58	37.49	41.76	61.35
	SEM	6.23	7.45	5.31	8.14
10	M	5.96	13.89	10.12	6.58
	SEM	2.42	3.67	3.25	2.32
15	M	3.46	1.77	2.07	0.08
	SEM	2.14	1.22	1.58	0.08
20	M	5.87	9.86	6.60	6.89
	SEM	2.72	4.69	4.48	4.12
25	M	4.72	30.23	28.06	24.02
	SEM	3.25	8.88	12.18	9.43
30	M	15.94	36.48	35.78	55.42
	SEM	5.78	10.21	8.21	10.54
35	M	11.16	27.51	39.00	81.55
	SEM	3.89	9.62	8.96	9.73
40	M	7.20	20.85	39.73	66.28
	SEM	2.53	7.06	10.24	12.77
45	M	5.50	9.03	28.63	39.43
	SEM	1.95	3.23	9.07	11.98
50	M	15.27	14.85	25.53	26.23
	SEM	7.26	5.94	8.18	8.99
55	M	6.94	4.20	10.04	29.15
	SEM	3.31	2.43	3.87	17.82
60	M	11.66	15.91	8.72	13.75
	SEM	6.75	9.18	4.17	11.62

Table 3-17

Licking Across Time in IL Groups

		IL/.5/Distractor	IL/.5/Empty	IL/1/Distractor	IL/1/Empty
5	M	16.99	16.84	32.88	61.76
	SEM	4.78	5.20	7.75	7.42
10	M	9.23	9.76	8.43	10.79
	SEM	3.01	3.74	3.05	3.02
15	M	1.56	1.64	2.40	0.14
	SEM	1.03	0.90	1.42	0.12
20	M	5.01	2.86	7.34	11.00
	SEM	4.10	2.86	3.37	7.54
25	M	8.35	11.20	22.36	33.97
	SEM	5.39	6.18	8.66	13.90
30	M	11.57	15.09	34.08	39.26
	SEM	4.58	8.99	8.46	12.47
35	M	12.79	10.99	43.18	38.82
	SEM	4.94	3.44	8.41	11.21
40	M	3.81	7.80	37.49	36.51
	SEM	2.31	4.03	7.87	12.02
45	M	11.39	4.71	51.91	27.19
	SEM	6.59	3.46	10.30	14.37
50	M	15.71	10.67	27.18	36.41
	SEM	7.13	4.37	8.52	13.86
55	M	9.43	4.12	19.31	27.59
	SEM	3.93	1.54	9.01	12.18
60	M	10.65	10.31	7.81	17.09
	SEM	3.53	3.57	3.85	6.72

Table 3-18

*Licking Time*Concentration*

		1%	0.50%
5	M	49.44	21.97
	SEM	3.43	3.32
10	M	8.98	9.71
	SEM	1.60	1.55
15	M	1.17	2.11
	SEM	0.65	0.63
20	M	7.96	5.90
	SEM	2.29	2.22
25	M	27.10	13.63
	SEM	4.58	4.44
30	M	41.13	19.77
	SEM	4.56	4.42
35	M	50.64	15.61
	SEM	4.10	3.97
40	M	45.00	9.92
	SEM	4.09	3.96
45	M	36.79	7.66
	SEM	4.41	4.27
50	M	28.84	14.12
	SEM	4.33	4.19
55	M	21.52	6.17
	SEM	4.21	4.08
60	M	11.84	12.13
	SEM	3.38	3.27

Table 3-19

*Licking Time*Distractor*

		Distractor	Empty
5	M	27.05	44.36
	SEM	3.34	3.42
10	M	8.43	10.26
	SEM	1.55	1.59
15	M	2.37	0.91
	SEM	0.63	0.65
20	M	6.21	7.65
	SEM	2.23	2.28
25	M	15.87	24.85
	SEM	4.45	4.56
30	M	24.34	36.56
	SEM	4.43	4.54
35	M	26.53	39.72
	SEM	3.98	4.08
40	M	22.06	32.86
	SEM	3.98	4.08
45	M	24.36	20.09
	SEM	4.29	4.40
50	M	20.92	22.04
	SEM	4.20	4.31
55	M	11.43	16.26
	SEM	4.09	4.20
60	M	9.71	14.26
	SEM	3.28	3.36

Table 3-20

*Licking Lesion*Distractor*

	M	*SEM*
IL/Empty	18.605	2.177
IL/Distractor	17.118	2.032
Sham/Empty	26.366	2.189
Sham/Distractor	16.095	2.224

Table 3-21

Acute-Phase CFPS in Each Experimental Condition

	M	*SEM*
IL/.5/Distractor	0.29	0.05
IL/.5/Empty	0.24	0.05
IL/1/Distractor	0.54	0.08
IL/1/Empty	0.55	0.07
Sham/.5/Distractor	0.26	0.08
Sham/.5/Empty	0.45	0.07
Sham/1/Distractor	0.60	0.06
Sham/1/Empty	0.71	0.06

Table 3-22

*Acute-Phase CFPS Lesion*Distractor*

	M	*SEM*
IL/Empty	0.391	0.047
IL/Distractor	0.417	0.0438
Sham/Empty	0.582	0.0472
Sham/Distractor	0.429	0.048

Table 3-23

Acute-Phase Licking in Each Experimental Condition

	M	*SEM*
IL/.5/Distractor	13.11	2.75
IL/.5/Empty	13.30	3.13
IL/1/Distractor	20.65	4.04
IL/1/Empty	36.28	4.62
Sham/.5/Distractor	11.27	3.20
Sham/.5/Empty	25.69	4.64
Sham/1/Distractor	25.94	3.01
Sham/1/Empty	33.97	4.49

Table 3-24

*Tonic-Phase CFPS Lesion*Distractor*

	M	*SEM*
IL/Empty	0.433	0.0553
IL/Distractor	0.483	0.0516
Sham/Empty	0.678	0.0556
Sham/Distractor	0.489	0.0565

