Endocrine Response to Social Rejection: The Effect of Testosterone and Cortisol on Pain Sensitivity

by

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B.A., Simon Fraser University, 2014

Thesis Submitted in Partial Fulfillment of the
Requirements for the Degree of
Master of Arts

in the
Department of Psychology
Faculty of Arts and Social Sciences

SIMON FRASER UNIVERSITY

Spring 2017

Approval

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Degree:	**Master of Arts (Psychology)**
Title:	***Endocrine Response to Social Rejection: The Effect of Testosterone and Cortisol on Pain Sensitivity***
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Date Defended/Approved:	March 30/2017

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Abstract

An expanding body of literature suggests that common neural underpinnings governing physical and social pain are evolved adaptations that punish social disengagement by using pain as a signalling mechanism for social rejection. Such a mechanism is necessary in the face of fitness benefits afforded by group living from which a ubiquitous need to belong has grown. Salivary testosterone and cortisol were examined in the context of fluctuating pain sensitivity in response to a social evaluation with a confederate. It was expected that a greater evolutionary prescribed tendency to seek interpersonal support would result in physiological responses to rejection in females leading to reductions in pain sensitivity. While non-significance was found for cortisol, results implicate testosterone as an important factor in altering sensitivity after social interactions in men. This relationship between testosterone and pain may be a function of dominance and increased status seeking resulting from acceptance in a social interaction.

Keywords: Rejection; Need to Belong; Pain Sensitivity; Testosterone; Cortisol

To my parents, and to Dillon, for their unconditional support and unwavering belief

Acknowledgements

I would like to express my appreciation to my supervisor, Dr. Neil Watson, for his support throughout the process of writing this thesis, as well as the many helpful comments on the many drafts leading to the final product. In addition, I would like to thank the rest of my examining committee for taking the time to engage with my research and make it better. Finally, I would like to thank my fellow graduate students for the many helpful discussions and the innumerable pieces of knowledge you have imparted upon me.

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List of Acronyms

ACC	Anterior Cingulate Cortex
ACTH	Adrenocorticotropic Hormone
AI	Anterior Insula
CBG	Corticosteroid Binding Globulin
CRH	Corticotropin Releasing Hormone
CV	Coefficient of Variation
dACC	Dorsal Anterior Cingulate Cortex
ELISA	Enzyme-Linked Immunosorbent Assay
FSH	Follicle-Stimulating Hormone
GnRH	Gonadotropin Releasing Hormone
GRE	Glucocorticoid Response Element
HPA	Hypothalamic-Pituitary-Adrenal
HPG	Hypothalamic-Pituitary-Gonadal
LH	Luteinizing Hormone
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NRM	Nucleus Raphe Magnus
PAG	Periaqueductal Grey
PFC	Prefrontal Cortex
PKC	Protein Kinase C
PLC	Phospholipase C
PVN	Paraventricular Nucleus
RPS	Research Participation System
RVPFC	Right Ventral Pre-Frontal Cortex
SFU	Simon Fraser University
SI	Primary Somatosensory Cortex
SII	Secondary Somatosensory Cortex

Chapter 1.

Introduction

Emotional pain, such as that experienced after rejection from a valued relational partner, is widely regarded as something akin to a traumatic event, one that not only affects psychological but also physical well-being (Baumeister & Tice, 1990; Leary, 1990; Buckley, Winkel, & Leary, 2004). Experiences provoked by the loss of social bonds are believed to be fuelled by the ubiquitous desire of human beings to belong (Baumeister & Leary, 1995). Such a need is thought to be a fundamental human drive; one that may have evolved as a mechanism to encourage interpersonal connection. In turn, this connection acts to maintain group cohesiveness as a result of the powerful fitness enhancing benefits that group living may have afforded to ancestral humans. The need to belong is fulfilled by engaging in positive social interactions which are structured around a framework of temporal stability and mutual concern (Baumeister & Leary, 1995). To belong then, is to engage in long-term relationships consisting of frequent interactions with the same people that are characterized by positive emotions and care for the other person. The need to belong can theoretically be satisfied by any type of relationship in which there has been adequate time to develop mutual intimacy and concern; most often these develop between parent and child, close friends, or romantic partners (Baumeister & Leary, 1995). Consequently, it is not surprising that the loss of such relationships has very real consequences for health and behavior, which are reflected in an association between social exclusion and psychologically distressing states such as depression and anxiety (Baumeister & Leary, 1995; Seeman, 1996) Individuals who experience high levels of social exclusion have also been found to experience decreased physical well-being, in the form of higher incidences of coronary heart disease, poorer recovery from heart attacks and stroke, and increased risk of mortality (Seeman, 1996).

However, while it is recognized that social isolation and rejection have negative physical and psychological consequences, the term ‘pain’ to describe the immediate reaction to these circumstances has always been taken to be rather metaphorical. After all, pain is typically thought of as a sensory response to the physical damage of body tissue, not psychological distress resulting from social isolation or rejection. However, research on the ability of opioid pain relievers to calm distress vocalizations in rat pups that are separated from their mothers, has brought the validity of this distinction into question (Panksepp, 1998). As such, the degree to which social pain and physical pain can be linked in more than just a metaphorical sense is of considerable interest.

1.1. Hormones

Hormonal effects on behavior have long been recognized to be bidirectional in nature, such that behavior is influenced by hormonal fluctuations that are, in turn, shaped by behavior and other environmental stimuli. As a result, the relationship between behavior and hormones is a complex one whose components continually reinforce each other to create emergent and ever-changing dynamics (van Anders & Watson, 2006). One particularly interesting avenue of research involves the interaction between hormones and social behavior. Many of these interactions are believed to reflect their functional consequences for evolutionary fitness. As such we find hormonal influences on behaviors that are linked to survival and reproduction, such as competition and parenting activities (van Anders & Watson, 2006). Many of these behaviors are regulated by the actions of steroids; lipophilic hormones derived from cholesterol which are capable of moving across cellular membranes in order to bind intracellular receptors. These intracellular receptors often act as transcription factors, which bind to steroid response elements and alter gene expression. Steroid hormones, therefore, are capable of exerting genomic effects, the results of which may not be evident for hours or days (Sapolsky, Romero, & Munck, 2000; Simoncini & Genazzani, 2003). However, given the immediate nature of many social interactions thought to be influenced by steroidal hormones, a non-genomic mechanism of action that would allow tissue specific responses within seconds to minutes has also been postulated. These mechanisms may include G-protein coupled membrane receptors as well as interactions with intracellular second-messenger systems, such as

phospholipase C (PLC) and protein kinase C (PKC) (Falkenstein, Tillmann, Christ, Feuring, & Wehling, 2000; Simoncini & Genazzani, 2003).

1.1.1. Cortisol

Cortisol is a steroid hormone, released in humans in response to both physical and psychological stressors as well as in a pulsatile circadian rhythm (Kirschbaum & Hellhammer, 1989). The release of cortisol is the culmination of a regulated process involving the coordination of a variety of systems throughout the body that is collectively known as the hypothalamic-pituitary-adrenal (HPA) axis. The process begins with the release of corticotropin releasing hormone (CRH) from the neurosecretory cells of the paraventricular nucleus (PVN) of the hypothalamus. CRH is released into the hypophyseal portal system of the anterior pituitary where it stimulates the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary into the general circulation of the body. Circulating ACTH stimulates the release of glucocorticoids, the most important of which is cortisol, from the zona fasciculata of the adrenal cortex (Tsigos & Chrousos, 2002). Upon release from the adrenals, the majority of glucocorticoids are bound to blood-borne carrier proteins, such as corticosteroid-binding globulin (CBG). Only about 5-10% of the glucocorticoids released from the adrenal cortex are free to circulate throughout the body in an unbound state and it is only this minority that are free to act upon target tissues (Kirschbaum & Hellhammer, 1989). Glucocorticoids most often exert their effects upon target tissues through binding to receptors located in the cytoplasm of cells. These receptors act as ligand-activated transcription factors which, upon binding with a ligand, translocate into the nucleus of the cell where they interact with glucocorticoid response elements (GREs). These GREs are specific sequences of DNA that are responsive to the glucocorticoid receptor homodimer which binds to this region and regulates transcription. However, glucocorticoids are also capable of having fast, non-genomic actions that may be facilitated by a membrane-bound glucocorticoid receptor or may involve interactions with other transcription factors such as NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells; Tsigos & Chrousos, 2002, Yudt & Cidlowski, 2002). Finally, the output of the HPA axis is regulated by several negative feedback loops in which cortisol feeds back onto the anterior pituitary and the hypothalamus to inhibit the further release of ACTH and CRH respectively. This negative feedback loop is crucial in assuring that the body is

not exposed to elevated levels of glucocorticoids for an extended period of time (Tsigos & Chrousos, 2002).

