STRESS-INDUCED CHANGES IN SENSITIVITY TO THERMAL NOCICEPTIVE STIMULATION IN NORMAL RATS AND FOLLOWING EXCITOTOXIC SPINAL CORD INJURY

By

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2006

To my family and friends who supported me through these years. With your guidance and patience, I achieved a great accomplishment. I would have not been as successful without you. Thank you.

ACKNOWLEDGMENTS

I want to acknowledge family and friends who sustained me. In particular, I am extremely thankful for the guidance, love, and patience of my family. My father Richard, mother Sharren, stepmother Sally, sister Kelsey, and wife Natasha have been my rock. Each of them has played a special role in my life. With their inspiration and encouragement, I accomplished my goals and understood the importance of family especially during the tough times. I also would like to remember the individuals who have left our family including my mother Sharren, grandfather King, and grandmother Flint. They are loved and missed.

I also thank the professors who helped me during my graduate studies. I am fortunate to have worked with them. Dr. Caudle provided me with an opportunity to work in his lab, visit foreign lands, and further develop my research and academic proficiencies during a tough time in my life. Also, I appreciate Dr. Vierck's guidance and wisdom over the past few years. I would also acknowledge help and advice from Drs. Darragh Devine and Andre Mauderli about stress and the potential pitfalls of behavioral testing. Last but not least, I express my gratitude to Dr. Yezierski as an honest and supportive mentor. In the process of developing my training and research program, he was able to convey his knowledge about pain, and was very patient in educating me about writing. I am thankful to each of my professors for their involvement in my development as a scientist and a person.

In Drs. Yezierski's and Vierk's lab, I worked with several amazing individuals who also gave me technical and moral support. I would like to express my appreciation to my research backbone: my "rat ladies" Jackie, Karen, and Jean. I would not have accomplished my research goals without their dedication and assistance. They educated me on many things related to my research, and also showed that you are only as good as the people around you. I also like to thank Dr. Cannon for his computer, histology, and perfusion expertise; and for listening to my unending questions about these issues. Also, I thank Victoria Gority for administrative assistance and long talks about world problems. I also thank Sandra for her assistance, even though I have only known her for a short time.

Finally, I would like to thank my friends; especially my old roommate and lab mate Federico. I also like to thank Sara (a fellow graduate student in Dr. Yezierski's lab) for her support and friendship through our graduate training. Finally, I thank my God and my savior Jesus Christ for giving me a great family, friends, and an opportunity to develop into a scientist.

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STRESS-INDUCED CHANGES IN SENSITIVITY TO THERMAL NOCICEPTIVE STIMULATION IN NORMAL RATS AND FOLLOWING EXCITOTOXIC SPINAL CORD INJURY

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August 2006

Chair: Robert Yezierski
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Major Department: Medical Science-Neuroscience

The sensation of pain is a complex experience that requires processing of nociceptive stimulation by cortical structures. Various manipulations (including stress and injury to the nervous system) influence activity in these structures and thus influencing pain perception. To understand the effects of stress on nociceptive sensitivity, behavioral responses of normal (injury naïve) and spinal injured animals were evaluated before and after a 15 minute exposure to restraint stress. Two types of behavioral assessment strategies were used, including reflex (dependent on spino-bulbo-spinal processing) and operant (dependent on cerebral processing) responses to low-intensity thermal stimulation (44.0 to 44.5°C) that activates C-nociceptors. Excitotoxic spinal cord injury was accomplished by intraspinal injection of the AMPA/metabotropic receptor agonist quisqualic acid (QUIS). Additional features of

stress-induced changes in nociception were also investigated, including the impact of opioids and sympathetic-mediated thermoregulation of skin temperature.

Results suggest that restraint stress decreased thermal sensitivity of reflex responses by activating an endogenous opioid system, supporting previous reports of stress-induced hyporeflexia. Interestingly, low-dose morphine enhanced reflex lick/guard responses and opposed inhibitory effects of restraint stress on reflexes, suggesting a separate mechanism mediating these effects. In contrast, restraint stress increased thermal sensitivity to heat in the operant escape and thermal preference tests, which was opposed by tonic endogenous opioids and by exogenous opioid administration. Results provide evidence for stress-induced hyperalgesia, which was not observed the following day or during sessions at neutral temperatures (36.0°C) suggesting that this effect is specific to activation of C-nociceptors. Excitotoxic spinal cord injury also increased thermal sensitivity to heat in some animals, which was enhanced by stress in subsequent testing sessions.

In summary, results suggest that exposure to acute restraint stress has a differential effect depending on the behavioral assessment strategy. Furthermore, stress was found to enhance thermal hyperalgesia after excitotoxic injury. Finally, assessment of skin temperatures during thermal stimulation showed an association between the regulation of sympathetic vasoconstriction and enhanced sensitivity to heat on operant responses after stress and excitotoxic injury.

CHAPTER 1
INTRODUCTION AND LITERATURE REVIEW

The purpose of my study is to advance our understanding of the behavioral and pharmacological mechanisms responsible for modulating nociceptive responses after acute stress. Pre-clinical and clinical studies of pain have described changes to the psychological condition initiated by stressors that may lead to changes in nociceptive sensitivity and precipitate psychopathologies. Psychological stressors are encountered on a daily basis and appear to correlate with conditions of increased pain sensitivity in individuals with a variety of pain conditions, including chronic pain syndromes such as fibromyalgia, rheumatoid arthritis, and irritable bowel syndrome (Bennet et al., 1998; Blackburn-Munro and Blackburn-Munro, 2001; Davis et al., 2001; Mayer et al., 2001). Because these studies are limited, additional research is needed to understand the negative effects of stress on acute and chronic pain conditions. However, pre-clinical research has been hampered by poorly defined behavioral assessment strategies that focus on reflex responses dependent on spinal and brainstem processing of painful information. A well-defined, unambiguous animal model that demonstrates the stress-induced enhancement of pain is therefore required. Fortunately, operant escape task, a recently developed behavioral assay, can address these issues.

The Pain Experience

Based on the original hypothesis by Melzack and Casey (1968), the pain experience may be thought of in terms of a sensory discriminative component in which precise anatomical mapping of stimulus intensity, location, and modality are maintained. The

pain experience is also thought to have an affective motivational component (in which pain perception is modulated by the concurrent overlay of an emotional component as well as previously learned associations). This organization reflects the definition of the International Association for the Study of Pain (IASP) that pain is “an unpleasant sensory and emotional experience associated with actual or potential tissue damage” (Merskey and Bogduck, 1994, page 210).

Although complex, it is heuristically useful to consider that pain is a valuable response to potentially tissue damaging stimuli. Pain is detected in the periphery through the activation of primary Aδ or C-nociceptors, transmitted to the dorsal horn of the spinal cord, and transmitted to supraspinal structures through ascending pathways. Painful information is processed in the brainstem and cerebrum and results in the activation of descending modulatory pathways that inhibit or facilitate pain transmission in the spinal cord. Subsequent responses are organized through a complex interaction of neuroanatomical structures. These mechanisms encompass primary afferent transduction to spinal encoding, and finally supraspinal stimulus-response relationships. Within the nervous system, numerous structures and pathways (e.g., ascending and descending) transmit, process, and modulate information associated with the pain experience.

Ascending Pain Pathways

The complexity of processing sensory input in the spinal cord shows that it is a critical conduit for transmitting sensory nociceptive information. Studies have shown that nociceptive stimulation activates primary afferent nociceptors in the skin including fast conducting small diameter myelinated Aδ mechanoreceptors and slow conducting unmyelinated C polymodal fibers (Fleischer et al., 1983; Willis and Westlund, 1997).

Depending on the nature of the stimulus, Aδ- and C-nociceptors are activated differently. High and low intensity stimulation is required to excite Aδ- and C-nociceptors, respectively (Yeomans et al., 1996).

The initial location of nociceptive information processing occurs in the dorsal horn of the spinal cord. Nociceptive information is conveyed by afferent fibers that terminate on second order neurons located in the dorsal horn (e.g., superficial laminae I/II; Todd et al., 2000, 2002; Millan, 2002). Studies show the role of dorsal horn neurons in the rostral transmission of nociceptive information and descending modulation (Millan, 2002, 2003; Willis and Westlund, 1997). Several ascending nociceptive pathways have been identified in conveying nociceptive information including spinothalamic, spinomesencephalic, spinoreticular, spinocervical, and spinolimbic pathways (Burstein et al., 1987, 1990; Burstein and Giesler, 1989; Willis and Westlund, 1997; Yezierski, 1988). In addition to nociceptive transmission, dorsal horn neurons modulate other nociceptive projection neurons and motor neurons (Willis and Westlund, 1997). Nociception does not indicate pain perception (Le Bars et al., 2001; Vierck, 2006). Rather, pain perception requires cerebral processing of the nociceptive stimulus (Mauderli et al., 2000; Vierck, 2006).

Nociceptive input is transmitted along ascending pathways to supraspinal structures. Ascending pathways innervate brainstem (e.g., PAG, Bulbar reticular formation) and cortical (e.g., thalamus, hypothalamus, amygdala) structures involved in higher order processing of nociceptive information (Giesler et al., 1994; Price, 2000; Willis and Westlund, 1997). These cortical systems are important for the affective component of pain (Price, 2000). Spinothalamic tract (STT) cells are implicated in the

sensation of pain as a consequence of anterolateral cordotomies or spinal lesions (Price, 2000; Vierck and Light, 1999, 2002; Willis and Westlund, 1997; Yezierski, 1988). Spinothalamic tract (STT) cells are also activated in responses to thermal stimulation (Ferrington et al., 1987; Price et al., 1978).

Descending Pain Pathways

Previous studies show the presence of a complex endogenous inhibitory system that modulates spinal circuitry involved in nociceptive processing. Several supraspinal structures have been implicated in the modulation of spinal processing of nociception and nociceptive behavior. Cortical structures implicated in modulation include the amygdala, anterior cingulate cortex, insular cortex, and hypothalamus (Willis and Westlund, 1997; Price, 2000). In addition, brainstem structures have been shown to impact nociception including the locus ceruleus (LC), A7 catecholamine cell group, periaqueductal gray (PAG), reticular formation, and rostroventromedial medulla (RVM; Mitchell et al., 1998; Nuseir and Proudfit, 2000; Proudfit and Clark, 1991; Westlund and Coulter, 1980; Willis and Westlund, 1997). In addition, ascending projections from neurons expressing NK-1R influence the activation of descending pathways (Suzuki et al., 2002). This suggests that nociceptive stimuli activate a spino-bulbo-spinal system in which ascending projections provide afferent input to supraspinal loci. In turn, supraspinal neurons modulate spinal activity by descending projections. In conditions of pain and stress, enhanced nociceptive sensitivity most likely involves components of both ascending and descending projections among spinal cord, brainstem, and cortical regions.

Activation of descending inhibitory pathways suppresses nociceptive reflex responses, evidence for stimulation-induced hypoalgesia. An important bulbo-spinal circuit mediating the expression of hypoalgesia includes the connection between the PAG

and RVM. Other pathways include connections between the PAG and LC (Willis and Westlund, 1997). The PAG projects to nuclei of the RVM (Fields and Basbaum, 1999). From the RVM, descending pathways project to the spinal cord via the dorsolateral funiculus (DLF) and influence nociceptive neurons in the dorsal horn (Basbaum and Fields, 1984; Millan, 2002). Activation of neurons in the PAG and RVM by electrical stimulation or microinjections of opioids produces a decrease in the activity of nociceptive neurons and nociceptive reflexes to thermal stimulation (Carstens et al., 1979, 1980, 1981; Fields and Basbaum, 1999; Fields et al., 1988, 1991; Peng et al., 1996). STT cells that are implicated in transmitting pain sensations are particularly inhibited after stimulation of the PAG (Yezierski, et al., 1982).

Based on anatomical, electrophysiological and pharmacological evidence, RVM is thought to have a substantial role in the modulation of nociceptive responses and transmission of nociceptive input (Mason, 1999). In the RVM, cells have been characterized as “OFF”, “ON”, and neutral cells based on responses to thermal stimulation (Fields et al., 1991; Heinricher et al., 1989, 1997). The “OFF” cells are tonically active and pause in firing immediately before tail withdrawal from a noxious thermal stimulus and are thus thought to be involved in inhibition of spinal nociceptive neurons. The “ON” cells accelerate firing immediately before the nociceptive reflex and are directly inhibited by mu-opioid agonists; these cells are thought to produce facilitation of spinal nociceptive neurons (Fields et al., 1983, 1991; Heinricher et al., 1994; Urban and Gebhart, 1999). Both cell types project to dorsal horn (e.g., lamina I, II, and V) to modulate nociceptive transmission and responses to thermal stimulation (Fields et al., 1983; Morgan and Fields, 1994; Mitchell et al., 1998).

While traditional studies focused on descending inhibition from the RVM, several studies show that descending pathways exert facilitatory influences on nociceptive processing and responses through activity of RVM neurons in chronic pain states (Porreca et al., 2001, 2002; Urban and Gebhart, 1999). Descending pathways appear to facilitate nociception through activity of μ-opioid receptor expressing pronociceptive "ON" cells (Ossipov et al., 2000; Pertovaara et al., 1996). These observations led to hypothesis that spino-bulbo-spinal loop could contribute to the development and maintenance of exaggerated pain behaviors produced by noxious and non-noxious stimuli (Porreca et al., 2002; Urban and Gebhart, 1999). However, questions have been raised concerning descending pathways in the cortical processing of pain because these studies are based on reflex-mediated responses not dependent on cortical processing. Descending systems are also involved in regulating other physiological functions (autonomic, motor) especially to innocuous stimuli (Manson, 2005).

Descending bulbo-spinal pathways originating from the RVM are critical for expression of exogenous opioid anti-nociception as assessed by reflex responses (Fields et al., 1983; Gilbert and Franklin, 2002; Gebhart and Jones, 1988). Neurons responsible for descending pathways display high levels of opioid receptors and peptide expression (Marinelli et al., 2002). Microinjection of opioid agonists into discrete brainstem sites (e.g. PAG and RVM) produces reduced activity of dorsal horn neurons and nociceptive tail and hindpaw withdrawal responses to noxious stimulation (Jenson and Yaksh, 1986a, 1986b, 1986c; Jones and Gebhart, 1988; Yaksh, 1997, 1999; Yaksh et al., 1976). Furthermore, the hyporeflexic effects of systemic opioids appear to activate descending modulatory systems through these sites. For example, microinjections of opioid

antagonists into the PAG and RVM oppose the hypoalgesic effects of systemic morphine (Manning and Franklin, 1998; Yaksh and Rudy, 1978).

In addition, neural pathways associated with stress-induced changes in nociceptive reflexes include supraspinal neurons that exert a descending inhibitory effect on dorsal horn neurons including descending pathways from the RVM. Activation of the RVM during times of stress was shown to be critical for expression of morphine inhibition of reflex responses. In stressed rats, the enhancement of morphine inhibition of reflex responses was reduced by injections of lidocaine or muscimol into the RVM (Mitchell et al., 1998).

Supraspinal structures mediate morphine-induced inhibition of reflex responses through descending projections that are blocked by RVM and DLF lesions or inactivation of the RVM by lidocaine (Abbott et al., 1996; Basbaum and Fields, 1984; Fields et al., 1988, 1991; Gilbert and Franklin, 2002; Mitchell et al., 1998). The RVM is also implicated in the expression of stress-induced antinociception. Inactivation of the RVM by lidocaine attenuated reflexive behavior to heat in stressed rats (Mitchell et al., 1998). Damage to descending pathways in the DLF also reduced the development of stress-induced inhibition of reflex responses (Watkins and Mayer, 1982; Watkins et al., 1982).

At the spinal level, bulbo-spinal terminals release several neurotransmitters that modulate dorsal horn activity and nociceptive responses, including catecholamines, and opioid peptides (Schmauss and Yaksh, 1984**;** Takano and Yaksh, 1992). Activation of the bulbo-spinal descending inhibitory pathways are mimicked and enhanced by spinal application of α_2 and μ receptor agonists (Nuseir and Proudfit, 2000; Schmauss and

Yaksh, 1984**;** Takano and Yaksh, 1992). In contrast, the effects of activation of bulbo-spinal projections are reversed by spinal application of α_2 (e.g., phenotlamine) and μ (e.g., naloxone) receptor antagonists (Camarata and Yaksh, 1986; Yaksh, 1979; Yaksh and Rudy, 1977).

Animal Models of Pain

The sensation of pain provides important information to an organism about its internal and external environment in order to maintain homeostasis. In the presence of a painful stimulus, various systems are activated to avoid the stimulus and limit damage (Le Bars et al., 2001). Assessment of pain sensitivity in animal studies is inferred from a variety of behavioral responses to nociceptive stimuli as illustrated in Figure 1-1 (adapted from C. Vierck). These responses can be categorized hierarchically within the neuroaxis including segmental reflexes, supraspinal reflexes, and learned escape responses (Le Bars et al., 2001; Vierck, 2006).

As demonstrated in numerous studies, spinally mediated reflex responses are demonstrated by simple limb or tail withdrawal from a nociceptive stimulus (Franklin and Abbott, 1989). Reflex responses mediated by spino-bulbo-spinal circuitry are revealed by more complex responses including licking, guarding, vocalization, and jumping (Le Bars et al., 2001; Matthies and Franklin, 1992; Woolf, 1984). Finally, learned escape responses requires cerebral processing of nociceptive information and development of a proper strategy to terminate the stimulus (Mauderli et al., 2000). The concept of learning is not included in most tests of nociception that utilize reflex assays (Le Bars et al., 2001).

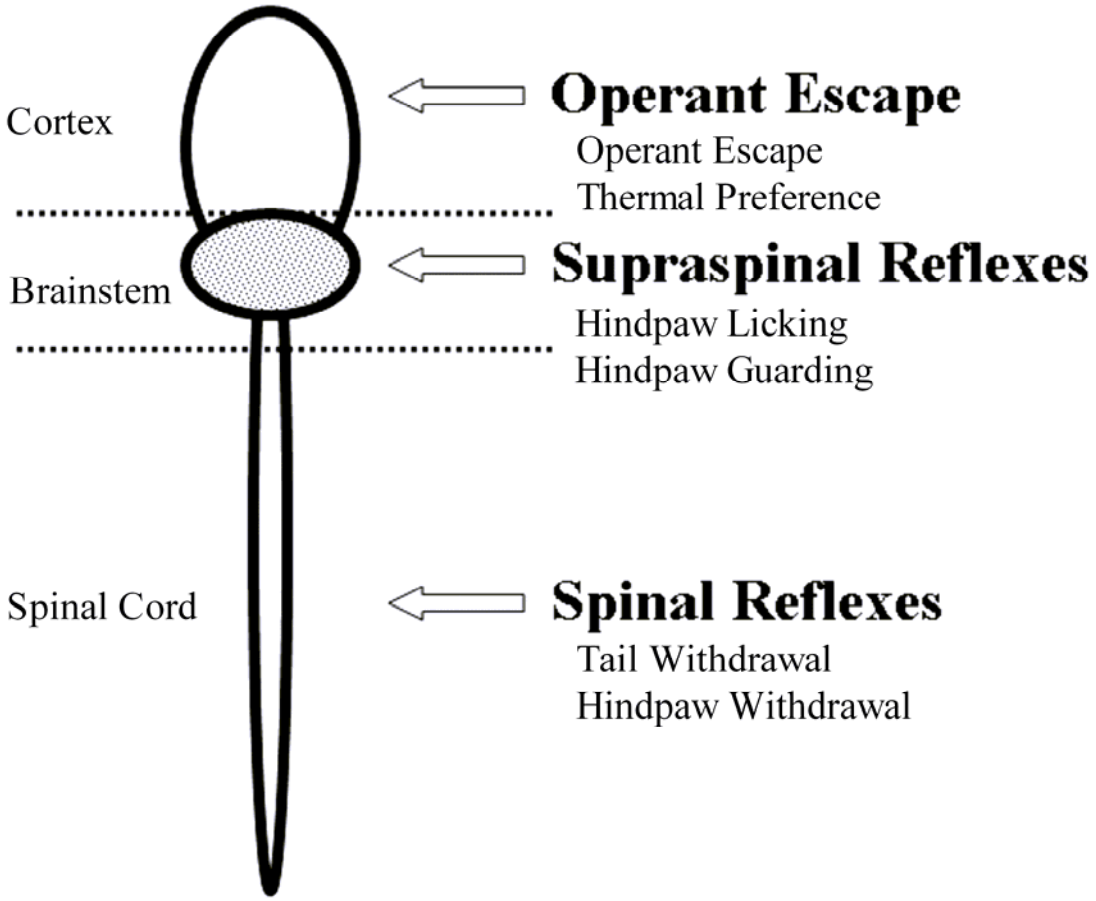


Figure 1-1. Hierarchical behavioral responses to nociceptive stimuli including spinal, supraspinal, and cortical mediated responses.

Nociceptive Responses Mediated by Spinal and Spino-Bulbo-Spinal Processing

In pre-clinical models, evaluation of sensory processing has utilized reflex based assessment strategies. These behavioral endpoints have been used to evaluate the presence of pain and assess alterations in nociception by various experimental manipulations. Segmentally organized spinal pathways regulate withdrawal of a rodent's tail or hindpaw from a nociceptive stimulus. Furthermore, segmentally-mediated tail or hindpaw withdrawal responses can be elicited in spinalized animals (Figure 1-1; Borszcz et al., 1992; Franklin and Abbott, 1989; Kauppila et al., 1998). In addition,

bulbo-spinal-mediated responses including paw-licking and vocalization can be elicited in decerebrate animals (Figure 1-1; Woolf, 1984; Matthies and Franklin, 1992). It is important to note that a majority of animal studies utilize brief high intensity thermal stimulation above 50.0°C that activates mylinated Aδ nociceptors (Yeomans et al., 1996). Finally, reflex responses represent an important measure of nociception and can be modulated by brainstem (e.g., LC, RVM) and cerebral structures (Le Bars et al., 2001; Vierck, 2006).

Experimental manipulations such as stress and nerve injury can alter both types of reflex responses. A heightened or diminished sensitivity to a noxious stimulus after an experimental manipulation illustrates two concepts in the field of pain: hypereflexia and hyporeflexia, respectively. For example, acute stress reduces nociceptive reflex responses presumably by endogenous opioid mechanisms (Bodnar et al., 1978b; Gamaro et al., 1998; Watkins and Mayer, 1982), and injury to the central nervous system heightens sensitivity to thermal stimulation (Acosta Rua, 2003; Yezierski et al., 1998). However, these end-points fail to account for the interactions between manipulations (stress) and higher order functions that are responsible for the affective dimension of pain.

Reflexive responses do not represent the conscious or clinical aspect of pain perception, but rather spinally and supraspinally mediated nociceptive responses to thermal stimulation (Mauderli et al., 2000; Le Bars et al., 2001). Studies utilizing reflex-mediated responses assume that changes in reflex responses are a consequence of altered sensory processing at different levels of the neuroaxis (Le Bars et al., 2001; Vierck, 2006). However, these studies may fail to address other non-sensory factors

affected by an experimental manipulation including changes in motor output, posture, motivation, attention, and cognition. Finally, pathways underlying reflex responses are associated with other physiological functions unrelated to nociception (Mason, 2005; Le Bars et al., 2001). Therefore, assessment of reflexive responses can lead to deceptive conclusions about their importance in the overall sensation of pain.

Nociceptive Responses Mediated by Cerebral Processing

A main feature working against reflex based assessment strategies is that these strategies do not take into account interactions between experimental manipulations and higher cortical activity that are critical for the perception of nociceptive stimuli. Therefore, in contrast to reflex responses, conscious and motivated responses to thermal stimulation are believed to characterize clinically relevant aspects of nociceptive perception dependent on higher order cerebral processing of nociceptive input. Consequently, operant responses are absent in decerebrate animals as they are dependent on cerebral processing of nociceptive input and environmental cues for the execution of appropriate escape responses (Figure 1-1; Mauderli et al., 2000; Vierck, 2006; Vierck et al., 2003, 2004).

Recently, an operant escape task was developed that evaluates thermal nociceptive sensitivity in awake, unrestrained, and conscious rats (Mauderli et al., 2000). This test overcomes the limitations inherent with reflex withdrawal responses and offers a strategy to evaluate changes in the affective dimension of pain. The escape test provides an opportunity to evaluate: a) the consequences of experimental manipulations on a non-reflexive behavioral outcome measure; b) the mechanisms involved in hyporeflexia (e.g., decrease in nociceptive sensitivity) and hyperaglesia (e.g., increase in nociceptive sensitivity); and c) the effects of these manipulations on behaviors dependent on spinal

and brainstem or cerebral processing of nociceptive input. More importantly, an opportunity exists to directly compare reflex and escape responses to similar levels of thermal stimulation.

Differences between reflex lick/guard and operant escape responses have been observed in several studies. Systemic injections of low dose morphine (0.5 to1.5 mg/kg) attenuate escape responses (e.g., increase response latencies and decreased duration) dependent on unmyelinated C-nociceptor activation (~44.0°C; Cooper et al., 1986; Vierck et al., 2002). By comparison, escape responses were not affected by morphine at temperatures activating Aδ-nociceptors. In contrast, reflex responses were augmented (e.g., decreased response latencies and increased duration) at the same temperature after morphine administration. Typically, suppression of reflex response is reported after injections of higher dose morphine (3 to 10 mg/kg; Holtman and Wala, 2005; O'Callaghan and Holtzman, 1975). Reflex responses were more sensitive to the hyporeflexic effects of morphine at temperatures lower than 50.0°C (Holtman and Wala, 2005).

Based on these and other studies, the difference in sensitivity to morphine that depends on the activation of Aδ or C-nociceptors illustrates an importance of the rate of cutaneous heating by a thermal stimulus. It has been shown that near threshold for nociceptor activation occurs at temperatures ranging from 43.0 to 45.0°C (Le Bars et al., 2001; Treede, 1995; Vierck et al., 2000). This temperature range preferentially activates C-nociceptors as a result of a slow rate of skin heating and is involved in human pain sensations. C-nociceptors are essential for the affective sensation of a painful stimulus and, therefore, important to the elicitation of operant escape responses (Cooper and

Vierck, 1986; Cooper et al., 1986; Vierck et al., 2000, 2004). In contrast, temperatures above 45.0°C produce a rapid rate of heating that activates Aδ- and C–nociceptors (Cooper et al., 1986; Yeomans and Proudfit, 1996; Yeomans et al., 1996). Clearly, the activation of C-nociceptors by gradual heating of the skin is important to overall pain sensation. The ability of low dose morphine to selectively suppress nociception mediated by C-nociceptors and not Aδ-nociceptors supports the idea that C-nociceptors are activated by sustained low intensity thermal stimulation.

Other manipulations demonstrate a difference in reflex and operant responses to thermal stimulation. Operant escape responses appear to be mediated in part by NK-1R neurons. Vierck et al. (2003) demonstrated that lesioning of NK-1R neurons with substance-P saporin reduced escape responses to low intensity thermal stimulation while reflex responses were not affected. Also, Vierck et al. (2005) reported that chronic constriction (CCI) of the sciatic nerve produced increase in cold sensitivity. Finally, other operant test may offer unique opportunities to evaluated responses dependent on cortical processing (Neubert et al., 2005, 2006).

Chronic Pain

While acute pain serves a protective function to the organism, chronic pain persists beyond its intended purpose as a result of abnormal activity in the central nervous system. In fact, chronic pain can last for a long period of time (> six months; Herr, 2004; Willis, 2002). Chronic pain is characterized as spontaneous, stimulus-independent, or evoked, stimulus dependent, pain sensations (Herr, 2004). Although the features of spontaneous and evoked pain will not be discussed, it appears that several mechanisms mediate these pain etiologies including sensitization (Willis, 2002; Willis and Westlund,

1997). Furthermore, a prominent feature of abnormal pain sensations is the presence of either allodynia (e.g., enhanced response to normally non-painful stimulus) or hyperalgesia (e.g., enhanced response to normally painful stimulus). Central pain conditions are initiated by injury to the central nervous system without involvement of peripheral nociceptors (Willis and Westlund, 1997). Central pain was defined by the IASP as "pain initiated or caused by a primary lesion of dysfunction within the CNS" (Merskey and Bogduk, 1994, page 211). In support of the definition, studies have shown that lesions of the central nervous system (e.g., spinal cord, brainstem, and brain) may result in central pain presumably through altered activity within nociceptive pathways. In particular, central pain after spinal cord injury will be discussed.

Spinal Cord Injury Pain

Spinal cord injury (SCI) is a challenging healthcare problem in terms of understanding the pathophysiology underlying the condition and treatment strategies. Spinal cord injury pain can develop immediately or over a period of time (Widertrom-Noga, 2002; Widertrom-Noga et al., 1999), and SCI pain can be either spontaneous or evoked (Siddall et al., 2002; Vierck et al., 2000; Yezierski, 2002). Several studies have reported between 60–90% of individuals with SCI experience pain of some type (Beric, 1997; Bonica, 1991; Kennedy et al., 1997; Mariano, 1992; Siddall et al., 2002; Widertrom-Noga et al., 1999). However, the development of chronic pain is higher in individuals with partial interruption of gray and white matter (e.g., incomplete SCI) compared to complete spinal injuries (Beric et al., 1988). In most cases, SCI pain is a major obstacle and overshadows other physiological consequences (e.g., impairment of motor functioning) based on the fact that SCI patients would forgo functional recovery for pain relief (Finnerup et al., 2001; Nepomuceno et al., 1979; Yezierski, 1996). In

addition, subsequent treatments strategies to treat SCI pain are limited and mostly ineffective (Davidoff et al., 1987; Yezierski, 1996).

Central pain after injury to the spinal cord is often characterized by abnormal sensations located in dermatomes at or below the level of injury. An increase in nociception in dermatomes or segments at or adjacent to the injury location is defined as at-level pain (Siddall et al., 2002; Vierck et al., 2000). In contrast, below-level pain after spinal cord injury is identified by an increase and spontaneous sensations in nociception in dermatomes caudal to the injury location (Siddall et al., 2002; Vierck et al., 2000). Another factor that distinguishes below-level pain is a delayed onset of weeks, months, or years. Other sensations are also reported in individuals suffering with chronic pain conditions. Abnormal sensations such as tingling, numbness, and itching are identified as either dysesthesias or paraesthesias (Herr, 2004). Several potential mechanisms have been hypothesized to mediate altered pain sensation after SCI (see blow). Some of these conditions include: a) abnormal activity (e.g., hyperactivity) of neurons associated with pain transmission in the spinal cord and loss of afferent input to rostral targets (e.g., deafferentation of thalamic and cortical areas), b) hypofunctioning of the endogenous opioid system, c) hyperfunction of glutaminergic excitatory systems, and d) loss of inhibitory mechanisms (Eide, 1998; Willis, 2002; Yezierski, 2002).

Mechanism of SCI Pain

Neuronal hyperexcitability after loss of inhibitory modulation, in areas above or below the lesion site, may also influenced pain sensation (evoked and/or spontaneous) in humans (Finnerup et al., 2003a, 2003b; Milhorat et al., 1996) and rodents (Vierck and Light, 1999, 2000; Yezierski and Park, 1993; Yezierski et al., 1998). In individuals with SCI, spinal and thalamic neurons show evidence of hyperexcitability that is characterized

as an abnormal increase in activity (resting and evoked; Lenz et al., 1987, 1994; Loeser and Ward, 1967, 1968). Additionally, in animals SCI models, the presence of neuronal hyperexcitability at spinal segments bordering the injury site is associated with at-level pain (Christensen and Hulsebosch, 1997; Drew et al., 2001, 2004; Hao et al., 1992a; Yezierski and Park, 1993). Using electrophysiological techniques, several studies have shown that neurons within pain pathways display abnormal spontaneous activity, expansion of receptive field, a diminish threshold for activation, an increased responses to stimulation, and extended afterdischarge (Eide 1998). Pharmacological investigations have demonstrated the role of neuronal hyperexcitability in altered pain sensitivity by administration of lidocaine (Loubser and Donvan, 1991) or NMDA antagonists (Hao and Xu, 1996; Hao et al., 1991b; Liu et al., 1997).

As mentioned previously, nociceptive information is conveyed from the spinal cord to rostral targets via ascending pathways including the spinothalamic tract (Willis and Westlund, 1997). Abnormal activity of STT pathway has traditionally been thought as a critical feature of central pain after SCI. Studies have supported this hypothesis after interruption of STT pathways (Vierck and Light, 1999, 2000) or lesioning of its rostral sites in the cerebral cortex particularly in post-stroke pain (Anderson et al., 1995; Boivie, 1994; Boivie et al., 1989). Although involvement of the STT pathway is important to the development of central pain, other factors appear to be equally important. In fact, some studies have reported similar damage to STT pathways in SCI patients with and without pain (Finnerup et al., 2003a, b). Using MRI methods, Finnerup et al. (2003a) demonstrated that individuals with central below-level pain, compared to patients without central pain, displayed similar damage to the STT pathways, but patients with pain had a

larger loss of gray matter. Damage to gray and white matter (e.g., interruption of the spinothalamic pathway) in the spinal cord appears to be critical factors in the development of below-level pain after SCI (Boivie et al., 1989; Vierck and Light, 1999). Thus, in support of other clinical studies implicating damage to STT pathways as critical factors in the development of central pain, damage to the spinal gray matter is also a critical factor.

Because the STT is the major ascending pathway to supraspinal targets, rostral sites, such as the thalamus and cerebral cortex, lose critical input if the STT is damaged (Loeser and Ward, 1967, 1968). Lesions of the anterolateral spinal cord after cordotomy produce spontaneous and evoked pain as a consequence of pathways originating from gray matter (Vierck and Light, 1999, 2000). This evidence supports the suggestion that central pain after interruption of the STT pathway is a consequence of deafferentation. Altered activity patterns are detected in deafferentated nuclei targeted by the STT pathways including the thalamus (Lenz et al., 1978, 1987; Weng et al., 2000) and cerebral cortex (Lenz et al., 1987, 1994). In addition, anterolateral cordotomies disrupt descending modulatory pathways, which also contributes to the enhancement of neuronal excitability in areas bordering the lesion (Vierck and Light, 2000). From these studies, it is clear that interruption of STT tract and changes to corresponding rostral targets are important for central pain. However, other factors will determine the expression of central pain including gray matter damage (Vierck and Light, 2000).

The endogenous opioid system is implicated in the pathophysiology of neuropathic pain (Edie, 1998; Hao et al., 1998; Ossipov et al., 1997; Porrecca et al., 2001). Based on several studies in rodents, the opioid system (e.g., PPD, PPE) is activated after injury in

spinal and supraspinal areas and appears to suppress abnormal pain sensation. But, dysfunctions of the opioid system lead to development of hypersensitivity to thermal and mechanical stimulation (Abraham et al., 2000, 2001; Xu et al., 1994). Finally, despite the unidentified pathophysiological mechanisms underlying SCI pain, psychosocial factors contribute this condition. Studies have identified a relationship between several psychological factors and SCI pain including depression, anxiety, fatigue, and stress (Kennedy et al., 1997; Mariano, 1992; Summers et al., 1991). Unfortunately, no pre-clinical studies have examined the impact of stress on SCI pain.

Animal Models of SCI Pain

Several pre-clinical models of SCI are used to examine pathophysiological mechanisms underlying alter sensitivity to nociceptive stimuli. Models such as hemisection (Christensen et al., 1997), photochemical lesions (Hao et al., 1991a, 1991b, 1992a, 1992b), contusion (Drew et al., 2004), and anterior lateral spinal cordotomy (Vierck and Light, 1999, 2000) have been employed to evaluate pathophysiological and behavioral changes occurring after SCI. Although these models will not be discussed, several reviews have compared and contrasted the models (Vierck et al., 2000).

While mechanisms underlying SCI pain are still unclear, evidence from experimental studies have demonstrated a relationship between abnormal pain sensitivity and several pathophysiological factors. Behaviorally, abnormal SCI pain in animals is evaluated by the presence of at-level or below-level changes in sensitivity. A common method to examine at-level pain sensations after SCI is assessing the presence of caudally directed grooming in dermatomes adjacent to the injury level. In addition, changes in nociceptive responses after SCI provide evidence for allodynia and hyperalgesia to

thermal and mechanical stimulation in dermatomes adjacent to or below the level of injury.

Excitotoxic Model of SCI

Recent research into SCI has demonstrated that trauma to the spinal cord produces damage to the gray matter through mechanisms of cell death. The release of excitatory amino acids (EAA) is implicated in the development of damage after SCI (Choi and Rothman, 1990). Subsequent release of glutamate after an insult activates AMPA and NMDA receptors initiating an excitotoxic cascade, which leads to neuronal cell loss within the gray matter of the dorsal horn (Berens et al., 2005; Gorman et al., 2001; Liu et al., 1991; Yezierski, 2002). The excitotoxic effect of EAAs is a critical initiating event for lesion progression and development of SCI pain. Furthermore, protection of neurons from the excitotoxic effects of EAA release has been minimized by the administration of NMDA and AMPA antagonists (Choi and Rothman, 1990; Liu et al., 1997) in addition to other treatments (agmatine; Yu et al., 2000, 2003).

The excitotoxic model of spinal cord injury utilizes an intraspinal injection of quisqualic acid (QUIS), an mGluR and ionotropic GluR agonist, to produce lesions of the gray matter (Berens et al., 2005; Caudle et al., 2003; Gorman et al., 2001; Yezierski et al., 1993, 1998). An important feature of the excitotoxic model is the occurrence of at-level and below-level pain, which is associated with neuronal loss (Yezierski et al., 1993, 1998; Berens et al., 2005). After an injection of QUIS, expression of spontaneous pain-like behaviors (e.g., overgrooming) is demonstrated at dermatomes corresponding to spinal segments near the lesion site. More importantly, overgrooming was prominent after sparing of the superficial dorsal horn (Berens et al., 2005; Yezierski et al., 1998). Superficial dorsal horn neurons (e.g., lamina I) are implicated in chronic pain conditions

(Ikeda at al., 2003). These cells also participate in the expression of injury induced overgrooming especially NK-1R expressing neurons (Khasabov et al., 2002). For example, Yezierski et al. (2004) reported that elimination of NK-1R neurons with a selective neurotoxin (e.g., substance-P saporin) reduced spontaneous pain-like behaviors after excitotoxic injury. Similar strategies have been used to reduce nociceptive responses to capsaicin (Mantyh et al., 1997) and nerve injury (Nichols et al., 2001).

By comparison, nociceptive responses to mechanical and thermal stimulation are augmented particularly in dermatomes adjacent to and below the lesion epicenter (Yezierski and Park, 1993; Yezierski et al., 1998). Evidence suggests a relationship between the enhancement of nociceptive responses and hyperexcitability of neurons (e.g., increased spontaneous activity, increased response to stimulation) bordering the area of neuronal loss (Yezierski and Park, 1993; Yezierski et al., 1998). Based on these and other lesion studies, a critical component of below-level spinal cord injury pain appears to be gray and white matter damage.

Additional factors impact the expression of heightened spontaneous and evoked nociceptive responses. In particular, these factors include the longitudinal progression, or the rostral-caudal distribution, of neuronal loss from the epicenter (~4.0 mm; Gorman et al., 2001; Yezierski, 1998). Furthermore, areas remote to the lesion epicenter also demonstrate changes after injury. Morrow et al., (2000) and Paulson et al. (2005) measured regional cerebral blood flow (rCBF), which indicates levels of neuronal activity in rodents. After excitotoxic injury, several supraspinal structures, which are targeted by rostral projecting pathways, were activated including forebrain (e.g., somatosensory cortex and thalamus). These areas are critical for the processing of pain and demonstrated

a remote effect of injury as a consequence of reorganization and/or deafferentation (Lenz et al., 1991). Other factors have already discussed including genetic factors (Brewer et al., 2001), sex hormones (Gorman et al., 2001), and endogenous opioid mechanisms (Abraham et al., 2000, 2001) are important for the expression of pain-like behaviors after excitotoxic injury.

Influence of Stress on Pain

Types of Stress

Stressors are characterized as either physical (systemic) or psychological (processive) and appear to activate different neural pathways. Systemic stressors (e.g., illness) primarily activate brainstem structures to restore homeostasis. By contrast, processive stressors (e.g. restraint) are processed by limbic structures and elicit emotional responses. Limbic activation by stress acts through hypothalamic and brainstem systems to initiate physiological and hormonal responses, and may modulate motor output through higher cortical centers (Herman and Cullinan, 1997; Herman et al., 1996). For example, the hypothalamus may not directly modulate a behavioral response, but rather modulates sensory input and the organization of learned responses driving the behavior.

Biological responses to stress

Several lines of research have suggested the ability of stress to modulate sensory perception in humans and reflex responses of laboratory animals. A stressor is defined as either an internal or external stimulus that presents an actual or perceived threat to the homeostasis of the organism (Herman and Cullinan, 1997). Exposure to a stressor induces a wide variety of adaptive stress responses including immune, hormonal, endocrine, physiological, and behavioral responses (Drolet et al., 2001; Herman and Cullinam, 1997). Ultimately, the stress response permits an individual to cope with the

stressor and maintain homeostasis under normal conditions, but after nerve injury, studies have suggested that stress can contribute to the development of psychopathologies and maintain the cycle of chronic pain (Herman and Cullinan, 1997; Melzack, 1999).

Modulation of Nociceptive Responses by Stress

Several studies have demonstrated that acute exposure to psychological stressors such as restraint produce attenuation of segmental and bulbo-spinal reflexive withdrawal responses to high intensity thermal stimuli as measured by both tail-flick and hotplate tests (Amir and Amit, 1978; Bodnar et al., 1978a, 1978b, 1978c, 1979; Calcagnetti and Holtzman, 1992; Calcagnetti et al., 1990, 1992; Gamaro et al., 1998), an effect referred to as stress-induced analgesia (SIA; Lewis et al., 1980). Reduction of reflexive responses to nociceptive stimuli is an adaptive response to acute stress exposure in order to cope with challenging situations. Transmitters regulating changes in nociceptive sensitivity on reflexive responses by stress include the endogenous opioid (Lewis et al., 1980; Porro and Carli, 1988), serotoninergic (Quintero et al, 2000), and noradrenergic systems (Watkins and Mayer, 1982). Interestingly, chronic stress exposure can increase sensitivity of reflex responses. For example, repeated exposure to an inescapable and uncontrollable stressor appears to induce sensitization of sensory neurons in the spinal cord. In addition, repeated exposure to cold-water swims produced a cutaneous thermal hyperalgesia as measured by reflex latencies (Quintero et al., 2000). Likewise, daily exposures to restraint stress over a forty-day period resulted in cutaneous thermal hyperalgesia as assessed by tail flick responses (Gamaro et al., 1998).

Modulation of Nociceptive Responses by Stress: Pharmacology

The antinociceptive effects of endogenous opioids, which are released after stress exposure, have been demonstrated after exposure to stressful stimuli. Opioid peptides are

derived from three separate precursor peptides and include enkephalin, endorphin, and dynorphin (Drolet et al., 2001; Yamada and Nabesima, 1995). These peptides interact with receptors distributed throughout the central and peripheral nervous system and are capable of modulating nociceptive sensations during stressful and painful stimuli (Kelley, 1982; Yamada and Nabesima, 1995). Threats to homeostasis induce the release of endogenous opioid peptides and are speculated to permit the organism to cope with the stressful situation (Amit and Galina, 1988; Terman et al., 1984).

Activation of the endogenous opioid system has been shown to parallel the induction of stress-induced hyporeflexia, or suppression of nocifensive reflex responses, by various stressors (Bodnar et al., 1978a; Gamaro et al., 1998; Madden et al., 1977). For example, a single exposure to foot-shock produced hyporeflexia, as measured by increasing time to elicit tail-flick responses to thermal stimulation. Stress-induced hyporeflexia also parallels increases in endogenous opioid levels in the central nervous system (Madden et al., 1977). Involvement of the opioid system in stress-induced changes in nocifensive responses was further characterized by: 1) naloxone, an opioid antagonist, which reversed stress-induced hyporeflexia in rats; and, 2) cross-tolerance between stress-induced hyporeflexia and morphine after repeated exposure to stress (Bodnar et al., 1978b; Girardot and Holloway, 1984; Lewis et al., 1980).

Restraint stress

In relation to other stressful stimuli, restraint stress is considered to be a psychological stressor and has been used to induce stress-induced hyporeflexia in rats (Tusuda et al., 1989; Calcagnetti et al., 1992; Gamaro et al., 1998). For example, Gamaro et al. (1998) demonstrated that exposure to a single session of restraint for one hour produced stress-induced hyporeflexia as assessed by tail-flick assay in both male

and female rats. Studies have also demonstrated the role of endogenous opioids in restraint-induced hyporeflexia on tail-flick and hindpaw withdrawal. The antinociceptive effects of μ-opioid agonists on reflexes (e.g., morphine, DAMGO) were potentiated after acute exposure to restraint stress (Abbelbaum and Holtzman, 1984; Abbelbaum and Holtzman, 1985; Calcagnetti et al., 1990; Calcagnetti and Holtzman, 1992; Calcagnetti et al., 1992). For example, restraint stress enhanced the hyporeflexive effects of opioids, indicated by increases in reflexive withdrawal latencies to thermal stimulation after systemic (Abbelbaum and Holtzman, 1984, 1985, 1986; Fleetwood and Holtzman 1989; Calcagnetti and Holtzman, 1990, 1992), intrathecal (Calcagnetti et al., 1992), and intracerebroventricular (Abbelbaum and Holtzman, 1985, 1986; Calcagnetti et al., 1990) administration compared to unstressed controls. These studies also suggest that both spinal and supraspinal opioid mechanisms contribute to stress-induced potentiation of opioids after i.t. and i.c.v. opioids on reflexive tests of nociception.

Additional evidence for the involvement of endogenous opioids in stress-induced hyporeflexia is observed after systemic injections of opioid antagonists. Administration of naloxone reverses the hyporeflexic effects of stress (Pilcher and Browne, 1983). Finally, evidence of endogenous opioids in stress-induced hyporeflexia is supported by the development of cross-tolerance between stress-induced hyporeflexia and morphine after repeated exposure to stress (e.g., habituation) or repeated exposure to morphine (e.g., tolerance). For example, the potentiation of the inhibitory effects of opioids by stress is reduced in habituated rats (Fleetwood and Holtzman, 1989) and morphine-tolerant rats (Torres et al., 2003). It is clear from these studies that restraint stress activates components of the endogenous opioid system and is involved in the

modulation of responses to nociceptive stimuli. While the impact of stress on nociceptive reflex responses has been appreciated, no previous studies have examined the effects of stress on operant responses.

Modulation of Nociceptive Responses by Stress: Chronic Pain

Stress is reported to increase nociceptive sensitivity in individuals with chronic pain (Ditor et al., 2003; Galvin and Godfrey, 2001). This effect of stress is especially significant, as stress has been linked to the onset and maintenance of numerous life threatening medical conditions, including those that severely compromise ones quality of life. Clinically, the presence of psychological stressors correlate with conditions of increased sensitivity in individuals with a variety of defined pain conditions, including fibromyalgia and those arising from nerve injuries (spinal cord injury). Furthermore, acute stress has been shown to increase pain sensitivity in chronic pain patients, and has been suggested to contribute to the development of chronic pain syndromes like fibromyalgia, rheumatoid arthritis, and irritable bowel syndrome (Bennet et al., 1998; Blackburn-Munro and Blackburn-Munro, 2001; Davis et al., 2001; Mayer et al., 2001). Even though patients with conditions such as spinal cord injury develop chronic pain, the effect of stress in clinical settings has not been adequately addressed. Likewise, pre-clinical models of chronic pain have not addressed the impact of stress on altered sensation after spinal injury.

Thermoregulation by Sympathetic Vasoconstriction

Numerous physiological mechanisms mediate behavioral responding to nociceptive stimulation including the sympathetic component of the autonomic nervous system. The autonomic system plays an essential function in mediating physiological responses to internal or external stimuli (McDougall et al., 2005). It also is implicated in pain

perception and affective/motivational states (Thayer and Brosschot, 2005). The regulation of heat (thermoregulation) is a consequence of sympathetic activity. Various manipulations can alter sympathetic tone, ultimately affecting the distribution of body heat and blood flow. For example, exposure to mental stress increases body temperature. In response to increase body temperatures, sympathetic-mediated vasoconstriction reduced peripheral temperature (cooling) by restricting blood flow (Cooke et al., 1990; Larsson et al., 1995; Nicotra et al., 2005).

Sources of sympathetic regulation are localized in the intermediolateral column of the thoracolumbar spinal cord (Hofstetter et al., 2005). Various neuroanatomical structures are involved in regulating the outflow of sympathetic preganglionic neuronal cell bodies including the hypothalamus, prefrontal cortex, amalgdala, and RVM (Dampney, 1994; Korsak and Gilbey, 2004; McDougall and Widdop, 2005; Nalivaiko and Blessing, 2001). Activation of the sympathetic nervous system is also accomplished by the HPA axis after exposure to nociceptive stimulation (Janig, 1995; Magerl et al., 1996) or stress (Herman and Cullinan, 1997; McDougall et al., 2005).

Activity of the sympathetic system can be evaluated indirectly by assessment of peripheral vasoconstriction during thermal stimulation (Shimodoa et al., 1998; Vierck, Unpublished Observations; Wakisaka et al., 1991; Willette et al., 1992). Overall, nociceptive stimulation decreases skin temperature ipsilaterally and contralaterally in non-stimulated areas. It appears that activation of nociceptors by stimulation triggers a sympathetic response (Magerl et al., 1996). Manipulations have been shown to increase sympathetic-mediated vasoconstriction, including stress (Larsson et al., 1995), peripheral injury (CCI: Kurves et al., 1997; Wakisaka et al., 1991), and spinal injury (Acosta-Rau,

2003). Furthermore, a relationship exists between the ability to demonstrate vasoconstriction and change in thermal sensitivity. If a manipulation blunts the expression to vasoconstriction, it will also display an enhanced sensitivity to thermal stimulation (e.g., increase escape response to heat). Reduction of vasoconstriction in response to thermal stimulation has been demonstrated after formalin (C. Vierck and R. Cannon, Unpublished Observations; C. Vierck and A. Light, Unpublished Observations) and excitotoxic injury to gray matter (Acosta-Rua, 2003). Thus, enhanced nociceptive responding is a consequence of peripheral and central injury that dramatically alters ability of the sympathetic nervous system to regulated cutaneous temperatures via vasoconstriction.

Summary

Efforts to study the effects of stress on sensory processing in pre-clinical models have frequently sought to employ reflexive behaviors as endpoints for assessing stress-induced alterations in nociception. It is clear that such reflex functions are mediated by systems that respond to environmental cues and previous experience. These end-points fail to account for the interactions between stress and higher order functions initiated by a particular stimulus condition. In contrast, operant escape responses reflect a higher order organizational function, presenting an approach by which we might establish a clinically relevant model of motivated behavioral responses to nociceptive stimuli that permits an evaluation of the affective component of pain. Though the importance of stressors on higher order function has been long appreciated, there are few studies that have examined the effects of stress on operant responses before and after injury to the central nervous system. The ultimate goal of the present proposal is to

understand the apparent differential modulation of sensory processing by stress in normal and after spinal cord injury on several tests of nociception.