Table 3-25

Tonic-Phase CFPS in Each Experimental Condition

	M	*SEM*
IL/.5/Distractor	0.29	0.07
IL/.5/Empty	0.24	0.05
IL/1/Distractor	0.67	0.08
IL/1/Empty	0.62	0.09
Sham/.5/Distractor	0.31	0.08
Sham/.5/Empty	0.46	0.09
Sham/1/Distractor	0.67	0.08
Sham/1/Empty	0.90	0.05

Table 3-26

*Tonic-Phase Licking Lesion*Distractor*

	M	*SEM*
IL/Empty	20.468	3.0093
IL/Distractor	21.164	2.808
Sham/Empty	31.473	3.026
Sham/Distractor	18.650	3.0736

Table 3-27

Tonic-Phase Licking in Each Experimental Condition

	M	*SEM*
IL/.5/Distractor	10.76	2.56
IL/.5/Empty	9.10	2.15
IL/1/Distractor	31.56	5.08
IL/1/Empty	31.84	6.46
Sham/.5/Distractor	10.52	2.17
Sham/.5/Empty	18.40	3.63
Sham/1/Distractor	26.78	4.23
Sham/1/Empty	44.54	4.98

Appendix C

Figure Captions

Figure 3-1 Photomicrographs of electrolytic lesion to the IL shown in coronal cross-section. Images from bottom-left to upper-right depict the most anterior extent of the damage to the most posterior extent of the damage.

Figure 3-2 Distance traveled (centimeters) in IL and sham conditions on each 10-minute habituation trial. All values presented as mean ± SEM. There was a general trend of decreasing distance traveled over time, but there were no statistically significant differences between lesion conditions on any habituation trial.

Figure 3-3 Time spent moving (seconds) in IL and sham conditions on each 10-minute habituation trial. All values presented as mean ± SEM. There was a general trend of decreasing movement over time, but there were no statistically significant differences between lesion conditions on any habituation trial.

Figure 3-4 Time spent in center zone (seconds) in IL and sham conditions on each 10-minute habituation trial. All values presented as mean ± SEM. Both groups decreased time spent in the center zone after the first day of habituation, but there were no statistically significant differences between lesion conditions on any habituation trial.

Figure 3-5 Time spent in periphery (seconds) in IL and sham conditions on each 10-minute habituation trial. All values presented as mean ± SEM. Both groups increased time spent in the periphery after the first day of habituation, but there were no statistically significant differences between lesion conditions on any habituation trial.

Figure 3-6 Mean Heading-to-Center Value (heading-to-center angle as measured by Figure 2-1). All values presented as mean ± SEM. There were no statistically significant differences

between lesion conditions in their orientation towards the center of the chamber on any habituation trial.

Figure 3-7 Distance Traveled on Test Day (seconds). All values presented as mean ± SEM. Data is depicted as Concentration/Distractor groups across Test Day time bins because of the significant interaction detected between concentration, distractor, and time. All pairwise comparisons were made relative to the opposite distractor condition in the same concentration condition. * indicates $p < .05$.

Figure 3-8 Time spent moving on Test Day (seconds). All values presented as mean ± SEM. Data is collapsed across concentration groups and depicted across Test Day time bins because of the marginally significant interaction detected between time and concentration. The high concentration of formalin showed increased locomotion at the beginning of the tonic phase of the test. * indicates $p < .05$.

Figure 3-9 Time spent moving on Test Day (seconds). All values presented as mean ± SEM. Data is depicted as Concentration/Distractor groups because of the significant interaction detected between concentration and distractor. The distractor increased movement in the high concentration, but not in the low concentration, possibly indicating increased attention to the distractor in the 1% condition. * indicates $p < .05$.

Figure 3-10 Time spent in center zone on Test Day (seconds). All values presented as mean ± SEM. Data is collapsed across distractor conditions and depicted across Test Day time bins. The distractor condition spent significantly less time in the center zone during the first 5 minutes, which is most likely due to a limited ability to enter the center zone. The distractor

condition also spent significantly more time in the center zone during the 35-minute time bin. *** indicates $p < .001$; * indicates $p < .05$.

Figure 3-11 Composite Formalin Pain Scores in all sham conditions across the entire 60-minute formalin test. All values presented as mean ± SEM. Green 1% conditions generally displayed higher acute and tonic-phase CFPS than blue .5% conditions. Light-colored distractor groups were generally lower than respective dark-colored empty groups. All pairwise comparisons were made relative to the matched group in the other distractor condition. # indicates a marginally significant difference $p < .072$, ms; * indicates $p < .05$.

Figure 3-12 Composite Formalin Pain Scores in all IL conditions across the entire 60-minute formalin test. All values presented as mean ± SEM. Green 1% conditions generally displayed higher acute and tonic-phase CFPS than blue .5% conditions. However, light-colored distractor groups were not significantly lower than respective dark-colored empty groups at any time bin. This suggests that the IL lesion attenuated distraction analgesia.

Figure 3-13 Composite Formalin Pain Scores collapsed across concentration conditions and depicted across entire 60-minute formalin test. All values presented as mean ± SEM. The 1% condition was associated with significantly higher CFPS than the .5% condition at all time bins in the acute and tonic phases of the formalin test. # indicates a marginally significant difference $p < .072$; *** indicates $p < .001$.

Figure 3-14 Composite Formalin Pain Scores collapsed across distractor conditions and depicted across entire 60-minute formalin test. All values presented as mean ± SEM. Relative to the empty condition, the distractor condition was associated with significantly lower CFPS at the 5-minute, 15-minute, and 40-minute time bins. * indicates $p < .05$.

Figure 3-15 Composite Formalin Pain Scores collapsed across entire 60-minute formalin test and depicted in Distractor/Lesion conditions. All values presented as mean ± SEM. CFPS was significantly different between IL and sham conditions in the empty chamber, indicating that the IL lesion decreased formalin pain scores. Critically, a significant difference was detected between Sham/Empty and Sham/Distractor conditions, but not between IL/Empty and IL/Distractor conditions, which suggests that the lesion attenuated the analgesic effect of distraction on CFPS. * indicates $p < .05$; ** indicates $p < .01$.