Basal levels of cortisol are determined by its pulsatile release in response to a 24-hour circadian rhythm characterized by inhibition and activation of the HPA axis at particular times during the day. The circadian pattern is such that cortisol levels reach their apex in the early morning and their nadir in the evening. Cortisol secretion is also released at a pulse frequency of 60-90 minutes in response to an ultradian rhythm (Young, Abelson, & Lightman, 2004). Therefore, varying levels of cortisol are released into the bloodstream throughout the day with, in general, the lowest levels occurring in the evening and the highest levels in the early morning. There are, however, alternating periods of activation and inhibition within that general pattern. These overlapping rhythms result in wide individual variation in glucocorticoid concentrations in the bloodstream at any given time during the day which may, in turn, affect individual responses to stressful stimuli.

In response to a stressor, the normal cycling of the HPA axis becomes upregulated, eventually resulting in increased concentrations of glucocorticoids released into the general circulation. Stress is defined as stimuli that threaten the complex and delicate balance of homeostasis within the body (McEwen, 2000). This increase in glucocorticoids in the blood stream helps the body to combat threats to homeostasis and, therefore, helps cope with stressful stimuli. Glucocorticoids do this by mobilizing energy resources via elevations in blood glucose through hepatic gluconeogenesis, suppressing glucose uptake in peripheral tissues, and the breakdown of fat and muscle (Dickerson & Kemeny, 2004; Sapolsky et al., 2000). This increase in glucose is particularly important for the bodily response to stress as it provides the organism with the metabolic resources necessary to deal with the stressor. Cortisol also has other important functions in relation to its role in the stress response. For example, it suppresses inflammation, facilitates increased heart rate and vasoconstriction, enhances memory, and inhibits immune functioning (Dickerson & Kemeny, 2004; Sapolsky et al., 2000).

There are, however, large individual differences in how people respond to stressful stimuli. One important factor in these differential responses to stress appears to be gender. For example, although women show higher rates of depression that have been

associated with higher basal cortisol levels, men typically show greater HPA axis responses to laboratory stress tasks (Stroud, Salovey, & Epel, 2002). Such gender differences may, in fact, reflect differences in orientation towards the task. For instance, most laboratory tasks used to measure stress are instrumental and achievement oriented. In a study measuring HPA responsiveness to achievement-oriented and social-rejection tasks, specific gender differences were found. In line with previous research, males experienced more stress and HPA activity in response to the achievement-oriented task while females were more reactive to the social-rejection task (Stroud et al., 2002). These proclivities arise from differences in personality tendencies, with men typically displaying a more instrumental orientation and women displaying a more interpersonal orientation. This focus on interpersonal interactions from a female perspective may reflect differential parental investment strategies between males and females, in which social interaction is particularly important for female evolutionary fitness. As such, a tend-and-befriend strategy in which interpersonal relationships are used to ensure the survival of self and offspring may be more relevant to females than a fight-or-flight strategy (Taylor et al., 2000). Alternatively, in many primate species males compete for dominant positions within a social hierarchy that will afford them greater access to resources and mates (Sapolsky, 2005). It may be presumed then, that social rejection may be salient to male primates in as much as it signals the loss of dominance that could reduce their fitness. There is, however, variation in the extent to which subordination in a social hierarchy actually produces physiological indices of stress (Sapolsky, 2005). In an unstable hierarchy in which dominance is continuously being contested, males relegated to subordinate positions may not experience this denial of status to be particularly stressful and may, in fact, be able to use alternative strategies to gain access to resources. Social rejection for females, however, is likely to consistently activate the HPA axis due to the importance of relationships in their response to threat. Assuming, then, that loss of interpersonal relationships would have had greater evolutionary consequences for females than for males, one might assume that social rejection or ostracism may trigger a greater biological stress response in females.

1.1.2. Testosterone

Testosterone is a hormone that is the principal member of a class of androgen steroids that are responsible for anabolic functions as well as organizing and maintaining masculine features (Mazur & Booth, 1998). Although testosterone is produced by the ovaries and adrenals of females, it is manufactured in far greater concentrations in the Leydig cells of the testes in males. This discrepancy between males and females is an important one, as testosterone plays an essential role in organizing masculine physiology and behavior both prenatally and during puberty. In utero, the previously bipotential gonad eventually becomes differentiated into testes or ovaries depending upon the chromosomal sex of the fetus (Mazur & Booth, 1998). Once established the fetal testes produce large concentrations of androgens which are responsible for masculinizing both the genitalia and the central nervous system. Importantly, the presence of testosterone in utero allows not only for the masculinization of the male fetus but also its defeminisation, the prevention of the development of feminine characteristics (Mazur & Booth, 1998). While prenatal testosterone has organizational effects on physiology, the testosterone surge during puberty is associated with the activation of these pre-existing structures and functions that are associated with the appearance of male secondary sexual characteristics and behavior.

Testosterone is the end product of the Hypothalamic-Pituitary-Gonadal (HPG) axis. The hypothalamus is responsible for the secretion of gonadotropin-releasing hormone (GnRH) which, when released, travels through the hypophyseal portal system to the anterior pituitary gland (Swerdloff, Wang, & Sinhahikim, 2009). The presence of GnRH in the pituitary signals the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the gonadotroph cells of the pituitary into general circulation. GnRH release is coordinated by a combination of neuronal inputs and circulating sex steroid concentrations, resulting in a pulsatile rhythm of increasing FSH and LH concentrations every 60-90 minutes (Swerdloff et al., 2009). LH and FSH also display a diurnal rhythm, at least in young adult males, in which concentrations are higher in the morning and lower in the evening. LH and FSH receptors are located in the gonads, where LH binds to receptors in the Leydig cells and FSH binds primarily to receptors in the Sertoli cells. Binding of LH to Leydig cells promotes the release of testosterone into the general

circulation where it has its physiological and behavioral effects. Similar to cortisol, approximately 1-10% of testosterone in serum is in an unbound, biologically active form, the rest is carried by serum proteins. Circulating testosterone regulates further release of LH and FSH from hypothalamic and pituitary sites through negative feedback mechanisms (Swerdloff et al., 2009).

In addition to its role during prenatal and pubertal development, recent work suggests that testosterone also plays a role in human social behavior (Eisenegger, Haushofer, & Fehr, 2011). For example, it has been well established that testosterone is associated with dominance behavior and social status seeking. Individuals higher in testosterone have stronger proclivities to seek high status positions, which can be achieved through either physical or non-physical forms of dominance behaviors (Mazur & Booth, 1998; Eisenegger et al., 2011). Furthermore, in line with the reciprocal relationship between behavior and hormones, testosterone fluctuation in response to social stimuli can affect subsequent social behavior. For example, after engaging in a competition against another participant, those who lost and experienced an increase in testosterone from pre-competition levels were more likely to decide to compete again against the same competitor (Mehta & Josephs, 2006). However, losing the competition coupled with decreasing testosterone was associated with the choice to engage in a non-competitive alternative task. These results equate the rise in testosterone in losing participants with a desire to regain status lost in the first competition while a decrease in testosterone was associated with avoidance of competition that could result in further loss of status (Mehta & Josephs, 2006). Testosterone may also be reactive to social situations involving rejection and acceptance by others. In a paradigm that involves including or excluding a participant in a virtual ball tossing game, both males and females experienced decreased testosterone concentrations after exclusion while inclusion produced increased testosterone in males. The *biosocial status theory*, which posits that social exclusion challenges dominance and social status motives and, furthermore, that these feelings are associated with decreased testosterone, was used to explain the changes in testosterone observed in this study (Seidel et al., 2013; Mazur & Booth, 1998; Eisenegger et al., 2011). Inclusion in social groups, meanwhile, may reinforce feelings of dominance and status that are associated with increased levels of testosterone after social acceptance. The fact that testosterone increase after inclusion was found only in males may suggest a stronger

propensity towards dominance and social status motives in males (Seidel et al., 2013; Mazur & Booth, 1998; Eisenegger et al., 2011).

The *dual hormone hypothesis* of Mehta and Josephs (2010) suggests that testosterone also regulates certain behaviors through its relationship with cortisol. In particular, cortisol is suggested to moderate the relationship that testosterone has with certain behaviors by inhibiting testosterone at high levels of cortisol. The result of this relationship is that testosterone has different effects on behavior depending upon whether cortisol levels are high or low. In relation to dominance, individuals with low cortisol showed a relationship between high testosterone and dominance (Mehta & Josephs, 2010). This relationship was either blocked or reversed in those with high cortisol, such that there was either no relationship between dominance and testosterone or high testosterone was associated with low levels of dominance (Mehta & Josephs, 2010). Besides dominance behavior, this cortisol-testosterone interaction has been shown to affect a wide range of human social behaviors; from social status seeking to violence and aggression, and from risk taking to empathy (Mehta & Prasad, 2015).