CHAPTER 2
EXPERIMENTAL METHODS AND DESIGN

The goal of this research is to increase our understanding of the effects of stress on sensory processing. In order to accomplish this goal, behavioral and physiological techniques were used to assess changes in nociceptive sensitivity in injury-naïve and spinally injured rats. Each behavioral testing session consisted of 2 consecutive testing trials in separate apparatuses that were constructed of plexiglass. Animals were exposed to a neutral temperature during the first trial (e.g., pre-test), which was used to normalize temperatures of the rodent's hindpaw and acclimate the animal to the apparatus. Thermal stimulation was delivered through a heated or cooled aluminum plate. During succeeding testing trials (e.g., test), animals were exposed to a range of non-nociceptive and nociceptive temperatures. The responses collected during the second trial were recorded through customized computer software. Assessment of nociceptive responses was accomplished by comparing reflex lick/guard, operant escape, and thermal preference responses before and after exposure to restraint stress and following spinal injury.

In order to produce stress-induced changes in nociception, restraint stress, a psychological stressor, was selected based on an extensive literature demonstrating that restraint activates limbic circuits and affects reflex responses to thermal stimulation. Restraint stress is a useful and convenient stressor, which can be delivered without difficulty, and does not present a direct thermoregulatory challenge to the animal compared to other stressors (e.g., cold water swim). Likewise, several studies have concluded that the underlying mechanisms mediating restraint-induced changes in

nociception is a result of activation of the endogenous opioid system. Based on these studies, pharmacological agents (e.g., naloxone and morphine) were used to determine the effect of the endogenous and exogenous opioids on stress-induced changes in nociception.

Furthermore, a common condition confronting individuals with spinal cord injury is chronic pain. In order to study the pathophysiology underlying SCI pain, the excitotoxic model of SCI that was developed by Dr. Yezierski shares similar pathophysiological consequences common after traumatic and ischemic SCI. This model provides an excellent platform to study altered pain processing after spinal gray matter damage. Behavioral manifestations of altered nociception after excitotoxic injury include spontaneous (e.g., at level grooming) and evoked (mechanical allodynia and thermal hyperalgesia) pain sensations (Gorman et al., 2001; Yezierski et al., 1998). Finally, experimental manipulations (e.g., stress and spinal injury) affect pain sensations by various mechanisms including modulation of cutaneous skin temperature by the autonomic nervous system.

Experimental Animals

Female Long Evans rats were housed in pairs and maintained on a 12-12 hour light-dark cycle with free access to food and water. The reasons for using female rats for behavioral testing were based on observations that females were easier to handle, less aggressive, and maintained their body weight over time. Also, chronic pain is more common in females compared to males. The rats were adapted to the testing apparatus and handled prior to behavioral training and baseline testing. All experiments were carried out according to the Guide for the Care and Use of Laboratory Animals and were

approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Florida (B193 and C013).

Behavioral Testing Procedures

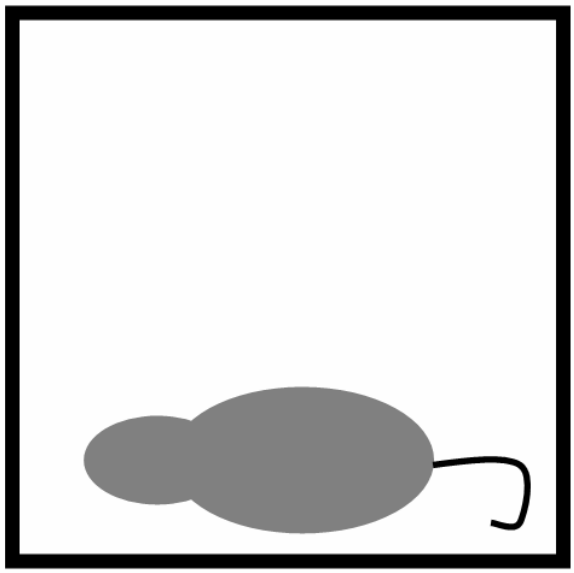
Assessment of Reflex Lick-Guard Responses

Reflex responses represent a supraspinally-mediated behavior. Lick responses were recognized as stereotyped lifting of one hindlimb, then holding and licking the hindpaw. Guard responses were scored when a hindlimb was raised from the platform and flexed in an exaggerated fashion. Guard responses were longer in duration than limb flexion that occurred during ambulation. Hindlimb reflex responses were measured during the second trial including frequency (number of responses during a trial), duration (total time spent licking or guarding during a trial), and latency to first lick-guard response.

Reflex apparatus

The apparatus used to evaluate lick-guard responses consisted of the reflex apparatus consists of a plexiglass box with a thermally regulated floor without an escape option (Figure 2-1). The enclosure was ventilated to permit airflow. Although no training is required for reflex responding, rats were familiarized with the apparatus and the testing procedure over 2-week period. Rats, which are not properly adapted to the testing environment, display stress-induced hypoalgesia due to the novel environment (Plone et al, 1996).

Two consecutive trials were used to assess reflex responses. Similar to escape testing, a 15 minute trial at 36.0°C (pre-test trial) was used to standardize foot temperatures, which was followed by a second 10 minute trial at 44.5°C (test trial).



Heat Plate

No Escape Platform

Figure 2-1. Reflex apparatus.

Because the animal cannot escape thermal stimulation, trial durations of 10 minutes were selected to prevent tissue damage. Reflex responses were exhibited during thermal stimulation at 44.0°C but not at 36.0°C.

Assessment of Operant Thermal Escape Responses

Operant escape apparatus

The escape apparatus incorporates a shuttle-box design, as described previously (Figure 2-2; Mauderli et al., 2000; Vierck et al., 2002). The escape test was carried out in a plexiglass box divided into two compartments by a hanging wall with an opening to permit rats to move freely between the compartments. The first compartment is dimly illuminated (0.5 foot candles) and includes a thermally regulated floor, which can deliver either non-nociceptive or nociceptive stimuli (43.0 to 47.0°C) to the paws during occupancy. Thermal stimulation was delivered by an aluminum plate regulated by a water bath (Neslab). The adjacent compartment contains a brightly illuminated (35-watt) halogen bulb above a thermally neutral escape platform. The platform provides animals an opportunity to escape nociceptive thermal stimulation. The dual compartment set-up provides a conflict between aversion to light and thermal nociception. As a consequence, rats will proportion their time on the platform in relation to the intensity of stimulation.

Operant escape training and assessment

Rats were trained over 3 weeks to learn to escape from thermal stimulation by climbing onto the neutral escape platform. During the training period, rats were familiarized with the testing procedure and trained to discriminate between gradually increasing floor temperatures (36.0, 40.0, 42.0, 44.0, 45.0, and 47.0°C) in the absence (first phase) and presence of bright light over the escape platform (second phase).

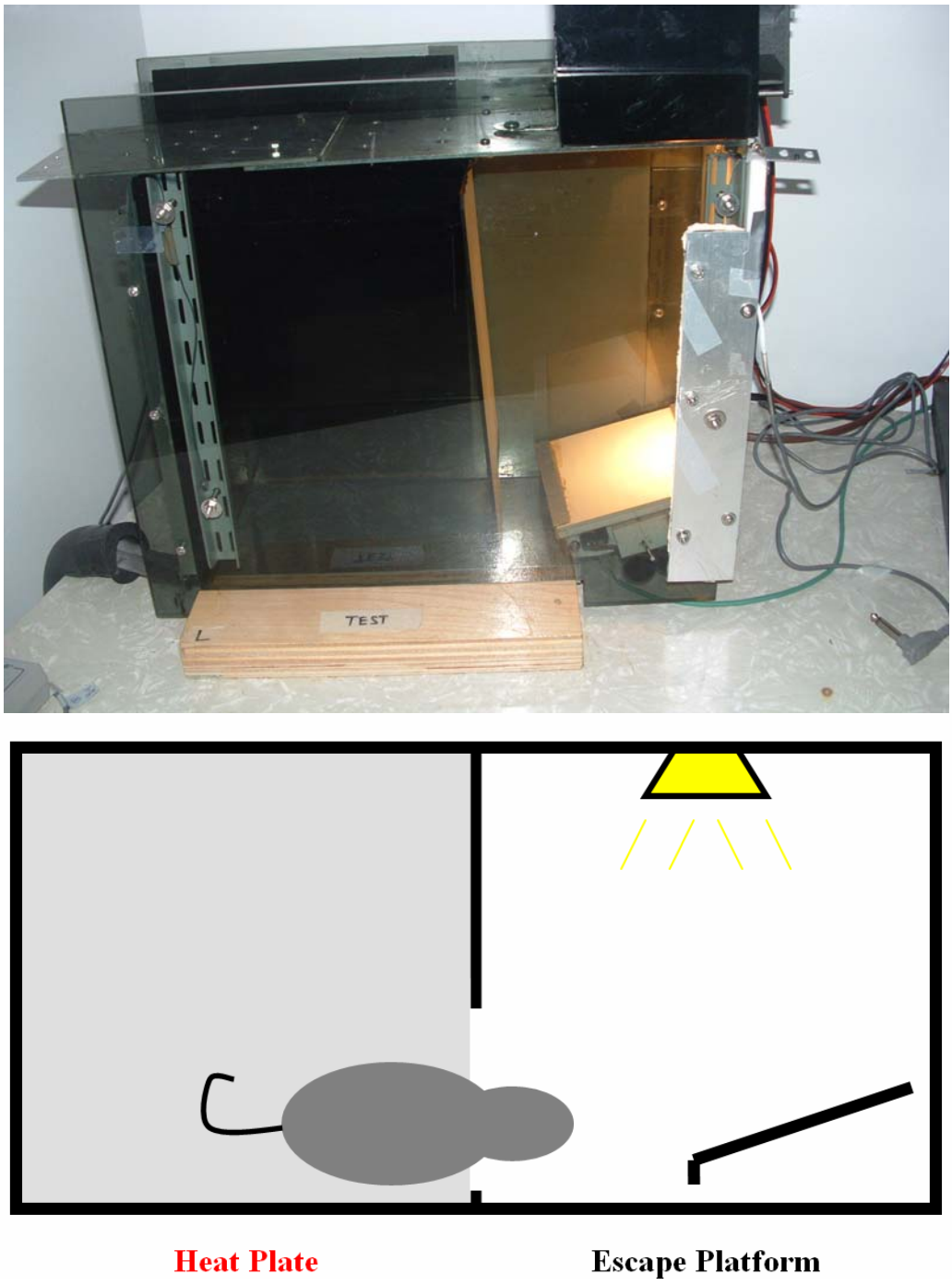


Figure 2-2. Operant escape apparatus.

Each training session consisted of two consecutive 15 minute trials. The first trial consisted of pre-test condition at 36.0°C, and the second trial consisted of a range of gradually increasing temperatures over successive daily sessions. The pre-test was used to standardize foot temperatures prior to testing, acclimate the rats to the apparatus, and extinguish avoidance behavior (e.g., occupancy of the escape platform unrelated to floor temperature).

After operant training, baseline escape responses were assessed over a 6 week period. Similar to training, rats were tested daily with two consecutive 15 minute trials at 36.0°C (pre-test) and then at 36.0, 44.0, or 44.5°C (test trial). Escape responses during the second (test) trial were assessed including frequency (number of responses during a trial), duration (total time occupying escape platform during a trial), and latency to first escape response.

Assessment of Operant Thermal Preference Responses

An additional operant assessment strategy included the thermal preference test (Mauderli et al., 2000; Vierck et al., 2002). This test can determine if an experimental manipulation (stress or injury) selectively affects cold (***first compartment***) or heat (***second compartment***) nociception. Preference of a thermal modality (cold or heat) will depend on the temperatures and experimental manipulation used. For example, if a manipulation affects sensitivity to heat nociception, the animal will spend less time on the heated compartment and more time on the cold compartment. In cases when both modalities are affected, animals will increase their preference for a modality less affected by the manipulation

Thermal preference apparatus

Similar to the operant escape test, thermal preference apparatus (Figure 2-3) uses a shuttle-box design that requires an animal to choose between two distinct compartments. However, unlike the operant escape test with one thermally regulated floor, both floors of the thermal preference are thermally regulated at different temperatures. The first and second compartments presented cold (0.3, 10.0, 15.0, or 36.0°C.) and heat (43.0, 44.0, 44.5, 45.0, 46.0 or 47.0°C.) nociceptive temperatures, respectively. In addition, the preference test was preceded by a pre-test at 36.0°C to standardize foot temperatures prior to placement into the testing apparatus.

Thermal preference training and assessment

Following one week of preference training, baseline responses were assessed over a 2 month depending on the stability of operant behavioral responses. It is important to note that only one cold and hot temperature, which are listed above, were used during a single testing session (e.g., 15.0 paired with 45.0°C.). The duration of a single thermal preference was 12 minutes to avoid tissue damage. Thermal preference responses were assessed by frequency (number of crossing during a trial), duration (total time spent occupying the escape platform during a trial), and latency to first thermal preference response.

Assessment of Darkbox Responses

The darkbox test was used to assess motivation to escape the light and to evaluate whether motor deficits (e.g., freezing behavior) were induced by experimental manipulations like restraint stress. The apparatus consisted of two compartments with a 2½ by 2½ inch opening in the dividing wall.

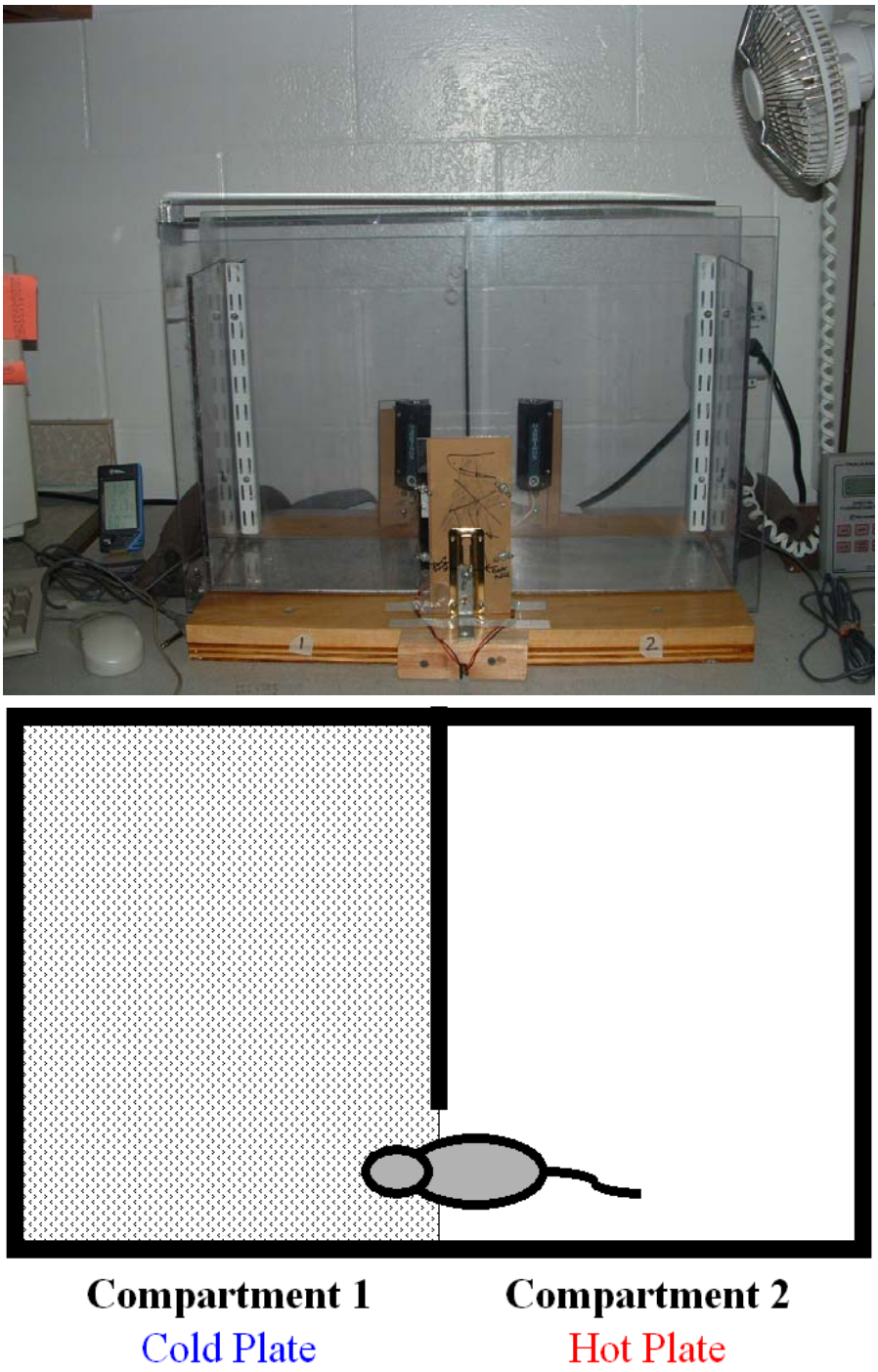


Figure 2-3. Thermal preference apparatus.

Each testing session began with ten-seconds of acclimation in which a computer identified the location of the rat by weight. Then, a 70-sec trial was initiated with presentation of light in both compartments. When the rat moved from the compartment it occupied at the start of the session to the adjacent compartment, the light was extinguished in the selected compartment for the remainder of the 70 second trial. At the end of the trial, both compartments were lit to initiate the next trial. Darkbox latency was defined as the time required for the rat to move to the adjacent compartment. Each session consisted of seven light escape trials over fifteen minutes. During stress testing, each rat was placed in the darkbox apparatus 15 minutes after termination of the stress exposure.

Assessment of Open Field Responses

The modified open field test consisted of a 90 cm x 90 cm square black Plexiglas container with an adjacent 20 cm x 20 cm start-box which allowed the animal to either remain in the start-box or enter into the open field (Figure 2-4; picture provided by Dr. Darragh Devine). A light fixture illuminated the open field about (5 to 150 Lux). A door separated the two boxes, which was opened via a rope and pulley system. Upon opening of the door, the rope was secured with a hook until the next trial. A camera, located above the box, recorded the animal's behavior. The trial duration for the open field was 5 minutes. After exposure to the field, the rat was returned to its homecage. The open field assesses anxiety-like responses in rats during exposure to a novel environment.

Restraint Stress Procedures

After stabilization of behavioral responses, rats were selected to receive an acute exposure to restraint stress (*stress condition*) or remained in their home cage until behavioral testing (*control condition*). Rats were removed from their home cages, and

rats were restrained for 15-minutes. The restraint tube (Figure 2-5; D. Devine, Personal Communications) is composed of a soft flexible sheet of plastic 11" X 7 ¾" mounted to a rigid plexiglas cradle (8 ½" X 3' X 3") by means of two small bolts with convex heads. There are ventilation holes at one end to allow unrestricted breathing, and the other end has a vertical slot to allow comfortable placement of the tail during the restraint process. The plastic sheet is then gently rolled around the animal and held securely in place with two 12' X 1" Velcro strips.

Groups either remained in their home cage until testing or received a 15 minute exposure to restraint stress (Figure 2-6). Then, each rat was removed from the restraint tube and placed in the pre-test apparatus at 36.0°C for 15 minutes. Rats were then placed in the adjacent test apparatus at 36.0°C (thermally neutral control temperature) or 44.5°C (testing temperature) for an additional 10 to 15 minutes depending on the behavioral test. Control rats followed the same protocol and did not receive stress on the day of testing. Both groups of rats remained in their home cages in a separate room until stress exposure or behavioral testing was complete. All temperatures were held constant over the two days of testing.

On successive testing weeks, exposure to restraint stress was switched to the group previously in the control condition. For example, group 1 received restraint stress while group 2 was not be exposed to stress, serving as a control. The following week group 2 was exposed to restraint stress and group 1 served as a control. The experiment was designed to expose animals to stress every two weeks with the aim of 1) avoiding adaptation and 2) using each animal as their own control. This testing protocol has been shown not to cause carry-over effects of stress (King et al., 2003).

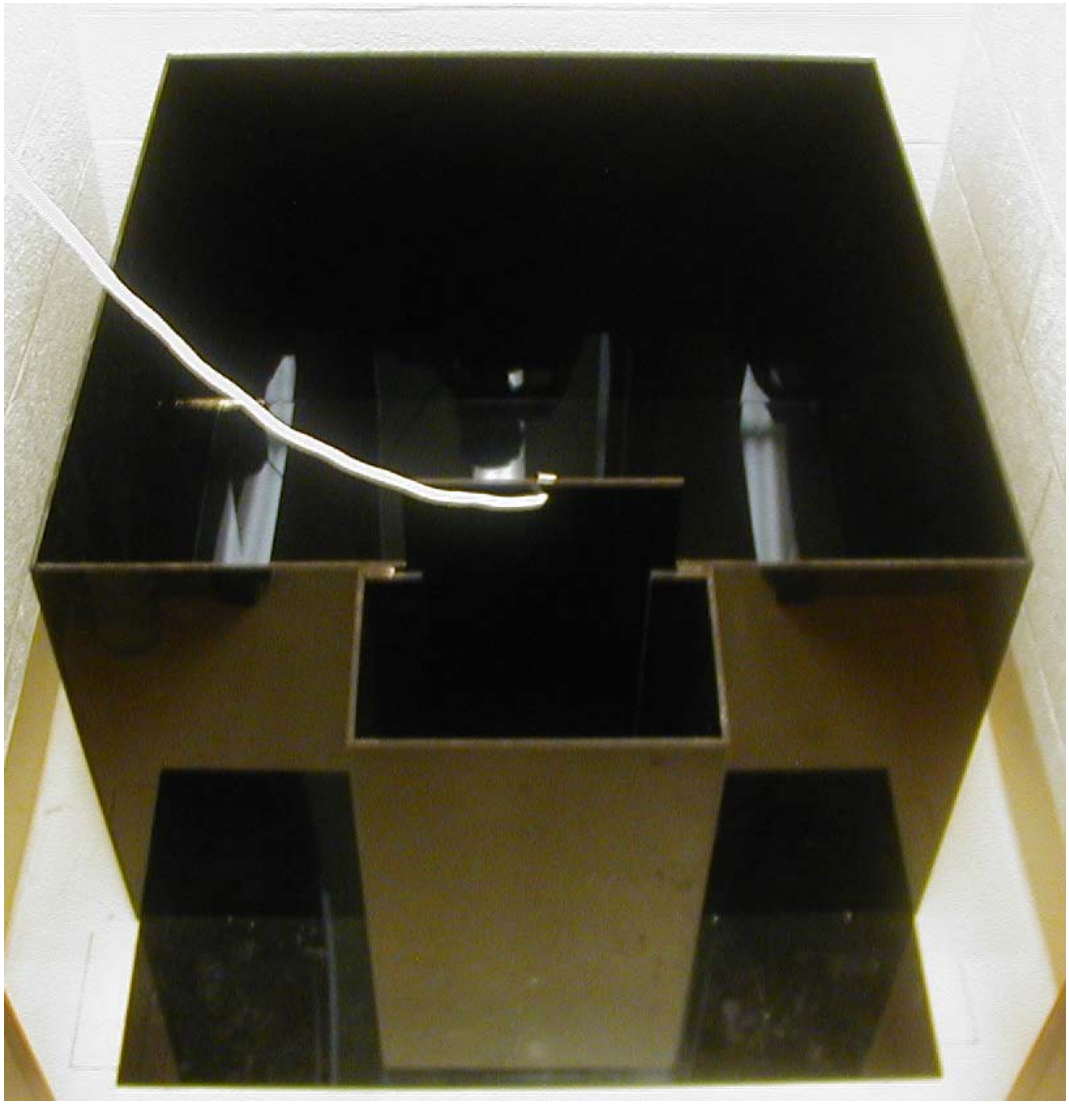


Figure 2-4. Open filed apparatus.

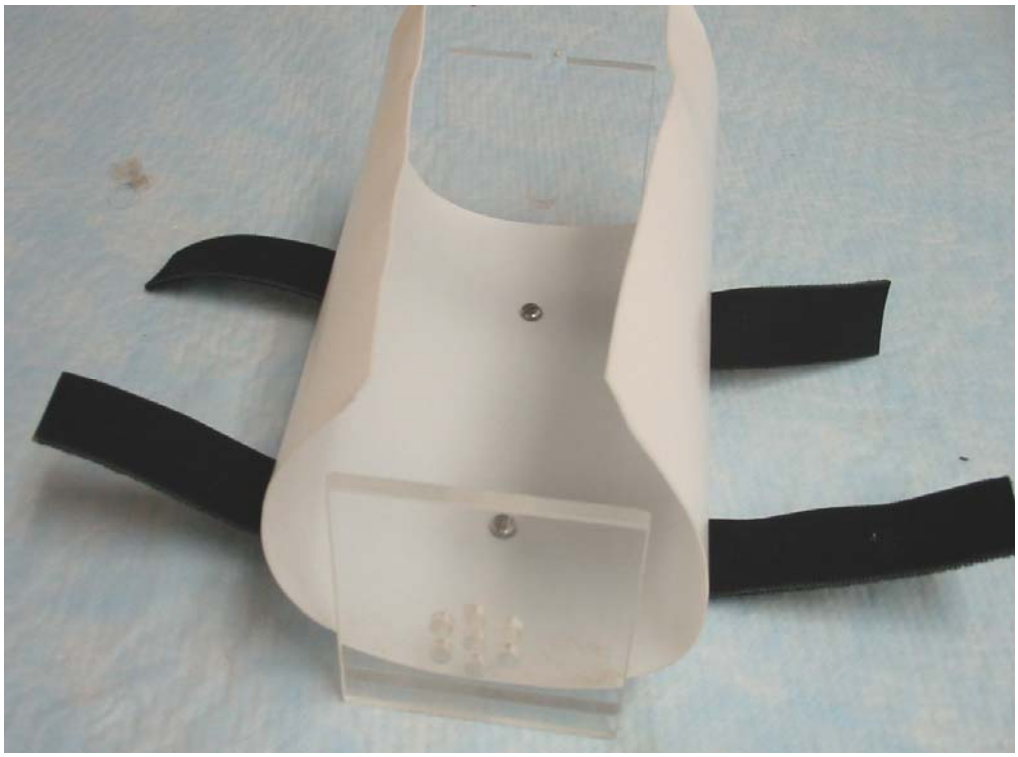


Figure 2-5. Restraint tube.

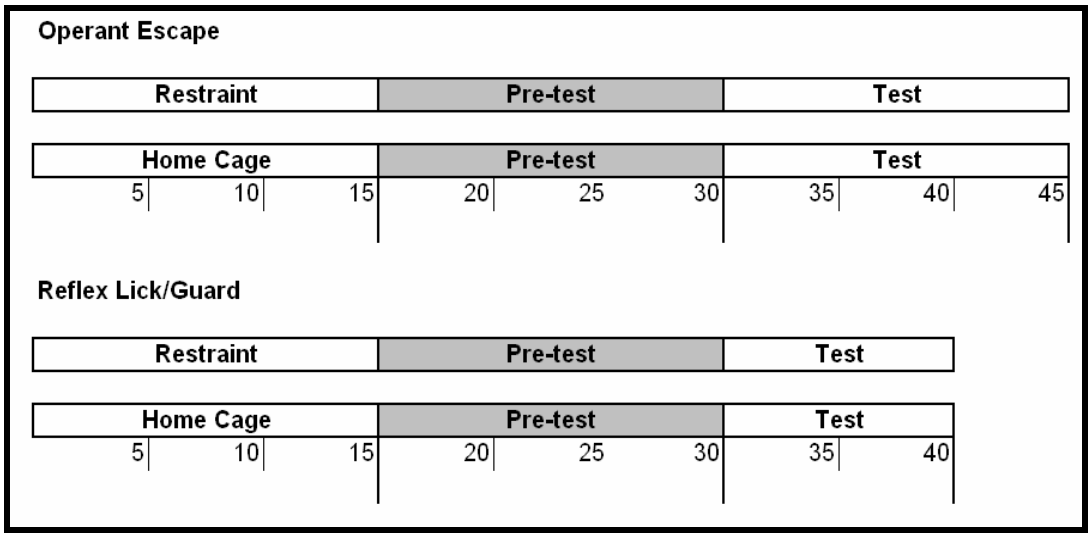


Figure 2-6. Behavioral testing sequence, stress exposure, and injection schedule for evaluation of operant and reflex lick/guard responses.

Drug Administration

In a separate group of animals, behavioral responses in normal animals were evaluated after an injection of naloxone and morphine. After stabilization of baseline responses, rats were randomly assigned to receive either stress or no stress before behavioral testing. After 15 minutes of restraint stress, rats were immediately injected with either an injection of morphine (opioid agonist, 1 mg/kg, i.p.), naloxone (opioid antagonist, 3 mg/kg, i.p.), or saline (1 mg/kg, i.p.). Then, rats were placed into the pre-test apparatus at 36.0°C for an additional 15 minutes. Behavioral responses were recorded during the second trial at 44.5°C. Control subjects remained in their home cage separate from stress subjects. Control subjects were injected and tested similarly to stress subjects, but control subjects did not receive stress on the day of testing.

Surgical Procedures

In this study, the effects of stress on operant responses were assessed after excitotoxic lesioning of mid-thorasic (T_8) or lumbar (L_2) segments of the spinal cord. Rats received an intraspinal injection of quisqualic acid (QUIS), which is a non-NMDA receptor agonist that interacts with AMPA and mGlu receptors. Previous studies have shown that nociceptive responses are enhanced after an intraspinal injection (Acosta-Rua, 2003; Gorman et al., 2001; Yezierski et al., 1998). The escape and thermal preference responses were recorded before and after surgery. Excitotoxic lesioning of the spinal cord was conducted after several weeks of baseline testing. Behavioral testing was resumed 2 weeks after surgery. At 8 weeks post-op, rats were removed from their home cages and placed in a restraint tube for 15 minutes. After stress exposure, rats were placed in the pre-exposure testing apparatus at 36.0°C. After fifteen minutes of

pre-exposure, rats were placed in the adjacent testing apparatus at 44.5°C. Both control animals and animals waiting to receive stress were kept separate from animals undergoing stress.

Intraspinal Injection of Quisqualic Acid (QUIS)

As mentioned previously, animals underwent excitotoxic injury as mentioned in other studies (Gorman et al., 2001; Yezierski et al., 1998). Rats were anesthetized with a combination of ketamine (3 ml), acepromazine (1 ml), and xylazine (1 ml) at 0.65 ml/kg administered subcutaneously. Level of anesthesia was evaluated by noxious pinch of the hindpaw. Body temperature was maintained at normal levels (36.5°C) during QUIS surgery and post-operative period. Several pathological features occur after QUIS injections including neuronal loss, cavitations, demylination, and alteration of glia (Yezierski et al., 1993, 1998; Berens et al., 2005). Following injections muscles were closed in layers, the skin closed with wound clips, and animals returned to their home cages.

Intraspinal Injection Procedures

After placement into a sterotaxic frame, the dorsal surface of the spinal cord was exposed via laminectomy corresponding between spinal segments T_8 to L_2. After removal of the dura and pia matter, QUIS was injected bilaterally into the spinal cord to target mid-thoracic and upper lumber spinal cord segments. Glass micropipettes (tip diameter 5 to 10μm) attached to a Hamilton microliter syringe (volume 5μl) are used for injections. The syringe was mounted on a microinjector attached to a micromanipulator. Injections were made between the dorsal vein and dorsal root entry zone at depths ranging from 500 to 1200 μm below the surface of the cord. To avoid white matter

damage, all intraspinal injections were placed in the middle of the gray matter (lumbosacral cord: T_8-L_2). Stock solutions of 125 mM QUIS (Sigma) was made fresh daily using sterile saline and buffered to physiological pH as needed. At each injection site 0.1-0.6 µl of QUIS was injected (over a 60 second time interval). The total volume of QUIS injected/animal was 1.2 to 1.5 µl per side. The standard injection consisted of three bi-lateral injection tracks separated by 0.5 mm.

In Vitro MRI Analysis of Spinal Cord

At the end of the study, animals were injected with sodium pentobarbital (1 ml, i.p.) and underwent transcardial perfusion with PBS followed by 10% formalin in PBS (Fischer Scientific). Spinal cord segments containing QUIS lesions were removed and placed into an MRI tube. All excised cords were subjected to in vitro three-dimensional MR microscopy (3D MRM). Images were acquired with a three-dimensional (3D) gradient echo pulse sequence using a TR = 150 msecs, TE = 10 msecs with NA = 2. The image FOV was 2 cm x 0.5 cm x 0.5 cm in a matrix of 512 x 128 x 128 in a total data acquisition time of 1.5 hours. Therefore, MR images were acquired with a resolution of ~40 microns x 40 microns x 40 microns. A 3D Fourier transformation was applied to the acquired data matrix to produce the 3D image. General image processing and analysis was performed using custom software written in the Interactive Data Language (IDL, from Research Systems, Boulder, CO).

Assessment of Core and Cutaneous Temperature

The effects of experimental manipulations could be a consequence of altered temperature regulation (core and cutaneous). In order to evaluate the impact of stress and spinal injury on thermoregulation, core and cutaneous temperatures were evaluated

before and after exposure to restraint stress. In addition, temperatures were also evaluated before and after excitotoxic spinal injury.

Core Temperature

An implantable thermal probe (IPTT-300; BMDS, Delaware) recorded core body temperature. After induction of anesthesia with isoflurane, the injection site for the probe was prepared by removing the hair on the animal's back followed by aseptic preparation of the site with alcohol and iodine wipes. The probe was injected subcutaneously with a specialized injector. Temperatures were recorded by portable reader (BMDS, Delaware) without any restraint of the rat. Temperatures were recorded before and after exposure to the first testing apparatus. Data are expressed in degrees Celsius.

Autonomic-Mediated Skin Temperatures

The autonomic nervous system (ANS) regulates skin temperature by changes in vasoconstriction through activity of the sympathetic nervous system. Experimental conditions, including stress, pain, and injury, may activate and potentially alter the ANS. In order to determine if these manipulations could modulate an animal's autonomic response to thermal stimulation, skin temperatures were recorded in the absence (resting conditions) and presence of heat stimulation (44.5°C; Figure 2-7). In order to assess skin temperatures, all animals were injected with diazepam (10 mg/kg, i.p.). Previous research has demonstrated that isoflurane negatively affected autonomic activity, which is counteracted by diazepam (C. Vierck, Personal Communication). 1% Isoflurane is used to induce (5%) and maintain (1%) anesthesia. A thermal heating blanket is used to maintain normal body temperature (36.0°C). Several sites were monitored to changes in temperatures including rectal core temperature, both forepaws, and both hindpaws. For

each paw, a thermocouple was applied to the skin with an adhesive foam pad. For the right forepaw, left forepaw, and right hindpaw, thermocouples session were applied to the plantar surface, and a thermocouple was applied to the top of the left hindpaw (stimulated paw). After stabilization of paw temperatures, a pre-heated thermode (44.5°C.) was applied against the left hindpaw for 10 minutes. Cutaneous temperature of each (non-stimulated) paw was recorded for 20 minutes during (10 minutes) and after stimulation (10 minutes; resting period) to permit skin and core temperatures to return to baseline.

For each manipulation, pre-stress or pre-operative skin temperatures were collected several weeks prior to restraint or excitotoxic injury. For the stress condition, animals were stressed for 15 minutes followed by induction of anesthesia with isoflurane. Skin temperature was assessed 15 minutes after the termination of restraint, which permitted stabilization of skin temperatures before testing and corresponding to 30 minutes after the onset of stress.

Temporal Profile of Skin Temperature: Effects of Restraint

Stress condition

In order to evaluate the effects of a pre-exposure to 36.0°C (pre-test) on skin temperature after restraint stress, a thermocouple was secured to the left and right hindpaw (plantar surface) to the skin with an adhesive foam pad. Temperatures were recorded over a 30 second period. Then, animals were placed into a restraint tube for 15 minute. Skin temperature was continuously recorded during the entire trial. Animals were removed from the tube at the end of the restraint period. Following removal of the thermocouples, animals were placed into a 36.0°C pre-test for 15 minutes. Then,

thermocouples were reattached to both hindpaws for 1 minute. Core temperature was also recorded before restraint and at 5 minute intervals thereafter. The sequence of events paralleled the testing conditions for operant and reflex testing.

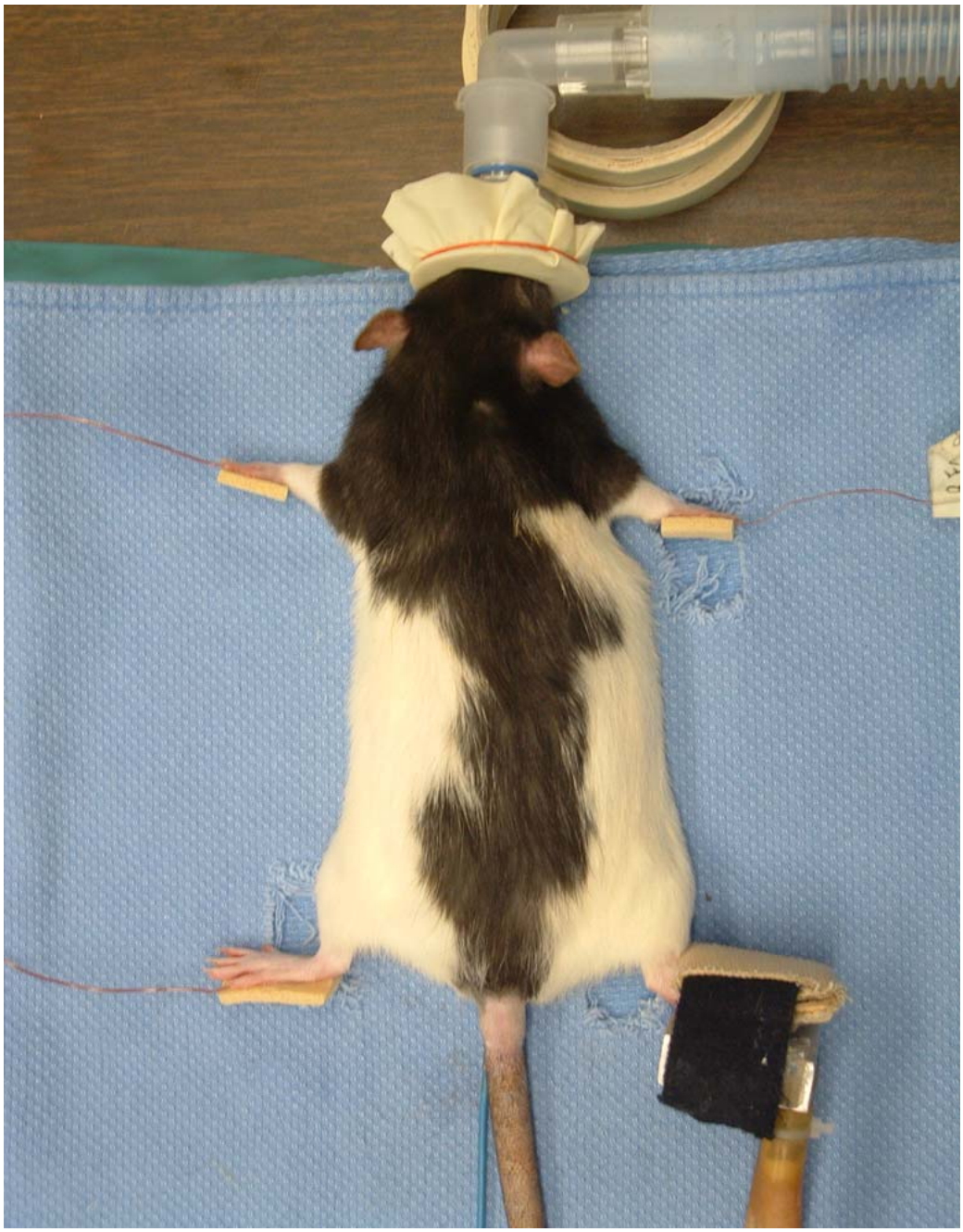


Figure 2-7. Skin temperature recording in anesthetized rats.

Control conditions

In addition, non-stressed control animals were tested before and after exposure to 36.0°C. Skin temperature was recorded for 1 minute prior to placement into the pre-test and then removed. After a 15 minute pre-test trial, animals were removed and thermocouples were reaffixed to both hindpaws.

Statistical Analysis

The frequency, latency, and duration of behavioral responses (escape, thermal preference, and licking/guarding, as appropriate) were collected by custom software (EVENTLOG, Autorat, Robot). The data are expressed in seconds, and values were represented as absolute group means ± S.E.M. Statistical analysis of behavioral responses between groups was performed by t-tests. Analysis of behavioral responses between groups was performed by an one-way analysis of variance (ANOVA) or two-way analysis of variance (ANOVA) with or without repeated measures followed by Newman-Keuls post-tests. P-values less than 0.05 were considered significant. Analysis was performed using GraphPad Prism version 4.00 for Windows, GraphPad Software, San Diego California USA (www.graphpad.com). Analysis of Covariate and correlations were performed by SSPS.

Study Design

The current experiments were based on previous literature and studies conducted in the labs of Drs. Vierck and Yezierski suggesting that nociceptive responses are mediated through different neuroanatomical pathways. Previous research has suggested that stressors inhibit reflex mediated nociceptive responses particularly to high intensity thermal stimulation. Inhibition of reflex responses has supported evidence for stress-induced analgesia (SIA). For example, acute exposure to restraint stress has been

shown to increase segmental (tail withdrawal) and spino-bulbo-spinal reflexes (hindpaw withdrawal or licking) to thermal stimulation. However, despite limit anecdotal and clinical evidence that stress enhances pain sensations, the effects of stress on thermal sensitivity in rats have not been examined by operant tests of nociception. Because pain is a complex sensation, it requires cortical structures to process and elicit appropriate actions. Thus, in order to study pain, proper behavioral strategies are required to examine these structures. Thus, the current study will determine effects of acute restraint stress on reflex lick/guard responses (a spino-bulbo-spinally mediated behavior) and operant escape responses (a cerebrally mediated behavior) in rats. It is hypothesized that an exposure to acute restraint stress produces a differential effect on reflex lick/guard and operant escape responses evoked by thermal stimulation.

In order to characterize the effects of stress on behavioral responses, the impact of various pharmacological agents and temporal profile were evaluated. First, the contribution of endogenous opioids to stress-induced changes in lick/guard and operant escape responses to thermal stimulation were evaluated by systemic administration of an opioid receptor agonist (morphine) or antagonist (naloxone) before behavioral testing. Previous studies have implicated endogenous opioid peptides as mediators of stress-induced hyporeflexia, as shown by the effects of either opioid agonists or antagonists on reflexive tests of nociception (Calcagnetti and Holtzman, 1992; Calcagnetti et al., 1990). Furthermore, stress has been shown to increase the release of endogenous opioids and modulate the physiological and psychological response to stressful and painful stimulation (Drolet et al., 2000; Madden et al., 1978). The effects of endogenous opioid peptides, however, after stress on responses dependent on cerebral

processing have not been examined. The inhibitory effects of stress on reflexive responses are modulated by the endogenous opioid system, but the system could oppose the facilitatory effects of stress on operant responses. It is hypothesized that the endogenous opioid system contributes to stress-induced reduction of spino-bulbo-spinal reflexes while opposing the excitatory effects of stress on cerebrally mediated operant escape responses to thermal stimulation.

Second, the temporal profile of stress on operant responses was assessed 15 minutes, 30 minutes, and 24 hours after the onset of stress. Previous stress literature has pointed out that magnitude of altered nociceptive responses is dependent on the duration and intensity of the stressor. Typically, long exposure to a stressor or a single exposure an intense stressor resulted in extended behavioral or physiological responses. Because the current stressor was only 15 minutes, it was hypothesized that the effects of restraint stress on operant responses would gradually disappear across time.

In addition, the effects of stress were examined on a well-established model of spinal cord injury (SCI), which results in enhanced expression of below-level behaviors. Stress triggers changes in several important physiological systems including the autonomic nervous system (e.g., sympathetic-adrenal system) and the hypothalamic-pituitary-adrenal axis (HPA axis). Clinically, psychological stress is associated with the progression and maintenance of several chronic pain conditions including fibromyalgia, irritable bowel syndrome, nerve injury, and rheumatoid arthritis. Likewise, physiological systems are altered in conditions of chronic pain.

In light of these clinical observations, only a limited number of studies have examined the effects of stress on altered nociceptive responses in chronic pain models,

particularly models of spinal cord injury. The current aim will use an excitotoxic model of SCI. In this model, animals underwent a bilateral injection of the AMPA/metabotropic receptor agonist quisqualic acid (QUIS) into the spinal cord. Operant responses were assessed before surgery (pre-op), after surgery (post-op), and after an exposure to restraint stress. It was hypothesized that excitotoxic injury of the spinal cord would produce an increase in thermal sensitivity and subsequent exposure to acute restraint stress will enhance injury-induced operant escape responses. Finally, the study examined a potential mechanism (thermoregulation) mediating altered thermal sensitivity by stress and excitotoxic spinal injury.

CHAPTER 3
EFFECTS OF RESTRAINT STRESS ON NOCICEPTIVE RESPONSES IN NORMAL SUBJECTS

Various experimental manipulations can influence processing of input from nociceptive afferents. Exposure to a stressor has been associated with both suppression (Abbelbaum and Holtzman, 1984; Borszcz et al., 1992; Gamaro et al., 1998) and enhancement (Borszcz et al., 1992; Huang and Shyu, 1987; Illich et al., 1995; King et al., 1996, 1999) of pain sensations. Based on several pre-clinical studies, stress inhibits reflex mediated responses (withdrawal of the tail or hindpaw) to thermal stimulation. However, some questions have been raised pertaining to relevance of reflex responses in the perception of pain, which is dependent on higher cortical processing. An important question can be raised concerning the effect of stress on pain sensations. Does stress affect responses dependent on operant processing of nociceptive information differently than reflex responses? Or does stress suppress reflex and operant responses similarly? In this chapter, the effects of restraint stress on reflex and operant (escape and thermal preference) responses to low intensity thermal stimulation, which activates C-nociceptor afferents by heat (44.0 to 44.5°C), were examined.

To control for confounding effects of stress (e.g., avoidance), control tests were also examined including operant escape responses at a neutral temperature (36.0°C) and darkbox responses. In order to further characterize the effects of stress on nociception, pharmacological conditions, which were based on previous research using reflex-based behavioral responses, were also investigated. Naloxone or morphine was administered to

determine the contribution of endogenous and exogenous opioids on normal and stress-induced changes in thermal sensitivity, respectively. Due to study limitations, opioid pharmacology was limited to reflex lick-guard and operant escape.

Effects of Restraint Stress on Reflex Lick/Guard Responses at 44.0°C

Behavioral responses of female rats (n=11) were assessed during a 3-day period, with baseline testing on Day 1 (baseline), post-stress or control testing on Day 2 (15 minutes), and evaluation of long-term stress effects Day 3 (24 hours). On Day 2, half the animals received 15 minutes of restraint stress, followed by a 15 minute pre-test and test trials as shown in Figure 3-1. Testing sessions included a 15 minute pre-test exposure to 36.0°C, followed by a test trial at 44.0°C. Reflex (Figure 3-1A) and operant escape (Figure 3-1B) responses were assessed for 10 and 15 minutes, respectively, during the testing trial. The control group remained in their homecage until behavioral testing.

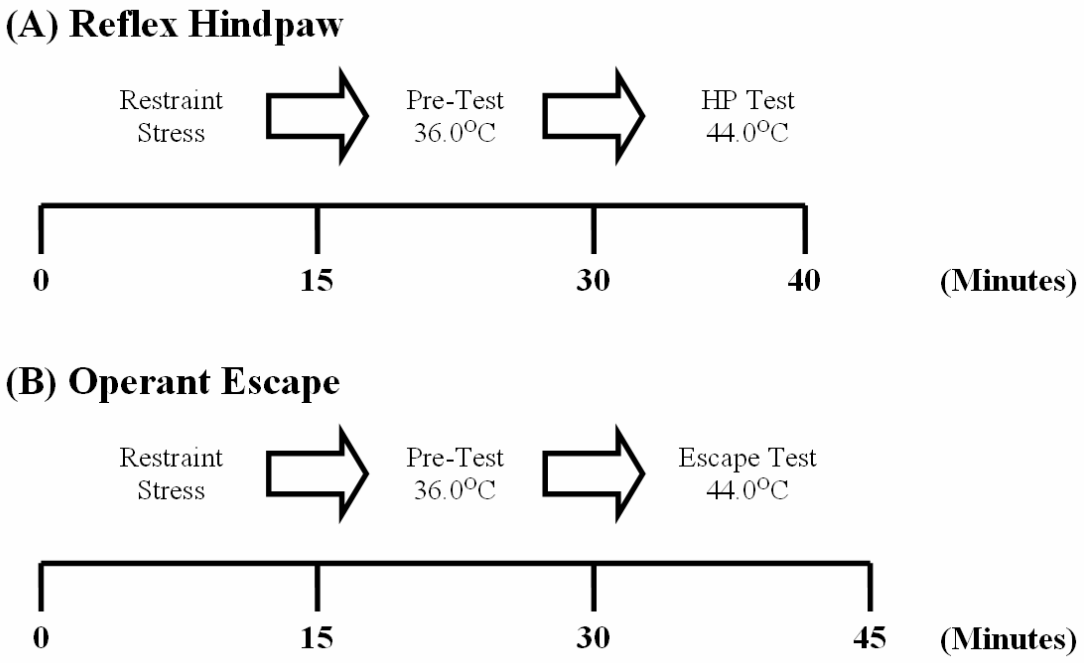


Figure 3-1. Behavioral testing sequence for the restraint group. (A) Reflex hindpaw. (B) Operant escape.

Reflex Lick-Guard Latency

The latency of first lick/guard responses to 44.0°C during baseline sessions (Day 1), testing sessions in which rats received 15 minutes of stress (Day 2; restraint group) or no stress (control group), and sessions 24 hours afterwards (Day 3) are presented in Figure 3-2. Latencies of lick/guard responses were significantly greater on Day 2 for the restraint group than for the control group (Figure 3-2A; F=24.61, P<0.001). Reflex response latencies were also greater for stressed rats on Day 2 than on days when the same rats were not stressed (Days 1 and 3; F=10.08; P<0.001). Difference scores between control and restraint groups revealed that reflex latencies were significantly higher after stress on Day 2 after stress (Figure 3-2B; F=8.78, P<0.001).

Reflex Lick-Guard Duration

The duration of lick/guard responses to 44.0°C were significantly lower for the restraint group than for the control group on Day 2 (Figure 3-3A; F=39.18, P<0.001). Reflex response durations were also significantly lower for the restraint group on Day 2 than on days when the same rats were not stressed (Days 1 and 3; F=13.61, P<0.001). Reflex responses for the control and restraint groups were stable on testing Days 1 and 3, demonstrating no adaptation to daily testing at 44.0°C. Difference scores between control and restraint groups revealed that reflex duration were significantly lower after stress on Day 2 after stress (Figure 3-3B; F=11.361, P<0.001).