Figure 3-16 Licking behavior in all sham conditions across the entire 60-minute formalin test. All values presented as mean ± SEM. Green 1% conditions generally displayed more licking behavior than blue .5% conditions. The magnitude of the distractor's effect was largest in the tonic phase of the 1% groups at the 35 and 40-min time bins. All pairwise comparisons were made relative to the matched group in the other distractor condition. # indicates a marginally significant difference $p < .072$, ms; * indicates $p < .05$; *** indicates $p < .001$.

Figure 3-17 Licking behavior in all IL conditions across the entire 60-minute formalin test. All values presented as mean ± SEM. Green 1% conditions generally displayed more licking behavior than blue .5% conditions. However, the light-colored distractor conditions were not consistently lower than the dark-colored empty conditions. The 5-minute time bin was the only bin to show a significant reduction of licking by the distractor, which only occurred in the 1% groups. The 45-min time bin unexpectedly showed that licking was significantly elevated in the IL/1/Distractor condition relative to the respective empty condition. All pairwise comparisons were made relative to the matched group in the other distractor condition. * indicates $p < .05$; ** indicates $p < .01$.

Figure 3-18 Licking behavior collapsed across each formalin concentration and depicted across the entire 60-minute formalin test. All values presented as mean ± SEM. The 1% condition was associated with significantly more licking behavior than the .5% condition at most of the time bins across the formalin test. * indicates $p < .05$; ** indicates $p < .01$; *** indicates $p < .001$.

Figure 3-19 Licking behavior collapsed across distractor conditions and depicted across the entire 60-minute formalin test. All values presented as mean ± SEM. The distractor condition was associated with significantly less licking then the empty condition at the 5-minute and 35-minute time bins. The distractor condition was also associated with marginally less licking then the empty condition at the 30-minute and 40-minute time points. This indicates that the distractor meaningfully decreased licking behavior over time. # indicates a marginally significant difference $p < .072$, ms; * indicates $p < .05$; *** indicates $p < .001$.

Figure 3-20 Licking behavior collapsed across time and depicted in Distractor/Lesion conditions. All values presented as mean ± SEM. The amount of licking behavior was significantly different between the IL and sham conditions in the empty chamber, indicating that the IL lesion decreased licking behavior. Critically, a significant difference was detected between the Sham/Empty and Sham/Distractor conditions, but not between the IL/Empty and IL/Distractor conditions, which suggests that the lesion attenuated the analgesic effect of distraction on licking behavior. * indicates $p < .05$; *** indicates $p < .001$.

Figure 3-21 Acute-phase Composite Formalin Pain Scores in each experimental condition. All values presented as mean ± SEM. The distractor significantly decreased acute-phase CFPS in Sham/.5 conditions, but not in the Sham/1, IL/.5, or IL/1 conditions. * indicates $p < .05$.

Figure 3-22 Acute-phase Composite Formalin Pain Scores collapsed across time and depicted in Distractor/Lesion conditions. All values presented as mean ± SEM. There was a significant difference between the IL and Sham groups in the empty chamber. Critically, distractor presence significantly decreased acute-phase CFPS in the sham animals, but not in the IL animals. * indicates $p < .05$.

Figure 3-23 Acute-phase licking behavior in each experimental condition. All values presented as mean ± SEM. The distractor significantly decreased acute-phase licking in Sham/.5 conditions. Unexpectedly, the distractor also significantly decreased acute-phase licking in IL/1 conditions, which was the only evidence that the distractor decreased formalin-induced behavior in IL animals. * indicates $p < .05$; ** indicates $p < .01$.

Figure 3-24 Tonic-phase Composite Formalin Pain Scores collapsed across time and presented in Distractor/Lesion conditions. All values presented as mean ± SEM. Mean tonic-phase CFPS was significantly different between the IL and sham conditions in the empty chamber, indicating that the IL lesion decreased mean tonic-phase CFPS. Critically, a significant difference was detected between Sham/Empty and Sham/Distractor conditions, but not between IL/Empty and IL/Distractor conditions, which suggests that the lesion attenuated the analgesic effect of distraction on mean tonic-phase CFPS. * indicates $p < .05$; ** indicates $p < .01$.

Figure 3-25 Tonic-phase CFPS in each experimental condition. All values presented as mean ± SEM. There was a marginally significant difference between the Sham/1/Empty and Sham/1/Distractor groups, indicating a marginally significant distraction analgesic effect. The distraction analgesic trend was also apparent in the difference between the Sham/.5/Empty and Sham/.5/Distractor conditions, but this difference did not reach statistical significance, $p = .16$, ns. In the IL conditions, there was no significant difference between IL/1/Empty and

IL/1/Distractor nor was there a significant difference between IL/.5/Empty and IL/.5/Distractor, indicating that there was no distraction analgesic effect in the IL condition. This suggests that the IL lesion attenuated the analgesic effect of distraction on mean tonic-phase CFPS. # indicates a marginally significant difference, $p = .054$, ms.

Figure 3-26 Tonic-phase licking behavior collapsed across time and depicted in Distractor/Lesion conditions. All values presented as mean ± SEM. Mean tonic-phase licking was significantly different between the IL and sham conditions in the empty chamber, indicating that the IL lesion decreased mean tonic-phase licking behavior. Critically, a significant difference was detected between Sham/Empty and Sham/Distractor conditions, but not between the IL/Empty and IL/Distractor conditions, which suggests that the lesion attenuated the analgesic effect of distraction on mean tonic-phase licking behavior. * indicates $p < .05$; ** indicates $p < .01$.