1.2. Pain

Pain is a subjective, complex phenomenon, which is typically associated with actual or potential tissue damage and serves as a signal to terminate behaviors associated with the sensation. Like other sensory modalities, painful stimuli are carried from the periphery to the central nervous system by way of primary sensory neurons. These neurons are stimulated by nociceptors that are sensitive to stimuli, such as pressure, heat, or irritating chemicals, that are associated with potential tissue damage (Julius & Basbaum, 2001). Pain sensation is unique in that different aspects of the sensation are carried on different sensory fibres. Aδ fibres are thinly myelinated nociceptive neurons that carry the rapid, sharp pain associated with the first response to a painful stimulus. C fibres are unmyelinated neurons that conduct pain information relatively slowly and are associated with the dull, long-lasting sensation associated with tissue damage (Julius & Basbaum, 2001). Primary sensory neurons carry pain stimuli to the CNS by synapsing on lamina I and II of the dorsal horn of the spinal cord. From there, several ascending pathways carry nociceptive information to brainstem and thalamic regions that then relay

this information to midbrain and cortical pain processing centers, including the primary and secondary somatosensory cortices (S1, S2), the anterior cingulate cortex (ACC), the insula, and the prefrontal cortex (PFC) (Al-Chaer, 2009; Steeds, 2013). In addition to receiving nociceptive information from afferent pathways, pain processing also involves descending modulatory controls which are capable of altering the original nociceptive signal. Several of these descending pathways, originating from the periaqueductal grey (PAG) of the midbrain and the nucleus raphe magnus (NRM) of the medulla, are capable of inhibiting nociceptive transmission at the level of the spinal cord (Steeds, 2013; Al-Chaer, 2009).

Despite a tendency to focus on its physical dimensions, pain is actually processed by several fundamentally different neuroanatomical substrates that respond to different qualities of the painful stimuli (Price, 2000). For example, *pain sensation* refers to the physical qualities of the pain, and provides information on the intensity and location of the stimuli. On the other hand, *pain affect* connotes the emotional distress that painful experiences can invoke and provides the motivation to engage in behaviors that reduce or eliminate this pain (Eisenberger, 2012; Price, 2000). While pain affect is often linked to pain intensity such that more intense pain is associated with more negative affect, this is not always the case. Pain affect also involves cognitive processing regarding the potential long-term consequences of pain (Price, 2000). Distinct neurological systems underlie these separate dimensions of pain and are, therefore, maximally activated by these discrete aspects of the pain experience. The physical, sensory aspects of physically painful stimuli are believed to be processed by the primary and secondary somatosensory cortex and the posterior insula. In contrast, the affective dimension of pain appears to be processed by regions of the ACC, specifically the dorsal portion of the ACC, as well as the anterior insula (AI) (Eisenberger, 2012; Peyron, Laurent, & Garcia-Larrea, 2000; Price, 2000; Rainville, 2002; Rainville, Duncan, Price, Carrier, & Bushnell, 1997).

Men typically report higher threshold and tolerance levels than do women; in other words, men are less sensitive to pain than women. In addition, women typically report greater incidences of chronic pain conditions than do men (Giles & Walker, 2000). While explanations for such sex differences have focused primarily on sociocultural expectations that bias females to report greater incidences of chronic pain conditions and baseline

sensitivity, newer research suggests that there may be an underlying biological reason for this difference (Giles & Walker, 2000). For example, there may be gender differences in response to specific aspects of the painful stimulus. Painful pressure stimuli resulting in tissue damage leads to the release of certain chemicals, such as prostaglandins, serotonin, and substance P, which are associated with the body's inflammatory response to injury and that result in increased sensitivity around the affected area (Al-Chaer, 2009; Giles & Walker, 2000). The manufacture and release of these substances is believed to be influenced by gender, suggesting that there may be sex differences in the body's immediate response to tissue damage. Furthermore, pain sensitivity may be susceptible to differences in sex hormone concentrations. There is some evidence that pain sensitivity is variable across the menstrual cycle, suggesting that fluctuating concentrations of estrogen and/or progesterone may be responsible for changes in the response to pain in women (Giles & Walker, 2000). Finally, sex differences in response to analgesic substances have also been found. These differences may be in response to the pharmacological properties of the drugs themselves. For example, men appear to exhibit greater analgesic responses to μ-opioid agonists while females are more responsive to κ-opioid agonists (Giles & Walker, 2000). The preceding evidence suggests that gender differences in pain sensitivity are more than just an artefact of sociocultural expectations but may in fact have a biological basis. Consequently, examining these gender differences is not just an essential aspect of understanding the body's response to pain, but is an interesting avenue of inquiry in itself.

1.2.1. Stress-Induced Analgesia

Under stressful conditions, there are a number of changes that affect the sensitivity and perceptual abilities of different biological systems. It has long been known that stress, both acute and chronic, can affect the functioning of the pain system. It has been shown that sustained exposure to stress can result in a hypersensitivity to painful stimulation in rats. On the other hand, acutely stressful experimental conditions, such as restraint stress or the forced swim test, produces analgesia in rats; a phenomenon known as stress-induced analgesia (Puglisi-Allegra & Oliviero, 1983; Terman, Shavit, Lewis, Cannon, & Liebeskind, 1984; Kavaliers & Innes, 1987; Schwandt, 1993). Attempts to block this stress-induced analgesia with opioid antagonists have been met with mixed success. For

example, naloxone reportedly blocks stress-induced analgesia in some experimental paradigms, but this effect is not reliably obtained, suggesting that there are likely both opioid and non-opioid mediated mechanisms contributing to stress-induced analgesia (Terman et al., 1984). In addition, stress-induced analgesia can be induced via stimuli other than physical stressors. Social isolation, in which rats are housed individually instead of in normal group housing, is capable of inducing analgesia in the form of higher nociceptive thresholds (Puglisi-Allegra & Oliviero, 1983; Kavaliers & Innes, 1987).

It has been shown that basal levels of glucocorticoids play an important role in the expression of stress-induced analgesia in rats, and may in fact act in a permissive capacity for opioid analgesia (Sutton, Fleshner, Mazzeo, Maier, & Watkins, 1994). However, stress-induced glucocorticoid secretion may also play an important role in the non-opioid form of analgesia. This is evident by the elimination of this type of stress-induced analgesia after the administration of dexamethasone, which suppresses glucocorticoid secretion, or following adrenalectomy, which eliminates it (Yarushkina, 2008). In addition, glucocorticoids may also be involved in stress-induced analgesia in humans (McEwen & Kalia, 2010). Taken together, this experimental evidence indicates that there is a relationship between stress and pain such that stimuli that evoke an acute stress reaction, and therefore a release of glucocorticoids, can reduce pain sensitivity.

1.3. Social Pain Theory

Social pain is defined as the feeling of psychological distress or ‘pain’ that results from real or potential social rejection, especially from valued relational partners (Eisenberger & Lieberman, 2004). Accordingly, the term pain to describe such an experience is more than just a metaphorical phrase, but rather describes the relationship between the systems that process both physical and emotional pain. This relationship is mediated by overlapping mechanisms in the neural circuitry for both types of pain and, as such, stimuli that activate this system have consequences for both physical pain sensitivity as well as emotional distress (Panksepp, 1998; Eisenberger & Lieberman, 2004; Eisenberger, Lieberman, & Williams, 2003). Such stimuli involve the feeling of being excluded or devalued by a relational partner or group or, alternatively, from the loss of social bonds as occurs with the death of a loved one. In other words, the stimuli that are

capable of activating the social pain system are the stimuli that frustrate the fundamental drive to belong; that is, to engage in long-term, meaningful relationships with a few other people. (Baumeister & Leary, 1995; Eisenberger, 2012). Given the importance of social bonds for health and well-being, situations that threaten these interpersonal relationships may be processed, at a basic level, as a significant adaptive threat (MacDonald & Leary, 2005). This threat may be so salient that even rejection from strangers may be capable of causing distress and social pain, perhaps because it signals a deficiency within the individual that may cause rejection from more important social relationships in the future (Baumeister & Leary, 1995). Therefore, it is reasonable to postulate that the pain that one feels after being socially rejected is an evolved adaptation, one that helps maintain necessary social engagement and signals threats to existing social relationships.

Evolutionary theory suggests that a social pain system could have been built off of a pre-existing neural pain structure (Nelson & Panksepp, 1998; Panksepp, 1998; MacDonald & Leary, 2005; Eisenberger, 2012). Due to the highly social nature of the human species as well as the aforementioned need to belong, such a system makes a certain amount of sense in terms of aiding individuals to keep track of social relations and whether or not they are being excluded. Evidence from other mammalian species suggests that group living confers significant advantages to individuals who have the social skills necessary to maintain cooperation and acceptance with other individuals. For example, the infants of socially integrated female baboons are more likely to survive the first year of life than are those infants born to less socially integrated females (Silk et al., 2009). Despite the stable nature of baboon matrilineal dominance hierarchies these results were independent of the dominance rank of the mother but were, instead, predicted by the number of social relationships she was actively engaged in. The reproductive benefits of maternal social integration are likely due to central proximity within the group during feeding and sleeping which affords protection from predators as well as the stress and conflict buffering effects that result from social alliances, particularly with kin (Silk et al., 2009). Isolation from group membership thus produces a significant disadvantage, both reproductively and in the form of reduced access to resources and greater threats to physical well-being (Baumeister & Leary, 1995). The importance of membership in groups and interpersonal relationships is also apparent in humans as seen by the negative

physical and psychological consequences of social isolation (Baumeister & Leary, 1995; Seeman, 1996).