Base on these observations on reflex latencies and durations, it can be concluded that exposure to restraint stress suppressed reflex responses. ***Stress-induced hyporeflexia*** was characterized by a longer latency to elicit a hindpaw response and a shorter time engaging in licking or guarding of the hindpaw. The effect was transient and did not

persist the following day. Thus, this data supports previous research suggesting that stress inhibits spino-bulbo-spinal reflexes to thermal stimulation.

Effects of Restraint Stress on Operant Escape Responses at 44.0°C

Operant Escape Latencies

To determine the effects of stress on operant responses, the same groups of animals (described above) was stressed on weeks interspersed between reflex testing (Figure 3-4). The first latency of operant escape responses during trials at 44.0°C were observed during baseline sessions (Day 1), testing sessions in which rats received 15 minutes of restraint stress (restraint group; Day 2) or no stress (control group), and session the following day (24 hours, Day 3). Previous studies have suggested that the first escape latencies are less dependable outcome measures compared to escape durations (Vierck et al., 2002; 2003). Consistent with these observations, no differences in escape latencies were observed between the restraint and control groups on any day of testing (Figure 3-4A; F=0.2570, P=0.7740). Difference scores revealed no significant effects (Figure 3-4B, F=0.818, P=0.447).

Operant Escape Durations

The duration of escape responses was significantly greater on Day 2 for the restraint group than for the control group on Day 2 (Figure 3-5A; F=38.48, P<0.001). Furthermore, the duration of escape was greater after stress exposure on Day 2 than it was for the same rats on unstressed days (Days 1 and 3; F=49.01, P<0.001). Therefore, acute restraint stress did not produce a long-term (24 hour) effect on escape duration. Difference scores revealed that escape durations were significantly higher than controls after exposure to stress (Figure 3-5B; F=11.305, P<0.001).

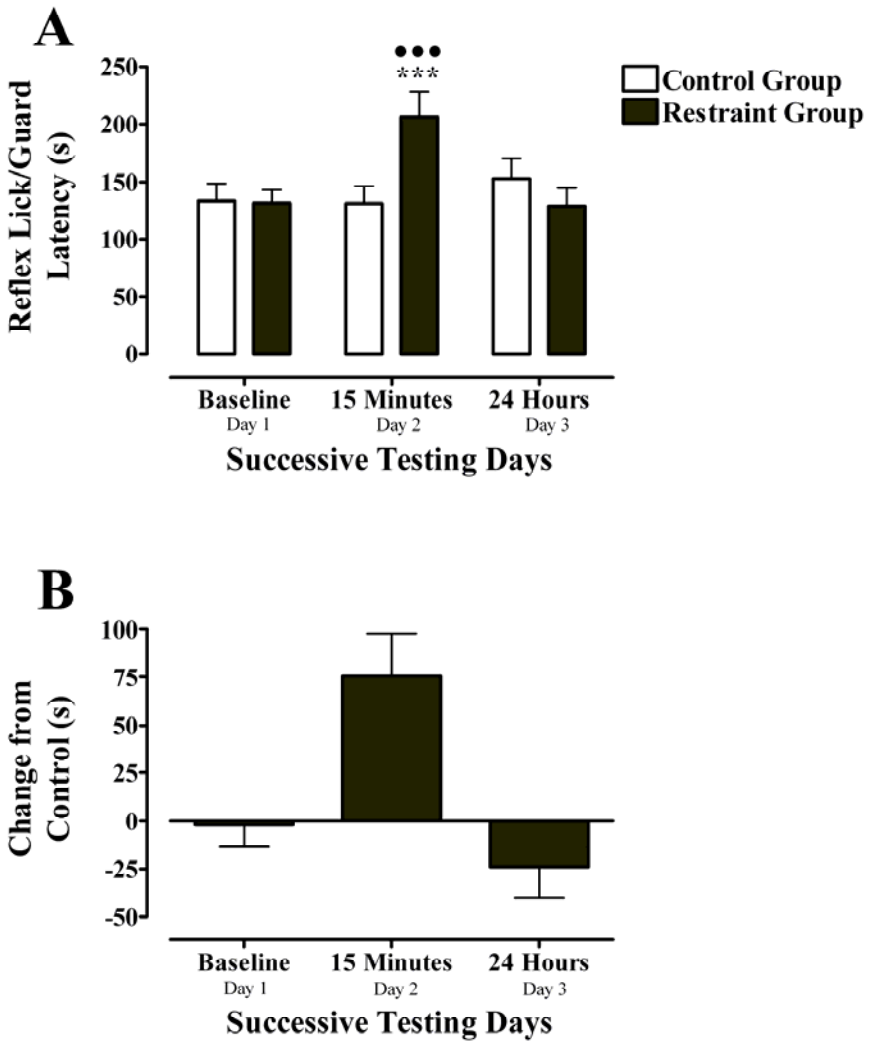


Figure 3-2. Reflex lick/guard latencies during testing trials at 44.0°C for control (open bar) and restraint (closed bar) groups during baseline sessions (Day 1), testing sessions in which rats received 15 minutes of restraint stress (restraint group; Day 2) or no stress (control group), and sessions the following day (Day 3). (A) Exposure to restraint significantly increased reflex latencies when tested fifteen minutes after stress (15 minutes; Day 2) compared to the control group and compared to unstressed trials on baseline and 24 hours after stress. (B) Difference scores confirmed that stress increased reflex latencies compared to controls (15 minutes; Day 2). Data are expressed in seconds and are represented as absolute group means ± S.E.M. Significant within-subject differences between trials 15 minutes after stress exposure and trials by the same rats on baseline and 24 hours are indicated as: *** $P<0.001$. Significant between-subject differences between the control and restraint groups on Day 2 are indicated as: ●●● $P<0.001$.

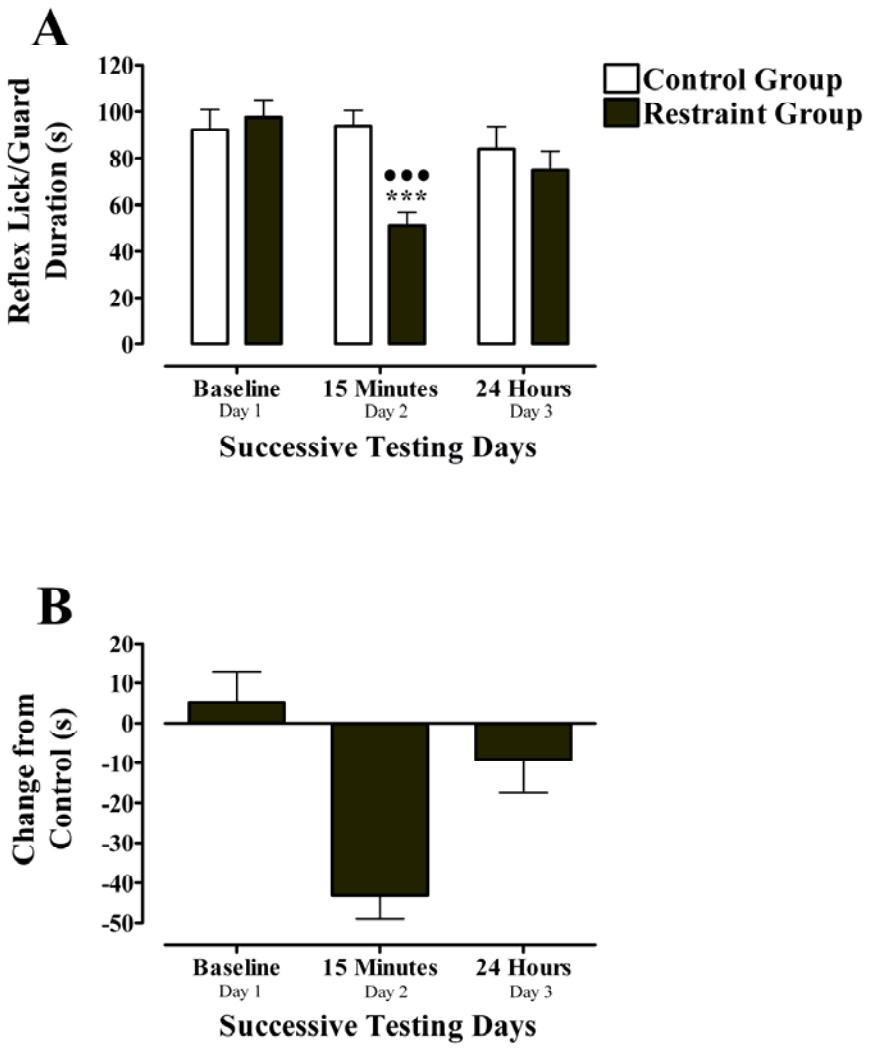


Figure 3-3. Cumulative reflex lick/guard durations during testing trials at 44.0°C for control (open bar) and restraint (closed bar) groups during baseline sessions (Day 1), testing sessions in which rats received 15 minutes of restraint stress (restraint group; Day 2) or no stress (control group), and sessions the following day (Day 3). (A) Exposure to restraint significantly decreased reflex durations when tested fifteen minutes after stress (15 minutes; Day 2) compared to the control group and compared to unstressed trials on baseline and 24 hours after stress. (B) Difference scores confirmed that stress decreased reflex durations compared to controls (15 minutes; Day2). Data are expressed in seconds and are represented as absolute group means ± S.E.M. Significant within-subject differences between trials 15 minutes after stress exposure and trials by the same rats on baseline and 24 hours are indicated as: *** $P<0.001$. Significant between-subject differences between the control and restraint groups on Day 2 are indicated as: ●●● $P<0.001$.

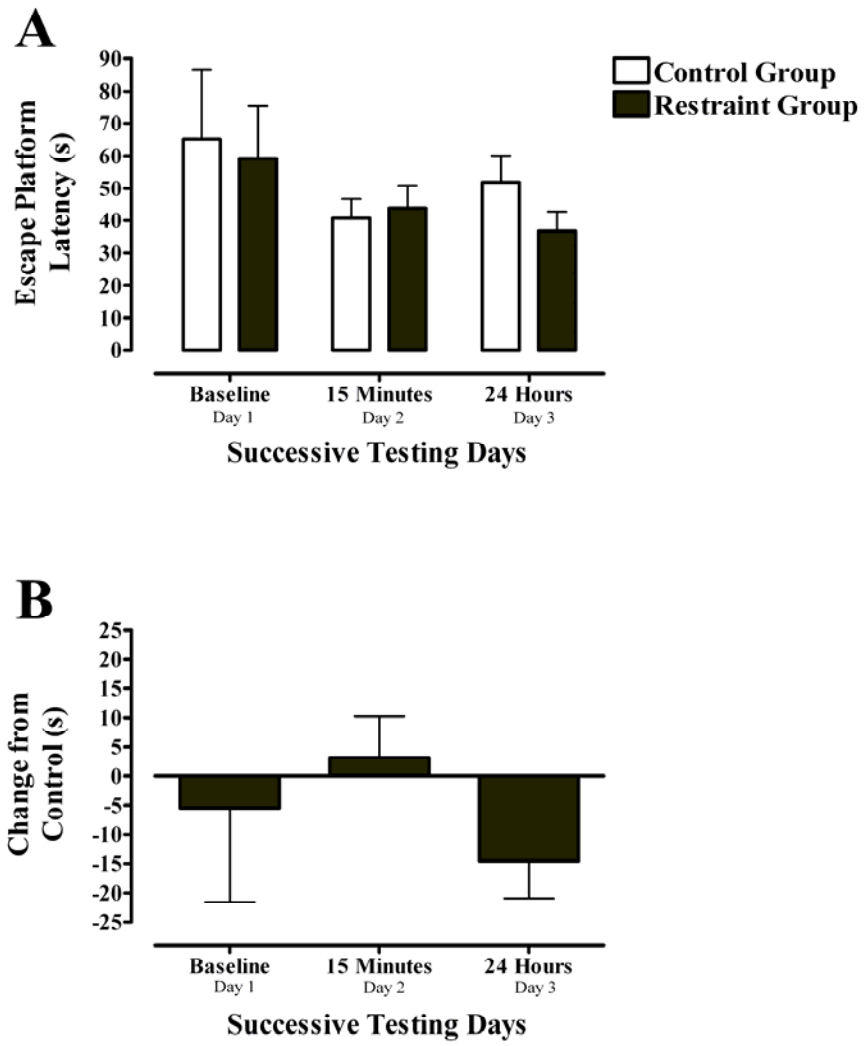


Figure 3-4. Escape latencies during testing trials at 44.0°C for control (open bar) and restraint (closed bar) groups during baseline sessions (Day 1), testing sessions in which rats received 15 minutes of restraint stress (restraint group; Day 2) or no stress (control group), and sessions the following day (Day 3). (A) Exposure to restraint did not affect escape latencies compared to the control group and compared to unstressed trials on baseline (Day 1) and 24 hours (Day 3) after stress. (B) Difference scores confirmed that stress did not alter escape latencies (15 minutes; Day 2). Data are expressed in seconds and are represented as absolute group means ± S.E.M.

A comparison of escape durations for the control group did exhibit a slight increase across the three testing days. Statistical analysis revealed no significant change across testing Days (F=2.968; P=0.0623). This effect has been observed previously in our lab as a consequence of repeated testing at the same thermal temperature.

Unlike reflex responses, operant responses are enhanced (increased heat sensitivity) after an acute exposure to restraint stress when assessed by durations. This effect, ***stress-induced hyperalgesia***, was characterized by a longer time spent on the escape platform during a trial, which were more reliable than latencies. Similar to reflex responses, this effect was transient and did not persist the following day. Thus, it can be concluded that stress produced a differential effect where reflexes were suppressed while nociceptive responses dependent on cortical processing of thermal stimulation were enhanced.

Sequence Analysis of Successive Operant Escape Durations

In addition to analysis of the total duration of escape, examinations of successive operant escape duration within trials presents an additional strategy to analyze the effect of experimental manipulations on behavioral responses. Successive operant escape plate (A, B) and platform (C, D) durations are shown in Figure 3-6 for control (left panel) and restraint (right panel) groups. In general, the maximum number of escape responses was twelve (12), but a majority of animals respond approximately 6 times.

Plate durations for control (A) and restraint (B) groups do not change across baseline (Day 1), 15 minutes (Day 2), and 24 hours (Day 3). Plate durations peaked between the 2nd and 3rd responses.

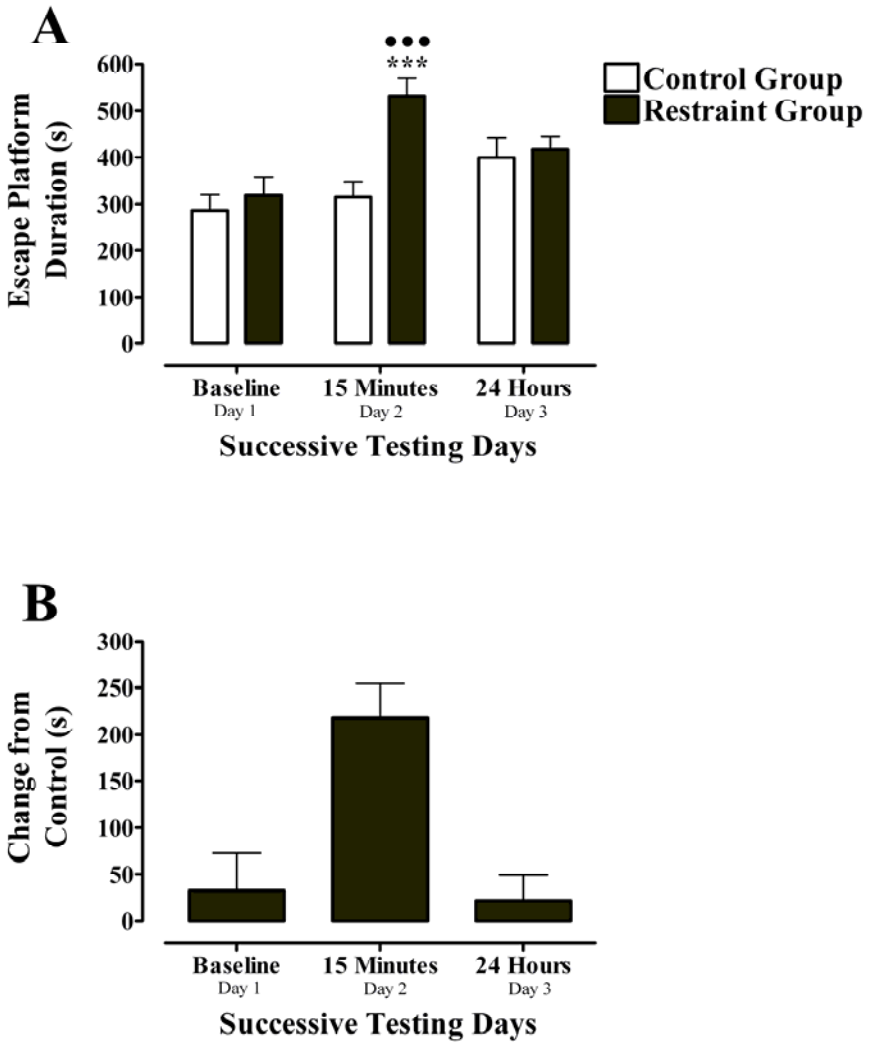


Figure 3-5. Cumulative escape durations during testing trials at 44.0°C for control (open bar) and restraint (closed bar) groups during baseline sessions (Day 1), testing sessions in which rats received 15 minutes of restraint stress (restraint group; Day 2) or no stress (control group), and sessions the following day (Day 3). (A) Exposure to restraint significantly increased escape durations when tested fifteen minutes after stress (15 minutes; Day 2) compared to the control group and compared to unstressed trials on baseline (Day 1) and 24 hours (Day 3) after stress. (B) Difference scores confirmed that stress enhanced escape durations compared to controls (15 minutes; Day 2). Data are expressed in seconds and are represented as absolute group means ± S.E.M. Significant within-subject differences between trials 15 minutes after stress exposure and trials by the same rats on baseline and 24 hours are indicated as: *** $P<0.001$. Significant between-subject differences between the control and restraint groups on Day 2 are indicated as: ●●● $P<0.001$.

In contrast, control and restraint groups displayed different patterns of responding during trials at 44.0°C on Day 2 (15 minutes) in which one group of animals was exposed to acute stress (restraint group) while the other group remained in their home cage (control group). Platform durations for the control (C) and restraint (D) groups were shorter than plate times in this group of animals and peak between the 3rd and 6th platform responses. On the day of stress, platform durations in the restraint group dramatically increased, which indicates an enhanced thermal sensitivity by restraint stress, compared to the control group. The peak of the effect occurs on the 3rd and persisted until the 9th platform response. Importantly, platform durations were comparable to baseline values when assessed the following Day (24 hours).

In summary, the average duration across of the first 6 responses for the control and restraint groups are compared (Figure 3-7). This number was selected because majority of animals quit responding after the sixth response. In the control group (Figure 3-7A), plate durations did not differ over the three consecutive testing sessions (F=0.793, P=0.453), but platform durations did change significantly (F=7.368, P=0.001). Platform durations were significantly higher than baseline during sessions on Day 3 (P<0.05) but not during the 15 minute testing period on Day 2 (P>0.05).

In contrast, the average duration for plate (Figure 3-7B; F=3.727, P=0.025) and platform (F=18.64, P<0.001) durations were different over the three consecutive sessions for the restraint group. In particular, plate durations gradually decreased over sessions (Days 1 through 3) with durations significantly lower than baseline during the Day 3 session only (P<0.05). Platform durations after restraint were significantly longer than baseline (P<0.001) but not 24 hours later (P>0.05). Furthermore, platform

durations were higher after restraint than the control group (F=38.004, P<0.001; Day 2). During sessions 24 hours after stress, platform durations were similar between the two groups (F=0.255, P=0.594; Day 3). Thus, analysis of successive operant escape responses revealed a transient hyperalgesia (enhancement of heat sensitivity) as indicated by increase in ***escape platform duration*** after restraint stress.

Effects of Repeated Stress Exposures: Adaptation

In order to avoid adaptation to repeated exposures to restraint stress (De Boer et al., 1999; Gamaro et al., 1998) at least 2 weeks elapsed between stress tests for each animal. The effectiveness of this strategy and the possibility that there might be carryover effects of repeated stress were evaluated by three types of statistical comparisons related to escape and lick/guard durations at 44.0°C, as shown in Table 3-1.

First, the effectiveness of stress was evaluated for the first and second administration of restraint prior to reflex or operant testing. Reflex durations were lower for stressed rats compared to unstressed rats on Day 2 for the first (F=9.15, P<0.01) and second stress sessions (F=5.06, P=0.01). Also, escape durations were greater for stressed rats compared to unstressed rats on Day 2 for the first (F =5.69, P<0.01) and second stress sessions (F=5.89, P<0.01).

Second, performance on day 1, 2, and 3 was compared for the first and second reflex and operant testing sessions, in order to determine whether there were cumulative effects of repeated stress on performance. None of these 12 comparisons revealed significant effects: for the control and restraint groups during either reflex or operant testing on each of the three days.

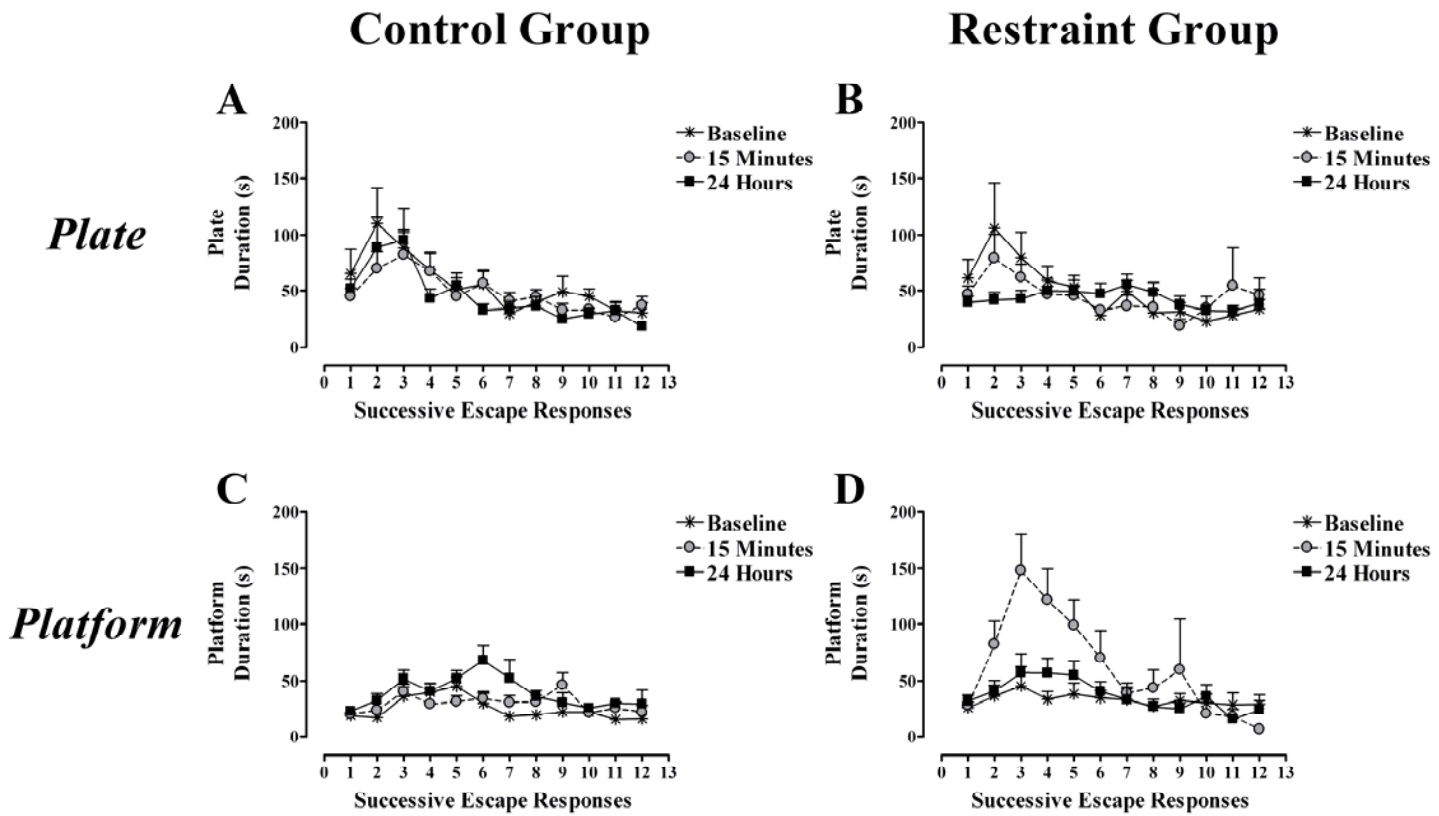


Figure 3-6. Sequence analysis of successive escape plate and platform durations during testing trials at 44.0°C for control (left panel) and restraint (right panel) groups during baseline sessions (asterisk; Day 1), testing sessions in which rats received 15 minutes of restraint stress (restraint group, gray circle; Day 2) or no stress (control group, gray circle), and sessions the following day (closed square; Day 3). Data are expressed in seconds and are represented as absolute group means ± S.E.M.

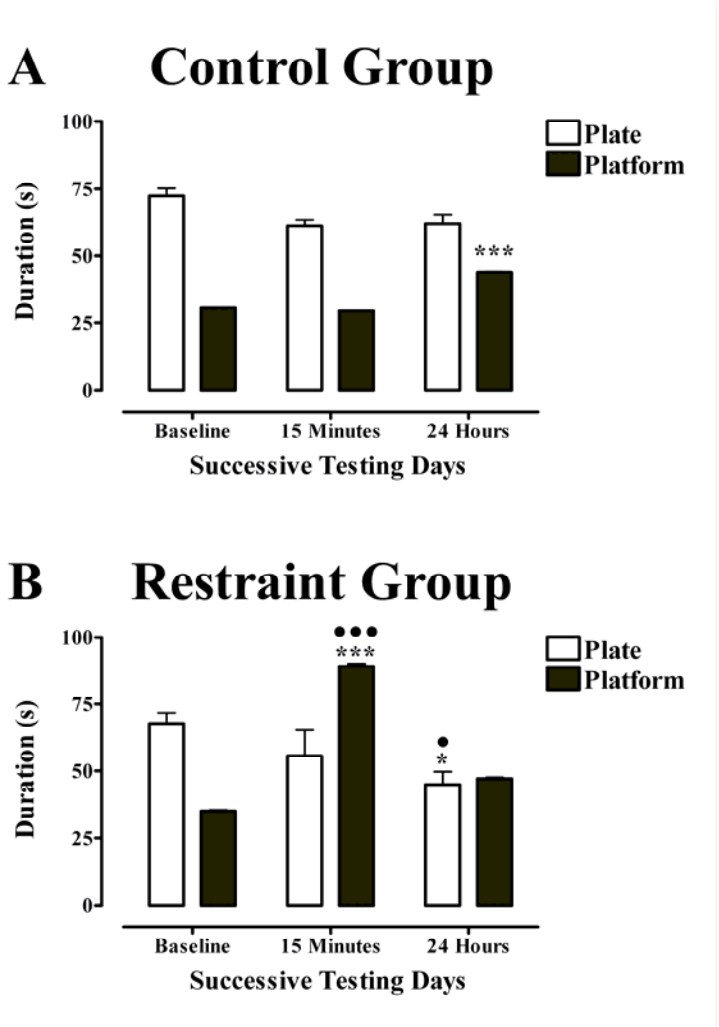


Figure 3-7. Average escape duration of the first six plate (open bar) and platform (closed bar) responses during testing trials at 44.0°C for control and restraint groups during baseline sessions (Day 1), testing sessions in which rats received 15 minutes of restraint stress (restraint group; Day 2) or no stress (control group), and sessions the following day (Day 3). (A) In the control group, no differences were observed in plate, but platform durations were slightly higher over repeated sessions. (B) Exposure to acute restraint stress significantly increased escape platform durations when tested fifteen minutes after stress (15 minutes; Day 2) compared to the control group and compared to unstressed trials on baseline (Day 1) and 24 hours (Day 3). Data are expressed in seconds and are represented as absolute group means ± S.E.M. Significant within-subject differences between trials 15 minutes and 24 hours after stress exposure or no stress and trials by the same rats on baseline and 24 hours are indicated as: * $P<0.05$ and *** $P<0.001$. Significant between-subject differences between the control and restraint groups during trials at 15 minutes and 24 hours are indicated as: • $P<0.05$ and ••• $P<0.001$.

Finally, neither escape (F=0.306; P=0.583) nor lick/guard (F=0.302, P=0.585) durations during baseline testing (Day 1) differed significantly across 4 weeks of testing at 44^{o}C (twice prior to the restraint group and twice prior to the control group). Overall, these data show that acute restraint stress remained effective with repetition at two (2) week intervals and did not accumulate (carryover) from one exposure to the next.

Table 3-1. Cumulative reflex lick/guard and operant escape durations over two sessions of restraint stress. Stress produced similar effects on reflex and escape durations to 44.0°C during the first and second exposures. The effects of stress were not diminished by repetition at 2-week intervals. The data are expressed in seconds, and values are represented as absolute group means ± S.E.M.

	44.0°C Reflex L/G		**44.0°C Operant Escape**	
	Baseline (Day 1)		***Baseline (Day 1)***	
	1st Session	***2nd Session***	***1st Session***	***2nd Session***
Control	91.1 ± 13.3	93.0 ± 12.1	314.5 ± 55.1	262.5 ± 41.9
Pre-Stress	102.8 ± 14.8	93.0 ± 7.0	271.4 ± 61.9	358.4 ± 50.6
	Test (Day 2)		***Test (Day 2)***	
	1st Session	***2nd Session***	***1st Session***	***2nd Session***
Control	94.9 ± 11.5	92.8 ± 8.1	344.4 ± 35.1	289.3 ± 56.3
Stress	50.4 ± 9.2	51.1 ± 8.2	565.9 ± 49.7	505.1 ± 53.3
	24 Hours (Day 3)		***24 Hours (Day 3)***	
	1st Session	***2nd Session***	***1st Session***	***2nd Session***
Control	76.4 ± 14.1	90.2 ± 13.4	411.4 ± 37.8	379.6 ± 67.1
Post-Stress	75.2 ± 13	81.1 ± 8.9	499.9 ± 37.3	383.6 ± 23.4

Time Course of Restraint Stress on Operant Escape Responses at 44.0°C

Previous studies have demonstrated that the effects of stress are detected after termination of the stressor and may last for several hours or days later (Calcagnetti et al., 1992; Drolet et al., 2001; Gamaro et al., 1998; Quintero et al., 2000; Tusuda et al., 1989). The duration and magnitude of stress effect is dependent on several factors including stressor intensity and the length of exposure. To examine the temporal profile of

stress-induced hyperalgesia, a separate group of female rats (n=12) were restrained for a period of 15 minutes followed by assessment of behavioral responses immediately (without a 15 minute pre-test at 36.0°C) after exposure to restraint stress as illustrated in Figure 3-8A.

In addition, the testing protocol used in experiments described above is shown in Figure 3-8B (with a 15 minute pre-test at 36.0°C). Finally, operant escape responses were assessed the following day (24 hours). The control group did not receive stress, but were tested under similar circumstances without or with a pre-test at 36.0°C.

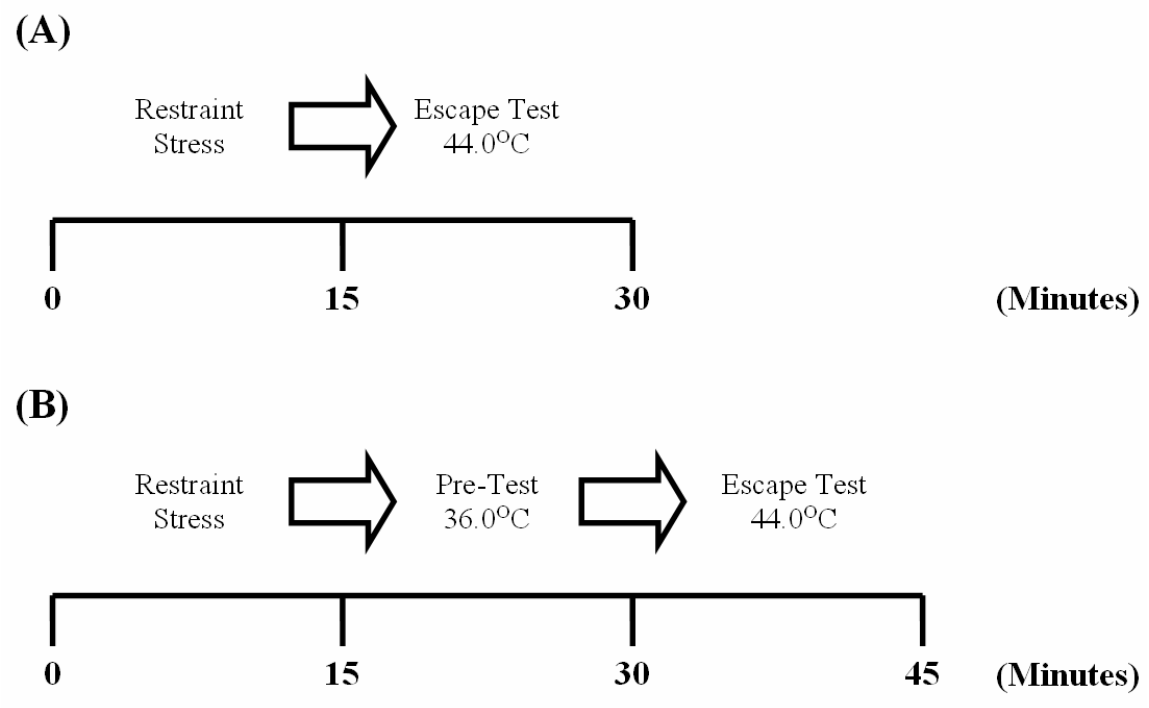


Figure 3-8. Temporal profile of restraint stress on escape responses during trials at 44.0°C. Responses were assessed 15 minutes (A), 30 minutes after the onset of stress (B), and 24 hours after restraint stress (not shown). The control group followed the same testing schedule but without exposure to restraint stress.

Operant Escape Latencies

To determine the effects of stress on escape latencies across multiple time points, animals received 15 minutes of restraint stress (restraint group) or no stress (control group) followed by testing sessions at 44.0°C immediately or 15 minutes after stress, and sessions the following day (24 hours; Figure 3-9). Escape latencies in the control group did not differ across testing sessions (Figure 3-9A; F=0.8555, P=0.4387). However, latencies were affected by restraint stress (F=4.279; P=0.0269). Escape latencies were shorter immediately and 15 minutes after restraint compared to the following day (P<0.05). Differences between the two groups (Figure 3-9B) revealed that latencies were shorter than controls when assessed immediately (F=12.7, P=0.002) and 15 minutes (F=6.358, P=0.019) but not 24 hours (F=0.137, P=0.714) after the termination of stress. Previous data suggested that escape latencies were unreliable and a poor outcome measure. Unlike the first set of experiments in a different group of animals, escape latencies were sensitive to stress. A reduction of escape latencies, which indicates a lower threshold to elicit an escape response, supports the conclusion of stress-induced hyperalgesia.

Operant Escape Durations

To determine the effects of stress on escape durations across multiple time points, responses in the same group of animals are presented in Figure 3-10. Similar to latencies, escape durations in Figure 3-10A did not differ across testing sessions in the control group (F=2.147, P=0.1407), but durations were significantly different in the restraint group (F=3.675, P=0.0420). Escape durations were significantly longer immediately and 15 minutes after restraint compared to the following day (P<0.05). Difference scores (Figure 3-10B) revealed that escape durations were longer than controls when assessed

immediately (F=16.035, P<0.001) and 15 minutes (F=5.989, P=0.023) but not 24 hours (F=0.518, P=0.479) after stress. In agreement with previous data (Figure 3-5), escape durations were influenced by stress. ***Stress-induced hyperalgesia*** was displayed immediately (greatest effect) after the termination of restraint as well as when evaluated 15 minutes after stress exposure. The effect was transient based on the fact that both groups showed similar responding 24 hours later.

Effects of Restraint Stress on Core Temperature

Various experimental manipulations such as restraint stress influence thermoregulation, which can impact behavioral responses to thermal stimulation. For example, restraint stress produces *hyporeflexia* and is associated with increased core temperature (~1.0°C; Chen and Herbert, 1995; Keim and Sigg, 1976; Le Bars et al., 2001; Thompson et al., 2003; Tjolsen and Hole, 1993). Furthermore, evidence suggests that changes in thermoregulation can influence the interpretation of behavioral responding (Le Bars et al., 2001; Tjolsen and Hole, 1993).

In the context of the previous study, the following question can be raised. Is the expression of hyperalgesia a consequence of increased body temperature after exposure to stress? Thus, a possible underlying reason for stress-induced changes in operant responses is altered body temperature. On the other hand, an increase in core temperature may not be a critical factor in the expression of stress-induced changes in nociception, but rather changes in skin temperature. In fact, skin temperature has been identified as a potential confounding variable (Tjolsen and Hole, 1993), and therefore, requires techniques to stabilize temperatures before behavioral assessment (e.g., pre-test trials at 36.0°C).

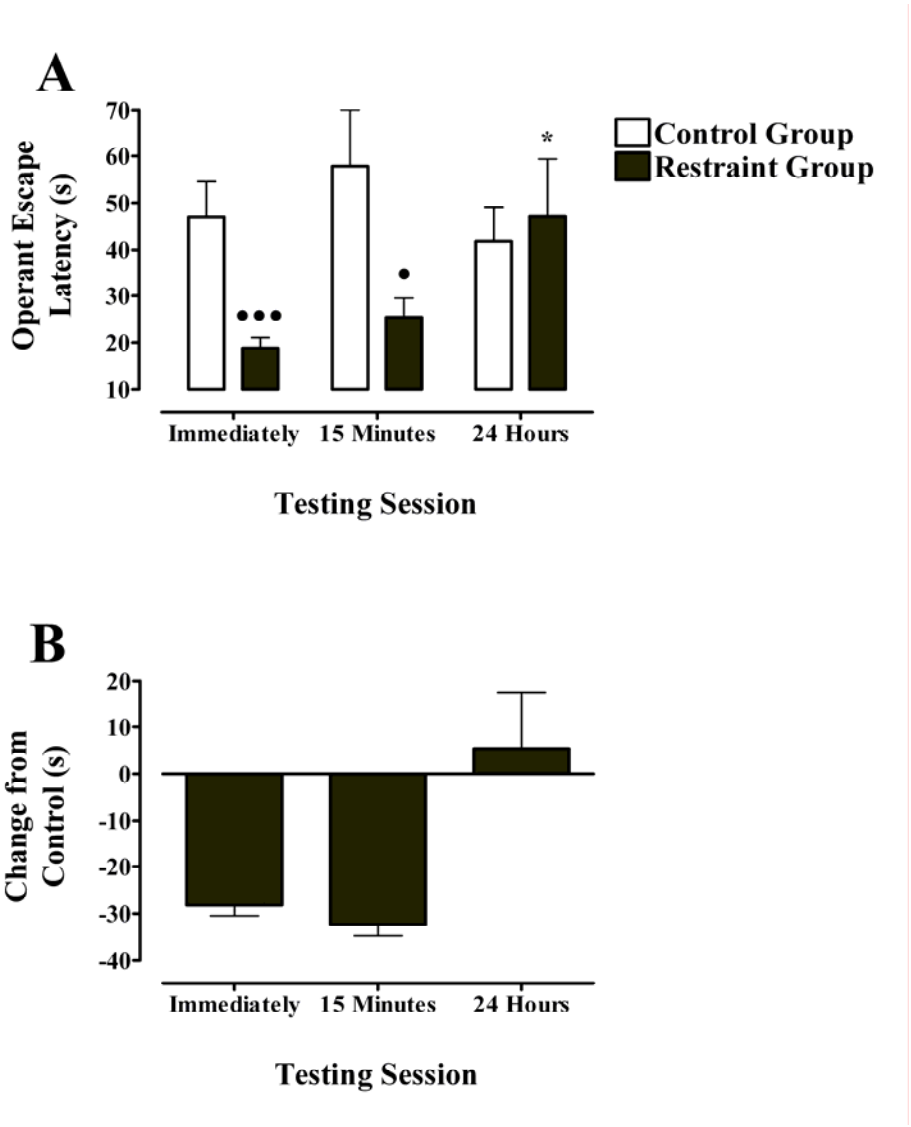


Figure 3-9. Escape latencies during testing trials at 44.0°C for control (open bar) and restraint (closed bar) groups when tested immediately, fifteen minutes, and 24 hours after exposure to restraint. (A) Escape latencies were shorter immediately and fifteen minutes after restraint stress compared to control groups. However, responses did not carry over to the following day. (B) Difference scores confirmed that stress reduced latencies compared to controls. Data are expressed in seconds and are represented as absolute group means ± S.E.M. Significant within-subject differences between 24 hours after stress exposure and trials by the same rats on baseline and 15 minutes are indicated as: * $P<0.05$. Significant between-subject differences between the control and restraint groups are indicated as: • $P<0.05$ and ••• $P<0.001$.

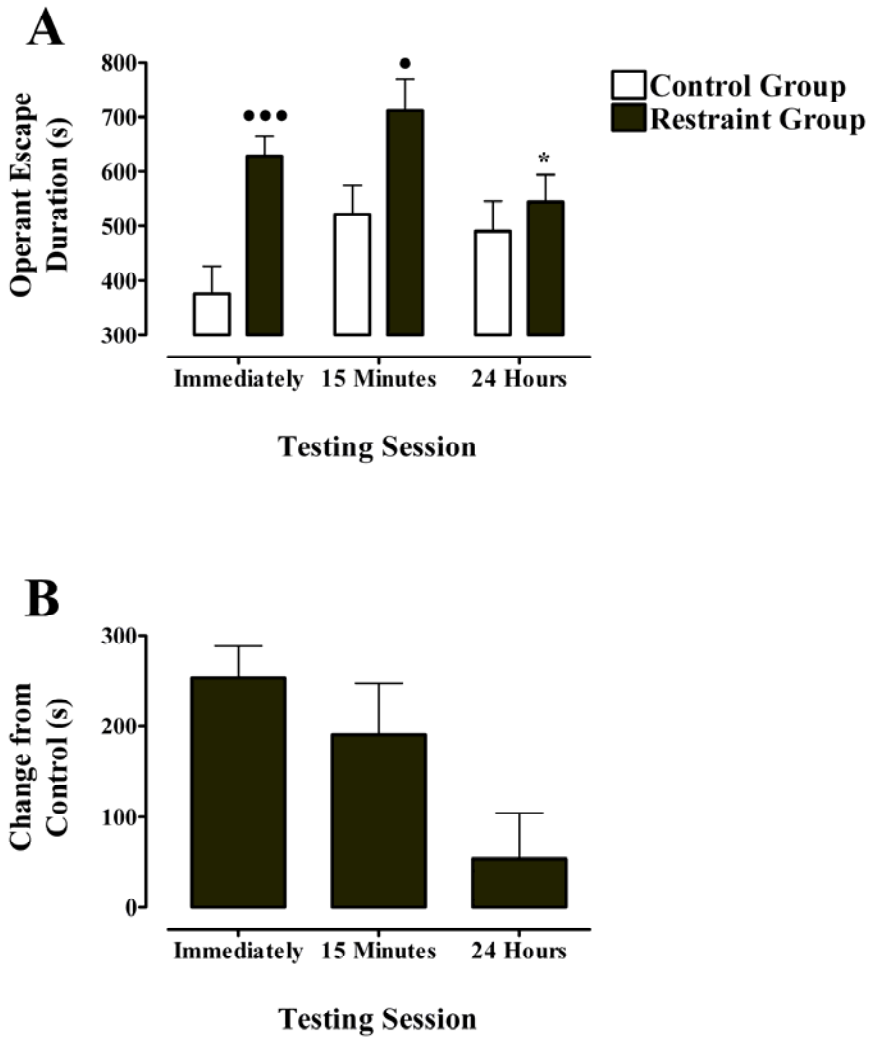


Figure 3-10. Escape durations during testing trials at 44.0°C for control (open bar) and restraint (closed bar) groups when tested immediately, fifteen minutes, and 24 hours after exposure to restraint. (A) Escape durations were immediately increased (stress-induced hyperalgesia) after restraint and when tested fifteen minutes after stress. However, responses did not carry over to the following day. (B) Difference scores confirmed that stress enhanced escape durations compared to controls. Data are expressed in seconds and are represented as absolute group means ± S.E.M. Significant within-subject differences 24 hours after stress exposure and trials by the same rats immediately and 15 minutes are indicated as: * $P<0.05$. Significant between-subject differences between the control and restraint groups are indicated as: • $P<0.05$ and ••• $P<0.001$.

Experiment 1

In order to determine the effect of restraint stress on thermoregulation, core temperatures of female rats (n=12) were recorded during separate sessions in which animals were exposed to no stress (control group) or stress (restraint group; Figure 3-11). Temperature recordings were assessed prior to trials at 44.0°C either immediately (Figure 3-8A), 15 minutes (Figure 3-8B), or 24 hours (not shown) after restraint stress. Control animals were tested under identical conditions without exposure to restraint.

In Figure 3-11A, body temperatures remained constant over the testing sessions in control group (F=0.047, P=0.954), but temperatures were significantly affected by stress (restraint group; F=18.06, P<0.001). Core temperatures were significantly greater when evaluated immediately (P<0.001) but not 15 minutes (P>0.05) or the following day (24 hours; P>0.05). Increased core temperatures could be a consequence of struggling during restraint, which consequently raises temperature. A feature of restraint stress is the uncontrolled restrictions imposed by the device that an animal initially tries to escape.

Differences between the two groups revealed that temperatures were significantly higher when assessed immediately (Figure 3-11B; F=30.807, P<0.001) and 15 minutes (F=7.506, P=0.043) but not twenty-four hours (F=2.012, P=0.170) after restraint. Thus, while restraint temporarily increased core temperatures (0.86 ± 0.09), they were slightly comparable to controls 15 minutes after the onset of stress. Changes in core temperature therefore cannot account entirely for the observation of stress-induced hyperalgesia on operant responses.

Experiment 2

An additional group of female rats (n=6) were used to examine the effects of stress on core (Figure 3-12A) and cutaneous temperatures (Figure 3-12B). Core temperatures were assessed over five minute intervals during restraint and exposure to a thermal plate at 36.0°C (similar to pre-test trials). Recordings during control and restraint groups were obtained in different sessions. In addition to core temperature, cutaneous temperatures were measured in rats in which animals were not stressed (control group) or stressed for 15 minutes (restraint group). For the restraint group, recordings were obtained before and after restraint and after exposure to a 15 minute pre-test trial at 36.0°C. Control animals were assessed before and after the pre-test.

Core body temperature

In Figure 3-12A, stress increased core temperature (restraint group, gray circle; $F=$ 9.420, $P<0.001$). Specifically, a significant increase in core temperatures was observed at ten ($P<0.001$) and 15 ($P<0.001$) minutes during restraint. Temperatures peaked at 38.62 ± 0.11^{O}C. These results are in agreement with Figure 3-11. But, after placement into the pre-test at 36.0°C, core temperatures gradually decreased. Temperatures were greater than baseline at five ($P<0.001$) but not after ten ($P>0.05$) or 15 ($P>0.05$) minutes during pre-test conditions. In the control group (asterisk with dashed line), temperatures increased slightly after placement into the pre-test, but this effect was not significant ($F=0.48$, $P=0.7$). Differences between groups (not shown) suggested that temperatures were higher after stress than control before placement into the pre-test at 36.0 ^{O}C ($F=14.511$, $P=0.003$) but did not differ five ($F=2.207$ $P=0.168$), ten ($F=0.000$, $P=1.0$), and 15 ($F=0.009$, $P=0.926$) minutes into the trial.

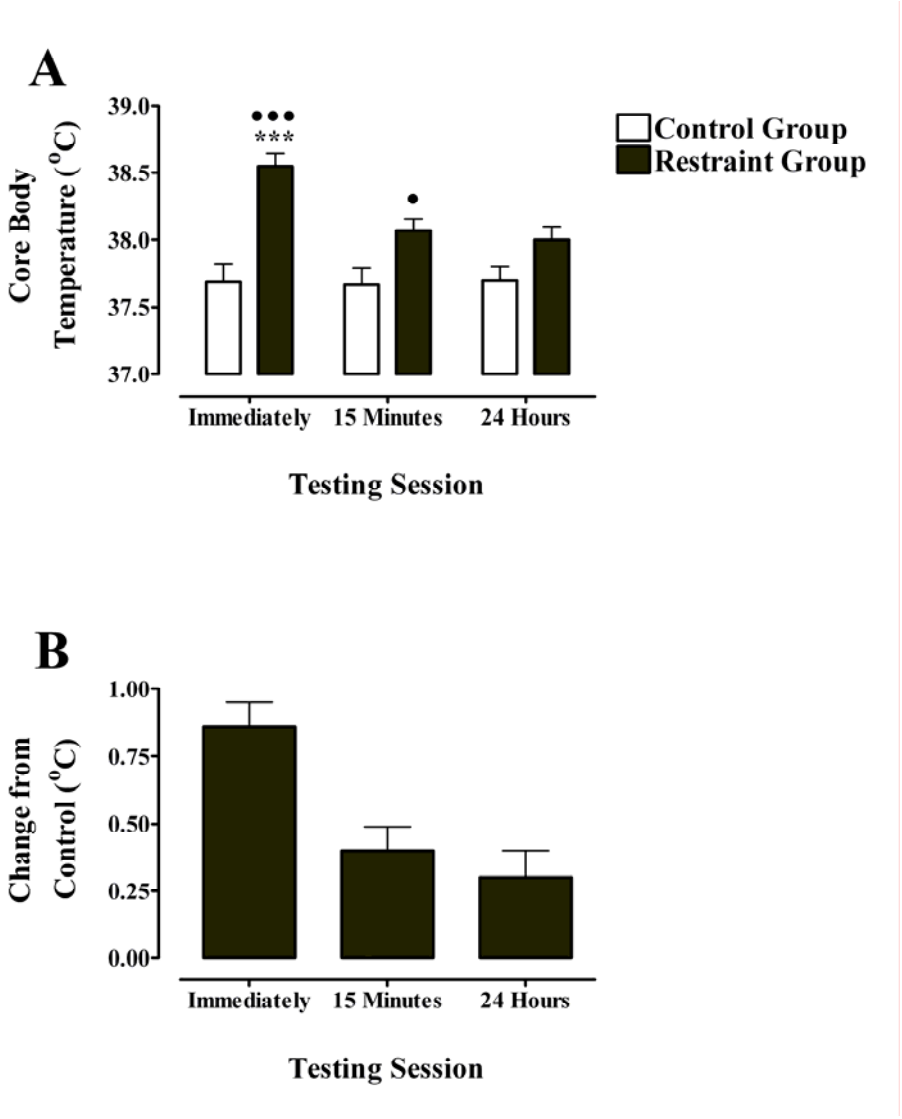


Figure 3-11. Core body temperatures during testing trials at 44.0°C for control (open bar) and restraint (closed bar) groups when tested immediately, fifteen minutes, and 24 hours after exposure to restraint. (A) Core temperatures were immediately increased (*stress-induced hyperthermia*) compared to the control group. Core temperature was still higher than controls fifteen minutes after stress. Core temperature did not differ the following day. (B) Difference scores revealed that stress-induced increase in temperature was most prominent immediately after stress and slowly returned to control values. Data are expressed in seconds and are represented as absolute group means ± S.E.M. Significant within-subject differences immediately after stress exposure or not stress are indicated as: *** $P<0.001$. Significant between-subject differences between the control and restraint groups are indicated as: • $P<0.05$ and ••• $P<0.001$.