Figure 3-27 Mean tonic-phase licking behavior in each experimental condition. There was a significant difference between the Sham/1/Empty and Sham/1/Distractor conditions, indicating a significant distraction analgesic effect. The distraction analgesic trend was also apparent in the difference between the Sham/.5/Empty and Sham/.5/Distractor conditions, but this difference did not reach statistical significance, $p = .18$, ns. In the IL conditions, there was no significant difference between IL/1/Empty and IL/1/Distractor nor was there a significant difference between IL/.5/Empty and IL/.5/Distractor, indicating that there was no distraction analgesic effect in the IL condition. This suggests that the IL lesion attenuated the analgesic effect of distraction on mean tonic-phase licking. ** indicates $p < .01$.

Figure 4-1 Sagittal cross-sections of the human brain depicting regions that displayed different frequencies of activation between high-intensity (“HIGH”) and low-intensity pain (“LOW”). High-

intensity minus low-intensity pain is indicated by the column labeled “HIGH vs. LOW.” Circles indicate the locations of peak differences between high-intensity pain and low-intensity pain. Shading of brain regions in rows A and C corresponds to the number of subjects displaying statistically significant activation at a given voxel (frequency). Shading of brain regions in rows B and D corresponds to z-score of the pain-intensity group analysis. Slice locations in rows A and B are -2 mm from the midline, and slice locations in rows C and D are 32 mm from the midline (reproduced without permission from Coghill, McHaffie, and Yen, 2003).

Figure 4-2 Arena and center zones overlaid on a top-down camera-view schematic image of two of the testing arenas. Image depicts hypothetical scenario in which the body of the rat is partially within the center zone, but the center-point of the rat would be detected in the perimeter. The center-point of the rat would be recorded in the center zone only when distance $X \leq 1.5$ cm. Yellow oval = scale representation of the body of the rat; Red dot = center-point of rat recorded by Ethovision; Thin red circle = Center Zone boundary; Orange circle = space occupied by falcon tube; Distance X = the distance between the falcon tube and rat.

References

Alhani, F. (2010). The effect of programmed distraction on the pain caused by venipuncture among adolescents on hemodialysis. *Pain Management Nursing : Official Journal of the American Society of Pain Management Nurses*, *11*(2), 85–91. doi:10.1016/j.pmn.2009.03.005

Bantick, S. J., Wise, R. G., Ploghaus, A., Clare, S., Smith, S. M., & Tracey, I. (2002). Imaging how attention modulates pain in humans using functional MRI. *Brain : A Journal of Neurology*, *125*(Pt 2), 310–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11844731

Beecher, H. K. (1946). Pain in men wounded in battle. *Annals of Surgery*, *123*(1), 96–105.

Berman, S. M., Naliboff, B. D., Suyenobu, B., Labus, J. S., Stains, J., Ohning, G., … Mayer, E. a. (2008). Reduced brainstem inhibition during anticipated pelvic visceral pain correlates with enhanced brain response to the visceral stimulus in women with irritable bowel syndrome. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *28*(2), 349–59. doi:10.1523/JNEUROSCI.2500-07.2008

Birrell, J. M., & Brown, V. J. (2000). Medial frontal cortex mediates perceptual attentional set shifting in the rat. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *20*(11), 4320–4. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10818167

Boyette-Davis, J. A., Thompson, C. D., & Fuchs, P. N. (2008). Alterations in attentional mechanisms in response to acute inflammatory pain and morphine administration. *Neuroscience*, *151*(2), 558–63. doi:10.1016/j.neuroscience.2007.10.032

Buhle, J. T., Stevens, B. L., Friedman, J. J., & Wager, T. D. (2012). Distraction and placebo: two separate routes to pain control. *Psychological Science*, *23*(3), 246–53. doi:10.1177/0956797611427919

Bushnell, M. C., Ceko, M., & Low, L. a. (2013). Cognitive and emotional control of pain and its disruption in chronic pain. *Nature Reviews. Neuroscience*, *14*(7), 502–11. doi:10.1038/nrn3516

Bushnell, M. C., Duncan, G. H., Dubner, R., Jones, R. L., & Maixner, W. (1985). Attentional Influences on Noxious and Innocuous Cutaneous Heat Detection in Humans and Monkeys. *The Journal of Neuroscience*, *5*(5), 1103–1110.

CDC. (2011). *Prescription Painkiller Overdoses in the US* (pp. 1–4). Retrieved from http://www.cdc.gov/vitalsigns/painkilleroverdoses/index.html

Chudasama, Y., Nathwani, F., & Robbins, T. W. (2005). D-Amphetamine remediates attentional performance in rats with dorsal prefrontal lesions. *Behavioural Brain Research*, *158*(1), 97–107. doi:10.1016/j.bbr.2004.08.011

Chudasama, Y., & Robbins, T. W. (2003). Dissociable contributions of the orbitofrontal and infralimbic cortex to pavlovian autoshaping and discrimination reversal learning: further evidence for the functional heterogeneity of the rodent frontal cortex. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *23*(25), 8771–80. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/14507977

Coderre, T. J., Fundytus, M. E., McKenna, J. E., Dalal, S., & Melzack, R. (1993). The formalin test: a validation of the weighted-scores method of behavioural pain rating. *Pain*, *54*(1), 43–50. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8378102

Coghill, R. C., McHaffie, J. G., & Yen, Y.-F. (2003). Neural correlates of interindividual differences in the subjective experience of pain. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(14), 8538–42. doi:10.1073/pnas.1430684100

Corbetta, M., & Shulman, G. L. (2002). Control of goal-directed and stimulus-driven attention in the brain. *Nature Reviews. Neuroscience*, *3*(3), 201–15. doi:10.1038/nrn755

Crombez, G., Eccleston, C., Van den Broeck, a, Van Houdenhove, B., & Goubert, L. (2002). The effects of catastrophic thinking about pain on attentional interference by pain: no mediation of negative affectivity in healthy volunteers and in patients with low back pain. *Pain Research & Management : The Journal of the Canadian Pain Society = Journal de La Société Canadienne Pour Le Traitement de La Douleur*, *7*(1), 31–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16231065

Dalley, J. W., Cardinal, R. N., & Robbins, T. W. (2004). Prefrontal executive and cognitive functions in rodents: neural and neurochemical substrates. *Neuroscience and Biobehavioral Reviews*, *28*(7), 771–84. doi:10.1016/j.neubiorev.2004.09.006

Derbyshire, S. W. G., Jones, a K. P., Creed, F., Starz, T., Meltzer, C. C., Townsend, D. W., … Firestone, L. (2002). Cerebral responses to noxious thermal stimulation in chronic low back pain patients and normal controls. *NeuroImage*, *16*(1), 158–68. doi:10.1006/nimg.2002.1066

Derogatis, L. (1977). *SCL-90-R Manual I*. Baltimore, MD: Johns Hopkins University School of Medicine.