Given the benefits of engaging in social relationships, mechanisms that motivate individuals to maintain such relationships and avoid their dissolution are likely to be adaptive. Motivation to belong begins during infancy in which the extended period of immaturity of the human species necessitates the development of a social bond through which the infant receives nourishment and care (Nelson & Panksepp, 1998). This motivation is maintained throughout adulthood, providing individuals with the various benefits of group living while attempting to maintain satisfying relationships (Baumeister & Leary, 1995). A signal that alerts the individual that their membership in a group is being threatened or revoked by interpersonal rejection will likely motivate behaviors to reduce this likelihood. The pain signal, co-opted by the social attachment system, is therefore hypothesized to serve as such a signal to alert individuals to the threat of social rejection (Nelson & Panksepp 1998; Panksepp 1998; MacDonald & Leary, 2005; Eisenberger, 2012).

While physical pain is mediated by separate sensory and affective components, it is likely that a pain signal resulting from episodes of social rejection or exclusion arises from the activation of the affective components of the pain system (MacDonald & Leary, 2005). The unpleasant, affective qualities of this activation then serve as a signal for the individual to engage in behaviors that reduce this affective unpleasantness. The neurological region associated with the processing of the affective component of pain as well as the painfully aversive experience of social rejection is the dorsal anterior cingulate cortex (dACC) (Eisenberger et al., 2003; Eisenberger & Lieberman, 2004; MacDonald & Leary, 2005; Eisenberger, 2012; Kawamoto et al., 2012). Conceptualization of such a role for this region is predicated on results that have found patterns of activation in response to social rejection within the dACC that are identical to those found in response to physically painful stimuli (Eisenberger et al., 2003). Furthermore, not only was greater dACC activity associated with greater distress upon exclusion, but exclusion was associated with the activation of a region, the RVPFC, often associated with the regulation of the distressing aspect of painful experiences. The dACC is hypothesized to act as a neural alarm system that becomes engaged in response to environmental cues that signal

the real or potential threat of social exclusion (Eisenberger & Lieberman, 2004; Eisenberger, 2012). Conceptualizing the dACC as an integral unit in the response to social rejection does not negate the common finding of a role for the dACC as a discrepancy or conflict monitor, but instead incorporates its role in error detection as part of this process (Braver, Barch, Gray, Molfese, & Snyder, 2001; Eisenberger & Lieberman, 2004; Eisenberger et al., 2003). As such, the neural alarm system proposed to be mediated by the dACC engages in discrepancy detection to determine when deviation from goals, such as social acceptance, has occurred. Once this stimulus is detected, pain affect acts as an alarm system that draws attention toward the socially rejecting stimulus in order to motivate behavior to correct the problem (Eisenberger & Lieberman, 2004; Eisenberger, 2012).

An overlapping neural system that mediates both social and physical pain has consequences beyond just common activation in response to painful situations, socially and otherwise. Instead, common activation suggests that responses to physical pain and social rejection are intimately intertwined, such that the presence of one type of stimuli should have consequences for both aspects of the system. For example, individuals who report greater baseline sensitivity to physical pain, or lower pain threshold, also experience greater distress after a socially rejecting episode (Eisenberger, Jarcho, Lieberman, & Naliboff, 2006). In addition, individuals who were more distressed after being rejected also reported greater sensitivity to a physically painful stimulus. Common regulation of both types of pain is further supported by the finding that socially mediated distress can be alleviated by a common physical pain medication. For example, individuals who ingested 1000 mg of acetaminophen every day for three weeks reported daily hurt feelings to a lesser degree than those who were given placebo (DeWall et al., 2010). In addition, participants taking acetaminophen showed less dACC and right anterior insula activation in an fMRI scan in response to rejection than those who took placebo. Such results are likely due to the central effects of acetaminophen which act to reduce neural activity associated with both social and physical pain (DeWall et al., 2010).

Despite the intuitive expectation that sensitivity to pain increases after social rejection, the opposite, seemingly paradoxical result is sometimes the case. Participants led to believe that a personality test predicted that they would lead a lonely life actually

experienced higher pain thresholds and a lower sensitivity to pain. They also experienced an emotional numbness and a significant decrease in their ability to empathize with others (DeWall & Baumeister, 2006). This result was replicated in a social rejection manipulation that was not as severe as a life lived alone, and was more in line with the kind of social rejection people experience in everyday life (Borsook & MacDonald, 2010). Both physical pain and social exclusion elicit stress in humans, and therefore activate the hypothalamic-pituitary-adrenal (HPA) axis. In other words, pain resulting from physical stimulation as well as social exclusion, results in a cascade of hormones culminating in the release of cortisol (Dickerson & Kemeny, 2004). This release of cortisol may be, at least partially, responsible for the stress-induced analgesia reported after periods of social rejection. This theory is supported by the finding that adrenalectomy in rats and the obstruction of HPA axis function in humans eliminates this stress-induced analgesia (Yarushkina, 2008). A role for cortisol in acutely reducing pain sensitivity seems intuitive in light of the hormone's role in the HPA axis response to stress. Such reduced pain sensitivity would be adaptive to effectively deal with a potential threat without the distracting and debilitating element of pain. In addition to reducing physical pain, the intimate connections between the physical and social pain systems are such that the pain reducing effects of cortisol should also reduce social pain as well.

The picture, however, becomes further complicated when taking gender differences into account. As mentioned previously, men and women differ with regard to their sensitivity towards pain as well as the frequency with which they are diagnosed with chronic pain conditions (Giles & Walker, 2000). In addition, there are also differences in the way that men and women respond to stressful stimuli. Given that females may have a greater, evolutionarily prescribed tendency to seek interpersonal support when faced with a threat, the loss of such relationships may have significant consequences for their fitness (Taylor et al., 2000; Stroud et al., 2002). Therefore, a biological stress response prompted by ostracism or social rejection may be expected in females to signal a threat to their survival. This may manifest itself in everyday life as a greater response of the HPA axis to signals of interpersonal rejection in females. Furthermore, given the ability of glucocorticoids to influence pain sensitivity as well as the connections between the physical and emotional pain systems, as described by the social pain theory, we may

expect that females will become more insensitive to physically painful stimuli after experiencing social rejection.

1.4. Hypotheses

The preceding evidence suggests that in addition to creating unpleasant emotional experiences that most people would like to avoid, social rejection may also activate psychophysiological pain mechanisms in ways that parallel pain from physical trauma. As a result of the relationship between these two types of pain, as well as their underlying ultimate explanations, several predictions can be made regarding the effects of social rejection on the physical pain system. Firstly, it is expected that research participants exposed to experimental social rejection will experience an increase in cortisol as opposed to subjects in a social acceptance condition. Secondly, it is also anticipated that participants in the rejection condition will experience increases in pain tolerance and threshold measurements from baseline to post-manipulation compared to those in the acceptance condition. Thirdly, it is predicted that social rejection will result in greater increases in cortisol as well as pain threshold and tolerance in females rather than males. Finally, it is expected that changes in cortisol as a result of the manipulation will be predictive of differences in post-manipulation pain threshold and pain tolerance.

Chapter 2.

Methods

2.1. Participants

Participants for this experiment were drawn from the undergraduate student population of Simon Fraser University. They were primarily recruited through the Psychology Department's Research Participation System (RPS) and received 2 credits towards their psychology courses for their participation. A small subset of participants (2 female, 6 male) were recruited through the use of an advertisement for the experiment which required them to contact the experimenter with their interest. These participants were paid $10 at the completion of their participation. There was, initially, a total of 101 participants in this experiment. However, 8 participants expressed suspicion regarding the validity of the social interaction with the confederate. The data from these participants were dropped from all analyses, therefore leaving a total of 93 participants (males= 46, females= 47). Participants ranged in age from 17 to 27, with a mean age= 19.7. The sample was 49.5% Asian, 22% South Asian, 19.8% Caucasian, 5.5% mixed race, and 3.3% Middle Eastern. Approximately half (46.2%) had graduated high school, while 48.4% had completed at least one year of university, 4.3 % had graduated college or university, and 1.1% was unspecified.

2.2. Protocol

Upon arrival participants were given the consent form (Appendix A) to read and sign. Next, an initial saliva sample (2-3 ml) was collected; using a passive drool procedure, in order to establish a baseline measurement of cortisol and testosterone levels. Baseline measurements of participants' pain threshold and tolerance was then assessed by using a pressure algometer, as described below. Next, the participant completed several psychological measures including the Positive and Negative Affect Schedule (PANAS) as a baseline measurement of their mood (Watson, Clark, & Tellegen, 1988; Appendix C),

The Rosenberg Self-Esteem Scale (Rosenberg, 1965; Appendix D), and the Perceived Stress Scale-14 item (Cohen, Kamarck, & Mermelstein, 1983; Appendix E). Following these measures, participants engaged in a modified version of the Relationship Closeness Induction Task (Sedikides, Campbell, Reeder, & Elliot, 1999; Appendix F; described below) as adapted by Borsook and MacDonald (2010). Following this task, the participants were given a post-manipulation battery of measures including an attribution questionnaire to check for participants that may be suspicious about the manipulation (Appendix G), a second PANAS, a demographics form (Appendix H), and the Interpersonal Reactivity Index (Davis, 1983; Appendix I). A second saliva sample was then taken (2-3ml) as well as a second measure of pain threshold and tolerance using the same procedure as above. Participants were then given a debriefing form (Appendix B) to read and sign before being thanked and dismissed.