Thus, stress-induced increases in core temperature are most prevalent during exposure to stress. Temperatures quickly return to levels comparable to controls during a pre-test trial at 36.0°C. This data supports the idea that altered body temperature is not responsible for the expression of stress-induced hyperalgesia when animals are tested at 44.0°C (consistent with data shown in Figure 3-11).

Peripheral cutaneous temperature

In general, cutaneous temperatures were lower than body temperatures (27.97±0.48 vs. 37.53±0.27°C; F=300.836, P<0.001). Cutaneous temperatures were significantly different across testing sessions (Figure 3-12B; F=19.621, P<0.001). Recordings were greater than baseline when assessed after termination of restraint (P<0.01) and exposure to 36.0°C (P<0.001). In the control group, skin temperatures also increased (F=90.886, P<0.001). Temperatures increased after the pre-test trial at 36.0°C in the absence of stress because of increased activity of the animal and exposure to thermal stimulation that was provided by the heated plate. No differences in temperatures were observed between control and restraint groups during baseline before stress (F=1.019, P=0.337) or after exposure to 36.0°C (F=0.554, P=0.474). However, recordings after restraint were higher than control before placement into the pre-test (F=15.689, P=0.003).

In summary, while stress increased skin temperatures (***stress-induced hyperthermia***), this effect does not persist, and quickly returned to levels comparable to controls. Because temperatures in control and restraint groups were equalized by 36.0°C before placement into the second trial at 44.0°C, core and cutaneous temperatures did not play an important role in the expression of stress-induced hyperalgesia.

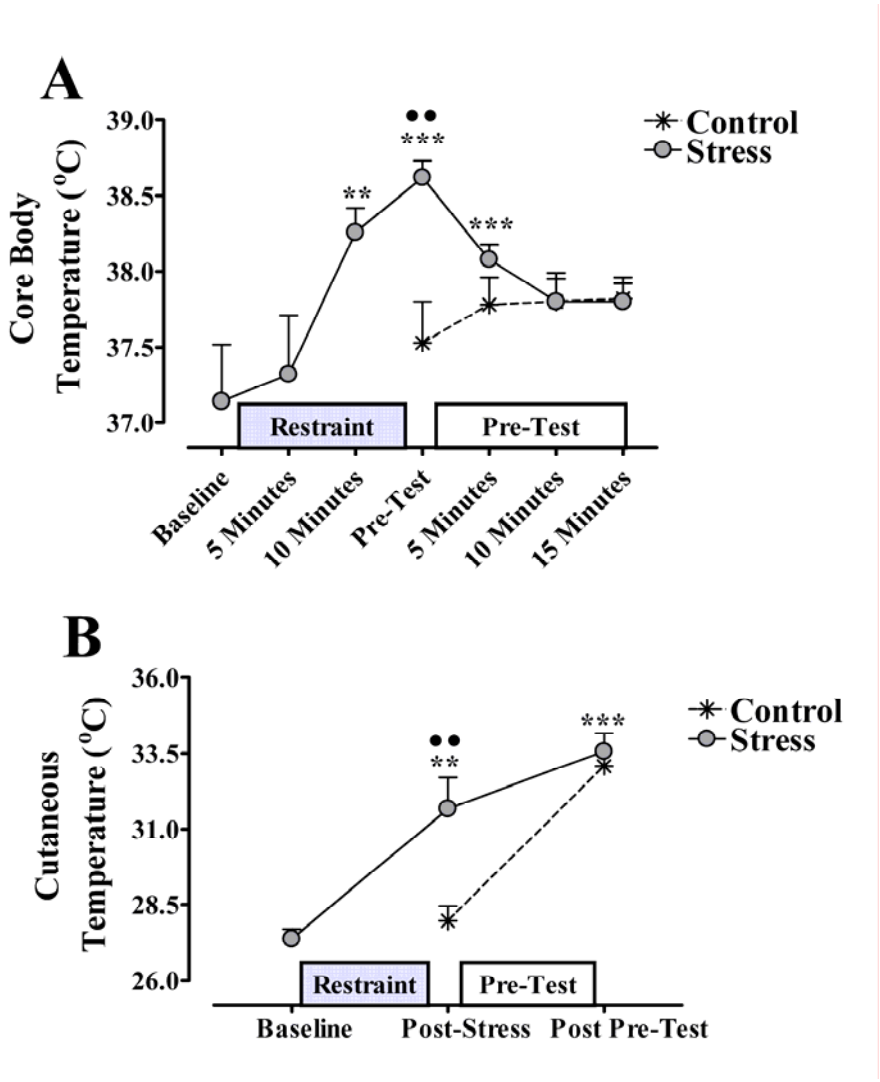


Figure 3-12. Core and cutaneous hindpaw temperatures for control (asterisk with dashed lines) and restraint (gray circle) groups. (A) Core temperature was increased during a fifteen-minute exposure to restraint stress. After termination of restraint, animals were placed into a pre-test at 36.0°C in which temperatures progressively returned to levels comparable to controls. (B) Cutaneous temperatures were greater after restraint, but exposure to pre-test normalized skin temperatures. Data are expressed in seconds and are represented as absolute group means ± S.E.M. Significant within-subject differences between temperature assessed during and after restraint compared to pre-stress baseline values are indicated as: ** $P<0.01$ and *** $P<0.001$. Significant between-subject differences between temperature assessed before and after a pre-test trial are indicated as: •• $P<0.01$.

Effects of Restraint Stress on Control Responses

Operant Escape Responses at 36.0°C

Behavioral responses during 36.0°C trials were obtained in female rats (n=11) during baseline sessions (Day 1), testing sessions in which rats received 15 minutes of restraint stress (restraint group; Day 2) or no stress (control group), and sessions the following Day (24 hours; Day 3). Assessment of escape responses during 36.0°C trials is an important strategy to determine if a particular experimental manipulation produces avoidance, or increased platform duration unrelated to thermal stimulation.

Operant escape latencies

As illustrated in Figure 3-13A, escape latencies were not affected by restraint stress (F=0.23, P=0.795). Durations were not different between control and restraint groups (Figure 3-13B; F=1.124, P=0.33). Response latencies were highly variable at 36.0°C, a non-noxious temperature, due to exploratory behaviors and the inability of this temperature to elicit escape behavior.

Operant escape durations

Similar to latencies during 36.0°C trials, escape durations were also not affected by restraint stress (Figure 3-14A; F=2.599, P=0.0869). Durations were not different between control and restraint groups (Figure 3-14B; F=0.993, P=0.382). At this neutral plate temperature, platform occupancy was short and the duration of operant escape response did not change significantly after restraint stress. Because responses were not affected, stress does not induce avoidance during the escape test.

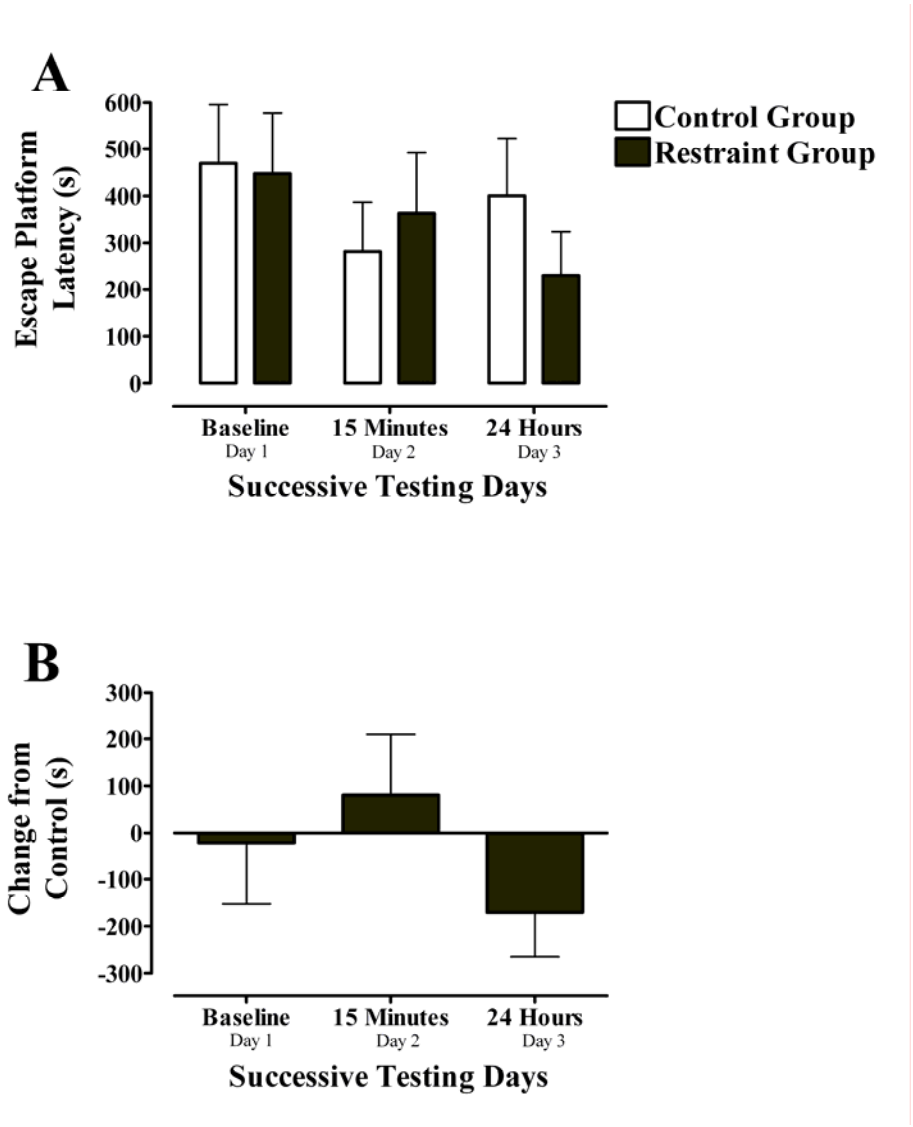


Figure 3-13. Escape latencies during testing trials at 36.0°C for control (open bar) and restraint (closed bar) groups during baseline sessions (Day 1), testing sessions in which rats received 15 minutes of restraint stress (restraint group; Day 2) or no stress (control group), and sessions the following day (Day 3). (A) Exposure to restraint did not affect escape latencies compared to the control groups and compared to unstressed trials on baseline and 24 hours after stress. (B) Difference scores confirmed that stress did not alter escape latencies (15 minutes; Day 2). The data are expressed in seconds and are represented as absolute group means ± S.E.M.

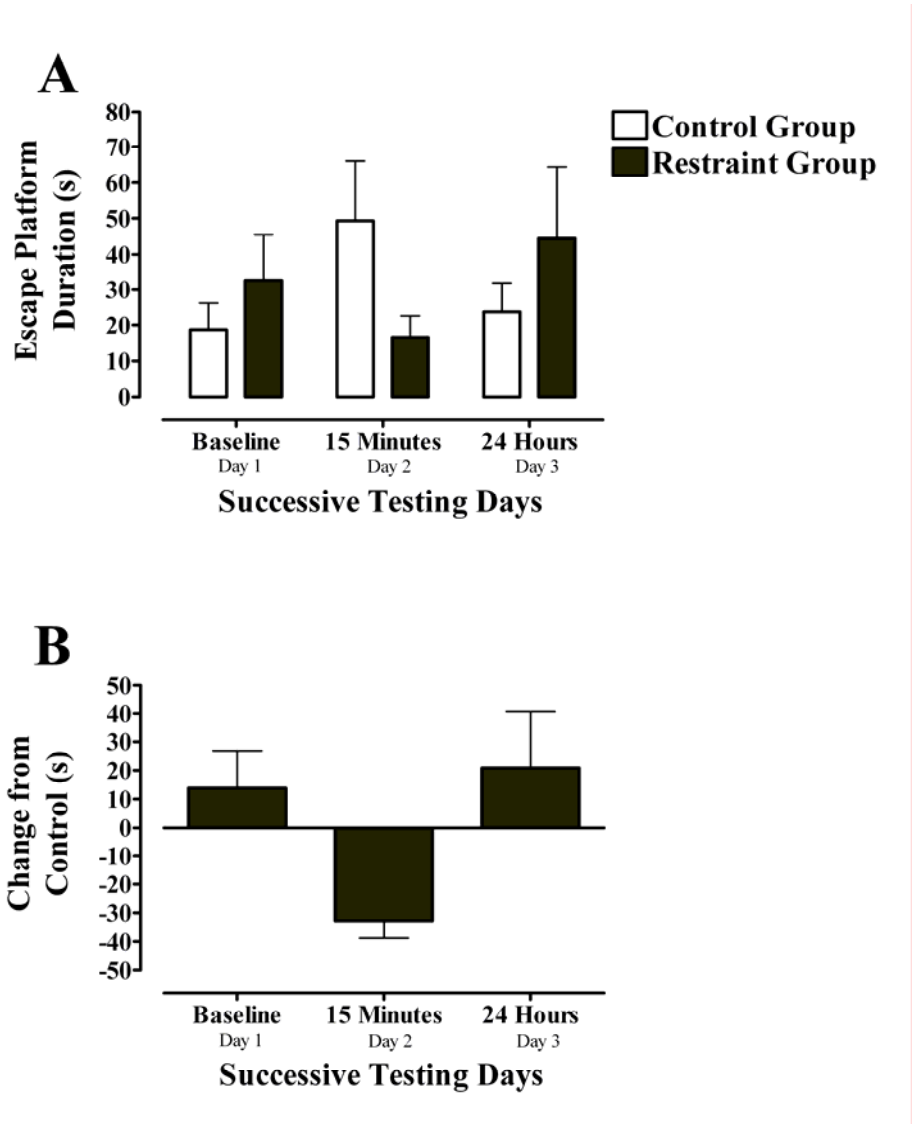


Figure 3-14. Cumulative escape durations during testing trials at 36.0°C for control (open bar) and restraint (closed bar) groups during baseline sessions (Day 1), testing sessions in which rats received 15 minutes of restraint stress (restraint group; Day 2) or no stress (control group), and sessions the following day (Day 3). (A) Exposure to acute stress produced no significant alteration of escape durations during 36.0^{O}C trials. (B) Difference scores confirmed that stress did not significantly alter escape durations (15 minutes; Day 2). Data are expressed in seconds, and values are represented as absolute group means ± S.E.M.

Sequence analysis of successive operant escape durations

In addition to analysis of the total duration of escape within trials at 36.0°C, successive escape plate (A, B) and platform (C, D) durations were examined between control (left panel) and restraint (right panel) groups (Figure 3-15). Overall, compared to plate responses at 44.0°C, plate times were longer at 36.0°C. For both groups, plate durations were higher during the beginning of the trial and gradually decreased. Plate durations for control (Figure 3-15A) and restraint (Figure 3-15B) groups did not change across the baseline, test, or 24 hours periods. In contrast, platform durations remained stable throughout the trial and were substantially shorter than plate durations for control (Figure 3-15C) and restraint (Figure 3-15D) groups. Although restraint stress did not influence platform durations, the number of responses was reduced compared to baseline. In support of the cumulative platform durations represented in Figure 3-16, restraint did not significantly affect escape responses at neutral temperatures. .

In summary, the average duration of the first 6 responses during trials at 36.0°C for the control and restraint groups are compared in Figure 3-16. In the control group, neither plate (Figure 3-16A; F=1.306, P=0.2859) nor platform (F=2.382, P=0.1096) durations changed over the three consecutive sessions. Similar to controls, stress did not affect plate (Figure 3-16B; F=2.256, P=0.1233) or platform (F=1.112, P=0.3422) durations over the three consecutive sessions.

Thus, stress does not produce an increase in platform duration at neutral temperatures suggesting that the expression of stress-induced hyperalgesia at 44.0°C, which activates C-nociceptors, is dependent on higher levels of sensory processing. Furthermore, ***stress-induced hyperalgesia*** is not a consequence of avoidance.

Darkbox Responses

In order to control from changes in motor functioning and motivation, darkbox responses were evaluated in female rats (n=11) for control and stress conditions (Table 3-2). Latencies of escape from bright light in the darkbox test were unaffected by prior stress (F=1.633, P=0.2358). Therefore, acute restraint stress did not significantly alter aversion to light or produce motor effects (e.g. freezing) that interfered with escape performance.

Table 3-2. Darkbox latencies for control and restraint groups. Responses were unaffected by stress. Data are expressed in seconds, and values are represented as absolute group means ± S.E.M.

	Baseline		**15 Minutes**		**24 Hours**
Control	12.7 ± 1.3	Control	13.4 ± 1.5	Control	15.3 ± 1.6
Pre-Stress	16.4 ± 1.8	Stress	15.3 ± 1.8	Post-Stress	12.6 ± 1.5

Effects of Restraint Stress on Operant Thermal Preference

Based on the preceding results, restraint stress produces a heightened sensitivity to heat (e.g., decreased plate durations; increased escape platform durations). But, is stress-induced hyperalgesia specific to the escape paradigm? Or, does acute restraint stress produce a generalized heightened sensitivity to heat? To determine if this observation was limited to the escape paradigm or could be generalized to other operant paradigms, an additional operant paradigm (thermal preference) was used to clarify this issue. Enhanced heat sensitivity would be indicated by an increase in duration (preference) for the cold compartment. In these experiments, the floor was cooled to 15.0°C in one compartment and heated to 45.0°C in the adjacent compartment. Responses were recorded during a 12-minute trial preceded by a pre-test trial at 36.0°C (15 minutes).

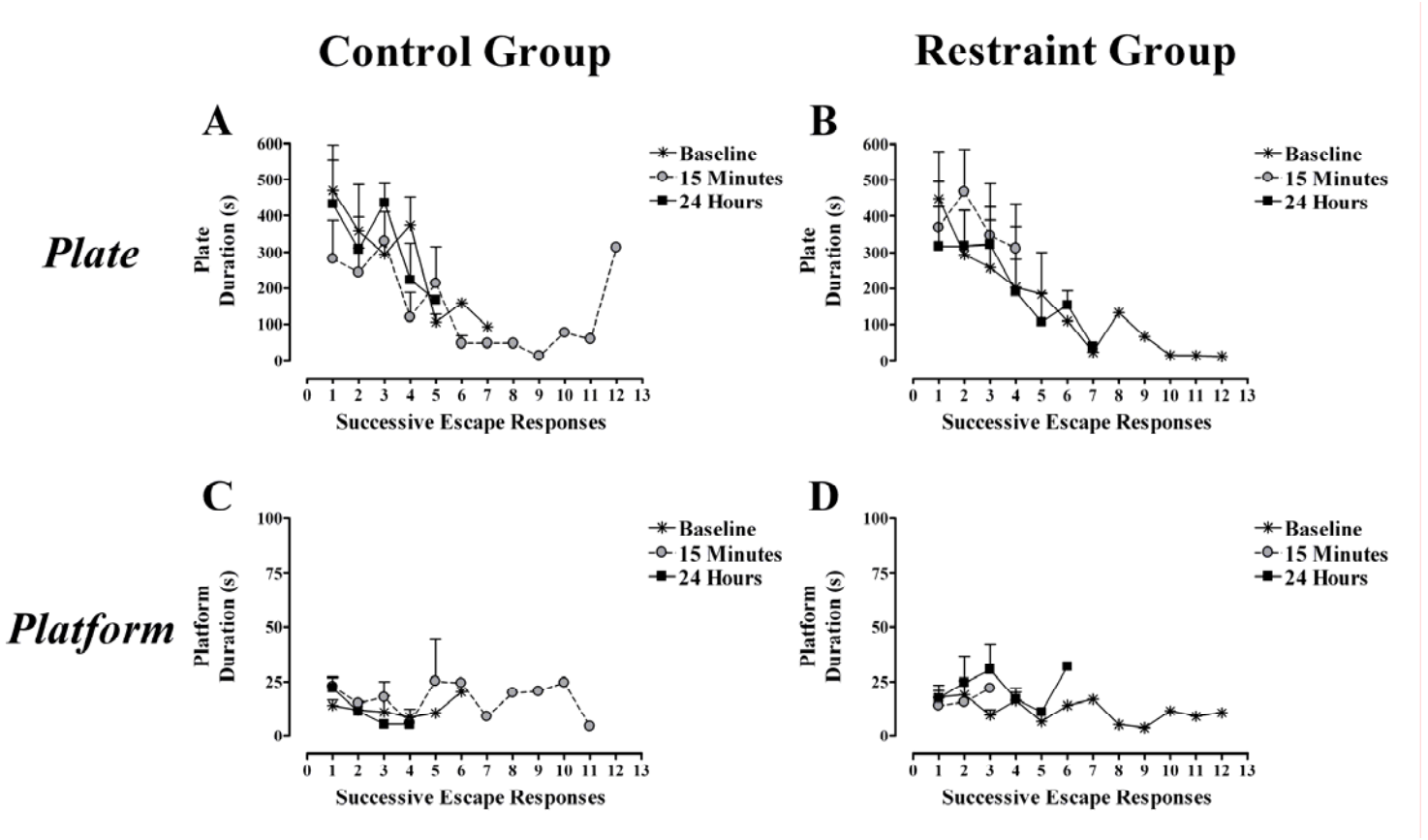


Figure 3-15. Sequence analysis of successive escape plate and platform durations during testing trials at 36.0°C for control (left panel) and restraint (right panel) groups during baseline sessions (asterisk; Day 1), testing sessions in which rats received 15 minutes of restraint stress (restraint group, gray circle; Day 2) or no stress (control group, gray circle), and sessions the following day (closed square; Day 3). Data are expressed in seconds and are represented as absolute group means ± S.E.M

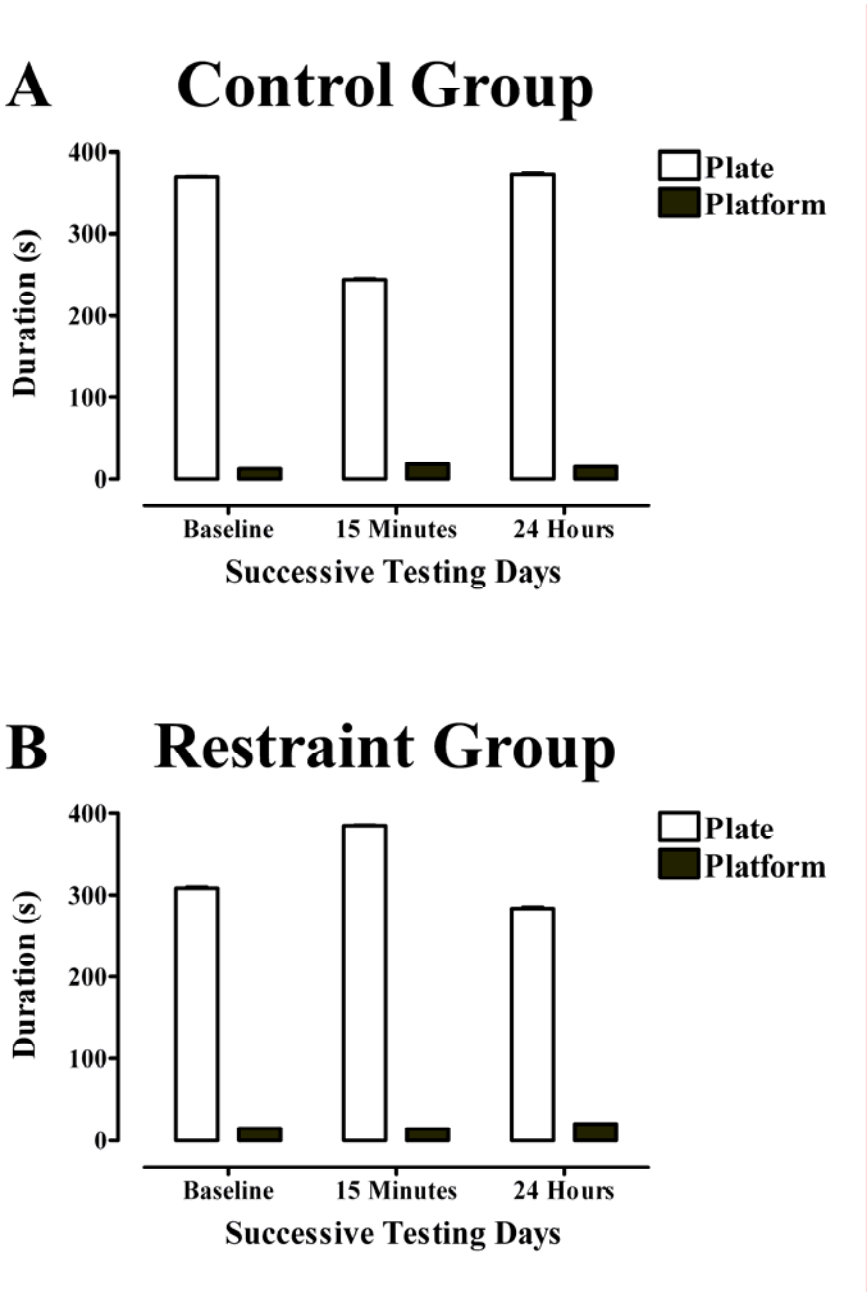


Figure 3-16. Average escape duration of the first six plate (open bar) and platform (closed bar) responses during testing trials at 36.0°C for control and restraint groups during baseline sessions (Day 1), testing sessions in which rats received 15 minutes of restraint stress (restraint group; Day 2) or no stress (control group), and sessions the following day (Day 3). In both groups, plate durations were longer than platform durations. In addition, no differences were observed in plate and platform durations over sessions. The data are expressed in seconds and are represented as absolute group means ± S.E.M.

Thermal Preference Durations

To determine the effects of stress on the thermal preference test, baseline responses (n=20; Day 1) were compared to sessions in which all animals underwent restraint stress for 15 minutes and testing 15 minutes (test; Day 2), 24 hours (Day 3) and 48 hours (Day 4) after restraint (Figure 3-17). Similar to the operant escape test, thermal preference responses were continuously assessed during the testing trial. In Figure 3-17A, restraint stress influenced thermal preference responses (F=2.865, P=0.041). Cold preference was significantly higher than baseline after stress (Day 2; P<0.05) but not 24 (Day 3; P>0.05) or 48 (Day 4; P>0.05) hours after stress. Conversely, heat preference was lower after stress (Day 2; P<0.05) but not 24 (Day 3; P>0.05) or 48 (Day 4; P>0.05) hours later.

Difference scores (Figure 3-17B) revealed that the effect of stress was most prominent when thermal preference was assessed 15 minutes after stress. Responses quickly returned to levels comparable to baseline during subsequent testing sessions. Thus, similar to escape (e.g., decreased time spent on the heat plate), restraint stress produced a heightened sensitivity to heat as indicated by an increase in cold preference and a decrease in heat preference.

Sequence Analysis of Successive Thermal Preference Durations

In addition to analysis of the cumulative preference responses, successive cold and heat durations within trials were compared. The average duration of the first 6 cold and heat preference responses were analyzed to determine the effect of stress on successive preference responses (Figure 3-18).

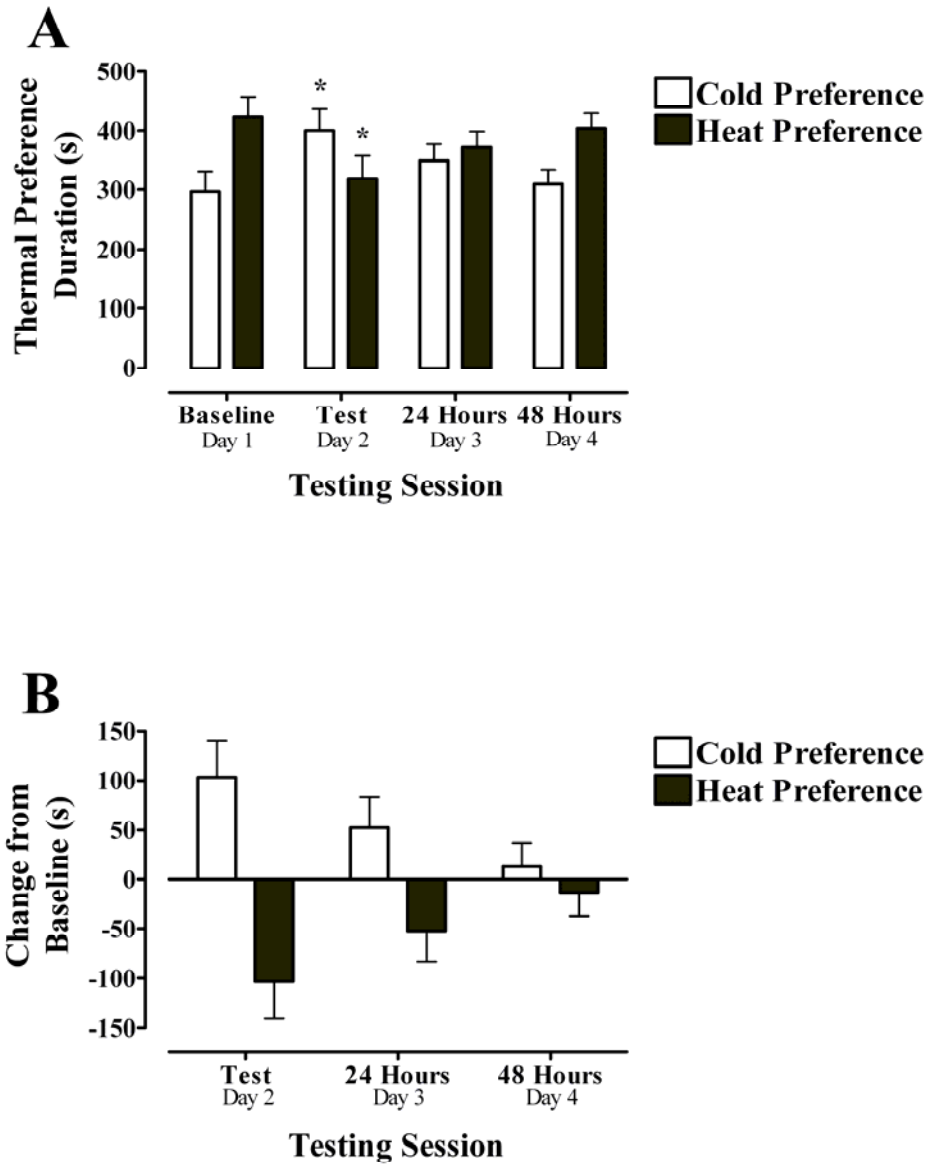


Figure 3-17. Cumulative thermal preference durations during testing trials at 15.0 (cold: open bar) and 45.0°C (heat: closed bar) during baseline sessions, testing session in which rats received 15 minutes of restraint stress, and sessions 24 and 48 hours after stress. (A) Under baseline conditions (Day 1), preference for heat was greater than the preference for cold. An acute exposure to restraint significantly decreased preference for heat on Day 2. However, preference for heat returned to pre-stress levels when assessed 24 (Day 3) or 48 hours (Day 4) hours after restraint stress. (B) Based on difference scores, stress produced a substantial change in preference that dissipated the following testing sessions. Data are expressed in seconds and are represented as absolute group means ± S.E.M. Significant differences between the control and restraint groups on Day 2 are indicated as: * $P<0.05$.

Restraint stress had an effect on cold (F=8.786, P=0.01) and heat (F=3.632, P=0.038) preference responses. The average duration of cold responses were significantly higher than baseline after exposure to stress (P<0.01) but not higher 24 (P>0.05) or 48 (P>0.05) hours later. Consequently, the average duration of heat responses were significantly lower than baseline after exposure to stress (P<0.05) but not higher 24 (P>0.05) or 48 (P>0.05) hours later. As shown on operant escape, stress produced a transient increase sensitivity to heat as indicated by an increase in cold preference to 15.0°C (longer duration) and a decrease in heat preference to 45.0°C (shorter duration).

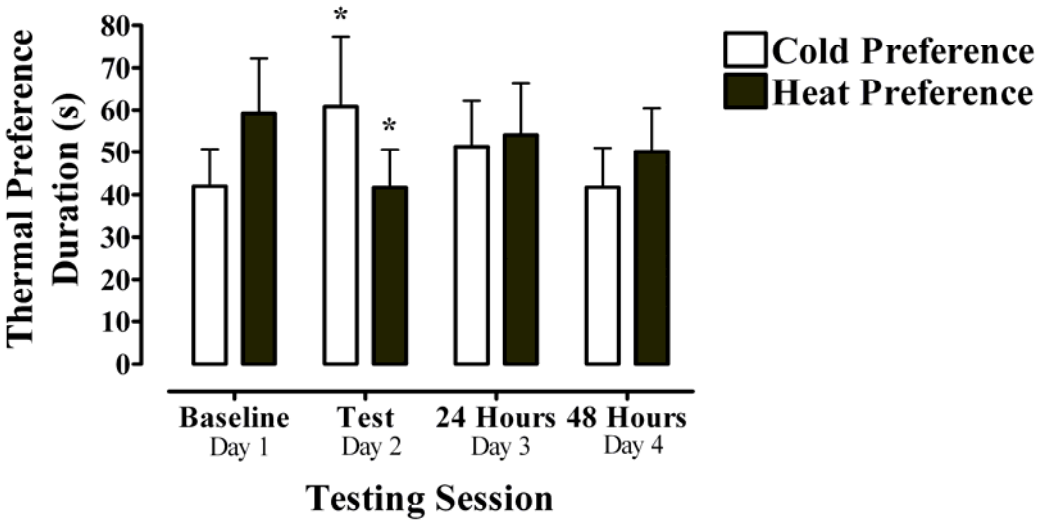


Figure 3-18. Average of the first six cold (open bar) and heat (closed bar) durations during baseline sessions, testing sessions in which rats received 15 minutes of restraint stress (test), and sessions 24 and 48 hours after stress. Restraint stress affected the average cold and heat preference responses. Cold responses were increased while heat responses were reduced. This effect did not continue during sessions assessed the following days (24 and 48 hours). Data are expressed in seconds and are represented as absolute group means ± S.E.M. Significant differences between restraint for cold and heat preference are indicated as: * P<0.05.

Effects of Endogenous Opioids on Stress-Induced Changes in Nociception

Previous studies suggest that restraint-induced changes in nociception are modulated by opioid mechanisms. Stress-induced changes in nociception are characterized as either opioid-dependent or non-opioid dependent (Lewis, Cannon, and Liebeskind, 1980; Porro and Carli, 1988; Drolet et al., 2001). Involvement of endogenous opioids, which are released during stress (Madden et al. 1977), in the expression of ***stress-induced hyporeflexia*** is confirmed by administration of an opioid antagonist (naloxone; Pilcher and Browne, 1983).

In the present study, injections of naloxone were used to determine the role of endogenous opioids in modulating effects of stress on reflex (*hyporeflexia*) and operant (*hyperalgesia*). It was hypothesized that the endogenous opioid system contributes to stress-induced reduction of reflexes while opposing the excitatory effects of stress on cerebrally mediated operant escape responses to thermal stimulation.

Reflex Lick/Guard Responses

As mentioned previously, restraint stress suppressed reflex lick/guard responses (increased latencies; decreased duration). To determine if the expression of stress-induced hyporeflexia was mediated by endogenous opioids, behavioral responses were assessed in a group of female rats during sessions in which rats received 15 minutes of restraint stress (restraint group; n=19) or no stress (control group; n=19) followed by an injection of saline (1.0 mg/kg) or naloxone (3.0 mg/kg). Then, animals were tested during a trial at 44.5°C for 10 minutes that was preceded by a pre-test at 36.0°C (15 minutes).

Reflex lick-guard latency

Reflex latencies are presented in Figure 3-19. Reflex latencies were significantly longer in the restraint group after an injection of saline compared to non-stressed controls (Figure 3-19A; F=10.065, P=0.003). Stress-induced increases of reflex latencies were reduced after administration of naloxone at 3 mg/kg (F=4.475, P=0.041). Naloxone did not affect reflex latencies in the control groups (F=0.517, P=0.477). Difference scores between control and restraint groups revealed that the reduction of reflex latencies after stress, a characteristic of ***stress-induced hyporeflexia***, was reduced by naloxone (Figure 3-19B, F=9.704, P=0.004).

Reflex lick-guard duration

The duration of reflex responses were also assessed in this group of rats during trials at 44.5°C (Figure 3-20). After saline injection, durations were significantly shorter after restraint than the control group (Figure 3-20A, F=7.526, P=0.009). Naloxone did not affect reflex durations in the control groups (F=0.013, P=0.723) but reversed stress-induced decreases of reflex durations (F=5.459, P=0.025). Reduction of reflex durations to thermal stimulation by stress, a feature of ***stress-induced hyporeflexia***, was reversed by naloxone as revealed by differences between both groups (Figure 3-20B, F=11.403, P=0.002).

Thus, the ability of naloxone to reduce expression of stress-induced hyporeflexia as assessed by both reflex latencies and duration provides evidence for the involvement of an endogenous opioid system in mediating these effects.

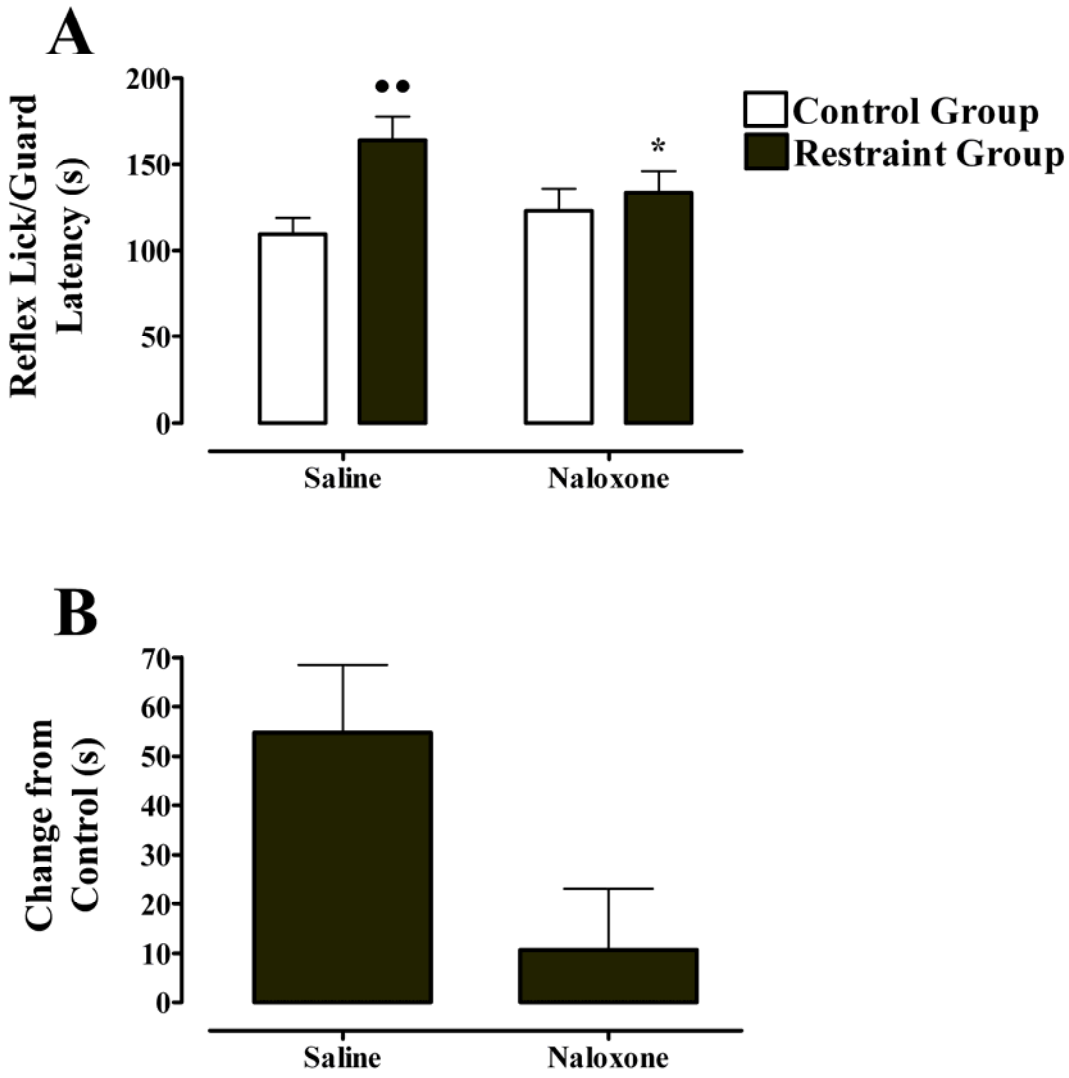


Figure 3-19. Reflexive lick/guard latencies at 44.5°C during testing sessions in which rats received 15 minutes of restraint stress (restraint group, closed bar) or no stress (control groups, open bar) followed by an injection of saline or naloxone. (A) Reflex latencies were longer after exposure to restraint stress than control group. Naloxone did not affect reflex latencies under control group, but naloxone reduced stress-induced inhibition of reflex latencies. (B) Difference scores between control and restraint groups revealed that naloxone reduced the increase in reflex latencies after stress. Data are expressed in seconds and are represented as absolute group means ± S.E.M. Significant within-subject differences after saline and naloxone injections are indicated as: * $P<0.05$. Significant between-subject differences between control and restraint groups are indicated as: ●●● $P<0.01$.

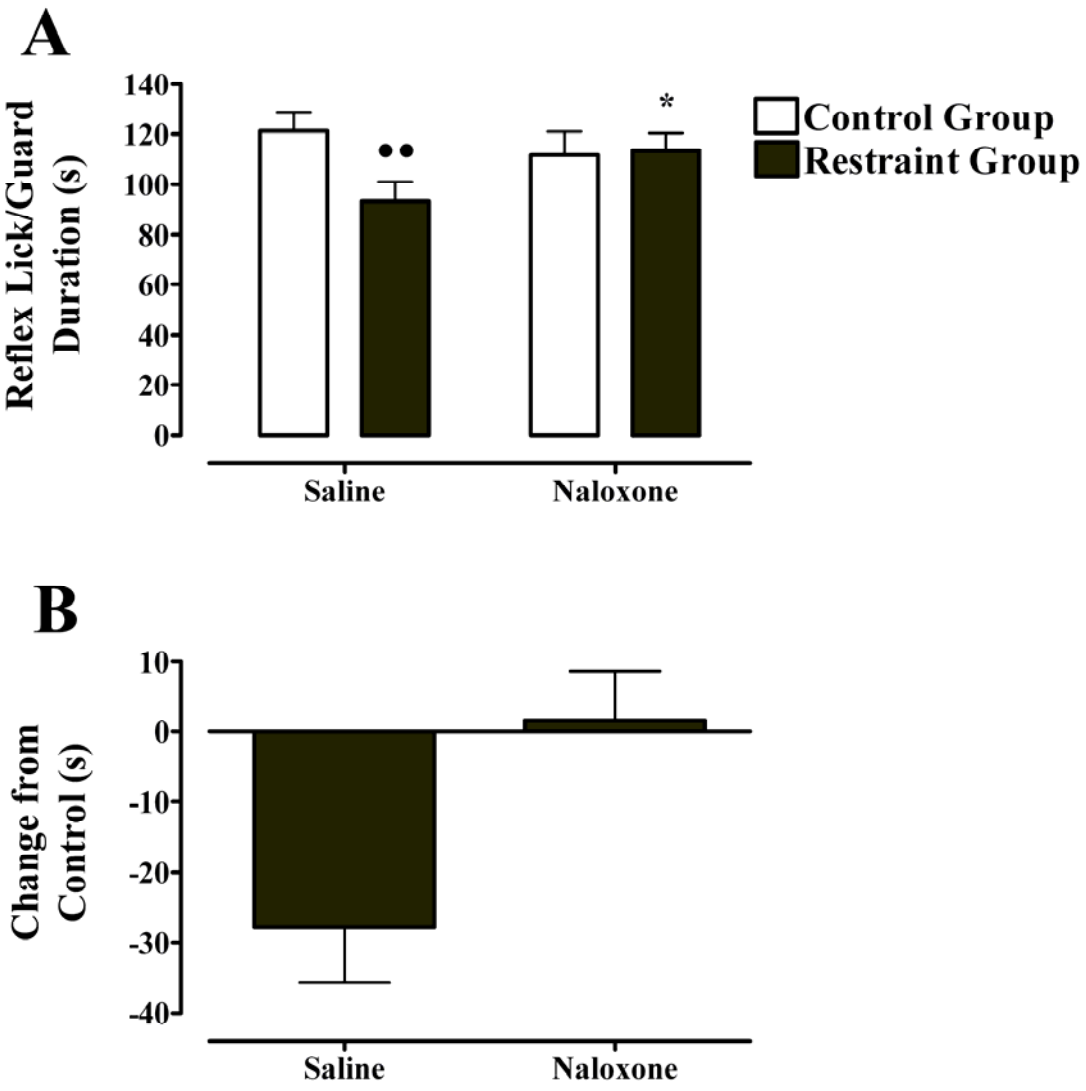


Figure 3-20. Cumulative reflexive lick/guard durations at 44.5°C during testing sessions in which rats received 15 minutes of restraint stress (restraint group, closed bar) or no stress (control groups, open bar) followed by an injection of saline or naloxone. (A) Reflex responses were reduced by restraint stress compared to control group. Similar to latencies, injection of naloxone did not affect reflex durations under control group, but naloxone reduced stress-induced inhibition of reflex durations. (B) Difference scores between control and restraint groups revealed that naloxone reduced the decrease of reflex durations after stress. Data are expressed in seconds and are represented as absolute group means ± S.E.M. Significant within-subject differences after saline and naloxone injections are indicated as: * $P<0.05$. Significant between-subject differences between control and restraint groups are indicated as: ••• $P<0.01$.

Operant Escape Responses

The expression of ***stress-induced hyporeflexia*** is mediated by the endogenous opioid system. However, the role of this system in mediating enhanced sensitivity on cortically mediated responses to thermal stimulation is unknown. To determine if the expression of stress-induced hyperalgesia was mediated by endogenous opioids, behavioral responses were assessed in a separate group of female rats during sessions in which rats received 15 minutes of restraint stress (restraint group; n=10) or no stress (control group; n=10) followed by an injection of saline (1.0 mg/kg) or naloxone (3.0 mg/kg). Then, animals were tested during a trial at 44.5°C for 15 minutes that was preceded by a pre-test at 36.0°C (15 minutes).

Figure 3-21 presents the cumulative duration of reflex responses that were assessed in a group of rats during trials at 44.5°C. Restraint stress enhanced thermal sensitivity (Figure 3-21A***, stress-induced hyperalgesia***). Specifically, durations were significantly greater after stress (saline; F=4.725, 0.043). Interestingly, naloxone increased escape durations in the control (F=4.080, P=0.050) and restraint (F=8.325, P=0.010) groups. Escape durations remained higher in the restraint group to the control group after injection of naloxone (F=5.209, P=0.035). However, difference scores (from controls) revealed that naloxone produced similar effects (e.g., increased duration) in both groups (Figure 3-21B, F=0.145, P=0.707).

The ability of naloxone to augment stress-induced hyperalgesia provides evidence for endogenous opioid system in mediating these effects. It appears that this system is important for mediating escape behavior and suppressing mechanisms underlying the expression of hyperalgesia.

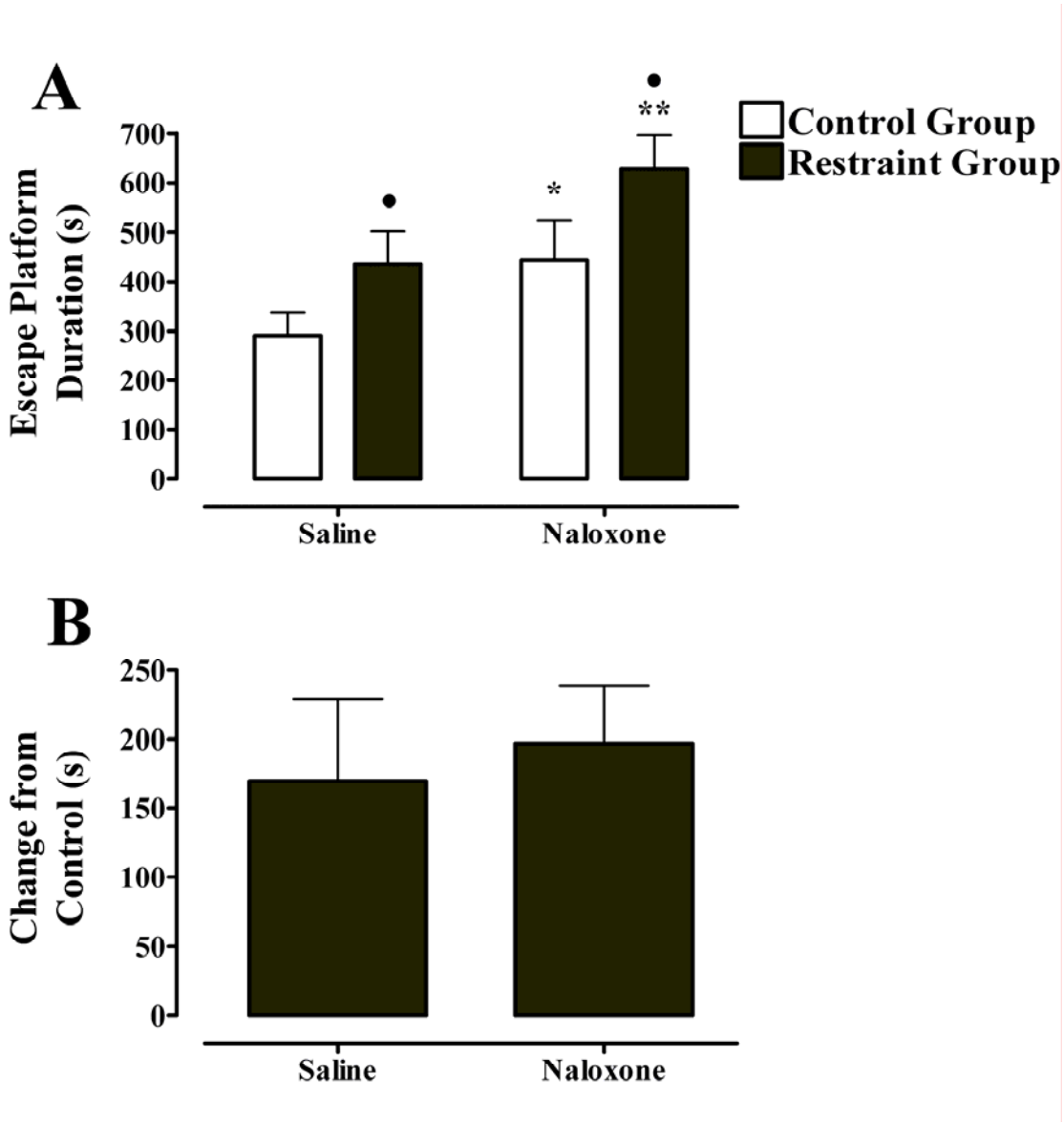


Figure 3-21. Cumulative escape durations at 44.5°C during testing sessions in which rats received 15 minutes of restraint stress (restraint group, closed bar) or no stress (control groups, open bar) followed by an injection of saline or naloxone. (A) Escape durations were significantly greater after an injection of naloxone when compared to control groups. Naloxone significantly increased escape durations after restraint stress. (B) Difference scores revealed that stress significantly increased escape durations in saline treated animals, which were slightly longer after naloxone. Data are expressed in seconds and are represented as absolute group means ± S.E.M. Significant within-subject differences after saline and naloxone injections are indicated as: * $P<0.05$ and ** $P<0.01$. Significant between-subject differences between control and restraint groups are indicated as: • $P<0.05$.