Dias, R., Robbins, T. W., & Roberts, A. C. (1996). Primate Analogue of the Wisconsin Card Sorting Test : Effects of Excitotoxic Lesions of the Prefrontal Cortex in the Marmoset. *Behavioral Neuroscience*, *110*(5), 872–886.

Donahue, R. R., LaGraize, S. C., & Fuchs, P. N. (2001). Electrolytic lesion of the anterior cingulate cortex decreases inflammatory, but not neuropathic nociceptive behavior in rats. *Brain Research*, *897*(1-2), 131–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11282366

Dubuisson, D., & Dennis, S. G. (1977). The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. *Pain*, *4*(2), 161–74. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/564014

Eccleston, C. (1995). Chronic pain and distraction: An experimental investigation into the role of sustained and shifting attention in the processing of chronic persistent pain. *Behaviour Research and Therapy*, *33*(4), 391–405.

Eccleston, C., & Crombez, G. (1999). Pain demands attention: a cognitive-affective model of the interruptive function of pain. *Psychological Bulletin*, *125*(3), 356–66. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10349356

Fields, H. L., & Basbaum, A. I. (1978). Brainstem Control of Spinal Pain-Transmission Neurons. *Annual Review of Physiology*, *40*, 217–248.

Ford, G. K., Moriarty, O., McGuire, B. E., & Finn, D. P. (2008). Investigating the effects of distracting stimuli on nociceptive behaviour and associated alterations in brain monoamines in rats. *European Journal of Pain (London, England)*, *12*(8), 970–9. doi:10.1016/j.ejpain.2008.01.002

Frankenstein, U. N., Richter, W., McIntyre, M. C., & Rémy, F. (2001). Distraction modulates anterior cingulate gyrus activations during the cold pressor test. *NeuroImage*, *14*(4), 827–36. doi:10.1006/nimg.2001.0883

Fuchs, P., & McNabb, C. (2012). The place escape/avoidance paradigm: a novel method to assess nociceptive processing. *Journal of Integrative Neuroscience*, *11*(1), 61–72. doi:10.1142/S0219635212500045

Fuchs, P. N., Roza, C., Sora, I., Uhl, G., & Raja, S. N. (1999, March 13). Characterization of mechanical withdrawal responses and effects of mu-, delta- and kappa-opioid agonists in normal and mu-opioid receptor knockout mice. *Brain Research*. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10064835

Gatchel, R. J., Peng, Y. B., Peters, M. L., Fuchs, P. N., & Turk, D. C. (2007). The biopsychosocial approach to chronic pain: scientific advances and future directions. *Psychological Bulletin*, *133*(4), 581–624. doi:10.1037/0033-2909.133.4.581

Gracely, R. H., Petzke, F., Wolf, J. M., & Clauw, D. J. (2002). Functional magnetic resonance imaging evidence of augmented pain processing in fibromyalgia. *Arthritis and Rheumatism*, *46*(5), 1333–43. doi:10.1002/art.10225

Gwilym, S. E., Keltner, J. R., Warnaby, C. E., Carr, A. J., Chizh, B., Chessell, I., & Tracey, I. (2009). Psychophysical and functional imaging evidence supporting the presence of central sensitization in a cohort of osteoarthritis patients. *Arthritis and Rheumatism*, *61*(9), 1226–34. doi:10.1002/art.24837

Handwerker, H. O., Iggo, A., & Zimmermann, M. (1975). Segmental and supraspinal actions on dorsal horn neurons responding to noxious and non-noxious skin stimuli. *Pain*, *1*, 147–165.

Hays, R., Prince-Embury, S., & Chen, H. (1998). *RAND-36 health status inventory.* San Antonio: The Psychological Corporation.

Heidbreder, C. a, & Groenewegen, H. J. (2003). The medial prefrontal cortex in the rat: evidence for a dorso-ventral distinction based upon functional and anatomical characteristics. *Neuroscience & Biobehavioral Reviews*, *27*(6), 555–579. doi:10.1016/j.neubiorev.2003.09.003

Hoffman, H. G., Chambers, G. T., Meyer, W. J., Arceneaux, L. L., Russell, W. J., Seibel, E. J., … Patterson, D. R. (2011). Virtual reality as an adjunctive non-pharmacologic analgesic for acute burn pain during medical procedures. *Annals of Behavioral Medicine : A Publication of the Society of Behavioral Medicine*, *41*(2), 183–91. doi:10.1007/s12160-010-9248-7

Hoffman, H. G., Patterson, D. R., Magula, J., Carrougher, G. J., Zeltzer, K., Dagadakis, S., & Sharar, S. R. (2004). Water-friendly virtual reality pain control during wound care. *Journal of Clinical Psychology*, *60*(2), 189–95. doi:10.1002/jclp.10244

Hoffman, H. G., Patterson, D. R., Seibel, E. J., Soltani, M., Jewett-Leahy, L., & Sharar, S. R. (2008). Virtual reality pain control during burn wound debridement in the hydrotank. *Clinical Journal of Pain*, *24*(4), 299–304.