2.3. Pressure Algometer

The pressure algometer is a handheld device, whose 1cm ^{2} round rubber pad is held perpendicularly on the dorsal interosseous muscle of the participants' middle finger. The rubber pad is then lowered onto the finger at a constant rate. The rubber pad is soft, causing only mild pain and no lasting effects or tissue damage (Kinser, Sands, & Stone, 2009; Borsook & MacDonald, 2010). In the first trial, participants were instructed to indicate when they first experienced pain by saying "now". The algometer was then retracted immediately and the pressure readout recorded as the participants' pain threshold. In the next trial, participants were instructed to indicate, by saying "stop", when the pain became too uncomfortable. At this point the pressure algometer was again retracted by the experimenter and the readout recorded as their pain tolerance. The two trials measuring threshold and tolerance were separated by a 90 second interval in order to control for habituation to the pain stimulus as recommended by De Wall & Baumeister (2006) and Bernstein & Claypool (2012).

2.4. Relationship Closeness Induction Task

Participants engaged in a social interaction with another person whom they believed was another undergraduate student taking part in the same experiment. This other 'participant' was, in fact, a confederate with explicit instructions on how to engage the participant in conversation throughout the task. To keep the gender of the interaction partner consistent, the confederate was always a male student from the psychology undergraduate population of SFU. There were four different confederates used over the length of the study. The task itself consisted of a list of questions that are designed to engage the participant in a conversation that involved divulging personal information about themselves to a stranger (Sedikides et al., 1999). The experimenter instructed the participants to take turns asking each other questions from this list with the ostensible purpose of getting to know each other. The experimenter then explained that she had flipped a coin to determine which 'participant' would begin each round of questions, however, the experimenter always assigned the confederate to this first position. The questions themselves were designed to become increasingly more intimate in order to get participants to divulge more than just surface information about themselves. This task was allowed to continue for 10 minutes at which point the experimenter interrupted the conversation and separated the participant and confederate.

2.4.1. Experimental Conditions

Confederates randomly assigned participants to a social acceptance or social rejection condition before meeting them and commencing the Relationship Closeness Induction Task. They did this by flipping a coin. Due to the fact that the confederate would have to know the participant's condition in order to behave appropriately during the task, and in the effort to keep the experimenter blind to condition, the confederate was tasked with assigning participants to their respective conditions. In the acceptance condition, the confederate used verbal and non-verbal tactics to display warmth and interest in the participant. These included smiling, nodding in response to the participant's answers, having an open and relaxed body posture, and responding in a positive way that involved agreeing with what the participant was saying and relating it to things in their own lives. The purpose of this condition was to make participants feel as though they are having a

positive interaction with another student and that this person liked and accepted them. In the rejection condition, the confederate used verbal and non-verbal methods to act in a standoffish and disinterested manner. These tactics included having a closed posture, avoiding eye contact and smiling, and responding with one word answers or showing disagreement with the participant's responses. Unlike the acceptance condition, this condition was designed to make the participant feel as though they were unliked and were being rejected by the other 'participant'.

At the end of this task, after the participant and confederate had been separated, the participant filled out a questionnaire (Appendix J) asking them to rate the social desirability and competence of the person they just interacted with. The participant was told that the other 'participant' was filling out the same questionnaire about themselves. They were also told that they would get to review each other's questionnaires, but that they would have no further contact with the other person, therefore they should try and be as honest as possible when answering questions about the other 'participant'. After finishing this questionnaire, the participant received the questionnaire they believed was completed by the other 'participant'. In reality, this questionnaire was pre-completed to match the condition they were in, such that participants in the acceptance condition received a questionnaire that confirmed the other 'participant' liked and accepted them while those in the rejection condition received the opposite. This Bogus Social Competence Scale was designed to reinforce the condition that the participants were initially placed in for the Relationship Closeness Induction Task.

2.5. Cortisol and Testosterone

Saliva used for hormonal analyses was collected via a non-invasive passive drool procedure in a plastic tube. For each sample, 2-3 ml of saliva was collected for 4-6 ml of saliva in total. Participants were instructed to avoid eating or drinking anything other than water and to avoid smoking for one hour prior to the beginning of the experiment in order to avoid contaminating the samples. The experimenter instructed the participant to allow the saliva to passively pool in their mouth before depositing the saliva into a plastic tube. One sample was taken at the beginning of the experiment to serve as a baseline measure of cortisol and testosterone concentrations. A second sample was taken 20 minutes after

the social acceptance/rejection manipulation to determine changes as a result of this interaction. Immediately after each sample was given, they were stored in a freezer at -20 °C. At a later time, samples were removed from the freezer, defrosted, and assayed using the enzyme-linked immunosorbent assay (ELISA) method (Engvall & Perlmann, 1972).

2.5.1. ELISA

ELISA is a competitive immunoassay technique designed to measure concentrations of substances, such as hormones, present in bodily tissues and fluids like serum and saliva. Through the use of antibodies against the analyte, or substance of interest, the concentration of this substance within a particular sample can be obtained. In this case, the ELISA technique was used to measure the presence of cortisol and testosterone in saliva samples. Cortisol and testosterone enter the saliva from the bloodstream in an unbound form, in concentrations unaffected by salivary flow rate. Thus, providing a reliable and consistent approximation of unbound cortisol and testosterone in serum. These hormones do not rapidly degrade in samples that are repeatedly thawed and frozen, making them ideal candidates for examination using the ELISA method. The principle behind this method involves the use of antibodies to cortisol and testosterone that are present within each well of a 96 well microtitre plate. All methods and materials for conducting the ELISA were obtained from Salimetrics, Inc (State College PA).

Samples were removed from the freezer on the day of analysis and allowed to thaw for 3 hours. All reagents as well as the microtitre plate were also allowed to thaw for up to 1.5 hours. Once defrosted, saliva samples were centrifuged for 15 minutes at 3000 rpm. This allowed the mucins in the saliva to separate and form a pellet of precipitate at the bottom of the tube. All standards, unknowns, as well as high and low controls were then transferred onto the active microtitre plate in a volume of 25 µl per well using a single channel pipette. Standards are samples with known concentrations of the analyte that are used to construct a calibration curve against which the concentrations of unknown samples, those obtained from participants, can be obtained. Controls are samples with high and low concentration ranges of the analyte that are used to calculate plate-to-plate consistency. Each sample was assayed in duplicate to allow for the calculation of an intra-assay coefficient of variation (CV). For cortisol, a 1:1600 dilution of the conjugate, which

contains cortisol linked to horseradish peroxidase, was created by adding 15 µl of the conjugate to 24 ml of assay diluent. 200 µl of this diluted conjugate was then added to each well of the microtitre plate using a multichannel pipette. For testosterone, a 1:1000 dilution of the conjugate was created by adding 19µl to 19 mL of assay diluent and adding 150 µl of the solution to each well. The plate was then mixed on a plate rotator at 500 rpm for 5 minutes at which time it was left to incubate at room temperature for 55 minutes. During this incubation period the hormone present within each sample competes with that present in the diluted conjugate for binding sites to the antibodies.

After this incubation period, the plate was washed in order to remove any unbound hormone and 200 µl of tetramethylbenzidine (TMB) was added to each well using a multichannel pipette. The plate was then rotated for 5 minutes at 500 rpm and then incubated, in the dark at room temperature, for an additional 25 minutes. TMB is a colorimetric substrate that reacts with the horseradish peroxidase within the conjugate to produce a blue color. The degree of color change after the addition of TMB depends upon the concentration of conjugate bound within each well. This concentration is, in turn, inversely proportional to the amount of hormone present within each sample. If the sample contained high concentrations of hormone, a relatively low proportion of the conjugate would be able to bind to the antibodies and most would be removed when the plate was washed. The TMB would have less conjugate to react with and the intensity of the color change would be weak. If the concentration of hormone in the sample was low, more conjugate would be bound and the intensity of the color change would be high. Therefore, the intensity of the color change is inversely proportional to the amount of hormone present within the sample. The reaction of TMB with the conjugate was stopped after the incubation period with the addition of 50 µl of 2M stop solution (sulphuric acid) to each well with a multichannel pipette. The plate was again mixed on a plate rotator for 3 minutes at 500 rpm at which time a color change from blue to yellow occurs in each well. The optical density of this color was then determined by reading the plate in a plate spectrophotometer.