Effects of Morphine on Stress-Induced Changes in Nociception

Under non-stressful conditions, morphine produces hyporeflexia at high doses (5 to 10 mg/kg), as indicated by *a longer latency to elicit a reflex response*, to high intensity stimulation (>50°C; for reviews see Drolet et al., 2001; Yamada and Nabesima, 1995). Studies have shown that morphine-induced hyporeflexia is enhanced by restraint stress (Abbelbaum and Holtzman, 1984, 1985; Calcagnetti and Holtzman, 1990, 1992; Fleetwood and Holtzman 1989). Paradoxically, low dose morphine (0.5 to 5.0 mg/kg) results in hypereflexia, which is demonstrated by a shorter latency and longer duration of reflex responses (Cooper and Vierck, 1986; Guirimand et al., 1995; Holtman and Wala, 2005; Vierck et al., 2002; Wiley, Personal Communication) presumably from excitatory effects on motor activity (Lee et al., 1978; Le Bars et al., 2001). Hyperactivity after low dose morphine was observed particularly in stereotyped behaviors and is mediated by μ-opioid (Negus et al., 1993; Weinger et al., 1991) and α_2 (Weinger et al., 1992, 1995) receptors. Mu-opioid receptors are widely distributed throughout the nervous system particularly in areas of the brainstem and cortex involved in sensory processing and motor output (Drolet et al., 2001; Yamada and Nabesima, 1995). Similar doses of morphine have been shown to reduce operant responses (morphine-induced hyporeflexia; Vierck et al., 2002).

Reflex Lick/Guard Responses

First response latency of reflex lick/guard responses to 44.5°C thermal was evaluated to determine if exogenous opioids could modulate the expression of stress-induced hyporeflexia. Behavioral responses were obtained during sessions in which rats received 15 minutes of restraint stress (restraint group) or no stress (control

group) followed by an injection of morphine. The drug was administered at 1 (n=19), 5 (n=13), 8 (n=10), and 10 (n=8) mg/kg in addition to saline (n=19).

Reflex lick-guard latency

In the control group, morphine significantly affected reflex latencies (Figure 3-22A, F=14.006, P<0.001). No differences were seen with 1 mg/kg (P>0.05), but reflex latencies longer than saline were observed after 5.0 (P<0.05), 8.0 (P<0.01) and 10.0 (P<0.01) mg/kg of morphine. In a similar protocol, morphine also significantly increased latencies after stress (F=26.600, P<0.001). Specifically, latencies were significantly longer after morphine at 5.0 (P<0.05), 8.0 (P<0.01) and 10.0 (P<0.01) but not 1 mg/kg of morphine (P>0.05). Difference between control and restraint groups (Figure 3-22B) revealed that reflex latencies were significantly longer after stress compared to the control groups for all doses of morphine: 1.0 (F=4.065, P=0.04), 5.0 mg/kg (F=6.030, P=0.02), 8.0 (F=7.947, P=0.011), and 10 (F=5.224, P=0.038) mg/kg. This supports previous data that stress enhanced the hyporeflexic effect of morphine.

Reflex lick-guard durations

The duration of reflex responses were also assessed in this group of rats during trials at 44.5°C after morphine administration (Figure 3-23). Reflex durations were significantly shorter after restraint than the control group, but morphine increased reflex durations in stressed animals suggesting that morphine countered an inhibitory effect of stress (Figure 3-23A, B). For example, reflex durations were shorter in the restraint group compared to the control group especially after injections of morphine at 1 (F=4.965, P=0.032), 5 (F=4.965, P=0.032) and 10 (F=5.395, P=0.039) mg/kg, but no significant differences were observed at 8 mg/kg (F=1.260, P=0.276) due to high degree of variability.

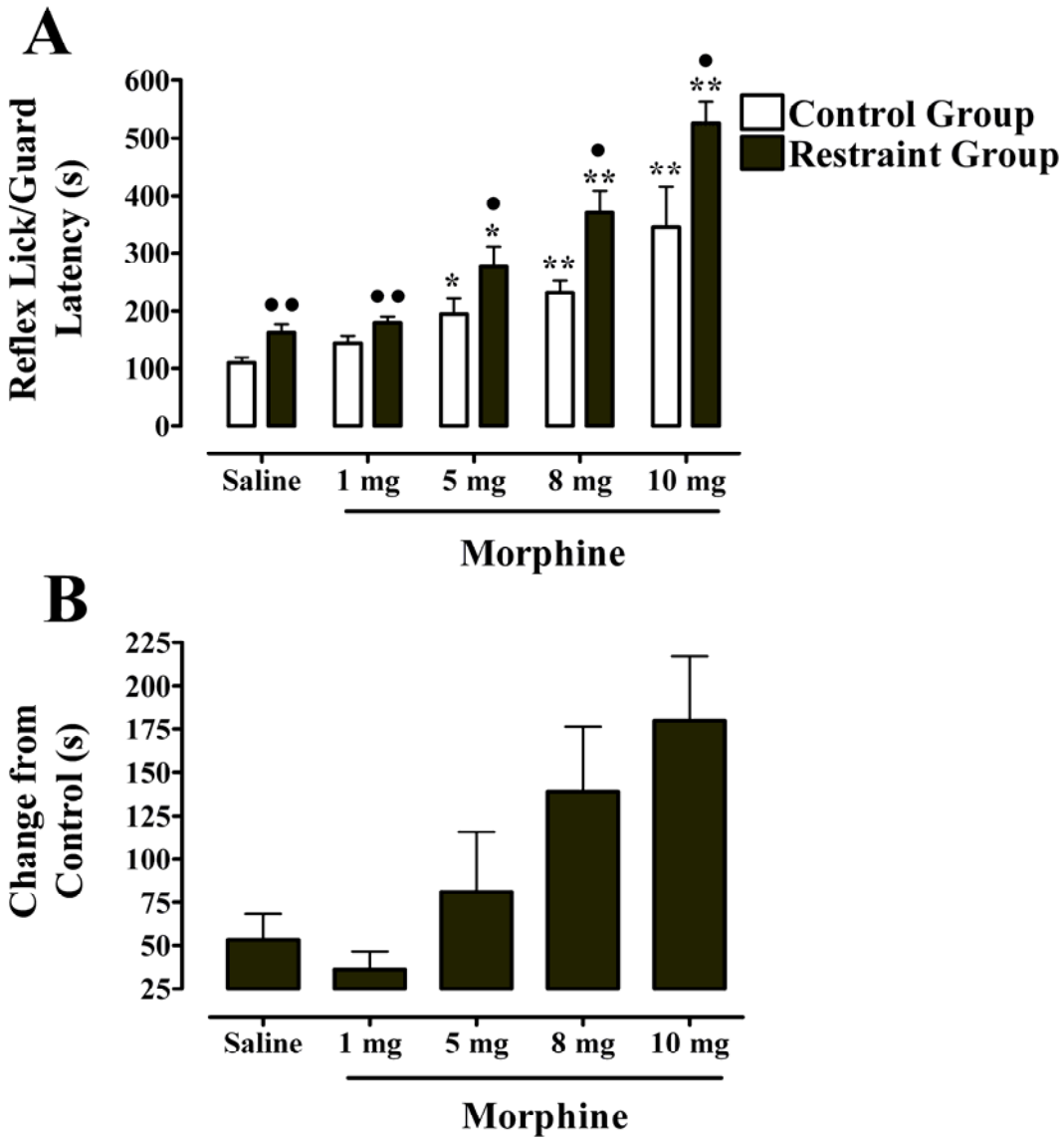


Figure 3-22. Reflexive lick/guard latencies at 44.5°C during testing sessions in which rats received 15 minutes of restraint stress (restraint group, closed bar) or no stress (control groups, open bar) followed by an injection of saline or morphine. (A, B) Reflex latencies were longer after exposure to restraint stress compared to the control group. Injections of morphine dose-dependently increased reflex latencies in both groups. Morphine potentiated stress-induced inhibition of reflex latencies compared to the control group. Data are expressed in seconds and are represented as absolute group means ± S.E.M. Significant within-subject differences after saline and morphine injections are indicated as: * $P<0.05$ and ** $P<0.01$. Significant between-subject differences between control and restraint groups are indicated as: • $P<0.05$ and •• $P<0.01$.

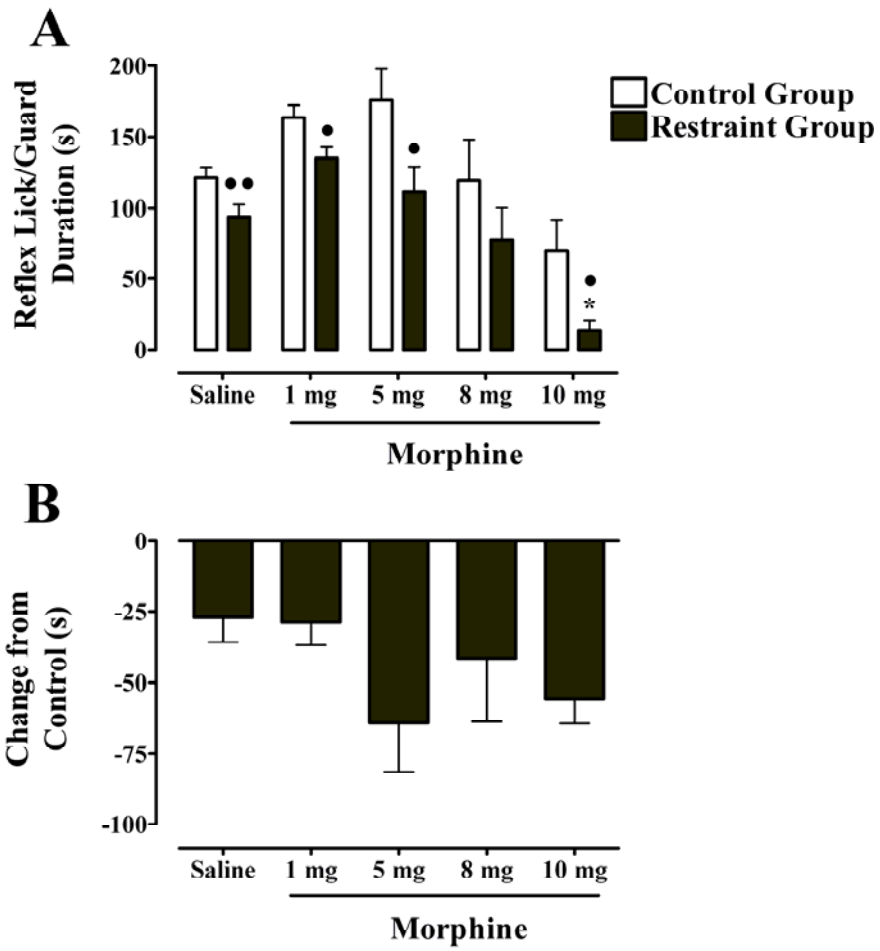


Figure 3-23. Reflexive lick/guard durations at 44.5°C during testing sessions in which rats received 15 minutes of restraint stress (restraint group, closed bar) or no stress (control groups, open bar) followed by an injection of saline or morphine. (A, B) Reflex durations were shorter after exposure to restraint stress compared to the control group. Injections of morphine produced a bi-directional effect depending on the dose of morphine. In the control group, reflex durations were longer after injections of 1.0 and 5.0, mg/kg of morphine compared to saline treated groups. Longer durations peaked at 5.0 mg/kg. Reflex durations were shorter after injections of 10.0 mg/kg of morphine. In the restraint group, reflex durations were shorter than morphine treated groups in the control group. However, reflex durations were longer after injections of 1.0 and 5.0 mg/kg of morphine compared to saline treated groups in the stress group. Reflex durations were shorter after an 8.0 and 10.0 mg/kg dose of morphine. Data are expressed in seconds and are represented as absolute group means ± S.E.M. Significant within-subject differences after saline and morphine injections are indicated as: * $P<0.05$. Significant between-subject differences between control and restraint groups are indicated as: • $P<0.05$ and •• $P<0.01$.

In summary, morphine produced a bi-directional effect of reflex response duration depending on the dose. Hypereflexia and hyporeflexia was observed at lower and higher doses, respectively. Stress-induced hyporeflexia was reduced by morphine. Furthermore, a discrepancy between reflex latencies and duration occurred. While reflex latencies were inhibited, durations were enhanced. It is important to note that a majority of studies use latencies as the behavioral outcome measure, but only a few groups use durations (Vierck et al., 2002). Studies that rely on reflex latencies failed to recognize changes in duration within a trial. Because previous studies used high intensity stimulation, trial durations were short and ranged from 10 to 30 seconds (Holtman and Wala, 2005). In the present experiment, the discrepancy may be a result of an initial decrease in reflex responding followed by a period of excitation.

Operant Escape Responses

Based on the ability of morphine to affect reflex responses in control and stress conditions, the role of exogenous opioids on the expression of stress-induced hyperalgesia was examined. Figure 3-24 (A, B) presents the effects of low dose morphine on operant escape durations during 44.5°C trials for control (n=10) and restraint (n=10) groups. In the control group, while durations were shorter after injection of morphine, but this effect was not quite significant (F=3.426, P=0.081). However, morphine significantly reduced durations in the stress groups (F=5.689, P=0.028). The effect of morphine was similar between control and stress groups (F=0.397, P=0.536). Morphine reduced the expression of stress-induced hyperalgesia (e.g. decreased escape platform duration; increased thermal plate duration).

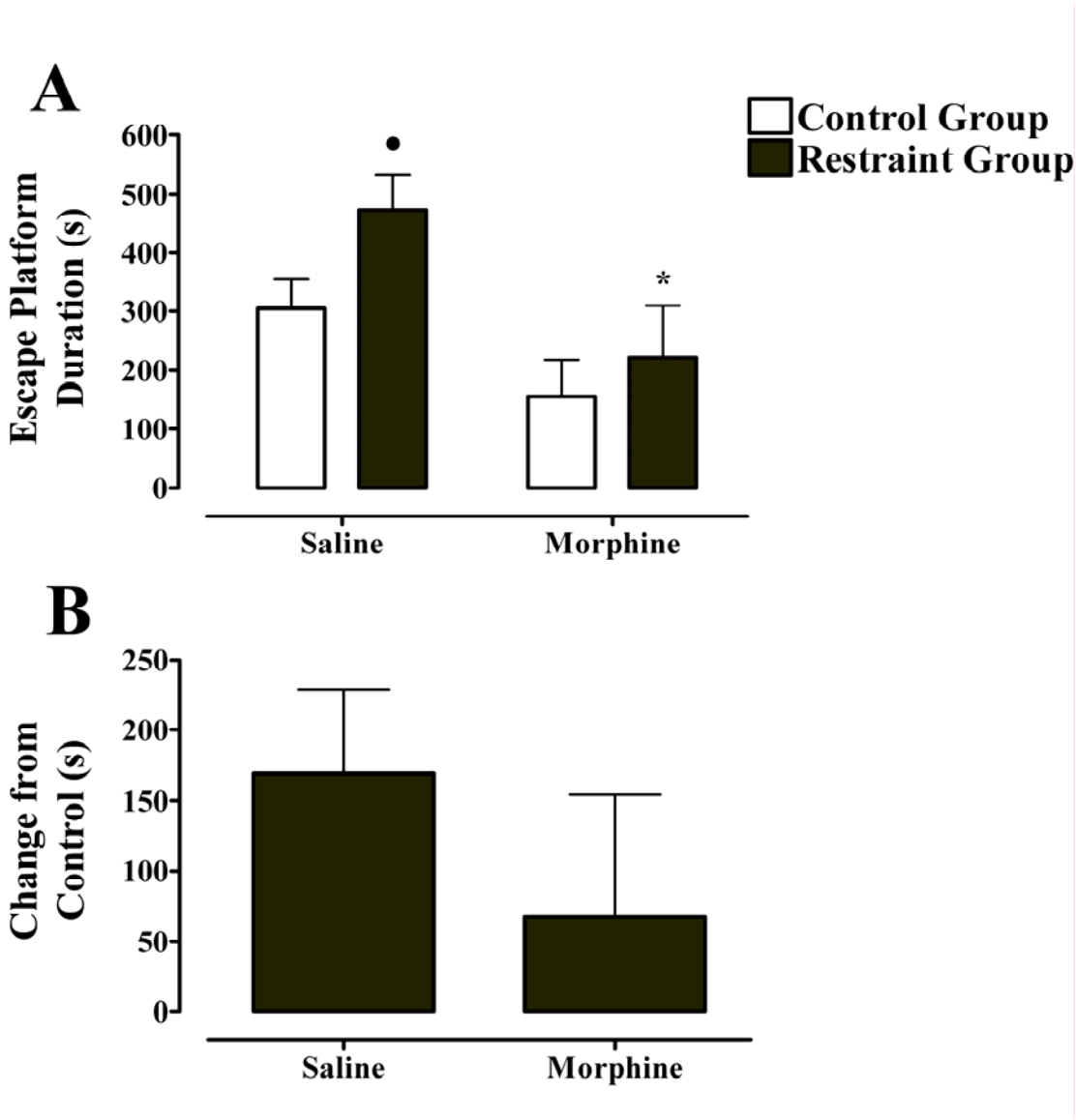


Figure 3-24. Cumulative escape durations during testing trials at 44.5°C during testing sessions in which rats received 15 minutes of restraint stress (restraint group, closed bar) or no stress (control groups, open bar) followed by an injection of saline or morphine. (A, B) Escape durations were significantly greater after restraint stress when compared to control groups. However, morphine reduced escape durations in control groups and reduced stress-induced excitation of escape durations. Data are expressed in seconds and are represented as absolute group means ± S.E.M. Significant within-subject differences after saline and morphine injections are indicated as: * $P<0.05$. Significant between-subject differences between control and restraint groups are indicated as: • $P<0.05$.

Summary and Discussion

Brief (15 minutes) exposure to restraint stress produced differential effects on reflex and operant responses to nociceptive thermal stimulation. Restraint stress depressed lick/guard responses, increasing response latencies and decreasing the time spent performing reflex responses during a trial. In contrast, stress enhanced escape responding to thermal stimulation. The latency of initial escape responses was not affected by stress in one group (Figure 3-4) but was affected in another group (Figure 3-9). Escape latency is a highly variable measure of nociceptive responsivity, due to factors that include a strong innate tendency to explore the confines of a test environment upon initial entry. Escape latency has been shown to be consistently less sensitive than response duration when measured against variations in stimulus intensity or experimental treatments other than stress (Vierck et. al., 2002, 2003).

In contrast to latency, rats consistently apportion their time on the escape platform according to the intensity of nociceptive stimulation, indicating that escape duration most reliably reflects the aversive magnitude of a stimulus. In addition, restraint stress produced a transient hyperalgesia in another operant escape test (the thermal preference) characterized by a decrease in time spent on a plate heated to 45.0°C. Based on these two operant behavioral assessment strategies, the primary consequence of stress is the enhancement of cortically mediated responses to heat (e.g., *stress-induced heat hyperalgesia*).

Role of the Endogenous Opioid System and Morphine

The expression of stress-induced changes in nociception was affected by endogenous and exogenous opioids. Studies have demonstrated exposure to restraint stress increases first response latency to engage in tail withdrawal, hindpaw licking, and

hindpaw guarding (Amir and Amit, 1978; Calcagnetti and Holtzman, 1992; Calcagnetti et al., 1992; Gamaro et al., 1998; Terman et al., 1986; Vidal and Jacobs, 1984). Reduction of reflex responses by stress has been interpreted as stress-induced hyporeflexia. Various mechanisms modulate the expression of stress-induced hyporeflexia including opioid mechanisms (Lewis et al., 1980; Porro and Carli, 1988; Yamada and Nabeshima, 1995).

In addition to regulating the stress response, opioid peptides regulate sensory and affective components of pain processing during painful and stressful situations through activity of μ-opioid receptors (Yamada and Nabesima, 1995; Zubieta et al., 2001). Several lines of evidence support the hypothesis that endogenous opioid peptides modulate stress-induced hyporeflexia. Release of opioid peptides and expression of stress-induced hyporeflexia share a similar temporal profile (Bodnar et al., 1978a; Madden et al., 1977). The contribution of endogenous opioids on the expression of stress-induced hyporeflexia is illustrated by its cross-tolerance with morphine and reversibility by naloxone (Pilcher and Browne, 1983; Terman et al., 1982).

In the current study, endogenous opioids have no tonic inhibitory effect on reflexes response as indicated by the inability of naloxone to influence reflex responses. However, cortical mediated responses (escape) are subjected to tonic inhibitory control by this system. In response to thermal stimulation, endogenous opioids are released (Cesselin et al., 1989; Kuraishi et al., 1984; Takagi, 1984) and consequently may regulate escape responses in the absence of stress. Administration of an opioid antagonist or other anti-opioid molecule (e.g., CCK) suppresses this system; endogenous opioids can no longer mediate responses, which should result in an increase sensitivity to heat stimulation.

Interestingly, the release of endogenous opioids under non-restraint conditions is an important factor in the expression of cererbrally-mediated responses, but not the neuroanatomical mechanisms underlying reflex responses. Under stress conditions, hyporeflexia was reversed by naloxone suggesting that the hyporeflexia was mediated by endogenous opioids. In contrast, stress-induced hyperalgesia was enhanced by naloxone suggesting that the expression of hyperalgesia was opposed by the same system. An increase in endogenous opioid release has been observed after stress (Larsen and Mau, 1994; Madden et al., 1977) particularly in areas traditionally involved in movement and drug addition (mesocorticolimbic systems; Kalivas and Abhold, 1987; Kalivas and Duffy, 1995).

In the current study, exogenous opioids (morphine) enhanced the expression of stress-induced hyporeflexia and hyperalgesia to low intensity suprathreshold thermal stimulation. However, depending on the behavioral outcome, differences between reflex responses were observed. In confirmation of previous studies, morphine and stress increased the latencies to elicit a reflex response. Previous studies have demonstrated that stress can enhance the hyporeflexic (e.g., increase reflex latencies) effects of μ-opioid agonists (e.g., morphine; Abbelbaum and Holtzman, 1984, 1985; Calcagnetti and Holtzman, 1990, 1992; Fleetwood and Holtzman 1989). These effects of stress are reduced in chronically stressed and morphine tolerant animals (Fleetwood and Holtzman 1989). In agreement with these studies, the hyporeflexic effect of morphine was enhanced by stress when assessed by reflex latencies. However, low dose morphine enhanced reflex responses (*morphine-induced hypereflexia*) and higher doses were required (> 8 mg/kg) to reduce reflex responses (*morphine-induced hyporeflexia*). If

latencies were only assessed, it would be falsely concluded that morphine produced hyporeflexia, which was stress enhanced, comparable to previous studies. But, evaluation of response durations provides evidence that morphine has pro-nociceptive properties presumably through the activation of NMDA receptors (Heinricher, et al., 1997, 2001; Holtman and Wala, 2005; Manning et al., 1996; Price et al., 2000). Furthermore, motor output is altered by opioids due to inhibition of GABAergic transmission in the cortex (e.g., substantia nigra and striatum; Turski et al., 1982) and brainstem (RVM; Weinger et al., 1991).

Based on reflex durations, morphine opposed the expression of stress-induced hyporeflexia and hyperalgesia, when assessed by response durations. These results suggest that morphine simultaneously activated descending inhibitory and facilitatory pathways, but activity within these pathways affected spino-bulbo-spinal and cerebrally mediated responses differently. The effects on facilitation (morphine-induced hypereflexia) were more significant than those on inhibition (stress-induced hyporeflexia). The effects on inhibition (morphine-induced hypoalgesia) were more significant than those on facilitation (stress-induced hyperalgesia).

Expression of Stress-induced Hyperalgesia

The expression of hyperalgesia is most noticeable when assessed immediately after stress. Escape responses were highest compared to controls during this time point. Although the effects of stress remained significant 15 minutes later, it was slightly smaller than responses assessed immediately after the termination of stress. Even though behavioral responses were not evaluated after 30 minutes, future studies could perform a more intensive assessment across multiple time points. Responses would be expected to

diminish over time based on the fact that responses were greater immediate after stress and returned to pre-stress levels the following day.

Evidence has suggested that the release of opioid peptides during exposure to a stressor regulates the stress response and allows an animal to cope with stressful situations (Amit and Galina, 1988; Curtis et al., 2001; Sumova and Jakoubek, 1989; Tanaka et al., 2000; Terman et al., 1984). But, the release of endogenous opioids does not explain the underlying mechanisms for ***stress-induced hyperalgesia***. Possible mechanisms may be related to changes in various physiological systems, which are most apparent immediately after stress. The release of excitatory or anti-opioid peptides could account for the increase in sensitivity. While the role of endogenous opioids was addressed, the current study did not evaluate additional neurochemicals previously identified to enhance nociceptive sensitivity such as cholecystokinin (CCK), noradrenaline (NE), and dopamine (DA). Evidence suggests that CCK possess anti-opioid properties. CCK mRNA is found in areas of opioid expression (Stengaard-Pedersen and Larsson, 1981, 1982). CCK is a contributing factor in the development of opioid tolerance after repeated morphine administration (Stanfa et al., 1994) and injury (Vanderah et al., 2001a, 2001b; Xie et al., 2005). Modulation of this system with agonists (CCK_8) or antagonist (L365, 260; proglumide) can reduce or enhance the hyporeflexic effects of morphine, respectively (Faris et al., 1993; Hawranko and Smith, 1999; Pu et al., 1994; Xie et al., 2005). Furthermore, stress-induced hyporeflexia is enhanced by CCK antagonists (Hawranko and Smith, 1999; Watkins et al., 1984, 1985).

Furthermore, the expression of hyperalgesia may be a consequence of hypervigilance or attentiveness to the environment. Stress has been known to increase vigilance in animals (Castilho et al., 1999; Hayes and Katayama, 1986; Maier et al., 1992) presumably through NE (Tanaka et al., 2000). Immobilization stress induces the release of NE (Glavin et al., 1983; Iimori et al., 1982; Keim and Sigg, 1976; Tanaka et al., 1982, 1983a, 1983b). Interestingly, exogenously administered opioids antagonists or agonists can increase (naloxone) or decrease (morphine) stress-induced release of NE in limbic and other cortical structures, respectively. It has been speculated that the release of NE by stress is a critical factor in the expression of anxiety (Tanaka et al., 2000). Opioid peptides, in turn, are responsible for the termination of the stress response (Valentino and Van Bockstaele, 2001), which also supports evidence that opioids regulate affective and motivational states during pain and stress (Zubieta et al., 2001). Other possible mechanisms include alterations in the DA system (Altier and Stewart, 1996, 1999a, 1999b; Beaulieu et al., 1987; De Souza and Van Loon GR, 1986; Kalivas and Duffy, 1995; Watanabe, 1984) and activity of the sympathetic nervous system (Elam et al., 1986), which will be addressed in later chapters.

Role of Thermoregulation

In support of other studies, body temperature was higher after restraint stress (e.g., stress-induced hyperthermia). Stress has been shown to increase body temperature (Chen and Herbert, 1995; Keim and Sigg, 1976; Le Bars et al., 2001; Thompson et al., 2003; Tjolsen and Hole, 1993), but these studies did not properly evaluate the impact of altered thermoregulation on nociceptive responding. Cutaneous temperatures (tail or plantar surface of the hindpaw) were identified as a confounding variable in the interpretation of reflex responses (Tjolsen and Hole, 1993). A relationship was also observed between

temperatures and tail (primary thermoregulatory organ) of the rat during withdrawal responses. If the tail was cooler, the latency to withdrawal was longer suggesting a reduced sensitivity. Temperatures can profoundly impact nociceptive responses and subsequently provide erroneous interpretations of those responses. Thus, it is important to determine if changes in behavioral responding is a consequence of altered thermoregulation.

In the current study, while restraint increased body temperature, animals quickly cooled down after termination of restraint and during pre-test trials at 36.0°C (neutral temperatures). The pre-test trial serves an important role to normalize temperatures in stressed animals with non-stressed animals prior to testing at low intensity thermal stimulation. In addition, cutaneous temperatures also were increased after stress. But, the increase in temperature can be attributed to postural factors. Because the animal is confined within the tube, their hindpaws remained close to their body. As a consequence, an increase in hindpaw temperatures was observed. Even though tail temperature was not included in the present study, it underwent a period of cooling during restraint stress. While this can be attributed to the tail being exposed to the environment, restraint stress reduced cutaneous temperature of the tail and may indicate activation of the sympathetic nervous system (Chapter 5). Similar to core temperatures, pre-test trials at 36.0°C brought cutaneous temperatures of the control group to levels comparable to the restraint group. This provides evidence that pre-exposure to a neutral temperature normalize hindpaw temperatures prior to placement into the testing apparatus. Thus, the expression of stress-induced hyperalgesia is not influenced by changes in core body temperature.

Future studies may compare the effects of restraint stress and exercise (e.g. wheel running), which is a not stressful, on changes to both body and thermal sensitivity.

Control Procedures

Several control procedures ensured that the effects of stress on escape responding were not the result of changes in the aversive qualities of bright light, which discouraged occupancy of the escape platform. Latencies to escape light in the darkbox test were not altered by prior stress, and neither latencies nor durations of platform occupancy were altered during trials at a neutral temperature (36.0°C). Long latencies and short durations of platform occupancy at 36.0°C also showed that animals had not learned to avoid thermal stimulation by occupying the escape platform regardless of plate temperature.

The increase in escape duration produced by acute stress has important implications in relation to an extensive animal literature that has reported elevated latencies or thresholds for innate, unlearned nociceptive responses after exposure to a variety of stressors (Bodnar et al., 1980a). The present study confirmed the usual result for reflex responses, using the same animals and the same nociceptive stimulus for lick/guard responses as for operant escape. The attenuating effects of acute stress on lick/guard and other innate reflex responses have been considered as evidence for stress induced analgesia (SIA), based on an assumption that stress acts at spinal levels to suppress both segmental reflex and rostral projection systems receiving nociceptive input. However, in light of our finding that acute stress diminishes reflex responses to nociceptive input while enhancing operant responding to the same stimulus, it appears that stress-induced hyporeflexia can coexist with stress induced hyperalgesia. This combination of effects may have adaptive significance. For example, in stressful environmental circumstances

such as a predator/prey interaction, repeated elicitation of stereotyped reflexes such as limb flexion or licking would interfere with intended motor actions required of the situation (Brandao et al., 1994, 1999; Coimbra et al., 2006; Leão-Borges et al., 1988). Under the same circumstances, pain would be an important motivator and could initiate fighting or fleeing. These defensive responses are independent of stress-induced hypoalgesia (Coimbra et al., 2006; Prado and Roberts, 1985) and appear to be modulated by different mechanisms (Brandao et al., 1999; Castilho et al., 1999; Maier, 1990).

Notes

1. Parts of this chapter were previously published.
2. Reprinted from Brain Research, King, et al., Differential effects of stress on escape and reflex responses to nociceptive thermal stimuli in the rat, Volume 987(2), 214-222, Copyright 2003, with permission from Elsevier.

CHAPTER 4
EFFECTS OF RESTRAINT STRESS ON NOCICEPTIVE RESPONSES FOLLOWING EXCITOTOXIC SPINAL CORD INJURY

While other complications impact the daily activities in SCI patients, pain is a relatively common, serious health problem that negatively affects the quality of life in these individuals. To better understand altered pain sensations after injury to the spinal cord, an animal model (excitotoxic model of SCI) was developed by Dr. Robert Yezierski to identify central mechanisms underlying SCI pain and develop novel preventative and treatment strategies. Excitotoxic injury to the spinal cord is accomplished by an intraspinal injection of the AMPA/metabotropic receptor agonist quisqualic acid (QUIS), which results in damage to the spinal gray matter.

Histological examination of spinal injured cords reveals a relationship between pain sensations (at- and below-level) and several anatomical changes (regional neuronal loss; longitudinal extent of injury). Behaviorally, animals display a heightened sensitivity to mechanical and thermal sensitivity as assessed by reflex mediated responses. As discussed previously, reflex responses to nociceptive stimulation do not represent cortical mechanisms or pain sensation, but rather spinal or spino-bulbo-spinal mediated responses to nociceptive stimuli.

Based on these observations, a question can be raised regarding the impact of spinal injury on pain sensations. First, are responses dependent on cortical processing of nociceptive thermal stimulation affected by spinal injury? If so, what potential mechanism(s) are involved? Therefore, the effects of excitotoxic lesions of the spinal

gray matter, confined to the thoracic and lumbosacral cord, on operant responses were examined. Two types of operant tasks were utilized to examine these effects including operant escape and thermal preference. It was hypothesized that operant responses would be affected by excitotoxic injury. Injury-induced hyperalgesia (e.g., increased sensitivity to heat) after QUIS could be demonstrated by change in operant behavior following injury.

Finally, while a positive relationship exists between clinical pain conditions and exposure to stress, limited studies have examined the effects of stress on nociceptive responses in chronic pain models, particularly models of spinal injury. Therefore, the effects of restraint stress on operant responses were examined after excitotoxic lesions to the spinal gray matter. Several weeks after injections of QUIS, animals were exposed to restraint stress for fifteen minutes followed by assessment of thermal sensitivity on the day of stress and several days afterward. Restraint stress was expected to further enhance operant responses after excitotoxic injury.

Effects of Excitotoxic Spinal Cord Injury on Operant Escape

Overall Effect of Spinal Injury on Escape Responses

The effects of excitotoxic lesions of the spinal gray matter on operant escape responses (n=15) during trials at 44.5°C are shown in Figures 4-1 through 4-5. Escape performances were evaluated by two different response measures: a) the number of responses elicited during a trial (count); and, b) the total time spent on the platform (duration). Preoperative responses were recorded over several months before spinal injury. After excitotoxic injury (QUIS), behavioral assessment resumed three weeks later and continued over 10 weeks.

Escape counts

The effects of QUIS on the number of escape platform responses are presented in Figure 4-1. The number of postoperative responses (QUIS) was slightly higher than preoperative responses (baseline), but platform responses were not significantly different after QUIS (F=0.945, P=0.329). Failure to reveal an injury-induced increase in the number of responses suggests that spinal injury did not alter thermal sensitivity on the escape test in this group of rats.

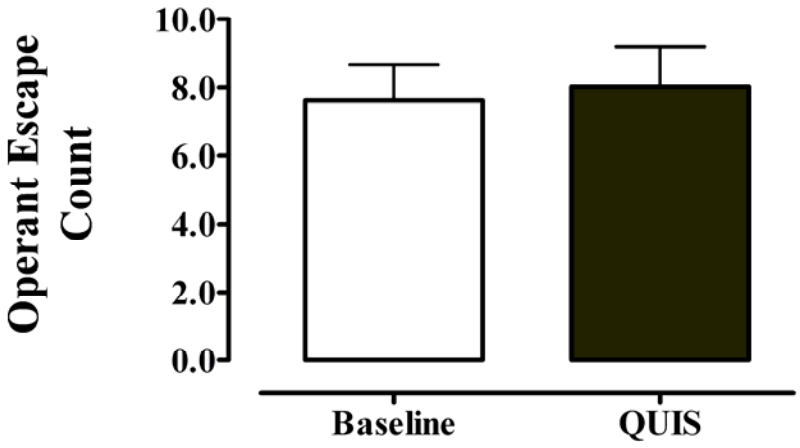


Figure 4-1. The number of escape platform responses during testing trials at 44.5°C before (baseline, open bar) and after (QUIS, closed bar) excitotoxic injury. Escape responses did not differ after QUIS. Data are expressed in seconds and are represented as absolute group means ± S.E.M.

Escape durations

A better evaluation of an experimental manipulation on operant responses is escape duration. Previous evidence has shown that animals display an enhanced sensitivity to heat after injury of the upper thoracic cord (Acosta-Rua, 2003). Injury-induced hyperalgesia was indicated by an increase in escape duration and a decrease in plate duration. It was hypothesized that operant responses would increase after excitotoxic

injury of the mid-thoracic and upper lumbar spinal cord. Figure 4-2 shows the effects of excitotoxic lesions on the duration of escape responses (platform).

In the current group of rats, an injury-induced hyperalgesia was not observed for platform duration (F=0.030, P=0.863). Conversely, the total time spent on the thermal plate was not different before or after QUIS (data not shown; F=0.029, P=0.865). The inability to alter thermal sensitivity assessed by operant responses (counts and duration) after QUIS suggests that cortically-mediated responses to thermal stimulation are not affected by QUIS injuries.

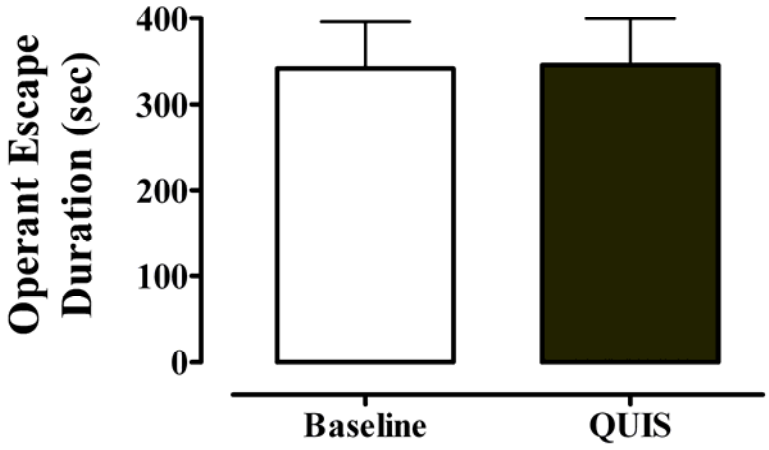


Figure 4-2. Cumulative escape platform durations during testing trials at 44.5°C before (baseline, open bar) and after (QUIS, closed bar) excitotoxic injury. Platform durations were affected by QUIS. Data are expressed in seconds and are represented as absolute group means ± S.E.M.

Sequence analysis of successive escape durations

To further examine the effect of QUIS on operant responses, successive escape plate and platform durations within a trial at 44.5°C are presented in Figure 4-3. A summary of the successive escape plate and platform durations is presented in Figure 4-4.

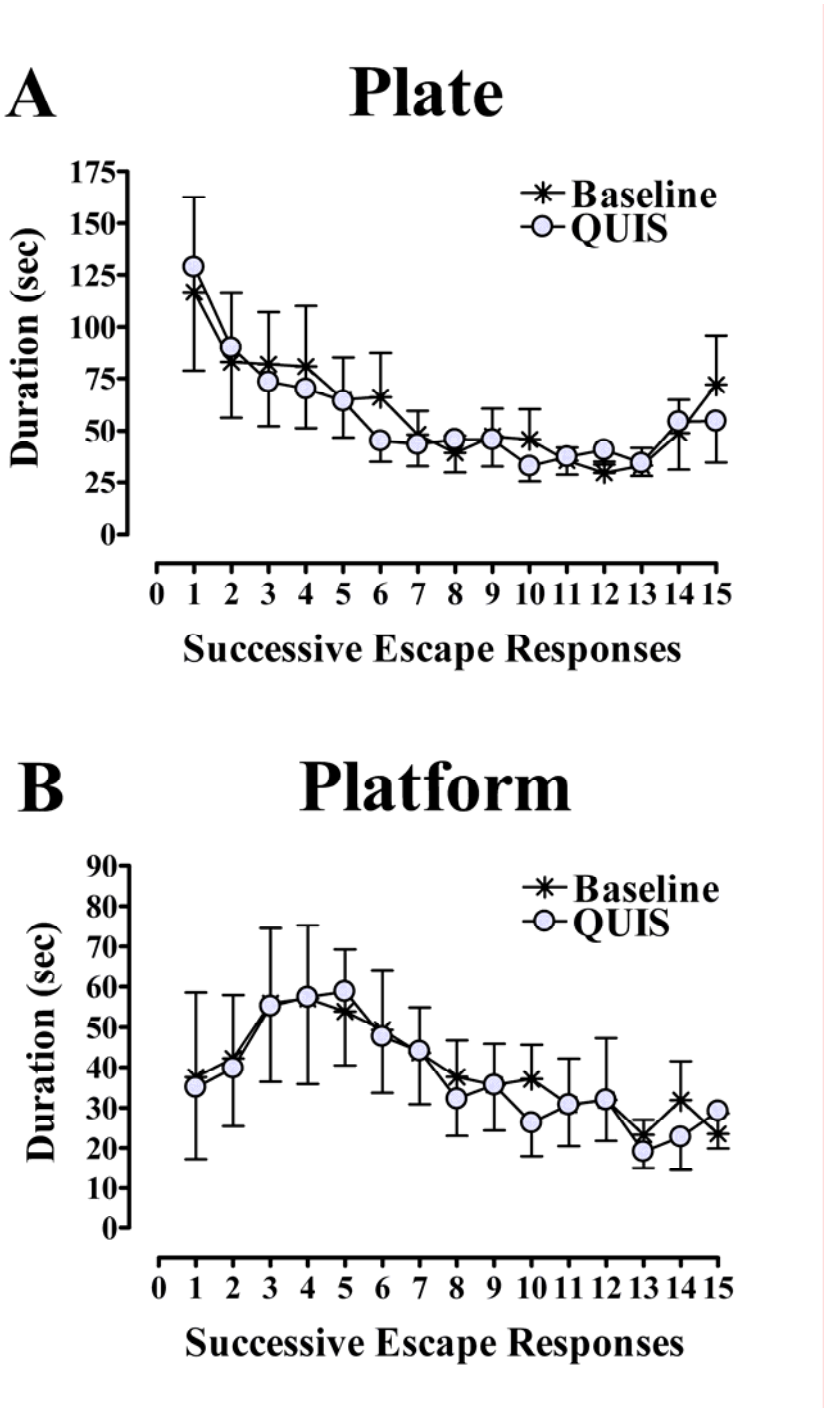


Figure 4-3. Sequence analysis of successive escape plate and platform durations during testing trials at 44.5°C before (baseline, asterisk) and after (QUIS, gray circle) excitotoxic injury. QUIS failed to influence successive operant escape plate (A) and platform (B) durations. Data are expressed in seconds and are represented as absolute group means ± S.E.M.

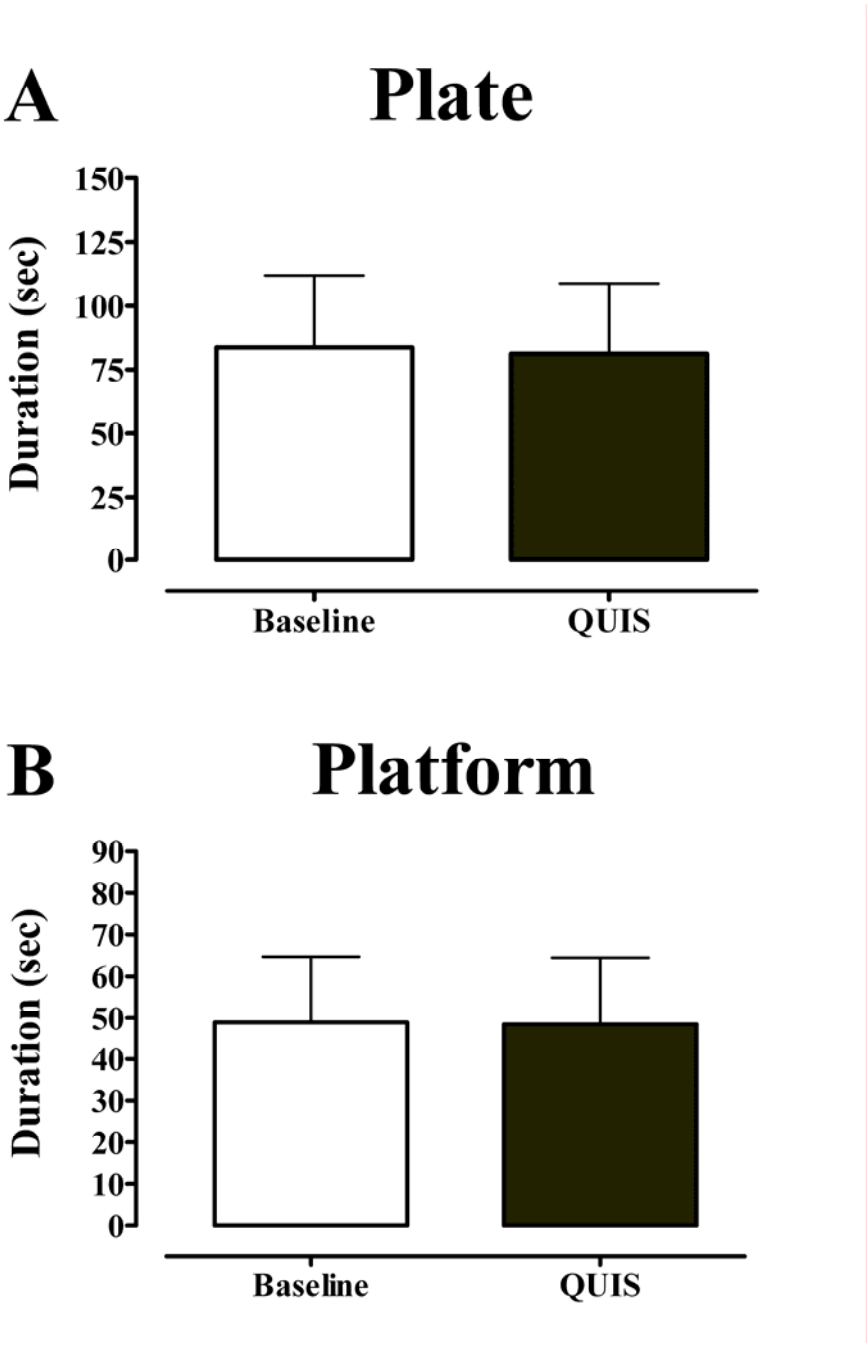


Figure 4-4. Average duration of the first six plate and platform responses during testing trials at 44.5°C before (baseline, open bar) and after (QUIS, closed bar) excitotoxic injury. No differences were observed in plate (A) and platform (B) durations between baseline and QUIS testing conditions. Data are expressed in seconds and are represented as absolute group means ± S.E.M.

In general, plate durations (Figure 4-3A) were initially higher than platform durations (corresponding to responses 1 through 6) and stabilized after the 7th response for the remainder of the trial. Excitotoxic injury did not affect plate durations, which appeared to be similar to baseline responses. In addition, platform durations (Figure 4-3B) appeared to increase slightly from the 1st response to the 5th response (peaking around 4th response). However, similar to plate responses, baseline platform durations were similar to these obtained following excitotoxic injuries.

The average durations of the first 6 durations are compared between baseline (open bar) and QUIS (closed bar). In agreement with cumulative escape durations, QUIS did not affect the average durations of plate (Figure 4-4A, F=0.106, P=0745) or platform (Figure 4-4B, F=0.064, P=0.800) responses. The average baseline plate and platform durations were similar between testing conditions.

Weekly assessment of escape durations

Assessment of weekly postoperative escape responses is shown in Figure 4-5 before (asterisk) and after QUIS (gray circle). Postoperative assessment of escape responses was reinstated three weeks after an injection of QUIS. Platform durations were variable across each week (peaking during the 7^{th} week). Statistical analysis revealed that QUIS did not affect escape responses across weekly testing sessions (F=0.507, P=0.851).

Effects of Spinal Injury in Individual Groups

In evaluating operant responses after injury, QUIS failed to enhance sensitivity to heat (no injury-induced hyperalgesia). However, two types of responses were identified in the population of animals with spinal injury.

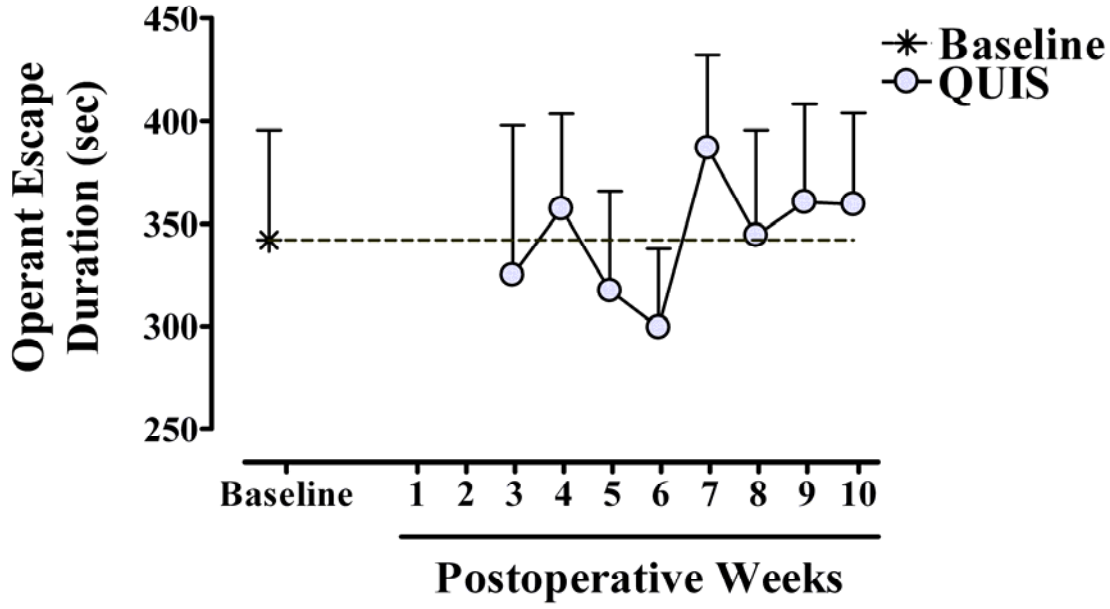


Figure 4-5. Weekly postoperative platform responses across several weeks of testing during trials at 44.5°C before (baseline, asterisk) and after (QUIS, gray circle) excitotoxic injury. Data are expressed in seconds and are represented as absolute group means ± S.E.M.