Hoffman, H. G., Patterson, D. R., Soltani, M., Teeley, A., Miller, W., & Sharar, S. R. (2009). Virtual reality pain control during physical therapy range of motion exercises for a patient with multiple blunt force trauma injuries. *Cyberpsychology & Behavior : The Impact of the Internet, Multimedia and Virtual Reality on Behavior and Society*, *12*(1), 47–9. doi:10.1089/cpb.2008.0056

Hoover, W. B., & Vertes, R. P. (2007). Anatomical analysis of afferent projections to the medial prefrontal cortex in the rat. *Brain Structure & Function*, *212*(2), 149–79. doi:10.1007/s00429-007-0150-4

Institute of Medicine. (2011). *Relieving Pain in America : A Blueprint for Transforming Prevention , Care , Education , and Research*. Washington DC: The National Academies Press.

Ji, G., & Neugebauer, V. (2011). Pain-related deactivation of medial prefrontal cortical neurons involves mGluR1 and GABA A receptors. *Journal of Neurophysiology*, *106*, 2642–2652. doi:10.1152/jn.00461.2011.

Johansen, J. P., Fields, H. L., & Manning, B. H. (2001). The affective component of pain in rodents: direct evidence for a contribution of the anterior cingulate cortex. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(14), 8077–82. doi:10.1073/pnas.141218998

Kabat-Zinn. (1982). An outpatient program in behavioral medicine for chronic pain patients based on the practice of mindfulness meditation: Theoretical considerations and preliminary results. *General Hospital Psychiatry*, *4*, 33–42.

Kabat-Zinn, J. (1983). The Body Part Problem Assessment Scale. In R. Melzack (Ed.), *Pain Measurement and Assessment* (pp. 227–231). New York, NY: Raven.

Kabat-Zinn, J., Lipworth, L., & Burney, R. (1985). The clinical use of mindfulness meditation for the self-regulation of chronic pain. *Journal of Behavioral Medicine*, *8*(2), 163–90. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3897551

Keefe, F. J., Huling, D. a, Coggins, M. J., Keefe, D. F., Zachary Rosenthal, M., Herr, N. R., & Hoffman, H. G. (2012). Virtual reality for persistent pain: a new direction for behavioral pain management. *Pain*, *153*(11), 2163–6. doi:10.1016/j.pain.2012.05.030

LaBuda, C. J., Donahue, R., & Fuchs, P. N. (2001). Enhanced formalin nociceptive responses following L5 nerve ligation in the rat reveals neuropathy-induced inflammatory hyperalgesia. *Pain*, *94*(1), 59–63. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11576745

LaBuda, C. J., & Fuchs, P. N. (2000). A behavioral test paradigm to measure the aversive quality of inflammatory and neuropathic pain in rats. *Experimental Neurology*, *163*(2), 490–4. doi:10.1006/exnr.2000.7395

Leary, S., Underwood, W., Anthony, R., Cartner, S., Corey, D., Grandin, T., … Patterson-kane, E. (2013). *AVMA Guidelines for the Euthanasia of Animals : 2013 Edition* (2013th ed.).

Lembo, T., Fitzgerald, L., Matin, K., Woo, K., Mayer, E. a, & Naliboff, B. D. (1998). Audio and visual stimulation reduces patient discomfort during screening flexible sigmoidoscopy. *The American Journal of Gastroenterology*, *93*(7), 1113–6. doi:10.1111/j.1572-0241.1998.00339.x

Leventhal, H. (1992). I know distraction works even though it doesn't! *Health Psychology : Official Journal of the Division of Health Psychology, American Psychological Association*, *11*(4), 208–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1396487

Levine, J. D., Gordon, N. C., Smith, R., & Fields, H. L. (1982). Post-operative pain: effect of extent of injury and attention. *Brain Research*, *234*(2), 500–4. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7059842

Lewis, G. N., Rice, D. a, & McNair, P. J. (2012). Conditioned pain modulation in populations with chronic pain: a systematic review and meta-analysis. *The Journal of Pain : Official Journal of the American Pain Society*, *13*(10), 936–44. doi:10.1016/j.jpain.2012.07.005

Longe, S. E., 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*(9), 2021–5. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11435940

Lorenz, J., Cross, D. J., Minoshima, S., Morrow, T. J., Paulson, P. E., & Casey, K. L. (2002). A unique representation of heat allodynia in the human brain. *Neuron*, *35*(2), 383–93. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12160755

Low, L. a, Millecamps, M., Seminowicz, D. a, Naso, L., Thompson, S. J., Stone, L. S., & Bushnell, M. C. (2012). Nerve injury causes long-term attentional deficits in rats. *Neuroscience Letters*, *529*(2), 103–107. doi:10.1016/j.neulet.2012.09.027

Lundeberg, T., Nordemar, R., & Ottoson, D. (1984). Pain alleviation by vibratory stimulation. *Pain*, *20*(1), 25–44. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6333660

Luongo, L., de Novellis, V., Gatta, L., Palazzo, E., Vita, D., Guida, F., … Maione, S. (2013). Role of metabotropic glutamate receptor 1 in the basolateral amygdala-driven prefrontal cortical deactivation in inflammatory pain in the rat. *Neuropharmacology*, *66*, 317–29. doi:10.1016/j.neuropharm.2012.05.047

Malloy, K. M., & Milling, L. S. (2010). The effectiveness of virtual reality distraction for pain reduction: a systematic review. *Clinical Psychology Review*, *30*(8), 1011–8. doi:10.1016/j.cpr.2010.07.001

Mauderli, a P., Acosta-Rua, a, & Vierck, C. J. (2000). An operant assay of thermal pain in conscious, unrestrained rats. *Journal of Neuroscience Methods*, *97*(1), 19–29. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10771071

McCaul, K. D., Monson, N., & Maki, R. H. (1992). Does distraction reduce pain-produced distress among college students? *Health Psychology : Official Journal of the Division of Health Psychology, American Psychological Association*, *11*(4), 210–7. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1396488

McCracken, L. M., Carson, J. W., Eccleston, C., & Keefe, F. J. (2004). Acceptance and change in the context of chronic pain. *Pain*, *109*(1-2), 4–7. doi:10.1016/j.pain.2004.02.006

McNair, D., Lorr, M., & Droppleman, L. (1971). *Profile of Mood States (POMS)*. (E. and I. T. Service, Ed.). San Diego.