Hormone concentration within each sample was determined by comparing obtained optical density values to a standard curve developed using densities of several known concentrations of the analyte in adjacent wells. Therefore, proper specification of

hormone concentrations depends upon the proper calibration of this standard curve. The coefficient of determination provides a measurement of this calibration by determining how well the standards map onto a theoretical curve. An R^2 value greater than or equal to 0.99 is typically considered an acceptable coefficient of determination. Reliability of the results of an ELISA are also determined by the values of the inter- and intra-assay coefficients of variability (CV). Coefficients of variability are dimensionless numbers that are obtained by dividing the standard deviation by the mean of a set of concentrations obtained from running an ELISA. In studies in which multiple plates of hormones are analyzed, the inter-assay CV provides a measurement for the degree of consistency between plates. The inter-assay CV is determined by the mean of the high and low controls that are provided on each plate. These high and low controls, therefore, must be contained within the range provided by the Salimetric's kit. An inter-assay CV of less than 15% is generally considered an acceptable value. The intra-assay CV represents the degree to which the duplicate measurements of each sample differ from each other and a value of less than 10% is generally acceptable.

2.6. Questionnaire Data

2.6.1. Positive and Negative Affect

For this experiment, positive and negative affect was measured using the PANAS scale (Watson et al., 1988). The scale consists of 20 emotion words with which the participant indicated the extent to which they are feeling on a 5-point Likert scale ranging from very slightly or not at all to extremely. Participants completed this scale twice, once at the beginning of the experiment to establish their baseline mood and once after the manipulation to quantify mood change.

2.6.2. Self-Esteem

Self-esteem in this experiment was measured using the Rosenberg Self-Esteem Scale (Rosenberg, 1965). This is a ten-item scale that is designed to determine how participants feel about themselves in terms of general self-esteem. Each item is ranked using a 4-point Likert scale ranging from strongly agree to strongly disagree.

2.6.3. Chronic Stress

The scale used to measure chronic stress in this experiment was the Perceived Stress Scale-14 item (Cohen et al., 1983). The PSS-14 is a 14 item self-report scale that is designed to determine the amount of stress the participant perceives they have been under for the past month. Each item is ranked with a 5-point Likert scale with answers ranging from never to very often.

2.6.4. Attributions

The participants' perceptions of the experiment were probed using an attribution questionnaire. The purpose of this questionnaire was to provide a check for any participants who may have harboured suspicions about the goals of the study or the legitimacy of the social interaction involved in the relationship closeness induction task. This questionnaire contained a question about whether the participant would consider working with the other 'participant' on a future hypothetical task, to which they could simply answer yes or no. The questionnaire also contained an open-ended request for feedback about the experiment, to which the participant was asked to provide two to three sentences.

2.6.5. Empathy

The Interpersonal Reactivity Index (Davis, 1983) was used to measure empathy. The IRI is a 28-item scale that requires the participants to read statements and indicate the extent to which that statement appropriately describes them. Answers come from a 5-point Likert scale ranging from does not describe me well to describes me very well.

2.6.6. Social Competence

The social competence scale was used to assess the degree to which the participant liked the confederate. This scale requires participants to read a number of statements regarding the confederate and indicate the extent to which they agree or disagree with these statements. The scale consists of 4 questions to which the participant could provide answers from a 5-point Likert scale ranging from disagree to agree.

Chapter 3.

Results

3.1. Statistical Analysis

All statistical analyses were conducted using SPSS (Version 19). Statistical significance, or the criterion for whether to reject the null hypotheses, was set to an α of 0.05. With the exception of the examples listed below, all variables were normally distributed as defined by a p value greater than 0.05 ($p > 0.05$) on the Shapiro-Wilk Test of normality and visual inspection of QQ plots. Outliers, defined as any value ± 3 standard deviations from the mean, were removed from the analysis (Gordis, Granger, Susman, & Trickett, 2006).

1. Post-Manipulation Pain Threshold: One participant had a value greater than 3 standard deviations from the mean. Therefore, this value was excluded from the analysis

2. Both the pre-manipulation and post-manipulation negative affect variables were non-normal ($p<0.05$). This may be a result of a floor effect, perhaps due to a tendency to misreport negative affect because of perceived undesirability. Despite attempts to transform in order to obtain normality, this variable remained very non-normal ($p<0.05$) As a result, these variables were assessed using non-parametric tests.

3. The chronic stress variable indicated non-normality ($p<0.05$). It was transformed using a square root transformation after which the variable was normally distributed. A number of participants ($n=4$) failed to complete the entire chronic stress measure. The missing portions were all greater than 25% percent of the scale, therefore, these values were removed listwise from the analysis.

4. The social competence scale displayed non-normality ($p<0.05$). Again, this is likely due to a ceiling effect that may be due to hesitation to assess another person too negatively. Despite attempts to transform in order to obtain normality, this variable also remained non-normal ($p<0.05$). Therefore, this variable was also assessed using non-parametric tests.

5. Both pre-manipulation and post-manipulation cortisol measures were non-normal ($p<0.05$). Cortisol values were log-transformed and used in all subsequent analyses.

6. Both pre-manipulation and post-manipulation testosterone measures were non-normal ($p<0.05$). Testosterone values were log-transformed and used in all subsequent analyses.

3.2. Pain Threshold

The first set of tests was used to determine whether there was any influence of the experimental manipulation or of gender on pain threshold. First, a two-way ANOVA was conducted to determine whether there were any statistically significant differences between conditions and genders for pre-manipulation pain threshold. Next, a three-way mixed ANOVA with repeated measures was conducted, with condition and gender as the between-subject factors and time (pre-and post-manipulation pain threshold) as the within subject factor. This analysis was used to determine whether there were any statistically significant changes in pain threshold from pre- to post-manipulation. Two values of post-manipulation pain threshold were outliers, therefore their data were removed from the analysis

1. Pre-Manipulation Pain Threshold: There were no statistically significant differences in pre-manipulation pain threshold between the acceptance and rejection conditions [$F(1, 89) = 0.2$, $p=0.664$], between males and females [$F(1, 89) = 1.8$, $p=0.181$, or the interaction between condition and gender [$F(1, 89) = 3.2$, $p=0.079$].

2. Change in Pain Threshold: The three-way interaction between condition, gender, and time approached significance [$F(1, 88) = 3.3$, $p=0.088$]. There was a statistically significant two-way interaction between condition and gender [$F(1, 87) = 4.3$, $p<0.05$; Figure 1]. All other two-way interactions were not statistically significant. Statistical significance of the simple main effect was accepted at a Bonferroni-adjusted alpha level of .025. There was a statistically significant simple main effect of gender in the acceptance condition [$F(1, 87) = 12.1$, $p< 0.05$, Male=15.3, Female=10.6], but not in the rejection condition [$F(1, 87) = 0.1$, $p=0.763$, Male=13.2, Female=12.7]. There was also a statistically significant main effect of time on pain threshold [$F(1, 87) = 10.5$, $p<0.05$, T1= 13.4, T2=12.4; Figure 2].