One group (QUIS *hyperalgesia*; n=7) displayed an optimal change in responding after injury that was characterized by an increase in platform escape duration. In the second group (QUIS *hypoalgesia*; n=8), platform escape durations were significantly reduced compared to baseline values. In order to characterize the groups, further analyses of platform durations as well as the number of platform responses were conducted and are presented in Figures 4-6 through 4-8.

Based on previous analysis, the number of platform responses was not affected by QUIS. However, based on the revised grouping, an effect of QUIS was revealed for the first group (QUIS *hyperalgesia*), which exhibited enhanced responding postoperatively, and the second group (QUIS *hypoalgesia*) that postoperative responding was marginally reduced after injury.

Escape counts

The number of baseline platform responses for these two groups are presented in Figure 4-6. Interestingly, the number of platform responses was lower in the *hyperalgesic* group compared to the *hypoalgesic* group. After injection of QUIS, platform counts were significantly higher in the *hyperalgesic* group (Figure 4-6A, $F=19.627$, $P<0.001$). By contrast, QUIS did not significantly affect the number of platform responses in the *hypoalgesic* group (Figure 4-6B, $F=2.057$, $P=0.153$).

Escape durations

The duration of escape responses did not differ before and after an injection of QUIS based on the original grouping. However, additional characterization revealed a difference in escape responses between the two groups (Figure 4-7). Platform durations (total time on the escape platform) were affected by QUIS. Platform durations in the *hyperalgesic* group were significantly higher during sessions after QUIS (Figure 4-7A; $F=3.869$, $P=0.05$) compared to baseline sessions. Conversely, plate durations were significantly lower during sessions after QUIS (data not shown; $F=3.903$, $P=0.05$) compared to baseline sessions.

In contrast to this group, QUIS produced an opposite effect in the *hypoalgesic* group, with platform durations significantly lower during sessions after QUIS (Figure 4-7B; $F=8.066$, $P=0.005$) compared to baseline sessions. Plate durations were significantly higher during sessions after QUIS (data not shown; $F=8.115$, $P=0.005$) compared to baseline sessions. Thus, QUIS produced either enhanced (decrease plate duration; increase platform duration) or reduced (increase plate duration; decrease platform duration) sensitivity to heat as represented in these two groups.

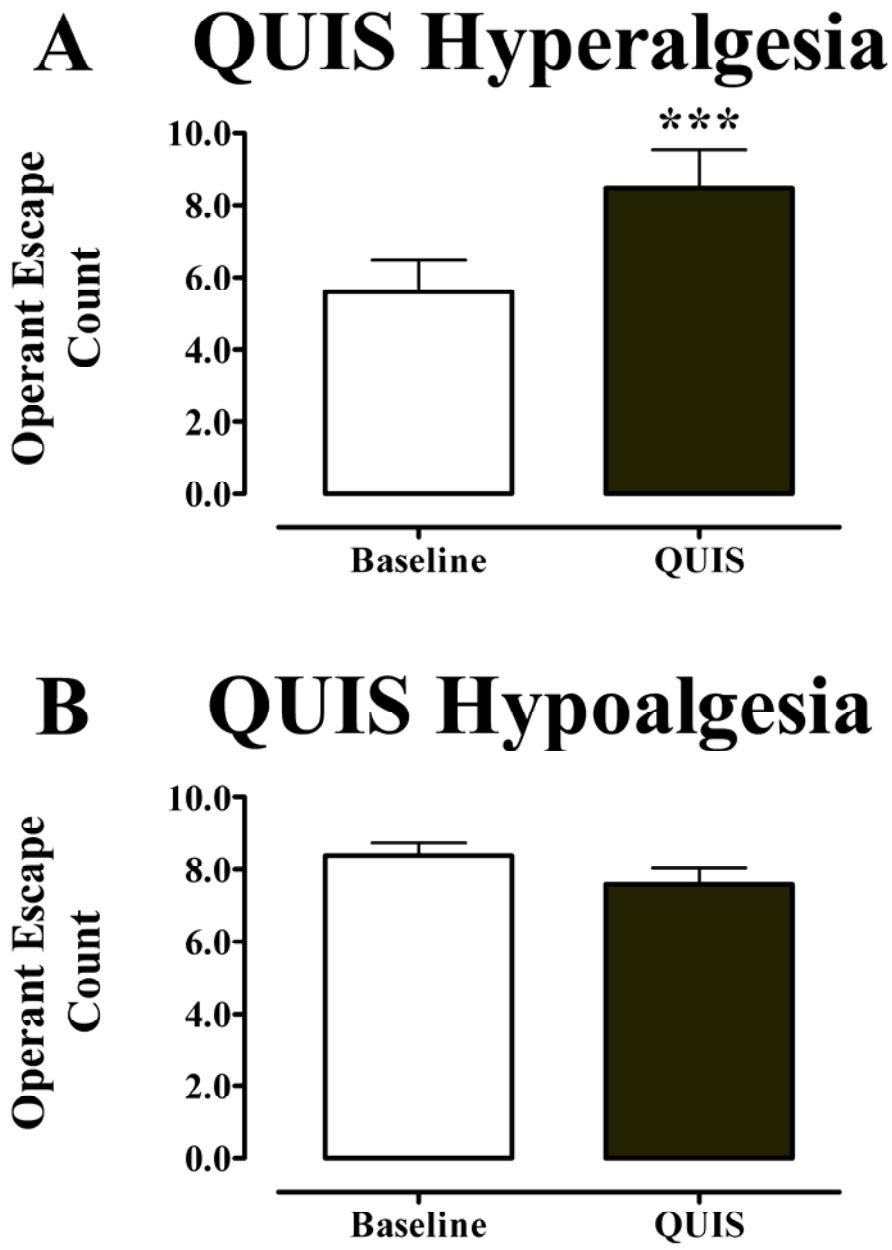


Figure 4-6. Number of escape platform responses during testing trials at 44.5°C before (baseline, open bar) and after (QUIS, closed bar) excitotoxic injury. While the number of responses in the *hyperalgesic* group (A) was increased, no effect was observed in the *hypoalgesic* group (B). Data are expressed in seconds and are represented as absolute group means ± S.E.M. Significant differences between baseline and QUIS testing periods are indicated by: *** $P<0.001$.

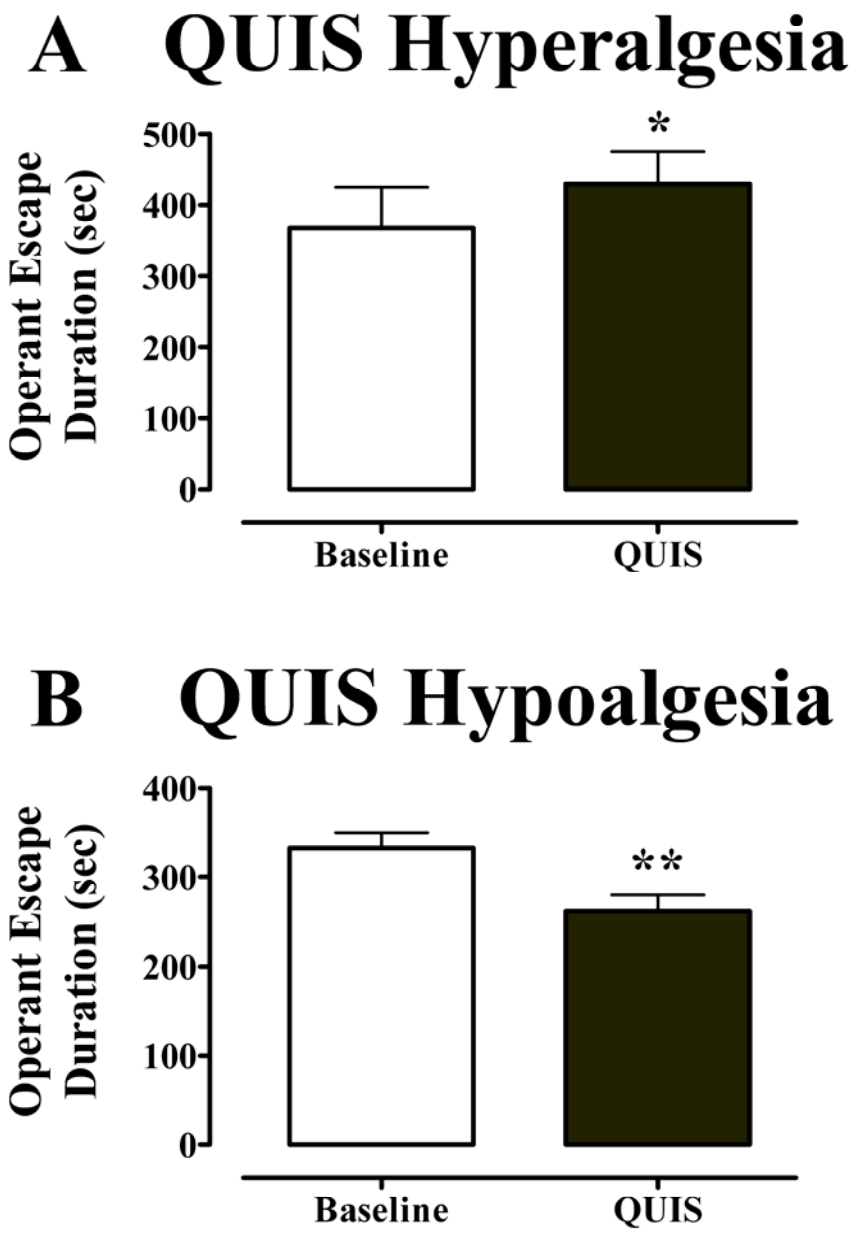


Figure 4-7. Cumulative escape platform durations during testing trials at 44.5°C before (baseline, open bar) and after (QUIS, closed bar) excitotoxic injury. The duration of platform responses was increased for the *hyperalgesic* group (A) but the *hypoalgesic* group (B) spent less time on the platform. Data are expressed in seconds and are represented as absolute group means ± S.E.M. Significant differences between baseline and QUIS testing sessions are indicated by: * $P < 0.05$ and ** $P < 0.01$.

Weekly assessment of escape durations

Assessment of weekly postoperative escape responses in *hyperalgesia* (A) and *hypoalgesia* (B) groups was compared before (asterisk) and after QUIS (gray circle) excitotoxic injury (Figure 4-8). QUIS enhanced escape responses to 44.5°C across several weeks after excitotoxic injury in the *hyperalgesic* group (Figure 4-8A). This enhancement appears to be greater during earlier weeks followed by a progressive return of responses to pre-QUIS levels. While QUIS enhanced platform responses in the *hyperalgesic* group, it decreased escape responses across several weeks in the *hypoalgesic* group (Figure 4-8B). Responses began to progressively return to baseline levels at the end of the testing period. Thus, QUIS produced two different effects: increased or decreased sensitivity to heat

Effects of Restraint Stress on Operant Escape Following Excitotoxic Injury

Overall Effects of Stress on Escape Responses after Injury

Behavioral responses dependent on cortical processing of thermal information are increased after a 15 minute exposure to restraint stress in normal uninjured animals (Chapter 3). Based on previous results suggesting that stress produces a heightened sensitivity to heat, how would animals with spinal cord injury respond to stress?While clinical evidence indicates that stress is an important factor in chronic pain, these effects have not been examined in an animal model. The excitotoxic model of SCI offers a unique opportunity to evaluate the effects of stress on altered sensation after spinal injury. The fact that animals have experienced injury to the spinal cord could be a critical factor in the development and/or enhancement of stress-induced increases in operant responding.

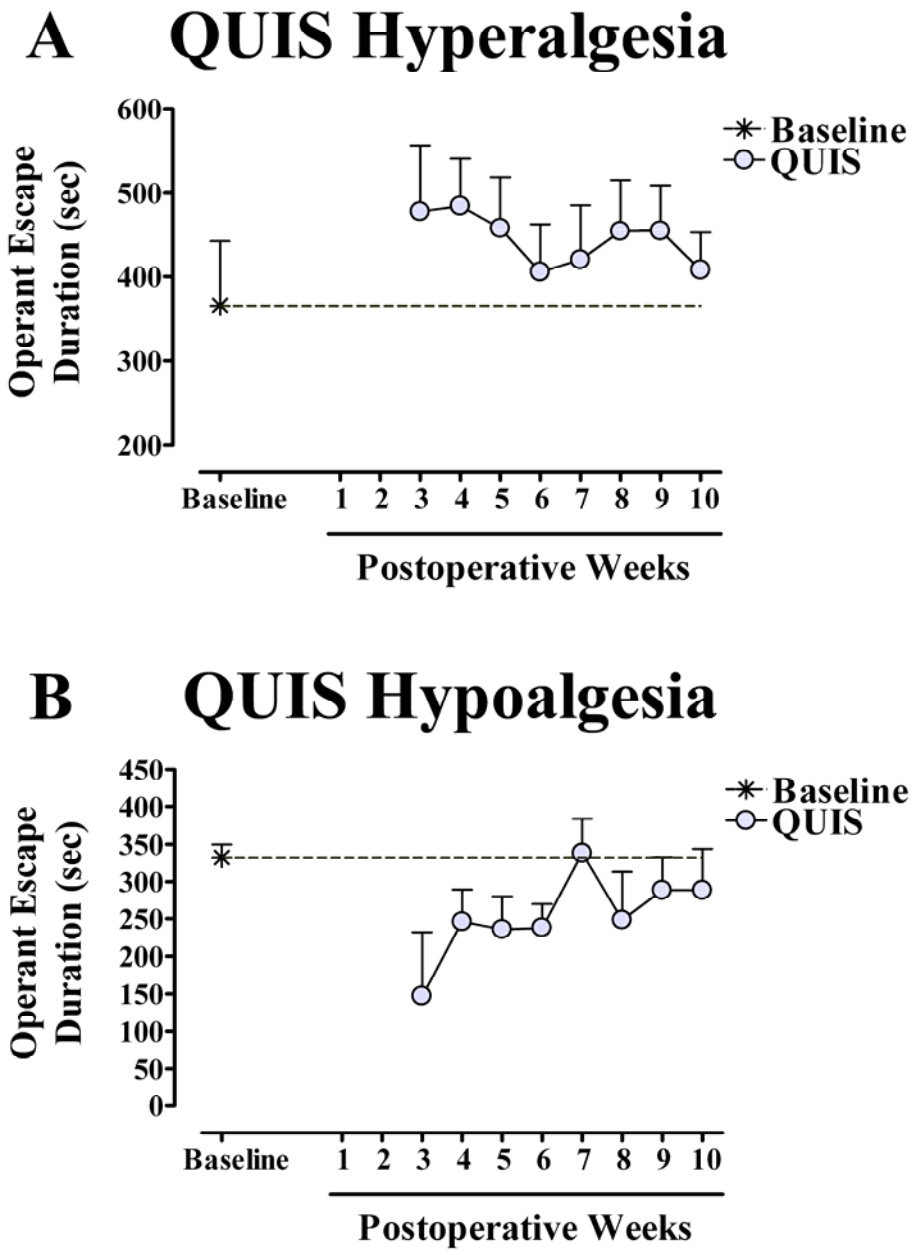


Figure 4-8. Weekly postoperative platform responses across several weeks of testing during testing trials at 44.5°C before (baseline, asterisk) and after (QUIS, gray circle) excitotoxic injury. Platform durations remained higher for the *hyperalgesic* group (A) and lower for the *hypoalgesic* group (B). Data are expressed in seconds and are represented as absolute group means ± S.E.M.

To examine if stress could enhance operant responses in spinally injured animals, animals were exposed restraint stress eight weeks after surgery, which was similar to the previous stress protocol in intact animals. Then, animals were placed into a pre-test for 15 minutes followed by a testing trial at 44.5°C. The effects of restraint stress were evaluated over three consecutive days including the day of stress (restraint stress), the following day (24 hours), and two days (48 hours) after restraint. In this initial analysis, animals were not subdivided into *hyperalgesic* or *hypoalgesic* groups.

In order to simplify the results from all animals that underwent escape testing, a summary of operant responding (escape counts; durations) after restraint stress is presented in Table 4-1. Restraint stress significantly reduced platform counts (F=7.347, P=0.007). On the following testing days, platform counts were not significantly lower, 24 (F=0.214, P=0.644) or 48 (F=0.789, P=0.376) hours after stress.

Platform escape durations were not significantly affected by restraint stress fifteen minutes (F=1.293, P=0.257), 24 hours (F=0.628, P=0.429), and 48 hours (F=0.002, P=0.967). Conversely, no significant differences were observed for plate durations fifteen minutes (F=1.284, P=0.258), 24 hours (F=1.284, P=0.258), and 48 hours (F=1.284, P=0.258) after stress.

Effects of Stress on Individual Groups

After extensive evaluation of behavioral responses, two types of behavioral responses after spinal injury emerged including animals displaying an increase or decrease to thermal stimulation. Based on these observations, analysis of stress-induced changes in operant responses was carried out on animals demonstrating an enhanced or a diminished response to thermal stimulation after QUIS. Based on the revised grouping,

these testing sessions were evaluated between *hyperalgesic* (increased responding after QUIS; left panel) and *hypoalgesic* (reduced responding after QUIS; right panel) groups.

Table 4-1. Number and duration of escape responses at 44.5°C before and during testing sessions in which animals were tested fifteen minutes (restraint stress), the following day (24 hours), and two days (48 hours) after stress. The data are expressed in seconds and are represented as absolute group means ± S.E.M.

	Count		***Thermal Plate Duration***		***Escape Platform Duration***	
QUIS	8.03 ± 0.29		554.12 ± 13.50		345.69 ± 13.50	
Restraint Stress	5.07 ± 1.17	$P<0.01$	494.35 ± 70.50	ns	404.67 ± 70.53	ns
24 Hours	7.53 ± 0.92	ns	514.15 ± 53.07	ns	385.53 ± 52.97	ns
48 Hours	7.07 ± 1.10	ns	556.24 ± 52.59	ns	343.60 ± 13.05	ns

Escape count in the hyperalgesic and hypoalgesic groups

The number of postoperative escape platform responses is illustrated in Figure 4-9 during sessions in which animals were tested after restraint stress (restraint stress; top panel) and one (24 hours; middle panel) or two days (48 hours; bottom panel) after stress. Restraint stress significantly reduced the number of platform responses in the *hyperalgesic* group (Figure 4-9A, $F=8.147$, $P=0.005$). However, the reduction of platform counts did not persist during sessions assessed 24 hours (Figure 4-9C, $F=0.287$, $P=0.593$) or 48 hours (Figure 4-9E, $F=0.395$, $P=0.531$) after stress.

In the *hypoalgesic* group, platform counts were not significantly different during sessions fifteen minutes (Figure 4-9B, restraint stress: $F=1.42$, $P=0.236$), 24 hours (Figure 4-9D, $F=0.018$, $P=0.894$), or 48 hours (Figure 4-9F, $F=0.354$, $P=0.553$) after stress.

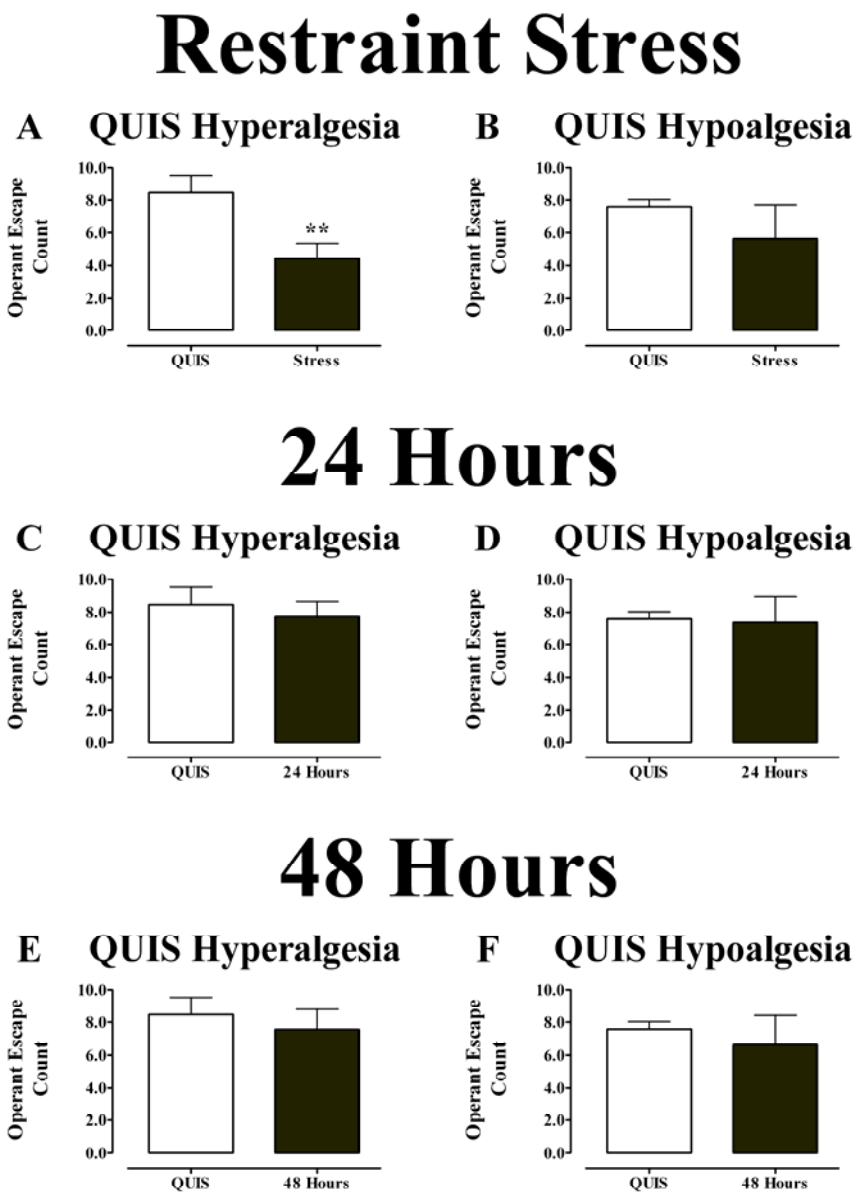


Figure 4-9. The number of escape platform responses at 44.5°C before (QUIS, open bar) and during testing sessions (closed bar) in which animals were tested fifteen minutes (restraint stress), the following day (24 hours), and two days (48 hours) after stress. Restraint stress decreased the number of platform responses for the hyperalgesic (A) and *hypoalgesic* (B) groups, but responses were only significantly reduced in the *hyperalgesic* group. In subsequent testing sessions, platform counts were comparable to pre-stress when assessed 24 (C, D) and 48 (E, F) hours after stress. Data are expressed in seconds and are represented as absolute group means ± S.E.M. Significant differences between baseline and QUIS testing periods are indicated by: ** $P<0.01$.

Escape durations in the *hyperalgesic* group

In Figure 4-10, the effects of restraint stress on the duration of plate and platform responses are compared during testing sessions, which animals were tested fifteen minutes (restraint stress), the following day (24 hours), and two days (48 hours) after stress. After excitotoxic injury, postoperative platform durations during 44.5°C trials for the *hyperalgesic* group were affected by stress. Restraint stress significantly increased platform durations (Figure 4-10A, F=5.981, P=0.016). Conversely, plate durations were lower after stress (data not shown; F=5.96, P=0.016).

Subsequent testing sessions that assessed the following day (24 hours) and two days (48 hours) after restraint revealed similar stress effect. For the *hyperalgesic* group, platform durations remained higher but were not significant at 24 (Figure 4-10B, F=2.765, P=0.099) or 48 (Figure 4-10C, F=2.765, P=0.099) hours. In addition, plate durations were not significantly lower during these sessions (24 hours, F=3.782, P=0.055; 48 hours, F=3.756, P=0.055).

Escape duration in the *hypoalgesic* group

In Figure 4-11, the effects of restraint stress on the duration of plate and platform responses were evaluated during testing sessions. Animals were tested fifteen minutes (restraint stress), the following day (24 hours), and two days (48 hours) after stress. Unlike the *hyperalgesic* group, stress failed to alter platform (Figure 4-11A, F=0.027, P=0.87) or plate (data not shown; F=0.027, P=0.87) durations during 44.5°C trials for the *hypoalgesic* group. Escape durations were similar to pre-stress responses.

Responses were comparable when assessed the following days. Platform durations did not differ from pre-stress responses during sessions 24 (Figure 4-11B, F=0.091,

P=0.764) or 48 (Figure 4-11C, *F*=1.656, *P*=0.201) hours after stress. Similar to platform responses, plate durations did not change across sessions 24 (data not shown; *F*=0.090, *P*=0.765) or 48 (data not shown; *F*=1.661, *P*=0.2) hours after stress.

Additional evaluation of escape responses

As mentioned previously, no effects were revealed following excitotoxic injury when evaluations included the entire group. Attempts were made to further characterize the response profile of these animals, which are discussed later in this chapter (e.g., histology, open field responses). However, no variables were able to further differentiate escape responses following injury. As a result, animals were characterized by changes in their escape responses. Animals showed either an enhanced (*hyperalgesic* group: increased duration; decreased latency: F=4.908, P=0.028) or reduced (*hypoalgesic* group: decreased duration; increased latency: F=6.564, P=0.011) thermal sensitivity following injury. In addition, restraint stress decreased and increased escape latencies in the *hyperalgesic* (F=1.123, P=0.292) *and hypoalgesic* groups (*F*=4.609, *P*=0.034), respectively. Latencies were not shown, but are presented here for comparison purposes.

Therefore, excitotoxic injury increased thermal sensitivity in some animals, which was also characterized by changes following restraint stress and during thermal stimulation. Stress significantly affected the hyperalgesic group but not the hypoalgesic group. Finally, changes in temperature regulation were more pronounced in the *hypoalgesia* group.

While this differentiation may appear artificial, further statistical analysis supports the subdivision of animals. Based on the Shapiro-Wilk analysis, test for normality revealed a nearly unequal distribution of escape durations (*P*=0.059) and latencies (*P*=0.06). However, a relationship exists between postoperative responses and other

conditions: responses following restraint stress and during baseline (preoperative) sessions. Further analysis revealed a significant association between postoperative and stress sessions (Figure 4-12) when assessed by escape latency (Pearson: r = 0.696, *P*=0.004) but not escape duration (r = 0.405, *P*=0.135).

In addition, evaluations of skin temperature in anesthetized rats (chapter 5) revealed additional association between postoperative escape responding and vasoconstriction (see Figure 5-4) when assessed after immediately (15 minute period; r = 0.5, *P*=0.05) or five minutes (20 minute period; r = 0.542, *P*=0.037) after the termination of 44.5°C (thermal stimulus).

Effects of Excitotoxic Spinal Cord Injury on Thermal Preference

In addition to operant escape, the effects of excitotoxic lesions (QUIS) on thermal preference (n=12) responses were evaluated preoperatively and postoperatively after the third week and continued over a 10-week period. Responses were obtained from a separate group of animals. The thermal preference test required an animal to decide between two thermal plates at 15.0 (cold) and 45.0°C (heat). Therefore, an increase in time spent in one thermal modality versus another indicates a change in sensitivity.

Thermal Preference Counts

The number of thermal preference responses (crossings between compartments) was not affected by QUIS (Figure 4-13). Following spinal injury, the number of preference responses did not change compared to preoperative responses. Thus, as with operant escape counts, no differences were seen with the number of responses before and after QUIS. While this may indicate the spinal injury did not produce altered thermal sensations, other outcome measures showed the effect of injury more reliably.

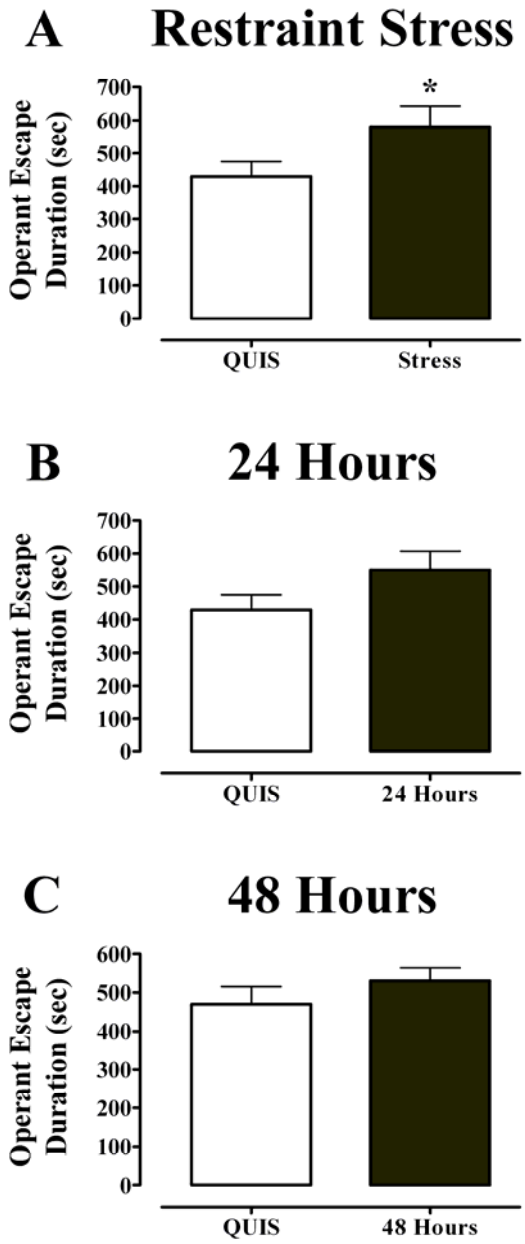


Figure 4-10. Cumulative escape platform responses at 44.5°C before (QUIS, open bar) and during testing sessions (closed bar) in animals tested fifteen minutes (restraint stress), the following day (24 hours), and two days (48 hours) after stress for the *hyperalgesic* group. Restraint significantly increased platform durations (A). While these effects were still present, platform durations were not significantly different from QUIS during sessions 24 hours (B) or 48 (C) hours after stress. Data are expressed in seconds and are represented as absolute group means ± S.E.M. Significant differences between baseline and QUIS testing periods are indicated by: * $P<0.05$.

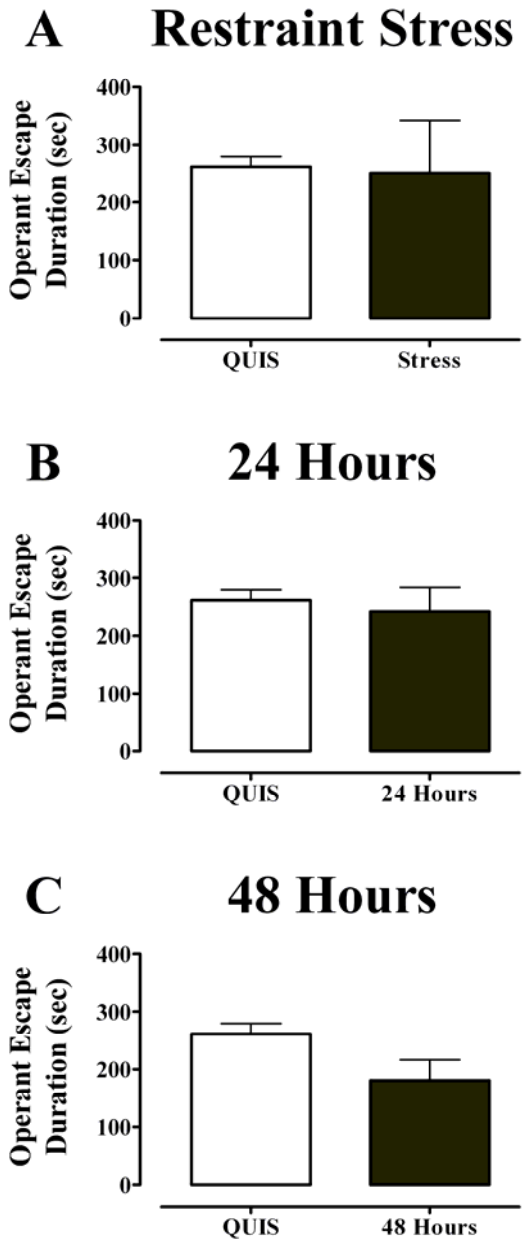


Figure 4-11. Cumulative escape platform responses at 44.5°C before (QUIS, open bar) and during testing sessions (closed bar) in animals tested fifteen minutes (restraint stress), the following day (24 hours), and two days (48 hours) after stress for the *hypoalgesic* group. Platform durations were not affected by restraint stress (A) and during sessions 24 hours (B) or 48 (C) hours later. Data are expressed in seconds and are represented as absolute group means ± S.E.M.

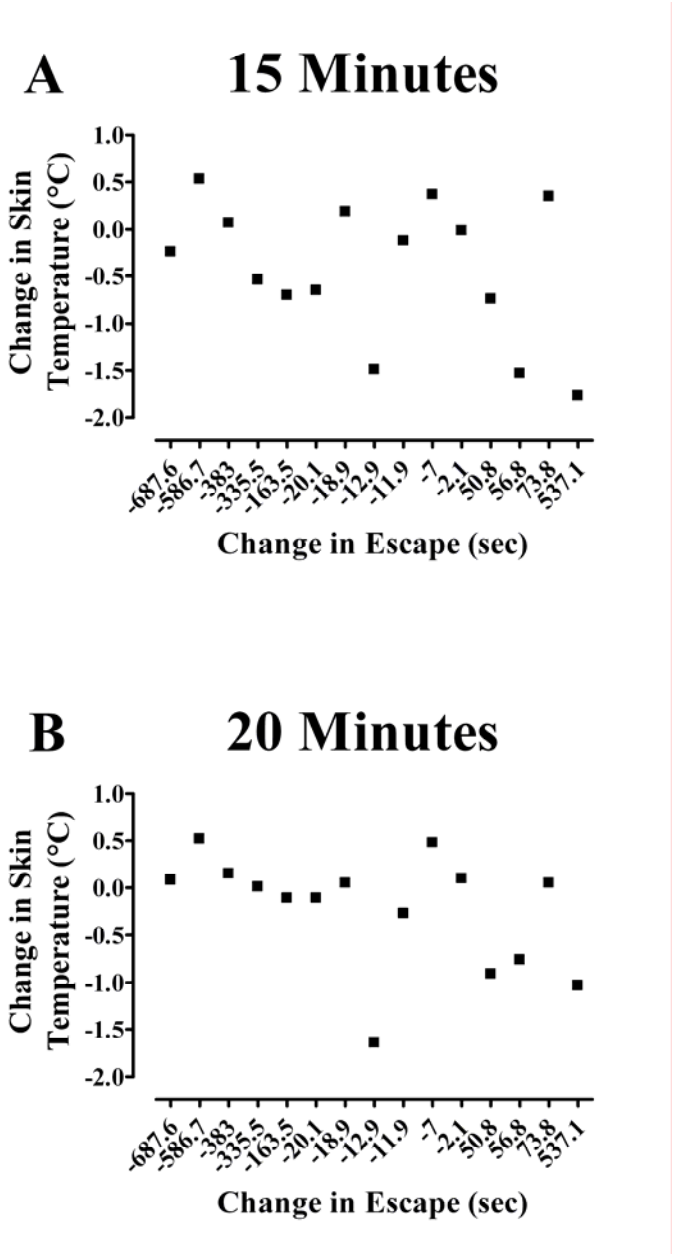


Figure 4-12. Correlation between postoperative responses following QUIS and change in skin temperature regulation during sessions following thermal stimulation of the left hindpaw. Data are expressed in seconds and are represented as absolute group means.

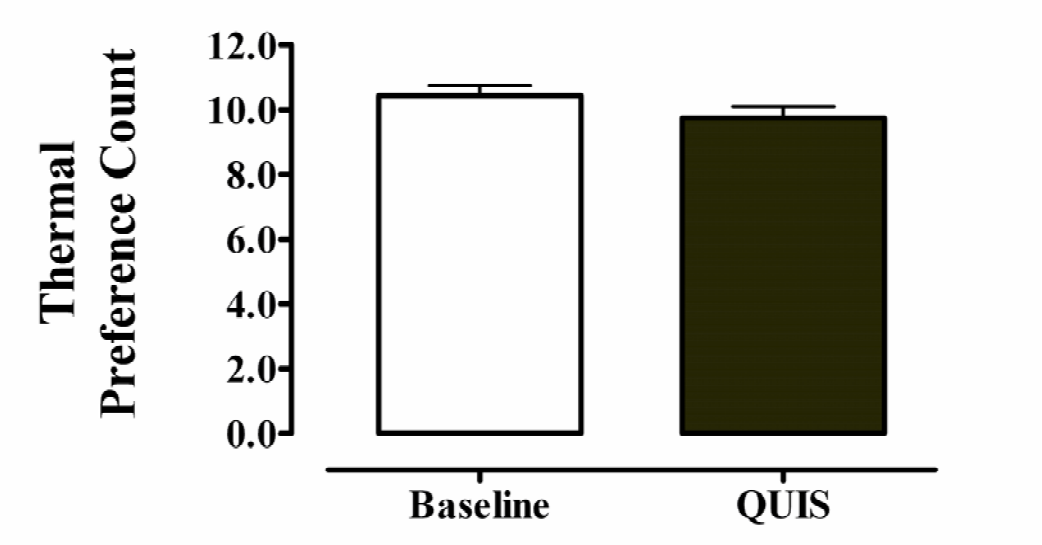


Figure 4-13. The number of thermal preference responses during testing trials at 15.0-45.0°C before (baseline, open bar) and after (QUIS, closed bar) excitotoxic injury. Thermal preference did not differ after QUIS. Data are expressed in seconds and are represented as absolute group means ± S.E.M.

Thermal Preference Durations

As shown in Figure 4-14, despite the number of responses being unaffected, QUIS had an impact on the total time spent on the cold versus heat plates. QUIS increased the time spent on the cold plate. Increased cold preference was significantly higher compared to baseline (Figure 4-14A; F=6.754, P=0.01). Conversely, QUIS reduced the duration spent on the heated plate, which was significantly different from baseline (Figure 4-14B; F=6.67, P=0.01). Thus, an increase in cold preference and decrease in heat preference indicates that spinal injury produced a heightened sensitivity to heat.

Sequence Analysis of Successive Thermal Preference Durations

To further examine the effect of QUIS on the thermal preference test, successive cold and heat durations are presented in Figure 4-15. In general, cold and heat preference responses peaked around the 4th and 5th responses.

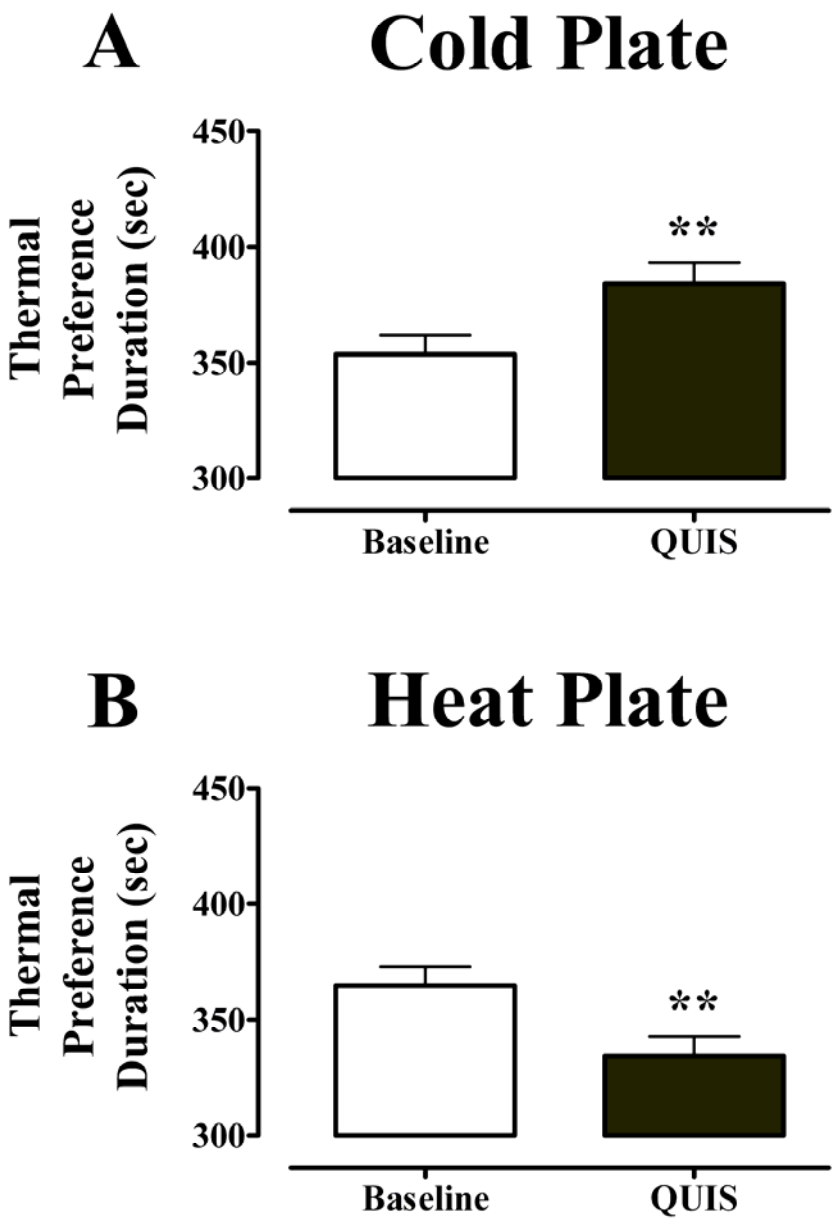


Figure 4-14. Cumulative durations of thermal preference responses during testing trials at 15.0-45.0°C before (baseline, open bar) and after (QUIS, closed bar) excitotoxic injury. (A) Cold preference was significantly higher while (B) heat preference was significantly lower after QUIS compared to baseline responses indicating a increase sensitivity to heat stimulation. Data are expressed in seconds and are represented as absolute group means ± S.E.M. Significant differences between preoperative and postoperative periods for cold and heat preference are indicated by: $^{**}P<0.01$.

As indicated by the cumulative preference durations, cold preference (Figure 4-15A) was initially higher, particularly in the beginning of the testing trial, after QUIS compared to baseline responses. On the other hand, QUIS lowered heat preference slightly compared to baseline values (Figure 4-15B). Figure 4-16 summarizes the average duration of the first six cold and heat preference responses that are compared between baseline (open bar) and QUIS (closed bar). QUIS did not significantly affect the average duration for cold (Figure 4-16A, F=1.025, P=0335) or heat (Figure 4-16B, F=0.010, P=0.921).

Weekly Assessment of Thermal Preference Durations

Assessment of weekly postoperative thermal preference responses is shown before (asterisk) and after QUIS (gray circle; Figure 4-17). Similar to animals in the escape paradigm, postoperative assessment of thermal preference responses was initiated three weeks after an injection of QUIS. Cold and heat preference progressively changed over time. The trend continued and was most evident at the 9th and 10th week postoperatively. Statistical analysis revealed that preference for cold (Figure 4-17A, F=2.225, P=0.025) and heat (Figure 4-17B, F=2.225, P=0.025) were significantly affected by QUIS across weekly testing sessions.

Effects of Restraint Stress on Thermal Preference Following Excitotoxic Injury

To examine if stress could enhance thermal preference responses in spinally injured animals, animals were exposed to acute restraint stress eight weeks after surgery, which was similar to the previous stress protocol in intact animals. Then, animals were placed into a pre-test for 15 minutes followed by testing trial at 15.0 and 45.0°C. Postoperative responses were examined over three consecutive days.

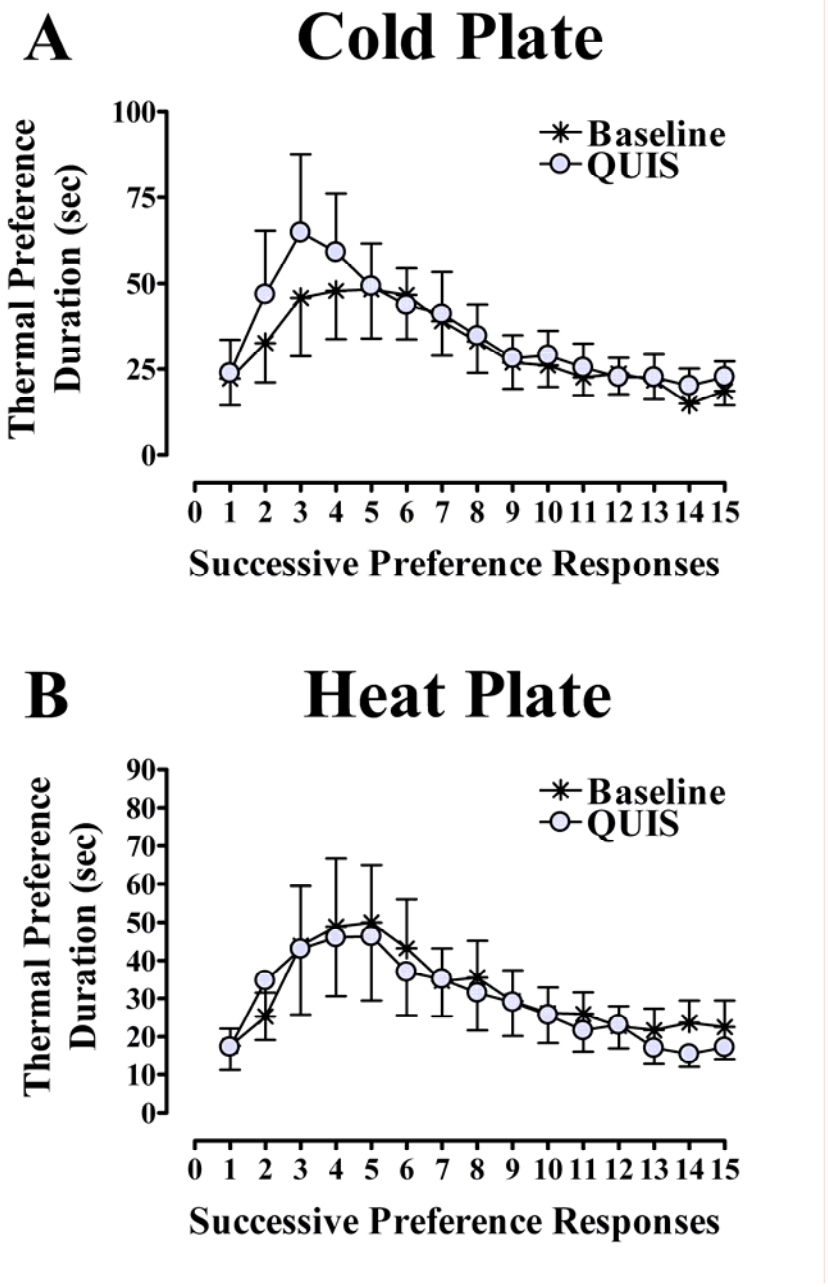


Figure 4-15. Sequence analysis of successive cold and heat preference durations during testing trials at 15.0-45.0°C before (baseline: asterisk) and after (QUIS: gray circle) excitotoxic injury. (A) Cold preference was slightly higher after QUIS throughout the trial especially at the beginning. (B) Heat preference was slightly decreased over successive response. Data are expressed in seconds and are represented as absolute group means ± S.E.M.

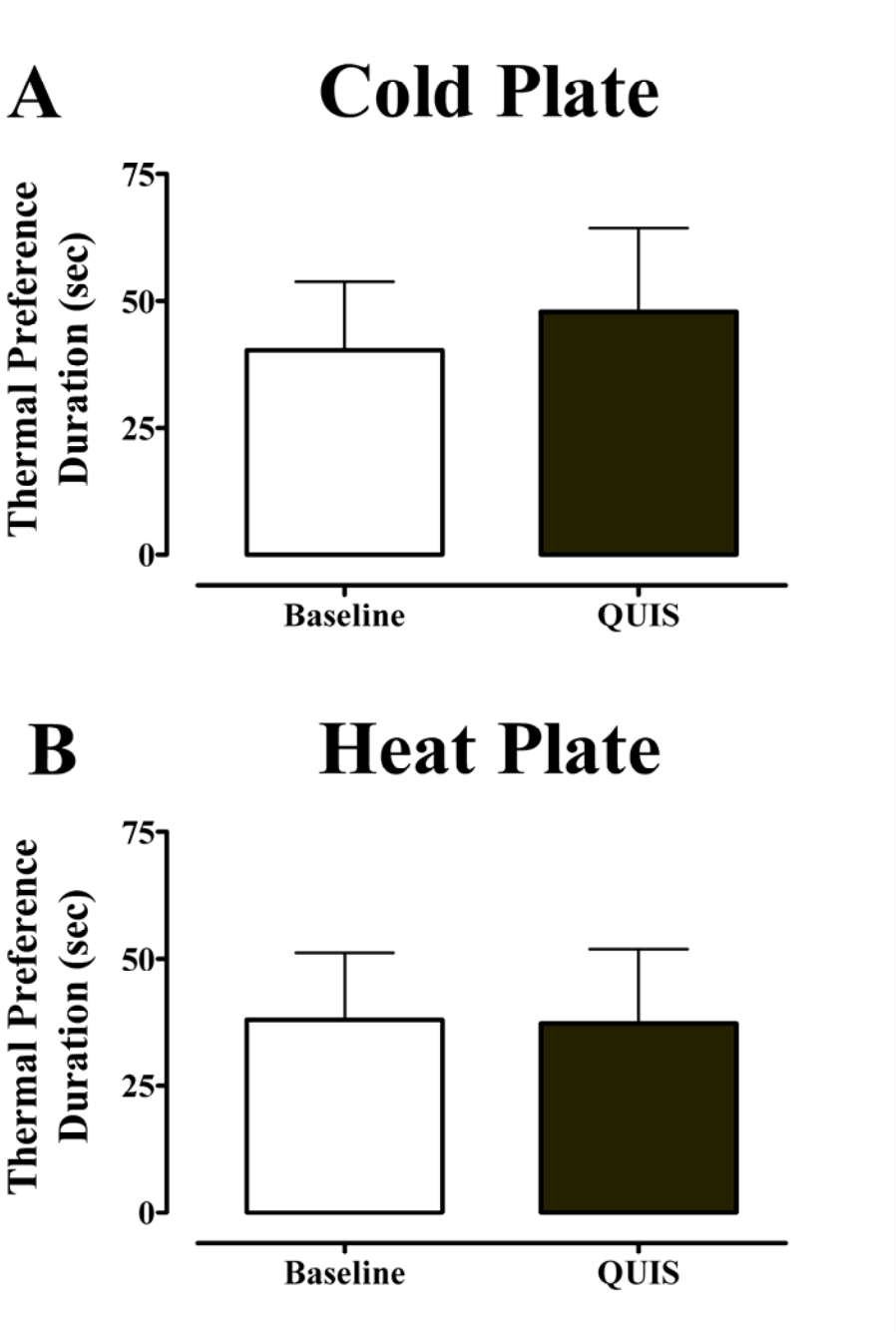


Figure 4-16. Average durations of the first six cold and heat preference responses during testing trials at 15.0-45.0°C before (baseline, open bar) and after (QUIS, closed bar) excitotoxic injury. Cold (A) and heat (B) preference was increased and decreased after spinal injury, respectively. However, the effects were not significantly different from baseline. Data are expressed in seconds and are represented as absolute group means ± S.E.M.