Melzack, R. (1975). The McGill Pain Questionnaire: Major Properties and Scoring Methods. *Pain*, (1), 277–299.

Melzack, R. (1987). The short-form McGill pain questionnaire. *Pain*, *30*(2), 191–197. doi:10.1016/0304-3959(87)91074-8

Melzack, R., & Casey, K. (1968). Sensory, Motivational, and Central Control Determinants of Pain. In D. Kenshalo (Ed.), *The Skin Senses, Proceedings of the First International Symposium on the Skin Senses, held at the Florida State University in Tallahassee, Florida* (pp. 423–443).

Melzack, R., & Wall, P. D. (1965). Pain Mechanisms : A New Theory. *Science*, *150*(3699), 971–979.

Metz, A. E., Yau, H.-J., Centeno, M. V., Apkarian, a V., & Martina, M. (2009). Morphological and functional reorganization of rat medial prefrontal cortex in neuropathic pain. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(7), 2423–8. doi:10.1073/pnas.0809897106

Millan, M. J. (2002). Descending control of pain. *Progress in Neurobiology*, *66*(6), 355–474. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12034378

Miron, D., Duncan, G. H., & Bushnell, M. C. (1989). Effects of attention on the intensity and unpleasantness of thermal pain. *Pain*, *39*(3), 345–52. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2616184

Moont, R., Pud, D., Sprecher, E., Sharvit, G., & Yarnitsky, D. (2010). “Pain inhibits pain” mechanisms: Is pain modulation simply due to distraction? *Pain*, *150*(1), 113–20. doi:10.1016/j.pain.2010.04.009

Morgan, M. a, Romanski, L. M., & LeDoux, J. E. (1993). Extinction of emotional learning: contribution of medial prefrontal cortex. *Neuroscience Letters*, *163*(1), 109–13. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8295722

Morley, S., Eccleston, C., & Williams, a. (1999). Systematic review and meta-analysis of randomized controlled trials of cognitive behaviour therapy and behaviour therapy for chronic pain in adults, excluding headache. *Pain*, *80*(1-2), 1–13. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10204712

Morone, N. E., Greco, C. M., & Weiner, D. K. (2008). Mindfulness meditation for the treatment of chronic low back pain in older adults: a randomized controlled pilot study. *Pain*, *134*(3), 310–9. doi:10.1016/j.pain.2007.04.038

Morris, L., Louw, Q., & Grimmer-Somers, K. (2009). The effectiveness of virtual reality on reducing pain and anxiety in burn injury patients: a systematic review. *Clinical Journal of Pain*, *25*(9), 815–826.

Neugebauer, V., Han, J. S., Adwanikar, H., Fu, Y., & Ji, G. (2007). Techniques for assessing knee joint pain in arthritis. *Molecular Pain*, *3*, 8. doi:10.1186/1744-8069-3-8

Ng, C.-W., Noblejas, M. I., Rodefer, J. S., Smith, C. B., & Poremba, A. (2007). Double dissociation of attentional resources: prefrontal versus cingulate cortices. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *27*(45), 12123–31. doi:10.1523/JNEUROSCI.2745-07.2007

Ohman, A. (1979). The orienting response, attention, and learning: An information-processing perspective. In H. Kimmel, E. Van Olst, & J. F. Orlebeke (Eds.), *The Orienting Reflex in Humans* (pp. 443–471). Hillsdale, NJ: Erlbaum.

Olds, J. (1962). Hypothalamic Substrates of Reward. *Physiological Reviews*, *42*, 554–604.

Ossipov, M. H., Dussor, G. O., & Porreca, F. (2010). Central modulation of pain. *Journal of Clinical Investigation*, *120*(11), 3779–3787. doi:10.1172/JCI43766.reduced

Pantelis, C., Harvey, C. a, Plant, G., Fossey, E., Maruff, P., Stuart, G. W., … Barnes, T. R. E. (2004). Relationship of behavioural and symptomatic syndromes in schizophrenia to spatial working memory and attentional set-shifting ability. *Psychological Medicine*, *34*(4), 693–703. doi:10.1017/S0033291703001569

Pastoriza, L. N., Morrow, T. J., & Casey, K. L. (1996). Medial frontal cortex lesions selectively attenuate the hot plate response: possible nocifensive apraxia in the rat. *Pain*, *64*(1), 11–7. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8867243

Pearce, J., & Morley, S. (1989). An experimental investigation of the construct validity of the McGill Pain Questionnaire. *Pain*, *39*(1), 115–21. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2812848

Petrovic, P., & Ingvar, M. (2002). Imaging cognitive modulation of pain processing. *Pain*, *95*(1-2), 1–5. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11790461

Petrovic, P., Petersson, K. M., Ghatan, P. H., Stone-Elander, S., & Ingvar, M. (2000). Pain-related cerebral activation is altered by a distracting cognitive task. *Pain*, *85*(1-2), 19–30. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10692599

Porreca, F., Ossipov, M. H., & Gebhart, G. F. (2002). Chronic pain and medullary descending facilitation. *Trends in Neurosciences*, *25*(6), 319–25. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12086751

Price, D. D. (1988). Classical and current theories of pain mechanisms. In D. Price (Ed.), *Psychological and neural mechanisms of pain* (pp. 212–231). New York, NY: Raven Press.

Price, D. D. (2000). Psychological and Neural Mechanisms of the Affective Dimension of Pain. *Science*, *288*(5472), 1769–1772. doi:10.1126/science.288.5472.1769

Prut, L., & Belzung, C. (2003). The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. *European Journal of Pharmacology*, *463*(1-3), 3–33. doi:10.1016/S0014-2999(03)01272-X

Reynolds, D. V. (1969, April 25). Surgery in the rat during electrical analgesia induced by focal brain stimulation. *Science (New York, N.Y.)*. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/4887743

Routtenberg, A., & Sloan, M. (1972). Self-stimulation in the Frontal Cortex of Rattus norvegicus. *Behavioral Biology*, *7*, 567–572.