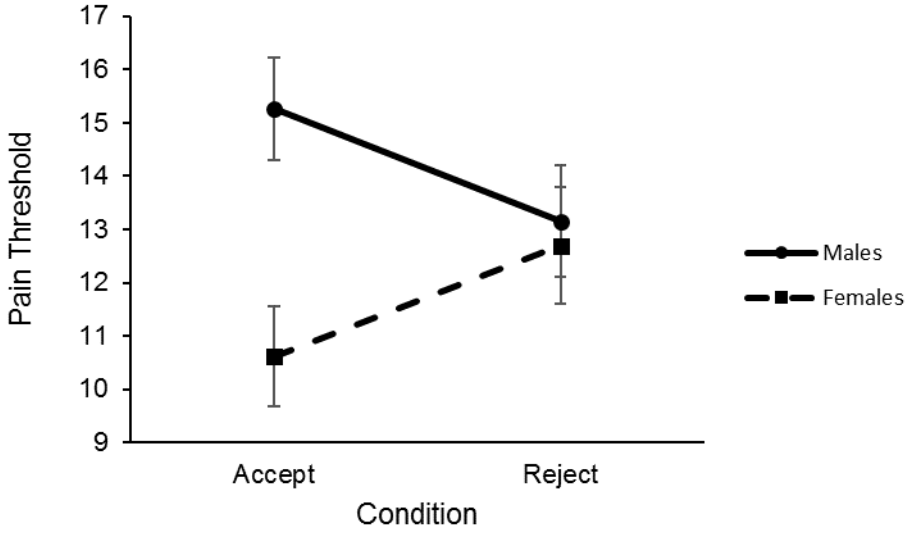

Figure 1 Gender differences in pain threshold between conditions

Note. Gender difference in acceptance condition significant at $p<0.05$

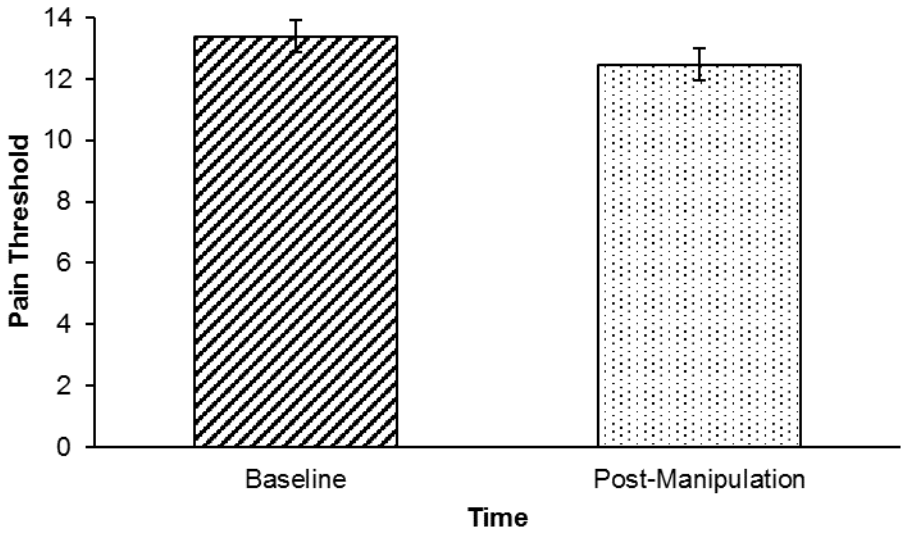

Figure 2 Difference in pain threshold from baseline to post-manipulation

Note. Significant at $p<0.05$

3.3. Pain Tolerance

The next set of tests was used to determine whether there was any influence of the experimental manipulation or of gender on pain tolerance. A two-way ANOVA was again conducted to determine whether there were any statistically significant differences between males and females and between the acceptance and rejection conditions on pre-manipulation pain tolerance. Next, a three-way ANOVA with repeated measures with condition and gender as the between-subjects factor and time (pre-and-post-manipulation pain tolerance) as the within-subjects factor, was conducted. Data for females in the acceptance condition were distributed non-normally for both the pre-manipulation ($p=0.013$) and post-manipulation ($p=0.027$) measures, but given the normality of the data in the rest of the groups and the robustness of the procedure, this was viewed as a relatively minor issue.

1. Pre-Manipulation Pain Tolerance: There were no statistically significant differences in pre-manipulation pain tolerance between the acceptance and rejection conditions [$F(1, 89) = 0.3$, $p=0.582$] or between males and females [$F(1, 89) = 3.9$, $p=0.052$] although this difference did approach significance. The interaction between condition and gender was also not significant [$F(1, 89) = 3.4$, $p=0.067$].

2. Change in Pain Tolerance: There was no statistically significant three-way interaction between time, gender, and condition. There was a statistically significant two-way interaction between condition and gender, [$F(1,89) = 4.3$, $p=0.041$; Figure 3]. All other two-way interactions are not statistically significant. Statistical significance of the simple main effect was accepted at a Bonferroni-adjusted alpha level of .025. There was a statistically significant simple main effect of gender in the acceptance condition [$F(1, 89) = 10.2$, $p= 0.002$, Male=22.2, Female=16.0] but not in the rejection condition [$F(1, 89) = 0.04$, $p=0.975$, Male=19.6, Female=19.6]. There was also a statistically significant main effect of time on pain tolerance [$F(1, 87)=16.4$, $p<0.05$, T1=20.0, T2= 18.7; Figure 4].

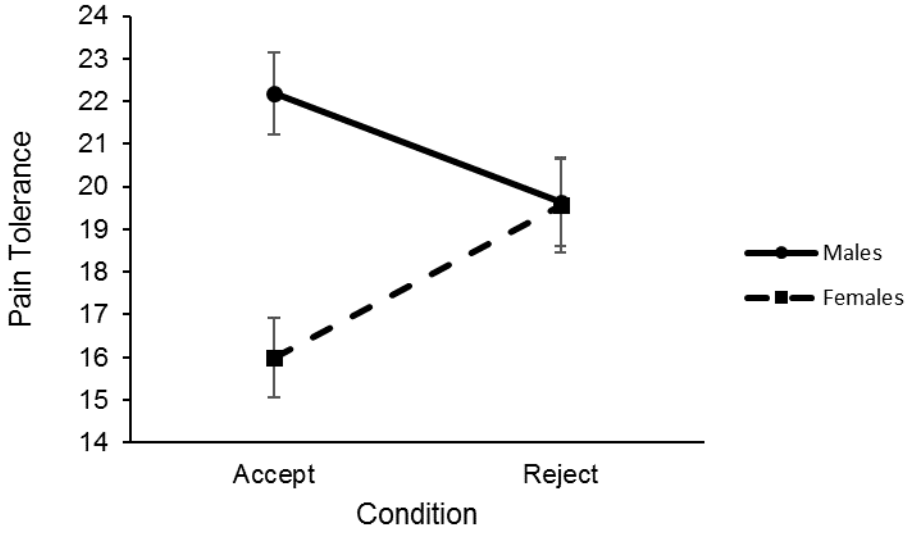

Figure 3 Gender differences in pain tolerance between conditions

Note. Gender difference in acceptance condition significant at $p<0.05$

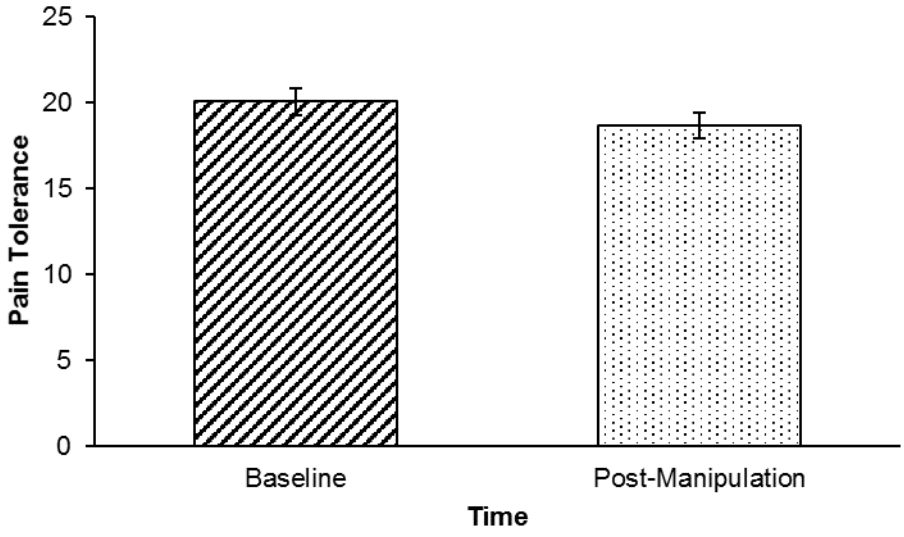

Figure 4 Difference in pain tolerance between baseline and post-manipulation

Note. Significant at $p<0.05$

3.4. Hormones

3.4.1. Cortisol

The next set of tests was used to determine whether there was any influence of gender or condition on cortisol. The inter-assay CV for cortisol was 3.30% and the intra-assay CV was 3.78%. First a two-way ANOVA, with condition and gender as the factors, was conducted to determine whether there were any statistically significant differences between conditions and gender for baseline cortisol. Next, a two-way ANCOVA was conducted on post-manipulation cortisol after controlling for baseline cortisol (baseline cortisol was included as a covariate in the model). The assumption of normality was violated for males in the acceptance group ($p=0.005$). Analysis was conducted anyway due to the presence of normality in the rest of the groups and the robustness of the procedure.

1. Baseline Cortisol: There were no statistically significant differences in baseline cortisol between gender [$F(1, 88) = 1.5$, $p=0.228$] or condition [$F(1, 88) = 3.7$, $p=0.058$], although condition did approach significance. There was also no statistically significant interaction between condition and gender on baseline cortisol [$F(1, 88) = 0.04$, $p=0.836$].

2. Post-Manipulation Cortisol: After adjustment for baseline cortisol, there were no statistically significant differences in post-manipulation cortisol between the acceptance and rejection conditions [$F(1,87) = 0.2$, $p=0.619$] or between males and females [$F(1, 87) = 0.0$, $p=0.982$]. There was also no statistically significant interaction between condition and gender on post-manipulation cortisol [$F(1, 87) = 0.9$, $p=0.357$].

3.4.2. Testosterone

The next set of tests was used to determine whether there was any influence of condition or gender on testosterone. The inter-assay CV for testosterone was 10.06% and the intra-assay CV was 5.69%. First, a two-way ANOVA was conducted to determine whether there were any statistically significant differences between conditions and genders for baseline testosterone. Next, a one-way ANCOVA, with condition as the factor, was conducted on post-manipulation testosterone after controlling for baseline testosterone (baseline testosterone was included as a covariate in the model). This

analysis was split by gender because of the significant difference between males and females in baseline testosterone.