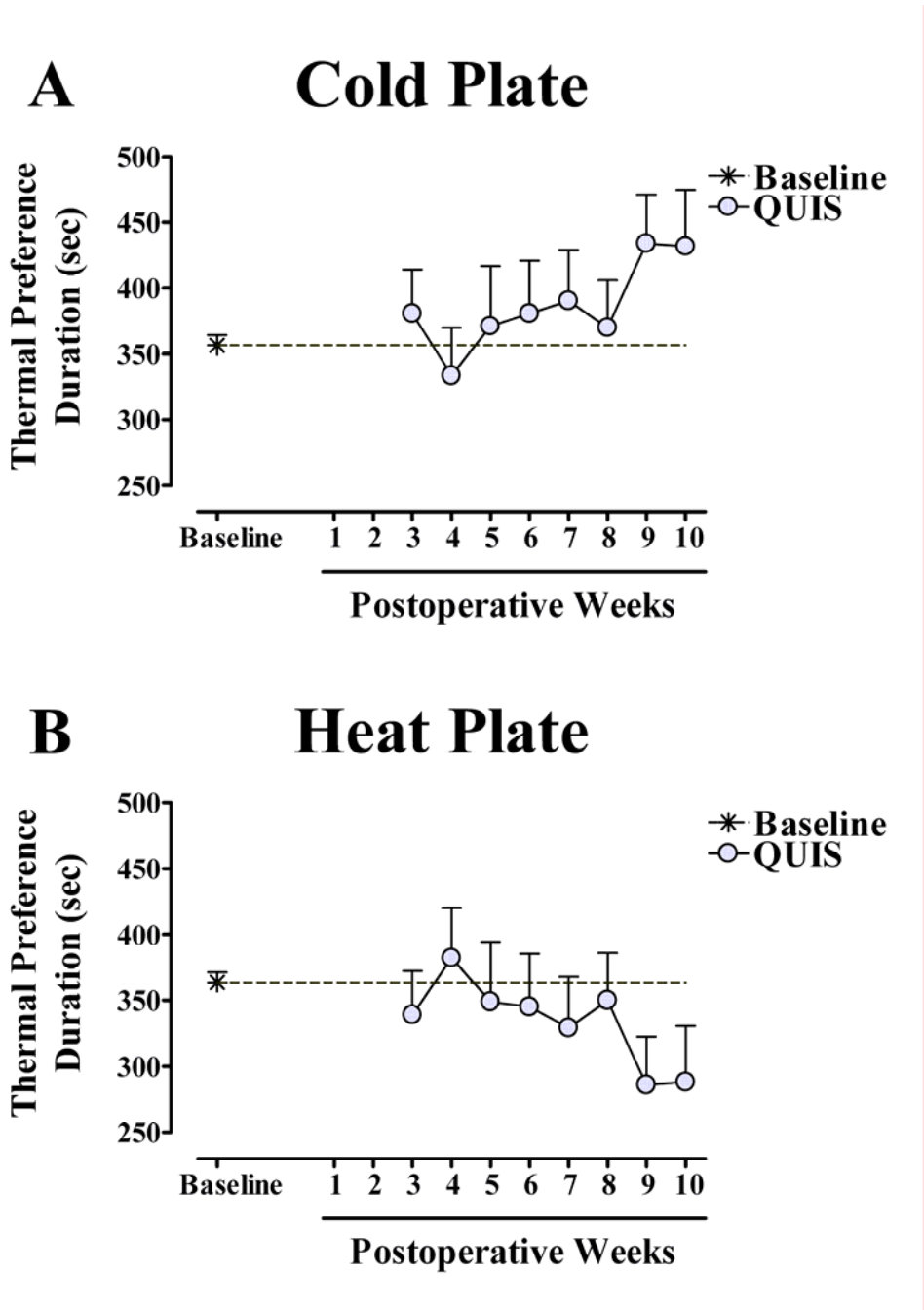


Figure 4-17. Weekly postoperative cold and heat preference responses across several weeks of testing during trials at 15.0-45.0°C before (baseline, asterisk) and after (QUIS, gray circle) excitotoxic injury. Data are expressed in seconds and are represented as absolute group means ± S.E.M.

Thermal Preference Counts

In Figure 4-18, the number of postoperative thermal preference responses before (QUIS) and after sessions in which animals were stressed and tested fifteen minutes (restraint stress), the following day (24 hours), and two days (48 hours) are presented. Thermal preference counts were not significantly different fifteen (Figure 4-18A, F=1.230, P=0. 269), 24 hour (Figure 4-18B, F=0.198, P=0.657), and 48-hour (Figure 4-18C, F=0.013, P=0. .908) hours after stress.

Thermal Preference Durations

In Figure 4-19, the duration of cold and heat preference were compared during sessions before (QUIS) and fifteen minutes (restraint stress), the following day (24 hours), and two days (48 hours) after stress. Preference for cold (Figure 4-19A, F=5.381, P=0.021) and heat (Figure 4-19B, F=5.540, P=0.019) was significantly affected by restraint. Specifically, cold durations were longer and, heat durations were shorter after exposure to restraint stress.

During assessment of subsequent testing sessions, stress-induced changes in thermal preference responses remained significantly different the following day (24 hours), but not two days (48 hours) after restraint. Statistical analysis revealed that cold preference during sessions the following day was significantly different compared to pre-stress responses (Figure 4-19C, F=8.911, P=0.003), but sessions two days later was not significant (Figure 4-19E, F=0.475, P=0.491). Conversely, preference for heat 24 hours afterwards was significantly different compared to pre-stress responses (Figure 4-19D, F=9.213, P=0.003), but sessions two days later were not significant (Figure 4-19F, F=0.507, P=0.477). Thus, restraint stress further enhanced injury-induced sensitivity to heat, which persisted the following day.

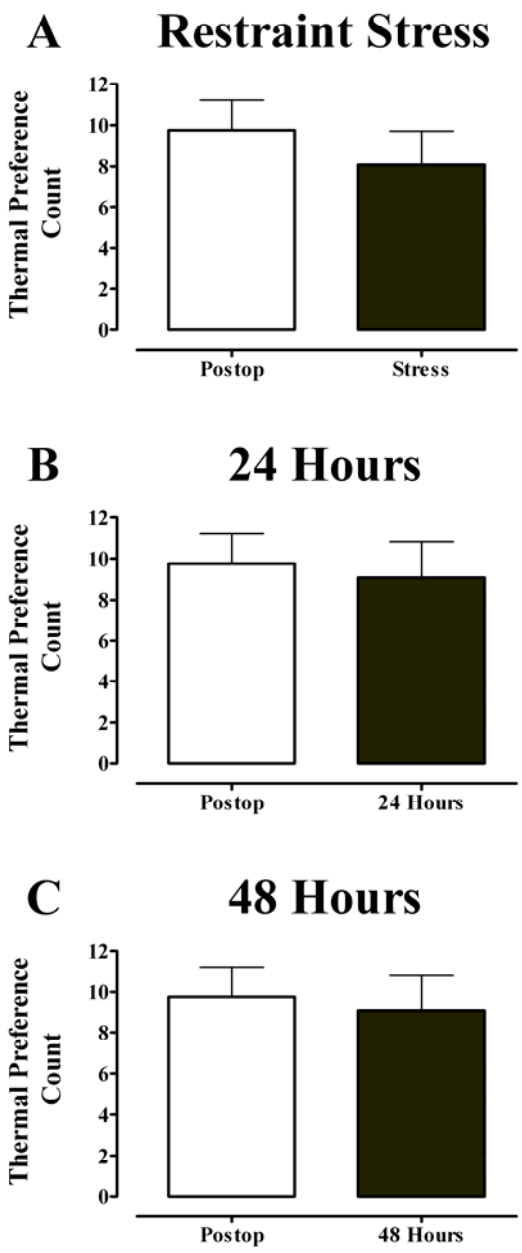


Figure 4-18. Number of thermal preference responses during testing trials at 15.0-45.0°C before (QUIS, open bar) and during testing sessions (closed bar) in which animals were tested fifteen minutes (restraint stress), the following day (24 hours), and two days (48 hours) after stress. Restraint stress did not significantly affect the number of responses 15 minutes (A), 24 hours (B), or 48 hours (C) afterwards. Data are expressed in seconds and are represented as absolute group means ± S.E.M.

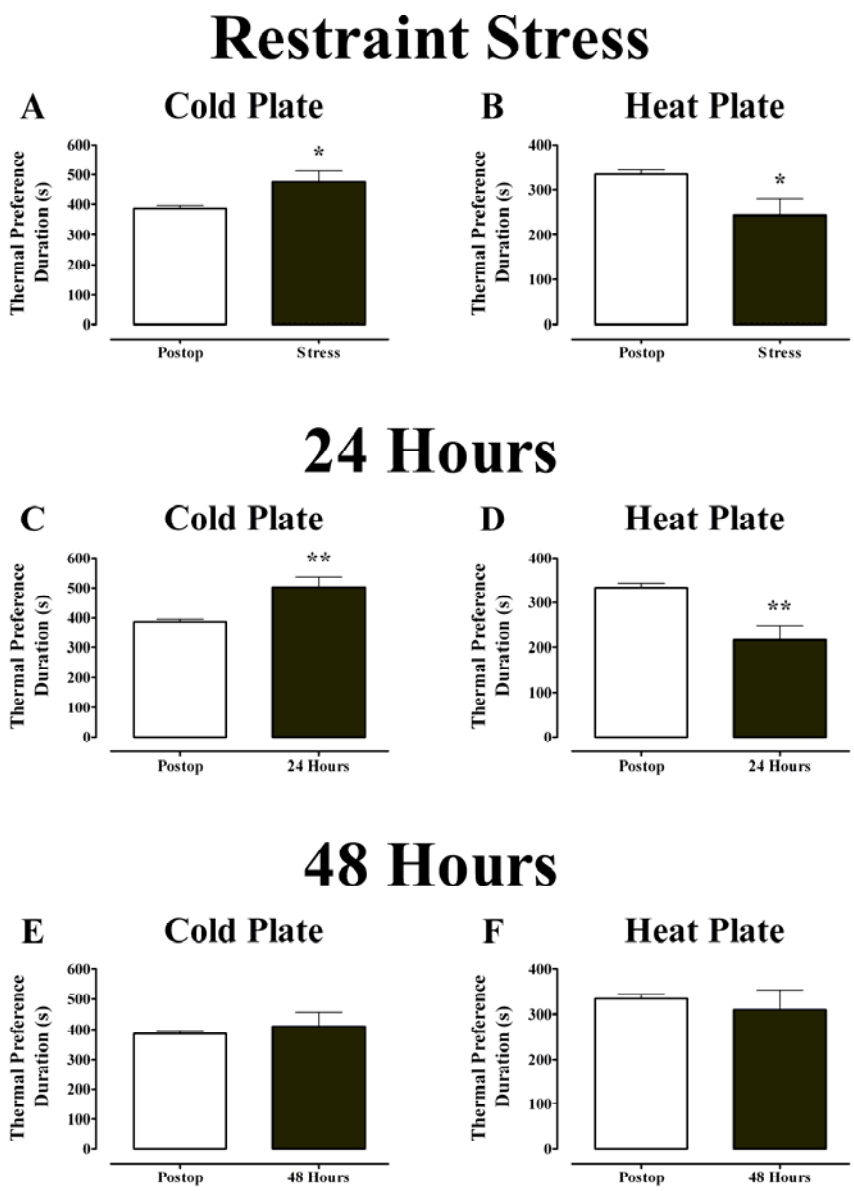


Figure 4-19. Cumulative cold (right panel) and heat (left panel) preference responses at 15.0-45.0°C before (QUIS, open bar) and during testing sessions (closed bar) in which animals were tested fifteen minutes (restraint stress), the following day (24 hours), and two days (48 hours) after stress. The duration of cold (A) and heat (B) preference were significantly higher and consequently lower after restraint stress indicating an increased sensitivity to heat. In subsequent testing sessions, preference remained higher for cold (C) and lower for heat (D) 24 hours after stress. However, preference for cold (E) and heat (F) were not different 48 hours after stress. Data are expressed in seconds and are represented as absolute group means ± S.E.M. Significant differences between baseline and QUIS testing periods are indicated by: * $P<0.05$ and ** $P<0.01$.

Prediction of Behavioral Responses Based on Open Field Responses

Another variable that could affect the expression of altered sensation is anxiety-like responses (assessed by the open field test). As mentioned previously, open field responses provide a measure of anxiety-like responses and determine stress responsiveness (Fernandez et al., 2004; Van den Buuse et al., 2001), which may be a predictor of individual sensitivity to thermal stimulation after stress (Jorum, 1989) or injury (Kontinen et al., 1999; Vatine et al., 2000). For example, stress sensitive animals are characterized by lower exploration of the open field (Kabbaj et al., 2000). During some preliminary sessions, a relationship was revealed in groups that displayed stress-induced hyperalgesia and anxiety-like response (e.g., shorter duration, longer latency).

To determine if responses in the open field could differentiate the behavioral groups after injury, open field responses were used as covariate including counts, durations, and latencies (Table 4-2). However, open field responses did not reveal significant effects of excitotoxic injury on behavioral responses

Table 4-2. Effect of open field responses on operant responses for groups after excitotoxic injury.

	Open Field		
	Count	***Duration***	***Latency***
Escape	*P=0.232*	*P=0.597*	*P=0.430*
Thermal Preference	*P=0.809*	*P=0.984*	*P=0.849*
	Inner Field		
	Count	***Duration***	***Latency***
Escape	*P=0.877*	*P=0.631*	*P=0.441*
Thermal Preference	*P=0.650*	*P=0.768*	*P=0.812*

Possible reasons for the inability of open field-test to predict an injury or stress response is the time between assessment of anxiety responses and other conditions (injury, stress). From the time animals were tested in the open field, several months had passed before excitotoxic injury and restraint stress. During this time, animals were extensively, acclimated, and trained in the operant escape tests. Future studies could use the open field test several weeks before excitotoxic injury for evaluation of the relationship between open field results and behavioral response to stress and spinal injury.

Comparison between Normal and Spinally Injured Animals

The effects of stress on QUIS animals were compared to responses in normal, uninjured animals. Difference scores from pre-stress values were used to determine significant effects. These comparisons between groups are illustrated for the escape (Figure 4-20) and thermal preference (Figure 4-21) testing sessions.

Differences in Escape Duration

For the escape test, restraint decreased plate (15 minutes; Figure 4-20A) and increased platform (15 minutes; Figure 4-20B) durations compared to pre-stress levels. In the QUIS condition, durations were slightly larger than responses in the normal condition but were similar between groups (plate and platform: F=0.576, P=0.458).

The effects of stress, which were characterized by a decrease in plate time and an increase in platform time, were observed in subsequent testing sessions. Plate and platform durations remained lower and higher than the normal condition, respectively, during sessions 24 (plate and platform: F=6.437, P=0.021) and 48 (plate and platform: F=5.470, P=0.032) hours after stress. In contrast, normal animals demonstrated no carry-over effects, and returned to pre-stress values 24 hours after stress.

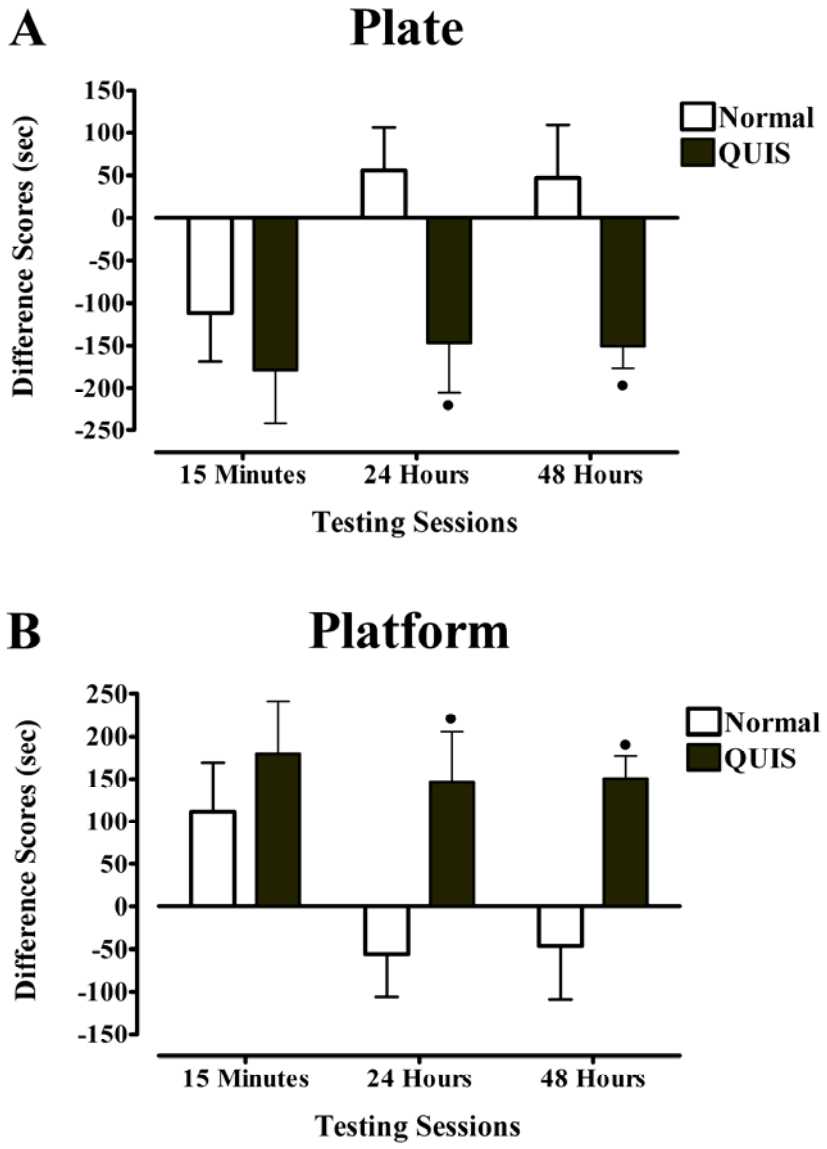


Figure 4-20. Difference scores for plate and platform durations during escape trials at 44.5°C in normal (open bar) and after excitotoxic injury (QUIS: closed bar) in which animals were tested for fifteen minutes (restraint stress), the following day (24 hours), and two days (48 hours) after stress. Restraint stress reduced plate (A) and increased platform (B) durations in both conditions. While the normal condition recovered 24 and 48 hours later (spending less time on the platform), the QUIS condition spent more time on the platform during the same time points. Data are expressed in seconds and are represented as difference scores from pre-stress values (means ± S.E.M). Significant differences between baseline and QUIS conditions are indicated by: • $P<0.05$.

Differences in Thermal Preference Duration

Restraint increased cold (15 minutes; Figure 4-21A) and decreased heat (15 minutes; Figure 4-21B) preference durations compared to pre-stress levels. In the QUIS condition, durations were slightly lower than responses in the normal condition but were similar between groups (cold and heat preference: F=0.118, P=0.732). In the normal condition, preference responses progressively decreased 24 and 48 hours after stress. In the QUIS condition, responses were significantly higher than the normal condition 24 hours (F=5.732, P=0.022) but only slightly higher 48 hours (F=0.024, P=0.876) after stress. Thus, carry-over effects were most prominent in spinally injured animals. These results show that stress further enhanced operant responding to low intensity thermal stimulation in spinally injured animals.

Histology

After the completion of behavioral testing, animals were euthanasized and purfused with PBS followed by 10% formalin. Spinal cords were removed and underwent further analysis by in vitro MRI microscopy (as mentioned in chapter 2). The use of this technique has been recently described (Berens et al., 2005). Images of spinal cord sections obtained from in vitro MRI confirmed the presence of pathological features (neuronal loss and cavitation) after QUIS. Intraspinal injection of QUIS has been shown to produce cavitation that is similar to conditions found in patients with syringomyelia (Berens et al., 2005; Yezierski, et al., 1993).

It has been shown that the release of excitatory amino acids (EAA) is responsible for development of excitotoxic injury and pain (Choi and Rothman, 1990; Yezierski, 2000, 2001). Based on histological data, excitotoxic injury resulted in pathology characterized in previous studies (Berens, et al., 2005; Yezierski, 2002).

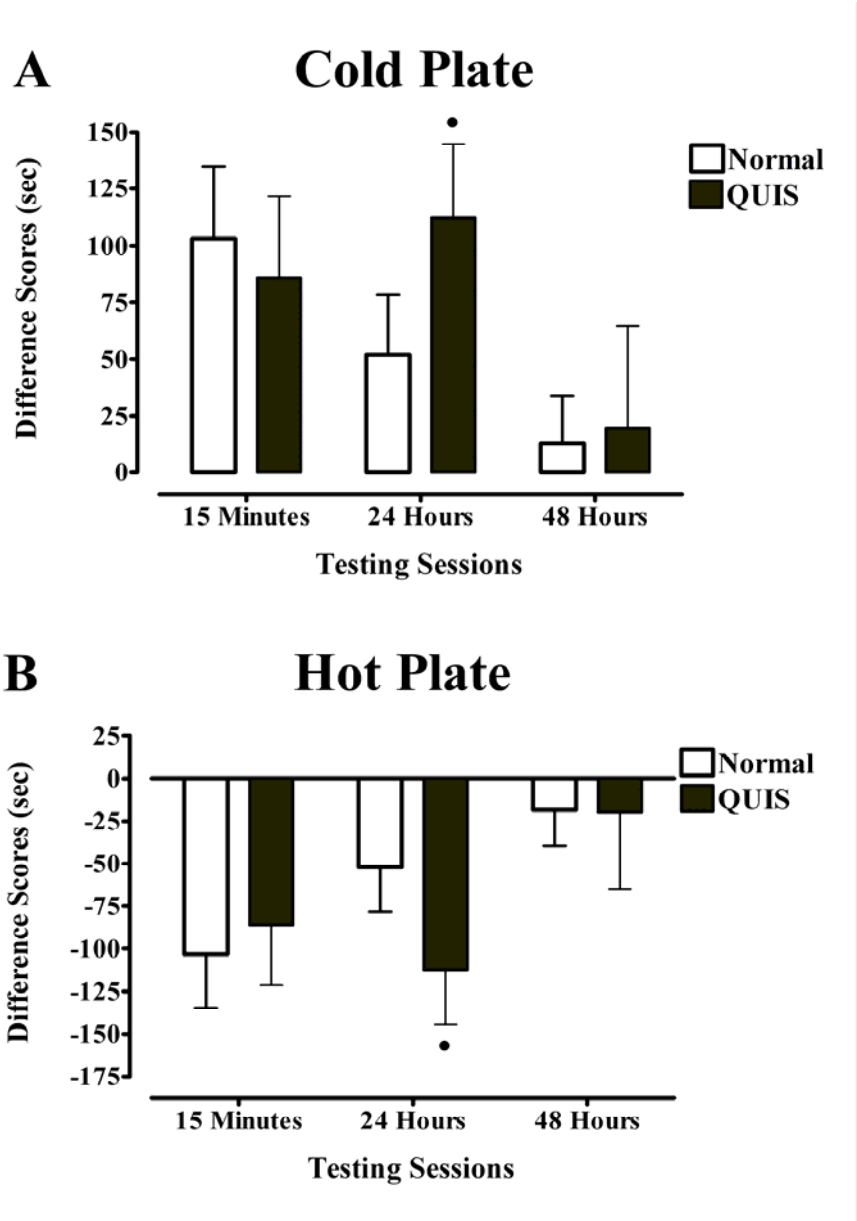


Figure 4-21. Difference scores for cold and heat preference durations during trial at 15.0-45.0°C in normal (open bar) and after excitotoxic injury (QUIS: closed bar) in which animals were tested for fifteen minutes (restraint stress), the following day (24 hours), and two days (48 hours) after stress. Similar to escape animals, restraint stress increased preference for cold (A) and reduced preference for heat (B) in both conditions. While the normal condition recovered 24 and 48 hours later (spending more time on the heated plate), the QUIS condition spent more time in the cold compartment during the same time points. Data are expressed in seconds and are represented as difference scores from pre-stress values (means ± S.E.M). Significant differences between baseline and QUIS conditions are indicated by: • $P < 0.05$.

In addition, in vitro MRI visualized the length and epicenter of damage within the spinal cord. Representative transverse images, which provide a better evaluation of neuronal loss, were assessed in spinal cord sections above and below level of excitotoxic injury (Figure 4-22).

Similar to findings from Berens et al (2005), white and gray matter are distinguishable. Two pathological features are observed in these images including evidence for hemorrhage (Figure 4-22; dark, hypotensive signal) and cavitation (Figure 4-22; light, hypertensive signal) particularly in the central canal. Based on these sections, spinal cords were evaluated for the presence of cavitations, the rostral-caudal extent of injury along the cord, degree of gray matter damage, and level of injury (Tables 4-3 and 4-4).

To illustrate some features that occur after excitotoxic injury to the spinal; cord, representative images from three animals are presented in Figure 4-23. Gray matter damage varied among animals after excitotoxic injury, which was either localized to the dorsal horn (Figure 4-23A; section 2) or included the ventral horn (Figure 4-23B, C; section 2). In addition, the formation of cavities after injury was variable. Cavitation was not present in some animals (Figure 4-23A; section 2) but present in others (Figure 4-23B; light signal in section 2).

Finally, longitudinal extent of damage following injuries was also variable (ranging from 4 mm to 10 mm). Representation of injury lengths is illustrated in sagittal images in which injury length was small (Figure 4-23A), moderate (Figure 4-23B), or extensive (Figure 4-23C).

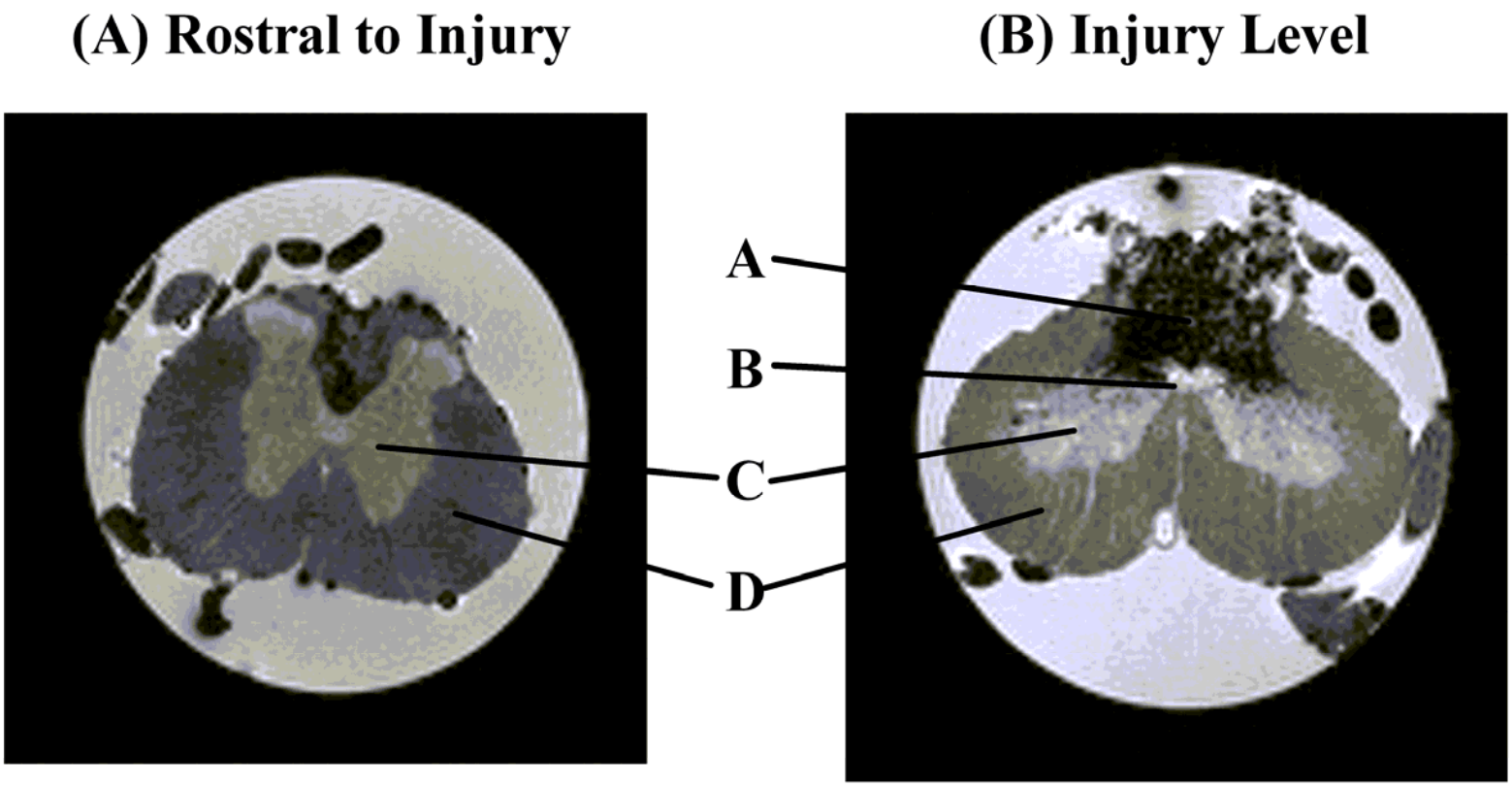


Figure 4-22. A comparison of *in vitro* MRI images in spinal cord section rostral (A) and at the level (B) of excitotoxic injury. Features of excitotoxic injury are identified as the following: A, hemorrhage; B, cavitation, C, gray matter; and D, white matter.

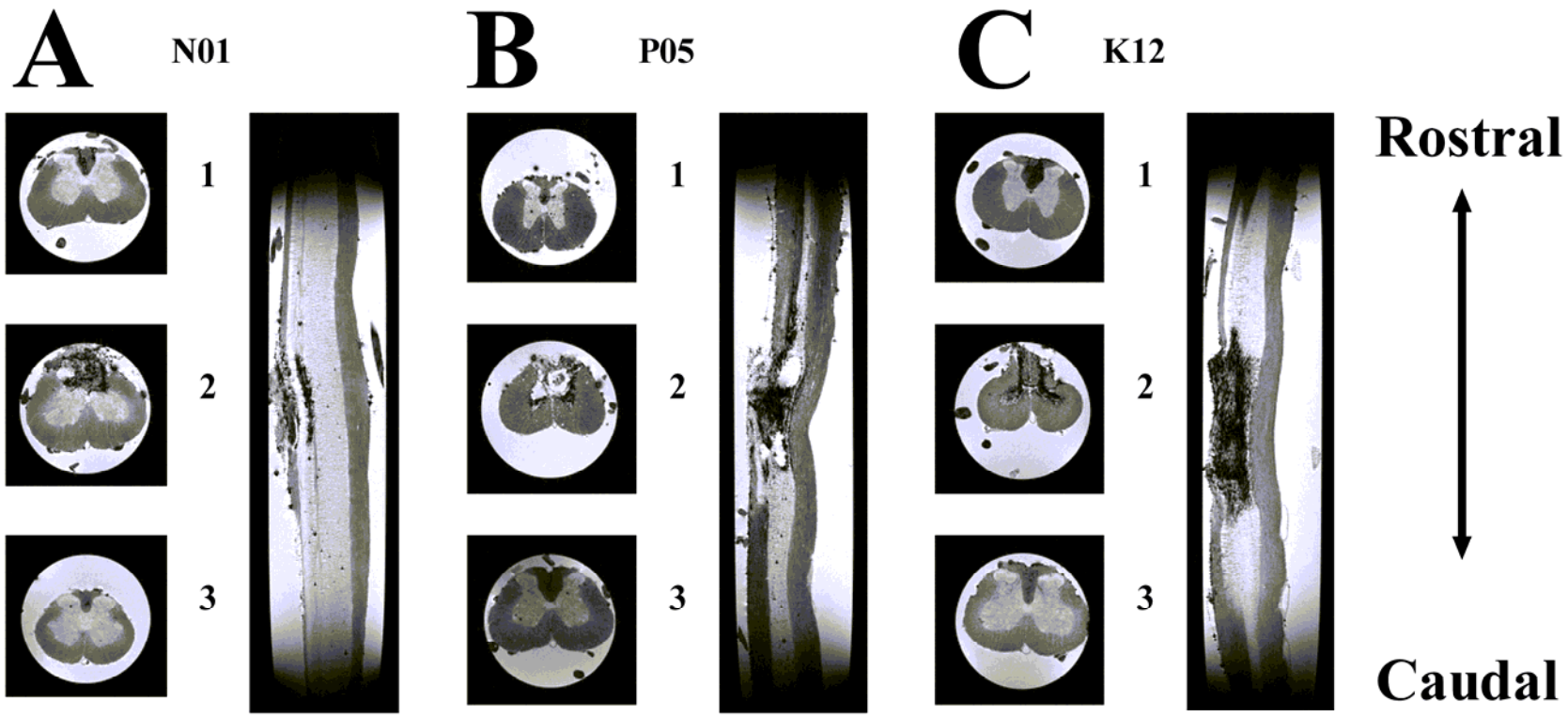


Figure 4-23. Summary of transverse and sagittal spinal cord images obtained through *in vitro* MRI after excitotoxic injury. Images are presented based on the rostral-caudal orientation, with rostral sections located at the top. Transverse sections are represented as follows: rostral (top image), injury level (middle image), and caudal (bottom image). Sagittal sections illustrate the length or rostral-caudal extent of injury.

In the present study, comparisons with behavioral responding were attempted. Tables 4-3 and 4-4 show the percentage of animals (each testing condition: escape, thermal preference) that exhibited the following pathology after excitotoxic injury: cavitation, longitudinal extent of damage, degree of gray matter damage, and level of injury. In regards to the frequency of cavitation after QUIS, groups differed. A majority (66.7%) of the animals in the escape group failed to develop cavities compared to a large number (41.7%) of thermal preference animals exhibiting an extensive amount of cavitation.

As for the extent of longitudinal damage, a majority of animals exhibited relatively moderate (4-7 mm) degree of damage along the spinal cord in both groups (escape: 60.0%; thermal preference: 41.0%). Interestingly, groups differed on the degree of gray matter damage. For the escape group, a majority of animals (40.0%) exhibited complete damage to the dorsal horn with some sparing of the ventral horn. However, the thermal preference group (58.3%) had extensive damage to the dorsal and ventral horn with involvement of the white matter. Finally, the level of injury varied between areas within the thoracic level to lower levels of the lumbar cord. A majority of animals in both groups possessed similar injury levels (escape: ~T_{13}; thermal preference: T_{11}-L_1).

To summarize the data in Tables 4-3 and 4-4, histological data was normalized into specific bins. For example, scores were assigned depending on the degree of cavitation (Table columns left to right: 1, cavitation; 2, minor expansion of the central canal; 3, major expansion of the central canal; 4, extensive cavitation within gray matter). Based on these values, averages were obtained and analyzed.

Table 4-3. Histological data for groups after excitotoxic injury that were behavioral assessed in the operant escape and thermal preference tests. Data are represented as percentage of animals within each condition.

Cavitation

	None	***Minor***	***Moderate***	***Severe***
Escape				
All Rats	66.7	13.3	13.3	6.7
Hyperalgesia	71.3	14.3	0	14.3
Hypoalgesia	62.5	12.5	25	0
Thermal Preference	16.7	25	16.7	41.7

Rostral-Caudal Extent of Injury

	4-6 mm	***6-7 mm***	***7-8 mm***	***8-9 mm***	***9-10 mm***	***>10 mm***
Escape						
All Rats	60	0	13.3	6.7	13.3	6.7
Hyperalgesia	71.4	14.3	0	0	14.3	0
Hypoalgesia	50	12.5	12.5	12.5	12.5	12.5
Thermal Preference	8.3	41.7	16.7	33.3	0	0

Table 4-4. Histological data for groups after excitotoxic injury that were behavioral assessed in the operant escape and thermal preference tests. Data are represented as percentage of animals within each condition.

Gray Matter Damage

	DH	***DH/VH (Minor)***	***DH/VH (Major)***	***Complete (Minor)***	***Complete (Major)***
Escape					
All Rats	13.3	13.3	40	20	13.3
Hyperalgesia	14.3	28.6	42.9	14.3	0
Hypoalgesia	12.5	0	37.5	25	25
Thermal Preference	0	16.7	8.3	16.7	58.3

Level of Injury

	T7-8	***T9-10***	***T11-T12***	***T13-L1***	***<L2***
Escape					
All Rats	26.7	0	20	46.7	6.7
Hyperalgesia	0	0	57.1	42.9	0
Hypoalgesia	50	25	12.5	12.5	0
Thermal Preference	16.7	25	33.3	16.7	8.3

Overall, cavitation was relatively minor in the escape group regardless of the subgroup (hyperalgesia ≈ hypoalgesia) but was extensive in the thermal preference group. The average length of damage along the longitudinal axis was 7-8 mm for both groups. Finally, the average level of injury for the escape and thermal preference group was located around T_{11}-T_{12} and T_{10}-T_{11}, respectively. In Table 4-5, operant responses were evaluated with the histological data as a covariate. Cavitation, length of injury, amount of gray matter damage, or level of injury did not influence behavioral responses.

Table 4-5. Effects of histological variables on operant escape and thermal preference responses after excitotoxic injury. A summary of histological data for groups after excitotoxic injury that was behaviorally assessed in the operant escape and thermal preference tests. Four pathological features (cavitation, rostral-caudal extent of damage, the amount of gray matter damage, and the level of injury) were used a covariate.

	Cavitation	***Rostral-Caudal Extent of Injury***	***Gray Matter Damage***	***Level of Injury***
Escape	*P=0.224*	*P=0.352*	*P=0.812*	*P=0.323*
Thermal Preference	*P=0.929*	*P=0.604*	*P=0.752*	*P=0.984*

Overall, histological data did not assist in explaining the behavioral responses from operant escape or thermal preference. One possible reason is that spinal cords were excised several months after excitotoxic injury and behavioral testing (4 months). In previous studies (Berens et al., 2005), damage to the gray matter increased over 30 days (end of study). In the current study, damage most likely progressed over time. It is possible that the relationship between operant responding and histological variables could not be established because analysis of these cords was several weeks after heightened sensitivity.

In order to address these issues in future studies; histological analysis should be collected during heightened sensitivity (e.g., within the first 10 weeks). A technique, *in*

vivo MRI, used in Berens et al. (2005) offers unique opportunity to examine the progression of injury over multiple time points in the same animal. While labor intensive, animals can undergo both image sessions during the assessment of their behavioral responses, which would provide a better opportunity to reveal a relationship between pathology and altered sensation. However, this option may not be feasible due to time restraints (imaging requires 3 hours per rat) and financial resources ($100 per hour; each session is $300 per rat). Therefore, spinal cords should be removed as mentioned in Chapter 2 within several weeks of heightened responding.

Also, it would be beneficial to examine the time course of damage over longer periods of time to determine the long-term progression regardless of behavioral responding. As mentioned previously, histological analysis of cords after QUIS has been limited to 30 days postoperatively (Berens et al., 2005). Furthermore, since the excitotoxic effect of EAA is critical for lesion progression and development of altered sensitivity after SCI, these effects could be minimized by administration of NMDA and AMPA antagonists during behavioral analysis as a possible treatment opposing injury-induced hyperalgesia (Choi and Rothman, 1990; Goda et al., 2002).

Summary

In pre-clinical models, behavioral consequences of excitotoxic SCI include allodynia and hyperalgesia to mechanical and thermal stimulation (enhancement of heat sensitivity). Depending on the operant task, injury-induced hyperalgesia is characterized by a reduction in the time spent on a heated plate. In the case of the operant escape test, increased platform durations were associated with reduction of thermal plate durations. Additionally, the thermal preference test revealed an increased cold preference and decreased heat preference. Thus, excitotoxic spinal cord injury produced an

injury-induced hyperalgesia to heat. Furthermore, the ability of stress to enhance operant responses (e.g. further increase in heat sensitivity) for a period of several days provides evidence that stress can enhance thermal sensitivity in animals that displayed an injury-induced hyperalgesia after spinal cord injury.

Several pathological features may contribute to heightened sensitivity to heat after damage to the gray matter For example, the longitudinal progression of neuronal loss from the epicenter (~4.0 mm) was found to be important in the development of at-level pain-like sensations (grooming; Vierck et al., 2000; Yezierski, 2000; Yezierski et al., 1998). But, based on the present histological data, no evidence was found to support this observation for the behavioral outcome measures used. All animals had injury that expanded from 4 to 10 mm. It is also possible that areas remote to the lesion epicenter may play a role in enhanced sensitivity. Increased blood flow in supraspinal structures, which are involved in processing of somatosensation (e.g., somatosensory cortex and thalamus), are observed after QUIS (Morrow et al., 2000; Paulson et al., 2005)

One interesting observation was made in animals tested in the operant escape test. Some animals exhibited an enhanced sensitivity to heat while a portion showed a reduction in sensitivity after excitotoxic injury. Possible explanations of these results include changes in the anxiety state, disruption of nociceptive pathways (ascending STT tract and descending modulatory pathways; Vierck and Light, 2000), and altered functioning of the endogenous opioid system. It has been shown that the opioid and anti-opioid (e.g., CCK) systems are activated after excitotoxic injury (Abraham et al., 2000, 2001).

Considering that injury-insensitive animals were identified in the escape test, it is important to acknowledge the difference between the two operant tests. While animals must decide between two nociceptive stimuli in the thermal preference test, the escape test presents only one nociceptive stimuli balanced by light. Under normal conditions, light is relatively unpleasant and an important factor in regulating responding. In fact, when the light is turned off, platform duration will significantly increase. It has been demonstrated that anxious animals (innate or due to stress) will avoid open, lighted areas (Fernandez et al., 2004). Anxiety can be evaluated in several tests like the open field (Van den Buuse et al., 2001). It is possible that the injury insensitive group spent less time on the platform due to an increased aversion to light. Therefore, assessment of motivational (darkbox) or anxiety (open field) would yield additional information regarding change in light sensitivity.

In other models of SCI, dysfunction of the opioid system is associated with hypersensitivity to thermal and mechanical stimulation (Abraham et al., 2000; Hao et al., 1998; Xu et al., 1994). For example, Xu et al (1994) reported that 50% of animals developed mechanical allodynia after ischemic SCI. It was speculated that the opioid system was dysfunctional in injury-sensitive animals but hyperactive in injury-insensitive animals. In confirmation of this hypothesis, the non- allodynic animals exhibited features of allodynia after administration of naloxone, an opioid receptor antagonist. Similar effects were reported after intrathecal injection of naloxone (Hao et al., 1998). Thus, in the current study, the endogenous opioid system was compromised in animals that develop abnormal sensitivity after SCI injury, but functional in animals with reduced sensitivity. This, in part, may explain the lack of stress-induced changes in the

injury-insensitive group. Future studies should use opioid antagonists such as naloxone to determine if the opioid system is overactive in animals that demonstrate a reduced sensitivity to heat.

A potential mechanism modulating pain sensation is cholecystokinin (CCK). Administration of a CCK antagonist (e.g., proglumide, L365,260) prevents morphine tolerance and enhances morphine anti-nociception (Heinricher et al., 2001; Millan, 2002; Stanfa, 1994; Valverde et al., 1994; Wiesnfield-Halilin et al., 1990). After injury, levels of CCK mRNA and peptides are elevated spinal and supraspinal structures involved in pain processing (Abraham et al., 2000, 2001; Ossipov et al., 1997; Vanderah et al., 2001a, 2001b). CCK, also, is implicated in mediating abnormal sensation after SCI (Brewer et al., 2003; Wiesenfeld-Hallin et al., 2002; Xu et al., 1994). For example, CCK mRNA levels were increased in the cortex and brainstem after excitotoxic injury. However, CCK was higher in animals that exhibited more spontaneous pain (grooming; Brewer et al., 2003). In another study, administration of a CCK_B antagonist reverses mechanical allodynia after ischemic SCI (Xu et al., 1994). Thus, up-regulation of CCK after SCI may suppress endogenous opioid activity that contributes to alterations in nociceptive responsiveness. The mechanisms may also contribute to the ineffectiveness of opioid therapy to treat SCI pain.

While no pre-clinical studies have examined the impact of stress on SCI pain, this is the first documentation that stress can augment sensitivity to heat assessed by cortically dependent responses. The ability of stress to increase sensitivity was similar between normal and injury groups. Unlike normal animals, the effects of stress persisted over several days. These results are comparable to clinical observations that stress can

exacerbate below-level pain (Ditor et al., 2003; Demirel et al., 1998; Galvin and Godfrey, 2001; Yezierski, 2002). Possible explanations for these observations include altered functioning of critical physiological system (autonomic nervous system; HPA), which have been identified on other pain conditions (Okifuji and Turk, 2002). The functioning of the autonomic system will be addressed in Chapter 5.

CHAPTER 5
EFFECT OF STRESS AND EXCITOTOXIC INJURY ON PERIPHERAL VASOCONSTRICTION

A possible mechanism mediating behavioral responses to heat is thermoregulation. Based on extensive literature, thermoregulation is regulated by the sympathetic nervous system (Willete et al., 1991). Various experimental manipulations alter sympathetic tone including stress, injury, and nociceptive stimulation (Janig, 1995; Herman and Cullinan, 1997; Magerl et al., 1996; McLachlan et al., 1992; Vierck, Unpublished Observations). One consequence of sympathetic activation is an increase in peripheral vasoconstriction resulting in cooling of the skin (described in Figure 5-1). A relationship exists between alterations in cutaneous temperature during thermal stimulation and enhanced sensitivity to thermal stimulation based on operant responses (Vierck, Unpublished Observations). Experimental manipulations (stress, formalin, excitotoxic injury) that affect an animal's sensitivity to mild nociceptive thermal stimuli can alter autonomic responses. Thus, if a manipulation attenuates peripheral vasoconstriction, the animal will lose the ability to counteract thermal stimulation. As a consequence, sensitivity to heat will increase as indicated by an increase in operant responses (hyperalgesia).

Recently, a method has been developed to assess peripheral vasoconstriction in rodents (Vierck, Unpublished Observations). Briefly, temperatures were recorded from the plantar surface of both forepaws and one hindpaw during sessions in which the remaining hindpaw is stimulated with low-intensity heat (44.5°C). During stimulation of

the hindpaw, temperatures of the non-stimulated paws diminished significantly within the first five minutes followed by a progressive increase in temperature.

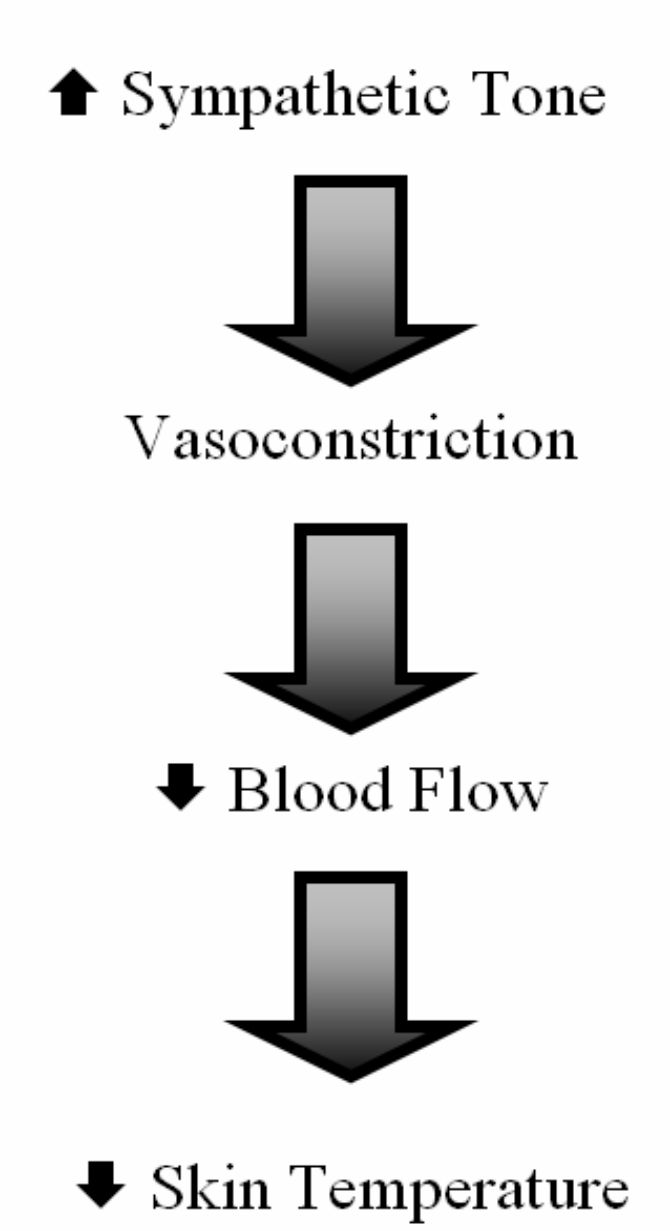


Figure 5-1. Reduction of skin temperatures by sympathetically mediated vasoconstriction.

To determine if altered cutaneous thermoregulation contributes to operant responding to heat, several groups of animals were tested during several sessions: stress only, QUIS only, and stress in spinally injured animals. Recordings were obtained during separate sessions: a) before and after stress in normal animals, b) before and after excitotoxic injury, and c) before and after stress in spinally injured animals. These manipulations can alter temperature regulation and subsequent responses to thermal simulation.

Effects of Restraint Stress on Peripheral Vasoconstriction

To determine the effects of stress on peripheral vasoconstriction, skin temperatures were recorded during baseline sessions and sessions in which animals were stressed for fifteen minutes (Figure 5-2). Skin temperatures during baseline and stress conditions were obtained during different sessions. After induction of anesthesia with isoflurane, skin temperatures were recorded exactly fifteen minutes after the termination of stress, corresponding to the behavioral paradigm used in Chapters 3 and 4. The hindpaw was stimulated for ten minutes with a temperature of 44.5°C.

As seen in figure 5-2A, skin temperatures during baseline conditions were lower during stimulation of the left hindpaw. Cooling of the skin suggests that stimulation increased sympathetic activity resulting in vasoconstriction (Figure 5-1). After the first 5 minutes of stimulation, non-stimulated paws demonstrated a progressive increase in temperature, which continued for the remainder of the recording period. On the other hand, exposure to restraint stress reduced the cooling of the non-stimulated paws. Despite a transient drop in temperature within the first three minutes of stimulation,

temperatures increased over time (e.g., warmer; vasodilatation) suggesting that a decrease in sympathetic activity.