Roy, M., Lebuis, A., Peretz, I., & Rainville, P. (2011). The modulation of pain by attention and emotion: a dissociation of perceptual and spinal nociceptive processes. *European Journal of Pain (London, England)*, *15*(6), 641.e1–10. doi:10.1016/j.ejpain.2010.11.013

Santos-Anderson, R. M., & Routtenberg, A. (1976). Stimulation of a Rat Medial or Sulcal Prefrontal Cortex During Passive Avoidance Learning Selectively Influences Retention Performance. *Brain Research*, *103*, 243–259.

Sharpe, L., Dear, B. F., & Schrieber, L. (2009). Attentional biases in chronic pain associated with rheumatoid arthritis: hypervigilance or difficulties disengaging? *The Journal of Pain : Official Journal of the American Pain Society*, *10*(3), 329–35. doi:10.1016/j.jpain.2008.10.005

Shibata, M., Ohkubo, T., Takahashi, H., & Inoki, R. (1989). Modified formalin test: characteristic biphasic pain response. *Pain*, *38*(3), 347–52. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2478947

Sullivan, M. J. L., Bishop, S. R., & Pivik, J. (1995). The Pain Catastrophizing Scale: Development and validation. *Psychological Assessment*, *7*(4), 524–532. doi:10.1037//1040-3590.7.4.524

Tjolsen, A., Berge, O., Hunskaar, S., Rosland, J., & Hole, K. (1992). The formalin test : an evaluation of the method. *Pain*, *51*, 5–17.

Torebjörk, H. E., LaMotte, R. H., & Robinson, C. J. (1984). Peripheral neural correlates of magnitude of cutaneous pain and hyperalgesia: simultaneous recordings in humans of sensory judgments of pain and evoked responses in nociceptors with C-fibers.

Journal of Neurophysiology, *51*(2), 325–39. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6707724

Tracey, I., Ploghaus, A., Gati, J. S., Clare, S., Smith, S., Menon, R. S., & Matthews, P. M. (2002). Imaging attentional modulation of pain in the periaqueductal gray in humans. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *22*(7), 2748–52. doi:20026238

Tsang, A., Von Korff, M., Lee, S., Alonso, J., Karam, E., Angermeyer, M. C., … Watanabe, M. (2008). Common chronic pain conditions in developed and developing countries: gender and age differences and comorbidity with depression-anxiety disorders. *The Journal of Pain : Official Journal of the American Pain Society*, *9*(10), 883–91. doi:10.1016/j.jpain.2008.05.005

Tzschentke, T. M. (2000). The medial prefrontal cortex as a part of the brain reward system. *Amino Acids*, *19*(1), 211–219.

Valet, M., Sprenger, T., Boecker, H., Willoch, F., Rummeny, E., Conrad, B., … Tolle, T. R. (2004). Distraction modulates connectivity of the cingulo-frontal cortex and the midbrain during pain--an fMRI analysis. *Pain*, *109*(3), 399–408. doi:10.1016/j.pain.2004.02.033

Vertes, R. P. (2004). Differential projections of the infralimbic and prelimbic cortex in the rat. *Synapse (New York, N.Y.)*, *51*(1), 32–58. doi:10.1002/syn.10279

Villemure, C., & Bushnell, M. C. (2009). Mood influences supra-spinal pain processing separately from attention. *Journal of Neuroscience*, *29*(3), 705–715. doi:10.1523/JNEUROSCI.3822-08.2009.Mood

Villemure, C., Slotnick, B. M., & Bushnell, M. C. (2003). Effects of odors on pain perception: deciphering the roles of emotion and attention. *Pain*, *106*(1-2), 101–108. doi:10.1016/S0304-3959(03)00297-5

Wager, T. D. (2005). The neural bases of placebo effects in anticipation and pain. *Seminars in Pain Medicine*, *3*(1), 22–30. doi:10.1016/j.spmd.2005.02.003

Wiech, K., Seymour, B., Kalisch, R., Stephan, K. E., Koltzenburg, M., Driver, J., & Dolan, R. J. (2005). Modulation of pain processing in hyperalgesia by cognitive demand. *NeuroImage*, *27*(1), 59–69. doi:10.1016/j.neuroimage.2005.03.044

Wong, D., & Baker, C. (1988). Pain in Children: Comparison of Assessment Scales. *Pediatric Nursing*, *14*(1), 9–17.

Wright, J. L., Hoffman, H. G., & Sweet, R. M. (2005). Virtual reality as an adjunctive pain control during transurethral microwave thermotherapy. *Urology*, *66*(6), 1320. doi:10.1016/j.urology.2005.06.123

Zeidan, F., Grant, J. a, Brown, C. a, McHaffie, J. G., & Coghill, R. C. (2012). Mindfulness meditation-related pain relief: evidence for unique brain mechanisms in the regulation of pain. *Neuroscience Letters*, *520*(2), 165–73. doi:10.1016/j.neulet.2012.03.082

Biographical Information

Christopher T. McNabb uses rodent models to study the interplay between cognition, emotion, and pain in the central and peripheral nervous systems. Prior to graduate study, Christopher earned a Bachelor's Degree in Music Education with a minor in Psychology from Texas Christian University. He received his Master of Science in Health Psychology in 2012 and his Ph.D. in Experimental Psychology in 2014 from the University of Texas at Arlington. His laboratory experience under the mentorship of Dr. Perry Fuchs provided extensive exposure to preclinical behavioral methodologies to assess pain, anxiety, memory, affect, and cognitive abilities in rodents. Through this experience, Christopher developed specific interests in the neural mechanisms which underlie the placebo effect, distraction analgesia, and other forms of cognitive and affective modulation of pain. Christopher has collaborated with faculty at the University of Texas Southwestern Medical School as well as the other members of the neuroscience faculty at UTA, and he has executed multiple contracted research projects for pharmaceutical and nutritional companies. In August of 2014, Christopher will begin a Postdoctoral Fellowship at the National Center for Complementary and Alternative Medicine at the National Institutes of Health in Bethesda, Maryland.