To evaluate changes in peripheral thermoregulation before and after stress, temperatures were collapsed over five minute periods (Figure 5-2B). Skin temperatures during baseline sessions were significantly lower than the stress condition during stimulation (5 minutes: F=452.72, P<0.001; 10 minutes: F=562.81, P<0.001). At the termination of stimulation, baseline temperatures remained significantly cooler than the stress conditions (15 minutes: F=545.67, P<0.001; ten minutes: F=11219.25, P<0.001). Thus, the expression of stress-induced hyperalgesia is a consequence of blunted peripheral vasoconstriction to thermal stimulation. Behaviorally, stressed animals are sensitive to heat because they lack the ability to compensate for stimulation

Effects of Excitotoxic Injury on Peripheral Vasoconstriction

Operant Escape Test

Overall vasoconstriction after spinal injury

In the operant escape test, it was predicted that animals would show an enhanced sensitivity to heat following excitotoxic spinal injury. But, as a group, no hyperalgesia was observed. Skin temperatures were recorded before (baseline) and after excitotoxic injury in order to determine the effects of QUIS on peripheral vasoconstriction (Figure 5-3). Similar to Figure 5-2A, stimulation of the left hindpaw at 44.5°C reduced skin temperatures, which was followed by a progressive increase during the later stages of and in the absence stimulation (baseline; Figure 5-3A).

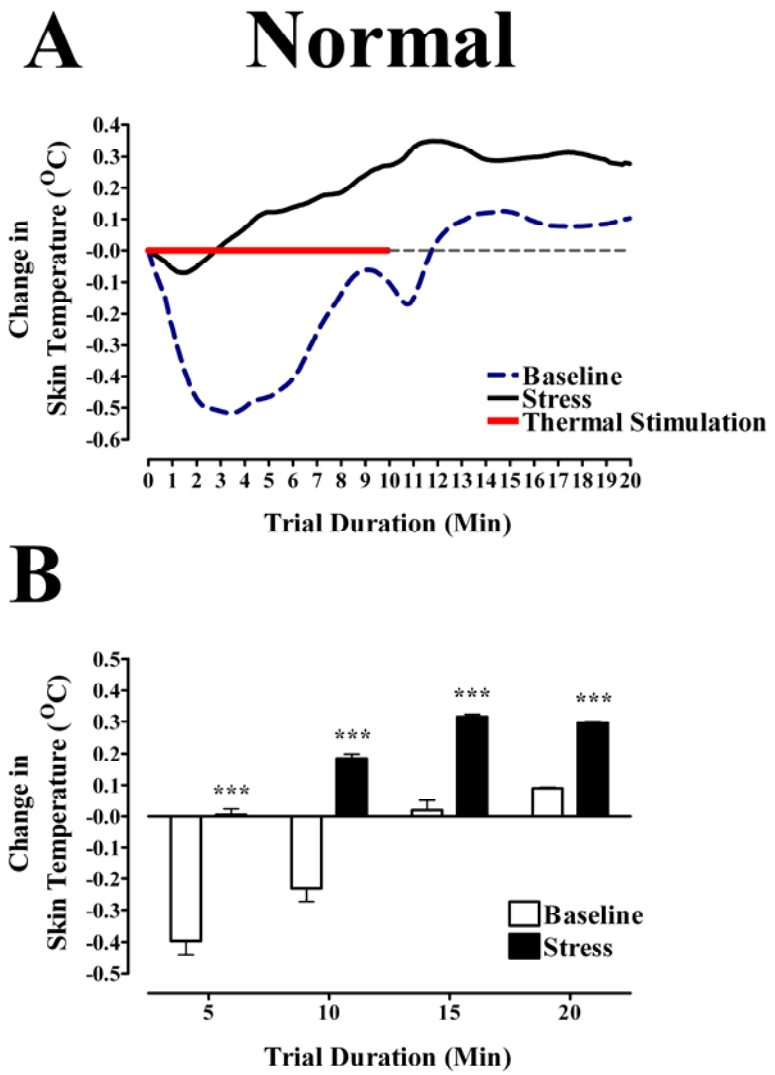


Figure 5-2. Skin temperature measurements from the plantar surface of non-stimulated paws during and after thermal stimulation of the left hindpaw at 44.5°C (thick red line) for 10 minutes for baseline (blue dotted line) and stress (black continuous line) conditions. (A) Baseline temperatures in non-stimulated paws were reduced during stimulation of the left hindpaw and returned to pre-stimulation levels at the end of stimulation. Restraint stress attenuated the cooling of paw temperatures (no reduction of temperature in non-stimulated paws). (B) Baseline recording were cooler than temperatures after stress. Restraint stress prevented the decrease in paw temperature. In addition, restraint stress increased skin temperatures after the first 5 minutes. Data are expressed as change from temperatures immediately prior to stimulation and subsequent temperatures during and after thermal stimulation. Significant differences between baseline and stress are indicated by: *** $P<0.001$.

Skin temperatures were not affected by excitotoxic injury (QUIS). In Figure 5-3B, skin temperatures were equally lower in both conditions during the first 5 minutes of stimulation (F=0.006, P=0.940) but recordings were significantly lower in the QUIS condition at 10 minutes (F=349.782, P<0.001). After stimulation ended, baseline temperatures rebounded while recordings in the QUIS group remained significantly cooler (15 minutes: F=317.619, P<0.001; 20 minutes: F=12512.69, P<0.001). Thus, QUIS failed to blunt peripheral vasoconstriction in this group, which can be a primary reason why injury-induced hyperalgesia was not observed in this group of animals. QUIS, on the other hand, appeared to prolong the cooling of paw temperatures even after termination of the stimulus.

Individual vasoconstriction after spinal injury

As a group, thermoregulation was not significantly affected by QUIS. But, two distinct groups were identified in Chapter 4 (QUIS *hyperalgesia* vs. QUIS *hypoalgesia*). These groups may have different sympathetic responses to thermal stimulation. Skin temperatures were further analyzed based on these categorizations (Figure 5-4). After excitotoxic injury, thermal stimulation of the left hindpaw lowered skin temperatures (e.g., cooling response) in the *hyperalgesic* (Figure 5-4A) and *hypoalgesic* (Figure 5-4B) groups. However, the *hyperalgesic* group showed a rebound (warming of non-stimulated paws) that paralleled a similar rebound in non-injured animals. The *hypoalgesic* group, on the other hand, did not recover.

Recordings were collapsed over 5-minute intervals for the *hyperalgesic* (Figure 5-4C) and *hypoalgesic* (Figure 5-4D) groups. During the first 5 minutes, skin temperatures were similar before and after QUIS for both groups (*hyperalgesic*: F=1.957, P=0.164; *hypoalgesic*: F=1.435, 0.233). But, temperatures between baseline and QUIS

conditions diverge after the first five minutes. Skin temperatures remained lower for the *hyperalgesic* group (10 minutes: F=576.720, P<0.001; 15 minutes: F=240.07, P<0.001; 20 minutes: F=5656.23, P<0.001). Similar responses were obtained for the *hypoalgesic* group at the end of stimulation. Temperatures were lower than baseline at 15 minutes (F=307.72, P<0.001), 20 minutes (F=21.092.7, P<0.001) but not during the earlier stages of stimulation (warmer vs. baseline at 10 minutes: F=108.58, P<0.001). The groups differed on the rates of recovery after stimulation was terminated. Skin temperatures in the *hyperalgesic* group recovered but cooler temperatures in the *hypoalgesic* group were prolonged even after cessation of stimulation.

Even after dividing animals into their respective groups, vasoconstriction remained active and was enhanced after excitotoxic injury. A difference was observed between the two groups. While temperatures of the *hyperalgesic* recovered, the *hypoalgesic* group failed to recover and remained cooler during the testing trial. Prolonged vasoconstriction may explain the observation that this group was less sensitive to heat (e.g., decreased platform duration; increased plate duration). The absence of an injury-induced hyperalgesia could be due to the inability of QUIS lesions to alter thermoregulation. Also, similar to histological analysis, evaluation of vasoconstriction was assessed several months after spinal injury. Thus, the effect of spinal injury on behavioral responses was no longer present.

Thermal Preference Test

In the thermal preference test, animals showed an enhanced sensitivity to heat (lower preference for heat; injury-induced hyperalgesia). To determine if QUIS altered thermoregulation, skin temperatures were recorded before (baseline) and after excitotoxic injury (Figure 5-5) similar to the escape group. While thermal stimulation of the left

hindpaw at 44.5°C reduced skin temperatures, excitotoxic injury (QUIS) diminished cooling of skin temperatures (Figure 5-5A). Recordings were higher during stimulation followed by a small drop at the end of stimulation (10 minutes).

In Figure 5-5B, baseline skin temperatures were significantly lower than recording in the QUIS conditions during the initial (5 minutes: F=91.148, P<0.001) but not the later period (10 minutes: F=3.588, P=0.06) of stimulation. At the end of stimulation, temperature in both conditions began to rebound in which baseline temperatures were significantly higher than QUIS conditions (15 minutes: F=435.18, P<0.001; 20 minutes: F=835.4, P<0.001). After excitotoxic injury, peripheral vasoconstriction was blunted in this group. The apparent consequence of altered functioning of the sympathetic system is the expression of injury-induced hyperalgesia.

Effects of Restraint Stress

Skin temperatures were recorded during sessions before and after restraint stress to determine the effects of stress on peripheral vasoconstriction after excitotoxic injury (Figure 5-6). Cooling of non-stimulated paws was significantly diminished by restraint stress (Figure 5-6A). Similar to normal conditions (Figure 5-2), recordings revealed a transient drop in temperature followed by an increase in temperature over time. After restraint stress (Figure 5-6B), temperatures were higher than pre-stress levels in QUIS animals during (5 minutes: F=196.883, P<0.001; 10 minutes: F=1123.85, P<0.001) and after (15 minutes: F=43.59, P<0.001; 20 minutes: F=68.427, P<0.001) thermal stimulation. Even after excitotoxic injury, altered peripheral vasoconstriction was affected by restraint stress. This supports behavioral data demonstrating that restraint enhances sensitivity to heat in spinally injured animals.

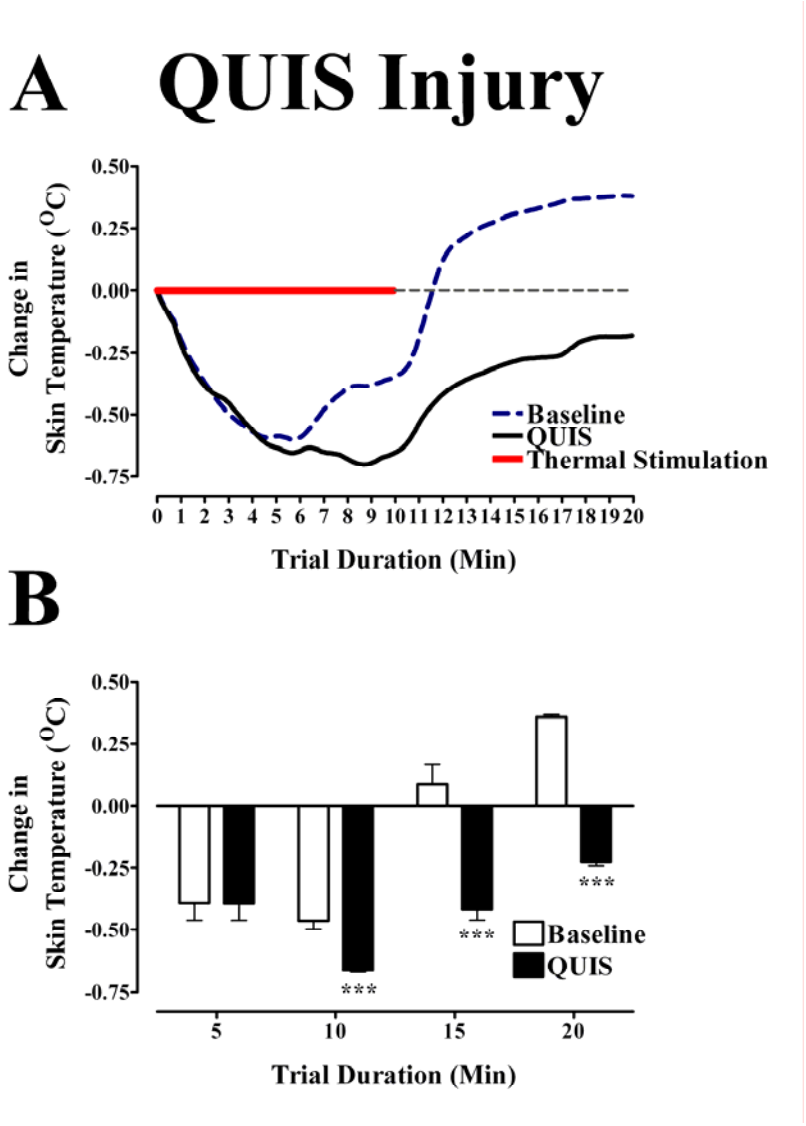


Figure 5-3. Skin temperature measurements from the plantar surface of non-stimulated paws during and after thermal stimulation of the left hindpaw at 44.5°C (thick red line) for 10 minutes before (baseline: blue dotted line) and after (QUIS: black continuous line) excitotoxic injury. (A) Baseline temperatures of non-stimulated paws were reduced during stimulation of the left hindpaw and returned to pre-stimulation levels at the end of stimulation. QUIS failed to attenuate the cooling of skin temperatures in the non-stimulated paws. (B) QUIS enhanced the cooling of paw temperatures, which continued after stimulation was terminated. Data are expressed as change from temperatures immediately prior to stimulation and subsequent temperatures during and after thermal stimulation. Significant differences between baseline and stress are indicated by: *** $P<0.001$.

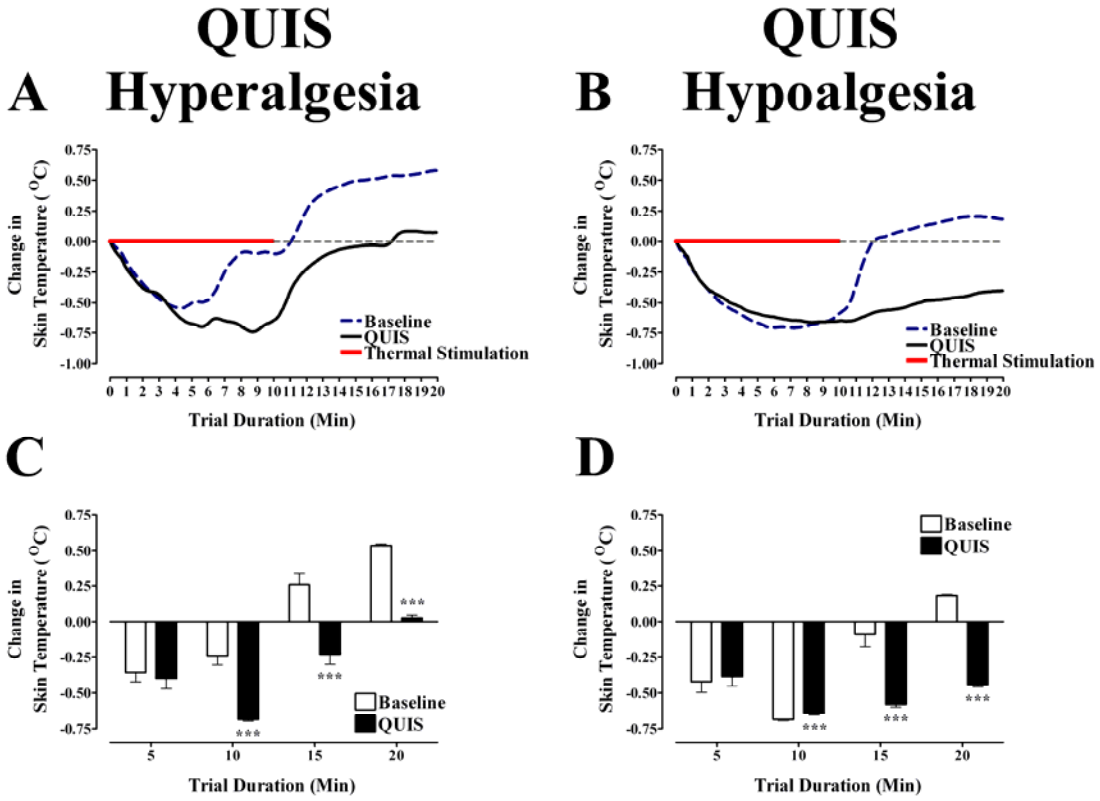


Figure 5-4. Skin temperature measurements from the plantar surface of non-stimulated paws during and after thermal stimulation of the left hindpaw at 44.5°C (thick red line) for 10 minutes before (baseline: blue dotted line) and after (QUIS: black continuous line) excitotoxic injury. (A, B) Baseline temperatures of non-stimulated paws were reduced during stimulation of the left hindpaw and returned to pre-stimulation levels at the end of stimulation. QUIS failed to attenuate the cooling of skin temperatures in the non-stimulated paws. (C, D) Cooling of paw temperatures was enhanced after QUIS. Reduction of paw temperatures continued in the *hypoalgesic* group but not the *hyperalgesic* group at the end of stimulation. Data are expressed as change from temperatures immediately prior to stimulation and subsequent temperatures during and after thermal stimulation. Significant differences between baseline and stress are indicated by: *** $P<0.001$.

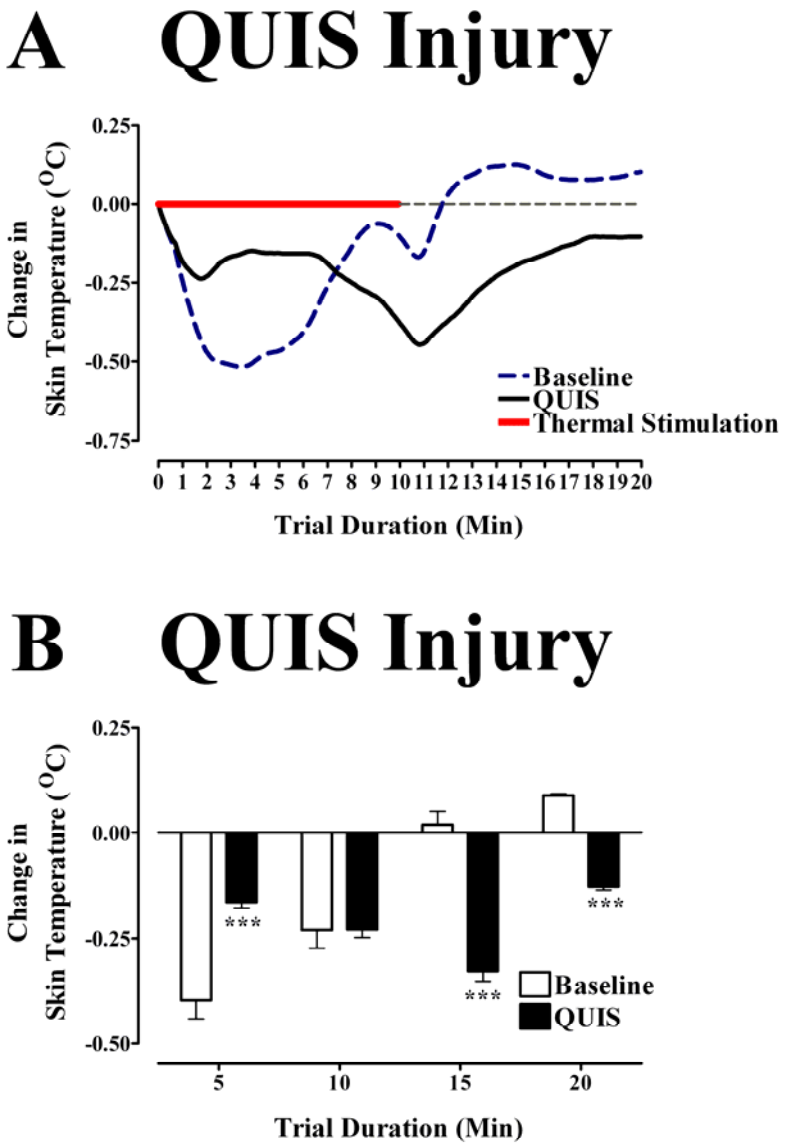


Figure 5-5. Skin temperature measurements from the plantar surface of non-stimulated paws during and after thermal stimulation of left hindpaw at 44.5°C (thick red line) for 10 minutes before (baseline: blue dotted line) and after (QUIS: black continuous line) excitotoxic lesioning. (A) Baseline temperatures of non-stimulated paws were reduced during stimulation of the left hindpaw and returned to pre-stimulation levels at the end of stimulation. QUIS attenuated cooling of paw temperatures in non-stimulated paws. (B) QUIS prevented the decrease in paw temperature within the first 5 minutes. In addition, paws displayed a slight decrease in temperature at the end of stimulation. Data are expressed as change from temperatures immediately prior to stimulation and subsequent temperatures during and after thermal stimulation. Significant differences between baseline and stress are indicated by: *** $P<0.001$.

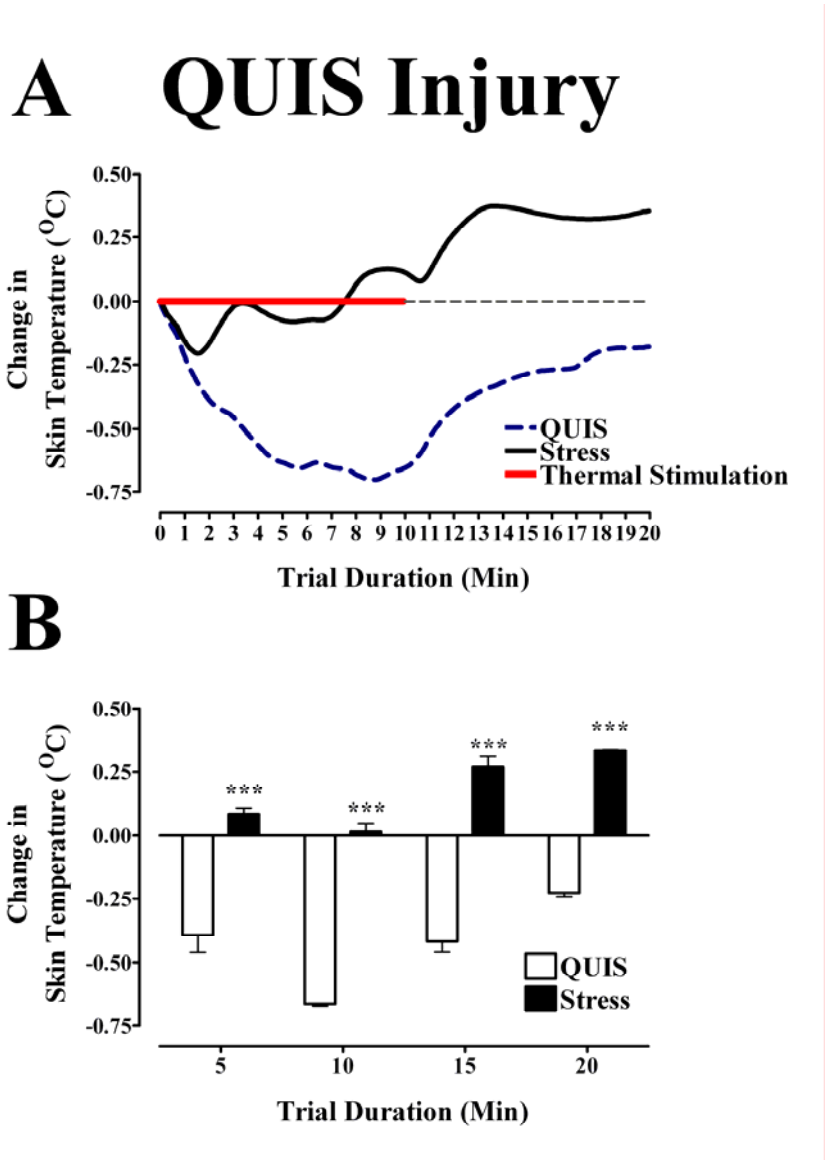


Figure 5-6. Skin temperature measurements from the plantar surface of non-stimulated paws during and after thermal stimulation of left hindpaw at 44.5°C (thick red line) for 10 minutes for baseline (blue dotted line) and stress (black continuous line) conditions after excitotoxic injury. (A) Restraint stress attenuated cooling of non-stimulated paws. (B) Restraint stress prevented the decrease in paw temperature. In addition, restraint stress increased skin temperatures after the first 7 minutes. Data are expressed as change from temperatures immediately prior to stimulation and subsequent temperatures during and after thermal stimulation. Significant differences between baseline and stress are indicated by: *** $P<0.001$.

Summary

Peripheral vasoconstriction is a potential mechanism that may mediate behavioral responses to thermal stimulation. A consequence of vasoconstriction due to a reduction in blood flow to the periphery is a cooling of skin temperatures. Under normal conditions, vasoconstriction permits the animal to compensate for heat (e.g., during exposure to a thermal plate). After restraint stress or spinal injury, enhanced sensitivity to heat (hyperalgesia; increased platform durations and decrease plate durations) may be a result of compromised sympathetic tone that reduces cooling of skin temperatures (e.g., vasoconstriction). Thus, behavioral hypersensitivity to heat (operant responses) appears to be a consequence of an impaired sympathetic vasoconstriction, which limits an animal's ability to counteract the effects of thermal stimulation.

Sympathetic Vasoconstriction after Stress

Some have speculated that the ANS is involved in the execution of context appropriate responses, goal-directed behaviors, and positive affective states based on an integrated processing of information by pre-frontal and limbic structures modulating "fight or flight" responses (Thayer and Brosschot, 2005). In fact, changes in blood flow have been associated with expression of defense responses (e.g., arousal, after an exposure to threatening environmental stimuli (Nalivaiko and Blessing, 1999; Vianna and Carrive, 2005). Similar effects are observed after electrical stimulation of selected brainstem structures (Nalivaiko and Blessing, 1999). Furthermore, a consequence following chronic activation of these systems by stress or injury results in a dysregulation that may be revealed by negative affective states, hypervigilance, impaired cognition, and a lower resistance to stress (Thayer and Brosschot, 2005). Clearly, the ANS is critical for the functional state of an animal during exposure to threatening stimuli.

The sympathetic nervous system has been shown to mediate temperature particularly in the periphery (Janig and McLachlan, 1992; Owens et al., 2002). Sympathetic vasoconstriction, or reduction in skin temperature, is observed after several manipulations. In addition, skin cooling is observed during thermal stimulation in humans (Karlsson et al., 1998, 2006; Krassioukov, 2005; Nicotra et al., 2005, 2006; Shimdoa et al., 1998; Vierck, Unpublished Observations; Willette et al., 1991) and animals (Vierck, Unpublished Observations). It is also seen after an acute exposure to stress in humans (Cooke et al., 1990; Larsson et al., 1995) and animals (Vianna and Carrive, 2005).

It has been known that activity within the sympathetic nervous system is responsible for "fight or flight" responses to a stressor (Barron and Van Loon, 1989) and is characterized by arousal and increases in heart rate, blood pressure, body temperature, and the release of catecholamines (Appelmaum and Holtzman, 1986; Chen and Herbert, 1995; De Boer et al., 1999; Thompson et al., 2003). Interestingly, while the temperatures of extremities (hindpaws) were cooler after exposure to a stressor, a warming of core temperature has been shown to occur simultaneously (Vianna and Carrive, 2005). Increased body temperature is a consequence of two factors: 1) increase metabolism (brain, muscles), and 2) decreasing vasoconstriction (Gordon, 1990; Vianna and Carrive, 2005). During stressful situations, blood flow from non-essential areas (gastrointestinal tract; skin) is redirected to areas critical to "fight or flight" structures including skeletal muscles and the central nervous system (Apler and Zink, 1994). It is hypothesized that reduction of skin temperatures through decreased blood flow to the extremities also serves as a protective mechanism to limit blood loss (Vianna and Carrive, 2005).

In the current experiment, peripheral vasoconstriction was reduced by restraint stress. A possible reason for this effect was illustrated by Cooke et al (1991) in which he used a mental stress paradigm (arithmetic) to stimulate sympathetic vascular tone. Differences were seen between males and females. During these tasks, males exhibited vasoconstriction while female subjects demonstrated vasodilatation (increase in blood flow) compared to baseline values. Females appear to possess higher resting levels of sympathetic vasoconstriction. During periods of sympathetic activation (mental stress), the additional release of adrenergic agents or vasoconstriction has a minimal effect due to a ceiling effect. In some cases, vasodilatation, or an increase in peripheral temperature, can be revealed (Vanhyoutte, 2003).

In the present experiment, restraint stress appeared to activate the sympathetic nervous system. Subsequently, further activation of this system resulted in a reduction of vasoconstriction that is normally observed under control conditions. Because stress heightened sympathetic vascular tone, thermal stimulation had a minimal effect and revealed an increase in skin temperature (e.g., vasodilatation). Thus, an inability to cool skin temperature during exposure to thermal stimulation appears to contribute to an enhancement of heat sensitivity.

Sympathetic Vasoconstriction after Spinal Cord Injury

Abnormalities of the autonomic (e.g., cardiovascular) nervous system are common after SCI caused by damage to descending sympathetic pathways. Especially, SCI patients exhibit abnormal sympathetic activity that can be characterized by a lack of cutaneous vasoconstriction (Karlsson, 2006; Krassioukov, 2005; Nicotra et al., 2006). For example, Nicotra et al (2005) reported that skin blood flow, determined by laser Doppler, was reduced in SCI compared to normal subjects after nociceptive stimulation

and mental stress. In addition, abnormal sympathetic tone was demonstrated by an overall decrease in sympathetic skin responses (SRR; Cariga et al., 2002; Curt et al., 1996, 1997). Assessment of sympathetic nervous system is accomplished by sympathetic skin responses (SSR), an electrophysiological procedure in which responses are induced by electrical stimulation. Lack of a sympathetic skin response (SSR) was most prominent below the lesion (hands vs. feet: Cariga et al., 2002) but not if the descending afferent tracts were spared (Nair et al., 2001). Based these studies, it can be concluded that the autonomic system is functionally abnormal after SCI particularly if descending projections (lateral funiculus; Krassioukov, 2005) are damaged.

Unlike the effects of restraint stress, excitotoxic injury either increased (operant escape group) or decreased (thermal preference group) peripheral vasoconstriction. This discrepancy maybe related to the analysis of histological variables considering assessment of vasoconstriction was several months after spinal injury. Bravo et al. (2002) found evidence that the sympathetic nervous system was diminished during the early stage following spinal injury. Thus, future studies should examine alterations in sympathetic vasoconstriction in parallel with behavioral testing especially during the first few weeks following injury.

However, based on these results, differences in cutaneous cooling may be related to behavioral responding. In the escape group, thermal stimulation produced a prolonged vasoconstriction. After spinal injury, loss of descending pathways and/or chronic activation of this system due to injury reduces resting sympathetic regulation of peripheral nerves. Consequently, nerves upregulate α-adrenergic receptors. During phasic stimulation, vascular hypersensitivity to circulating noradrenaline is observed and

results in an exaggerated vasoconstriction (McLachan and Brock, 2006). A pronounced vasoconstriction could reduce behavioral responses (hypoalgesia) to thermal stimulation.

Unlike the escape test, animals tested in the thermal preference test demonstrated a diminished vasoconstriction after spinal injury. Again, similar to restraint stress, abnormal vascular tone (e.g., higher resting level of sympathetic vasoconstriction) may contribute to reduced vasoconstriction to thermal stimulation. In this group of animals, spinal injury may have chronically activated the sympathetic nervous system. Additional stimulation had little effect.

Interestingly, restraint stress diminished peripheral vasoconstriction after spinal injury. At first glance, the reduced cooling response of cutaneous temperatures appeared to be greater in normal animals (exhibiting an increase in temperature due to vasodilatation). However, this observation is misleading because the QUIS group had a pronounced cooling response prior to stress. Clearly, additional research is needed to fully examine alteration in the sympathetic nervous system after spinal injury.

Future Studies

Additional research is required to directly evaluate changes in sympathetic nervous system. To accomplish this task, several techniques are available including radio-telemetric probes (measurement of heart rate, body temperature, blood flow, and general activity) and infrared thermography (measurement of radiating heat from body and extremities). Both strategies are advantageous because of the accuracy of measurements and practicality of assessing responses in conscious, freely moving animals without interference (Vianna and Carrive, 2005).

Furthermore, this system is also working in parallel with the HPA axis, as indicated by the secretion of gluocorticoids from the adrenal glands (Levine, 2000). Gluocorticoids

mediate the responses to a stressor (Herman and Cullinan, 1997) and indirectly affect the autonomic nervous system. Van Acker et al (2001) reported that restraint stress induced an increase in heart rate (sympathetically regulated) that was reversed by an intracerebroventricular injection of glucocorticoid antagonist. These results suggest that classical stress hormones significantly influence cardiovascular activity after stress through interactions with the autonomic system. However, no studies have examined the role of gluocorticoids in mediating vasoconstriction during stimulation (stress, injury). Future studies could examine the modulation of skin temperatures by gluocorticoids and other stress related hormones.

CHAPTER 6
CONCLUSIONS AND FUTURE STUDIES

Given the differential effects of restraint stress on reflex and operant responses to thermal stimulation in the present study, a variety of questions arise concerning the generality and mechanisms for these influences. That is, what aspects of stress and which modulatory systems are related to the suppression of innate reflexes and/or enhancement of nociceptive sensations dependent on cortical responses? For example, recent investigations indicate that an important determinant of stress effects on innate reflex responses is the duration of stress. Whereas acute stress attenuates licking and guarding (e.g., increase latencies), repeated presentation (chronic stress) is reported to enhance responsivity (e.g., decrease latencies; Gamaro et al., 1998; Quintero et al., 2000). Thus, suppression of innate reflexes may be unique to acute stress.

In addition to the importance of stress duration, there appears to be significant differences between the effects of processive and systemic stressors, particularly on sensations of pain. Some forms of acute systemic stress (e.g., vigorous exercise) attenuate pain sensitivity in humans (Vierck et al., 2001), but psychological stress can exacerbate experimental pain (Logan et al., 2001). In the present study, escape behavior may have been enhanced because restraint stress preferentially activated forebrain and limbic circuits (Herman and Cullinan, 1997; Herman et al., 1996). This raises the possibility that psychological stressors have greater effects on cerebral components of pain transmission systems than do systemic stressors that act predominantly through brainstem systems (Cullinan et al., 1995; Herman and Cullinan, 1997). Psychological

stress also stimulates forebrain catecholamine (e.g., noradrenalin; Tanaka et al., 2000) circuits, which project to supraspinal and spinal structures modulating innate reactions to nociceptive input (Millan, 2002). Therefore, licking and guarding are likely suppressed after restraint because psychological stress indirectly activates descending (brain stem to spinal) inhibitory systems (Calcagnetti et al., 1992; Gamaro et al., 1998; Tsuda et al., 1989).

It is important to note that presumed activation of descending modulatory systems by restraint stress did not inhibit nociceptive transmission from the spinal cord to the cerebrum, as assessed by escape duration. Not only was ascending nociceptive transmission spared, but it might have been accentuated at the spinal level by descending modulation. A potential mechanism for this combination of effects is that descending modulatory systems have both excitatory and inhibitory actions (Millan, 2000) that could suppress spinal motoneuron output while differentially activating cells of origin of pain transmission pathways.

In addition to differences in the type or duration of stress, how important is the form of nociceptive stimulation to the effects of stress? Post-stress effects have often been tested on behaviors elicited by intense and brief stimuli that activate A-delta nociceptors – inputs which more effectively and reliably elicit reflexes than selective activation of C-nociceptors (Cooper and Vierck, 1986; Cooper et al., 1986; Vierck et al., 2000). However, it is possible that stress enhances sensitivity to tonic input from C-nociceptors. For example, average escape latencies for 44.0°C stimulation (as in the present study) occurred when foot temperatures reached 44.0°C (Vierck et al., 2004), which approximates thresholds for activation of C-nociceptors (Fleischer et al., 1983;

Leem et al., 1993). Average lick/guard latencies were more than twice the escape latencies, and these behaviors occurred when skin temperatures and durations sufficient to activate A-delta nociceptors might have been attained. This raises the possibility that acute stress has opposite effects on nociceptive input from A-delta and C-nociceptors. In support of this hypothesis are studies showing stress-induced depression of reflex responses to intense thermal stimulation and demonstrations that operant escape from electrical stimulation is attenuated by acute stress (Bodnar et al., 1978c; 1979). Electrical stimulation provides an optimum model of A-delta nociception, because very high current levels are required to activate C-nociceptors (Cooper and Vierck, 1986; Cooper et al., 1986).

A key hypothesis of particular relevance to A-delta and C nociception is that acute stress can attenuate nociceptive reflexes through opioid mechanisms (Amir and Amit, 1979; Bodnar et al., 1978c; Gamaro et al., 1998; Lewis et al., 1980). Involvement of opioid mechanisms is indicated by blockade of stress effects by opioid antagonists, enhancement of stress effects by opioid agonists, and development of cross-tolerance with morphine after repeated exposures (Bodnar et al., 1978b; Lewis et al., 1980; Calcagnetti et al., 1992; Willer et al., 1981). However, doses as high as 10 mg/kg of systemic morphine are required to produce a reflex suppression comparable to that produced by stress (Bodnar et al., 1978b). Doses in this range are likely to produce a generalized behavioral/motoric suppression and are above threshold for attenuation of A-delta nociception (Cooper and Vierck, 1986, Mauderli et al., 2000). Higher dose morphine (3.0 to 5.0 mg/kg) attenuates A-delta nociception, whereas 0.5 mg/kg is sufficient to reduce operant responses to C-nociceptor input (Cooper and Vierck, 1986;

Vierck et al., 2002; Yeomans et al., 1995). Because systemic morphine preferentially attenuates C-nociception, the opioid hypothesis can be evaluated appropriately using low levels of nociceptive heat to selectively activates C-nociceptors (Yeomans and Proudfit, 1996; Yeomans et al., 1996), as in the present study. Accordingly, low dose morphine (0.5 mg/kg) suppresses escape responding to 44.0°C stimulation, but lick/guard responding to the same stimulus is enhanced by this dose of morphine (Vierck et al., 2002). Restraint stress had the opposite effect: enhancement of escape responding to 44.0°C and depression of lick/guard responses. Therefore, the opioid hypothesis is contradictory to stress effects on responses to C-nociceptor input that are sensitive to physiological levels of opioid agonists.

A probable reason for difficulties ascribing physiological levels of opioids or other transmitters to generalized effects of stress on nociceptive behaviors is that distinct responses are mediated by different central pathways, and each of these neural systems is subject to different patterns of modulation at multiple levels of the neuraxis. Licking is present in decerebrate animals but absent in spinal animals (Matthies and Franklin, 1992). Guarding, an elaboration of withdrawal reflexes that are present in spinal animals, is more difficult to interpret in terms of requisite neural circuitry but can be elicited after decerebration. Thus, both licking and guarding depend upon specialized spinal-brain stem-spinal loops that can be modulated at either of these levels. The tail flick response has characteristics of a strictly segmental reflex that can be modulated directly at spinal levels or indirectly by descending connections from the brain stem. Acute stress attenuates each of these responses. In contrast, several other innate responses (vocalizations and jumping) are present in decerebrate animals and have been reported to

be increased by acute stress (King et al., 1996, 1999; Simone and Bodnar, 1982). Therefore, the distinction between behaviors that are accentuated or depressed by acute stress appears not to be based entirely upon distinctions between operant and reflex effects or between actions at cerebral, brain stem or spinal levels. A possible commonality for rodent behaviors that are accentuated by stress is an adaptive significance for fight/flight situations in which survival is optimized by a compatible combination of intentional reactions and innate responses. Vocalization alerts other animals, and jumping can be effectively integrated into an escape strategy.

Future Directions

Several possible studies could be evaluated from the current set of experiments. Future studies could examine the effects of chronic stress or exposure of acute stress on lesioned animals with other chronic pain conditions (e.g., spinal nerve ligation, SNL; chronic constriction injury, CCI; formalin). In addition, the following studies were initially proposed for this dissertation. However, due to unforeseen limitations, the studies were not competed. These experiments would examine the neural circuitry and transmitter systems involved in producing stress-induced changes in nociceptive sensitivity in rats. Specifically, the role of ascending and descending inhibitory/facilitatory pathways in stress-induced alterations in operant and reflex responses to thermal stimulation could be examined. The contribution of these pathways to stress-induced changes in responses would be accomplished by: 1) intrathecal administration of adrenergic and opioid receptor agonists or antagonists; and, 2) using receptor specific neurotoxic lesions.

Spinal neurotransmitter systems in the expression of stress-induced changes in thermal stimulation

These experiments would examine the effects of intrathecal adrenergic and opioid agonists or antagonists on operant escape and reflexive responses to an acute exposure to restraint stress. In previous studies, spinally administered opioid or noradrenergic agonists reduced dorsal horn activity and reflex tail and hindpaw withdrawal responses to nociceptive thermal stimulation. The inhibitory effects of intrathecal agonists are mediated by spinal μ-opioid and α_2 adrenergic receptors (Clark and Proudfit, 1991; Takano and Yaksh, 1992; Yaksh, 1999). It is hypothesized that spinal adrenergic and opioidergic systems contribute to stress-induced effects on responses evoked by thermal stimulation. These receptors mediate the inhibitory effects of stress on reflexive responses and oppose the facilitatory effects of stress on operant responses through activity of descending brainstem projections to the spinal cord.

If the descending inhibitory effects of stress are mediated by opioid and noradrenergic receptors, activation of spinal μ-opioid and α_2-adrenergic will enhance the inhibitory effects of stress on reflex responses. In contrast, blockade of spinal μ-opioid and α_2-adrenergic will decrease the inhibitory effects of stress on reflex responses.

If the descending excitatory effects of stress are opposed by opioid and noradrenergic receptors, activation of the spinal μ-opioid and α_2-adrenergic receptors by i.t. DAMGO and clonidine, respectively, will decrease sensitivity of operant escape responses. In contrast, blockade of spinal μ-opioid and α_2-adrenergic receptors by i.t. naloxone and yohimbine, respectively, will increase the excitatory effects of stress on operant responses (see below for alternative results and future directions).

The results of the proposed experiments may result in little or no effects of stress and drugs on reflex or operant escape responses. First, noredranaline has a differential effect on behavioral responses to thermal stimulation depending on expression of adrenergic receptor subtypes. Due to the bi-directional activity of noredranaline in the spinal cord, the involvement of spinal α_1- (pronociceptive) and α_2- (antinociceptive) noradrenergic receptors in stress-induced changes in somatosensory processing can be examined by complementing α_2 adrenergic antagonists with intrathecal injections of prazosin (α_1-adrenergic antagonist). Blockade of spinal α_1-adrenergic (pronociceptive) receptors by i.t. prazosin will reduce the facilitatory effects of stress on escape and enhance the inhibitory effects of stress on reflex responses. The adrenergic α_1-adrenergic antagonist, prazosin, is ideal for these initial studies due the extensive literature on stress and modulation of nociception (Camarata and Yaksh, 1986).

The results of the proposed experiments may result in little or no effects of stress and drugs on reflex or operant escape responses. Secondly, the opioid and adrenergic receptors may not be involved in stress effects on operant responses, and therefore, neither μ-opioid or α_2-adrenergic receptor agonists (e.g., DAMGO, clonidine) nor antagonists (e.g., naloxone, yohimbine) will affect increases in sensitivity observed on these responses following stress. This possibility will necessitate the use of other pharmacological agents targeting transmitter systems thought to have a role in the descending modulation of nociceptive processing in the spinal cord (e.g., cholinergic, serotonergic, or cholecystokinin).

Considering recent reports by Zeitz et al (2002) suggesting the pronociceptive properties of spinal 5-HT_3 receptors, we could intrathecally administer a selective

5-HT_3R antagonist (ondansetron, 1-25μg) to reverse the facilitatory effects of these receptors on operant responses. Finally, it is possible that spinal neurotransmitter systems may not be critical in the stress-induced hypersensitivity. Future studies could examine the effects of intracerebroventricular (i.c.v.) injections in order to examine neural structures involved in the supraspinal processing of nociceptive information, which may produce a greater impact on escape responses as mentioned below.

Role neurokinin-1 receptor (NK-1R) expressing neurons on stress-induced changes in thermal responses

These experiments would examine the effects of a specific population of spinal neurons on somatosensory processing in non-stressed and stressed animals. NK-1R expressing spinal neurons are implicated in nociceptive transmission, controlling behavioral responses, and activation of descending pathways from the brainstem (Dolye and Hunt, 1999; Wiley and Lappi, 1997). These neurons would be lesioned by intrathecal injection of substance P-saporin after training and baseline testing. It is hypothesize that ascending pathways from spinal neurons expressing NK-1R contribute to stress-induced hypersensitivity of operant responses and hyposensitivity of reflex responses evoked by thermal stimulation.

If the excitatory effects of stress involve spinal neurons expressing NK-1R, we expect that ablation of these neurons will reduce operant escape responses in lesioned animals. If the inhibitory effects of stress involve spinal neurons expressing NK-1R, we expect that ablation of these neurons will reduce reflex responses in lesioned animals. These results are based on the hypothesis that an ascending pathway originating from NK-1R expressing neurons activates a descending excitatory as well as inhibitory feedback loop in the rostral medulla.

The reliability and specificity of neurotoxins are a potential problem. However, recent collaborations with Dr. Wiley and other labs have insured the proper use and viability of the neurotoxin. Low-doses of the neurotoxin will prevent non-specific damage to non-receptor expressing cells. If the neurotoxin lesions fail to alter stress-induced changes in nociception, moderate to high doses of the neurotoxin can be used. In addition, mechanical lesions to eliminate ascending (ALQ) and descending pathways (DLF) may be used instead. Elimination of these pathways will identify the location of critical pathways involved in producing stress effects. Depending on the intrathecal pharmacology, we could use specific lesions to eliminate influences by adrenergic and serotonergic systems in the spinal cord acting at nerve terminals (e.g., 5, 7-dihydroxytryptamine and 6-hydroxy DA).

Descending pathways mediating stress-induced changes in thermal responses

Finally, these experiments would examine the function of descending facilitatory and inhibitory pathways on somatosensory processing in non-stressed and stressed animals. Descending projections from the rostroventral medulla (RVM) originating from μ-opioid receptor-expressing neurons are implicated in the modulation of spinal nociceptive transmission and enhancement of nociceptive behaviors in rats. The origin of descending facilitatory pathways will be lesioned by bilateral medullary injection of dermorphin-saporin after training and baseline testing. It is hypothesized that descending pathways from the RVM contribute to stress-induced hypersensitivity of operant responses and hyposensitivity of reflex responses evoked by thermal stimulation.

If the excitatory effects of stress are mediated by neurons expressing μ-opioid receptor in the RVM, we expect that ablation of these neurons will reduce operant escape

responses in lesioned animals as a result of eliminating facilitatory pathways driving operant behavior. If the inhibitory effects of stress are mediated by neurons expressing μ-opioid receptor in the RVM, we expect that ablation of these neurons will reduce reflex responses in lesioned animals as a result of eliminating facilitatory pathways that normally antagonize descending inhibitory influences

The reliability and specificity of neurotoxins are a potential problem. However, a recent collaboration with Dr. Wiley and other labs has insured the proper use and viability of the neurotoxin. If the neurotoxin lesions fail to alter stress-induced changes in nociception, we could use moderate to high dose of the neurotoxin or inject lidocaine into the RVM. Finally, microinjection of lidocaine could be used to reduce neuronal activity in the RVM. Mitchell et al (1998) reported that inactivation of RVM neurons by lidocaine reversed morphine and stress-induced inhibition of reflexive responses.

Conclusions

The present studies demonstrated that restraint stress differentially affected innate reflex and operant escape responses to low intensity thermal stimulation, which is threshold activation for C-nociceptors. Reflex lick/guard responses were reduced after restraint stress (stress-induced hyporeflexia), which confirms previous studies of stress-induced hyporeflexia for Aδ-activation. In contrast, operant escape responses were enhanced in the same animals after stress (stress-induced hyperalgesia).

Additional characteristics of stress-induced changes in nociception were identified. First, control procedures showed that stress did not affect responding to non-nociceptive stimulation, did not influence aversion to light and did not enhance avoidance responding. Second, stress-induced changes in behavioral responses were greater when

assessed immediately after restraint. Third, the effect of stress on both responses was transient and did not undergo adaptation with repetition at 2 week intervals. Fourth, core body and cutaneous temperatures returned to levels similar to controls by fifteen minutes after stress termination. Finally, the expression of stress-induced changes in nociception was affected by endogenous and exogenous opioids. Stress-induced hyperalgesia (operant escape) is mediated by a non-opioid mechanism, which is counteracted by tonic endogenous opioids and exogenous opioid administration. In contrast, stress-induced hypoalgesia is mediated by endogenous opioid systems, which suppresses reflex lick/guard responses. Exogenous morphine enhances reflex responses and opposes effects of restraint stress on these reflexes through separate mechanisms. Taken together these observations suggest that acute exposure to a psychological stressor activates forebrain circuits mediating pain perception, producing hyperalgesia. Forebrain-limbic circuits in turn activate descending pathways to suppress nociceptive reflexes.

In addition, pain sensations are affected by damage to the spinal cord gray matter. Assessment of operant escape responses revealed that heat sensitivity was enhanced after Excitotoxic injury of the spinal cord by altering sympathetic-mediated peripheral vasoconstriction. It is clear that damage to the gray matter is a critical factor in the development of heightened pain sensation in humans (Finnerup et al., 2003) and may underlie observations that the autonomic nervous system is dysfunctional after spinal injury (Nicotra et al., 2005, 2006). Subsequent exposure to acute restraint stress enhanced injury-induced operant escape responses, which is consistent with anecdotal reports that stress increases clinical pain in humans especially after SCI (Ditor et al., 2003). Thus, the expression of stress-induced hyperalgesia is transient, dependent on the

preferential activation of C-nociceptors, not influence by changes in core body temperature but altered sympathetic regulation of peripheral vasoconstriction, and more prominent in spinally injured conditions.

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BIOGRAPHICAL SKETCH

Born in western Pennsylvania, I grew up outside of Pittsburgh with my father and mother. My childhood was like that of other normal children: playing sports, wandering in the woods, and watching several Pittsburgh professional sports teams. I attended a Catholic elementary school and a public high school. I was very active in sports during this time playing soccer and football. My parents divorced, but out of this unsettling situation I acquired a larger family after my parents remarried. I will never forget the day that my sister was born.

At the end of my senior year in high school, my mother passed away, leading to a rough period in my life. However, I found strength in my family, and I focused that strength into academic studies in college. I wanted to attend physical therapy school, but my attention shifted after taking Dr. Blustein's neuroscience class and working on several undergraduate projects at Beaver College. As my interest in neuroscience grew, I planned to attend graduate school, so I gained additional research experience in Dr. Caudle's lab. At the University of Florida, I was given that opportunity to fulfill my goal and continue my research interests in neuroscience. During my time in graduate school, I met and married the most wonderful woman in the world: Natasha. As my graduate career ends, I look forward to the future.