1. Baseline Testosterone: There were no statistically significant differences between the acceptance and rejection conditions in baseline testosterone [F (1, 89) =0.2, p=0.679]. Males had statistically significantly higher baseline testosterone levels than females [F (1, 89) =159.5, p<0.05; Figure 5]. There were no statistically significant differences in the interaction between condition and gender [F (1, 89) =0.02, p=0.881]

2. Post-Manipulation Testosterone: Males in the acceptance condition had statistically significantly higher post-manipulation testosterone levels than males in the rejection condition [F (1, 43) =4.2, p<0.05; Figure 6]. Females in the acceptance and rejection conditions did not differ in post-manipulation testosterone [F (1, 44) =0.8, p=0.370].

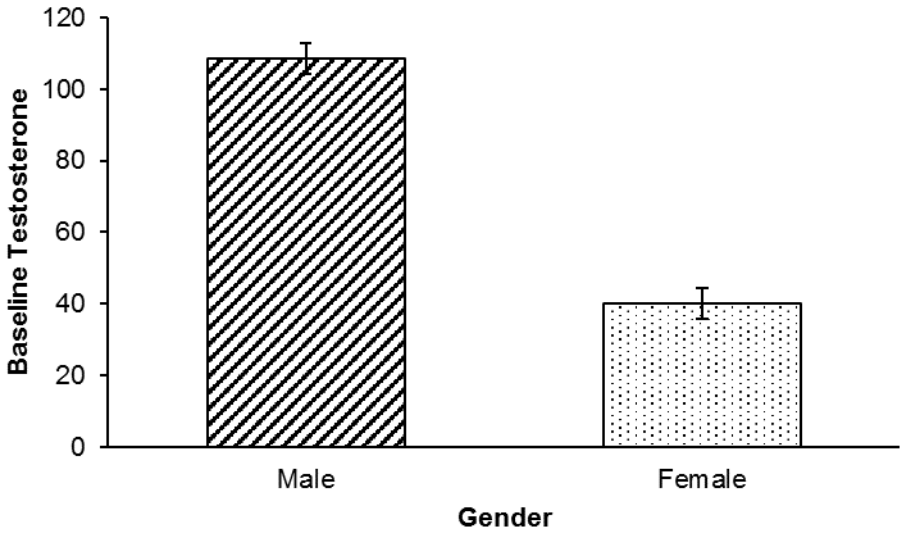

Figure 5 Mean gender differences in baseline testosterone

Note. Significant at p<0.05

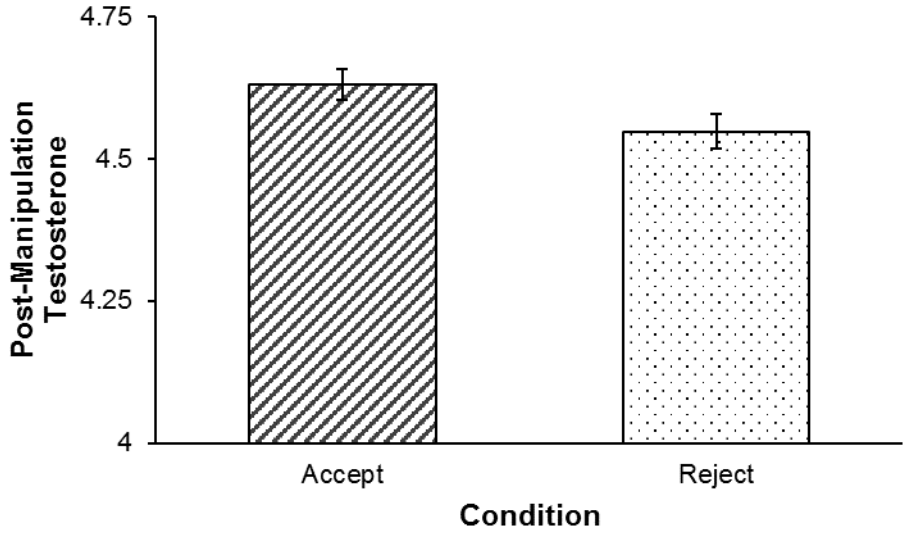

Figure 6 **Mean difference in post-manipulation testosterone for males controlling for baseline testosterone**

Note. Significant at $p<0.05$

3.5. Psychological Variables

3.5.1. Social Competence

A non-parametric test was used to determine whether judgments of the social competence of the confederate differed between the acceptance and rejection conditions. The distributions of the social competence scores for the acceptance and rejection conditions were not similar, as assessed by visual inspection. Social competence scores for the acceptance (mean rank=59.4) condition were statistically significantly higher than the rejection condition (mean rank=30.6), $U=405.5$, $z=5.1$, $p<0.05$.

3.5.2. Mood

The next set of tests was used to determine whether post-manipulation positive and negative affect differed between the acceptance and rejection conditions. First, a one-way ANCOVA was conducted on post-manipulation positive affect with baseline positive affect entered as a covariate. One participant had a positive affect score of greater than 3

standard deviations from the mean, therefore this value was removed from the analysis. Next, non-parametric tests were used to determine whether there were any statistically significant differences between conditions on post-manipulation negative affect.

1. Post-Manipulation Positive Affect: After controlling for baseline positive affect, post-manipulation positive affect was higher in the acceptance condition than in the rejection condition [F (1, 90) =21.7, p<0.05; Figure 7].

2. Post-Manipulation Negative Affect: Median negative affect was statistically significantly different between the acceptance (11.0) and rejection (13.5) conditions [U=1432, z=2.9, p=0.003; Figure 8].

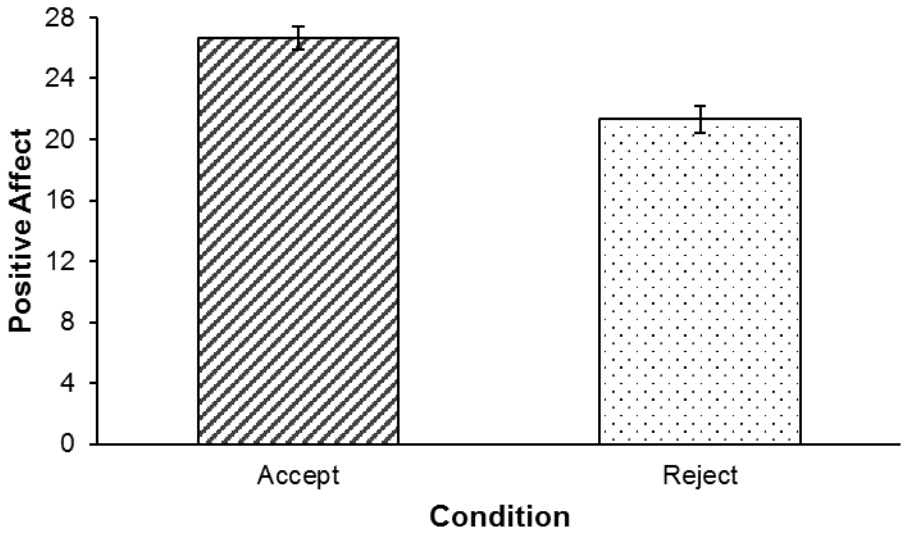

Figure 7 Mean difference in post-manipulation positive affect controlling for baseline positive affect

Note. Significant at p<0.05

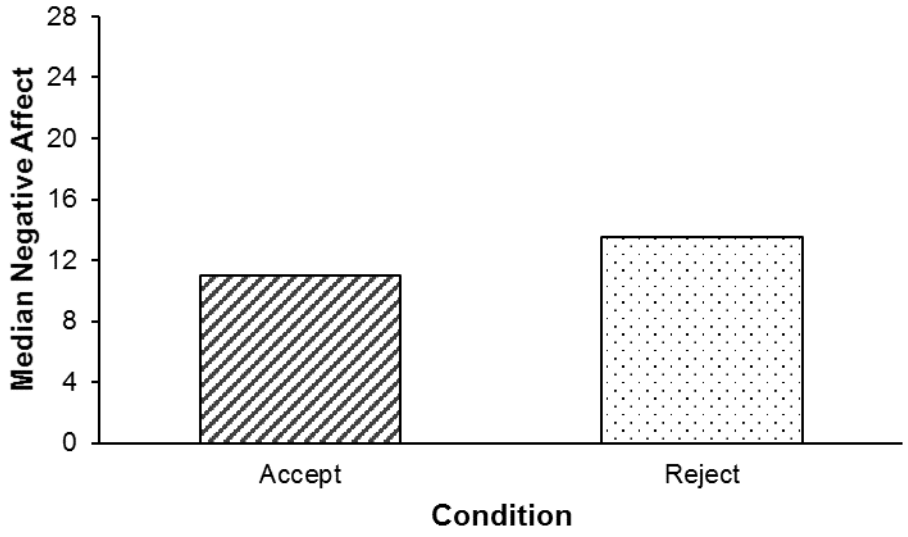

Figure 8 **Median difference in post-manipulation negative affect between conditions**

Note. Significant at $p<0.05$

3.5.3. Empathy

An independent samples T-test was conducted to determine whether there were differences in empathy between males and females. Females had higher levels of empathy (71 ± 12.703) than males (64 ± 12.803), a statistically significant difference [$t(91) = -2.6$, $p<0.05$; Figure 9].

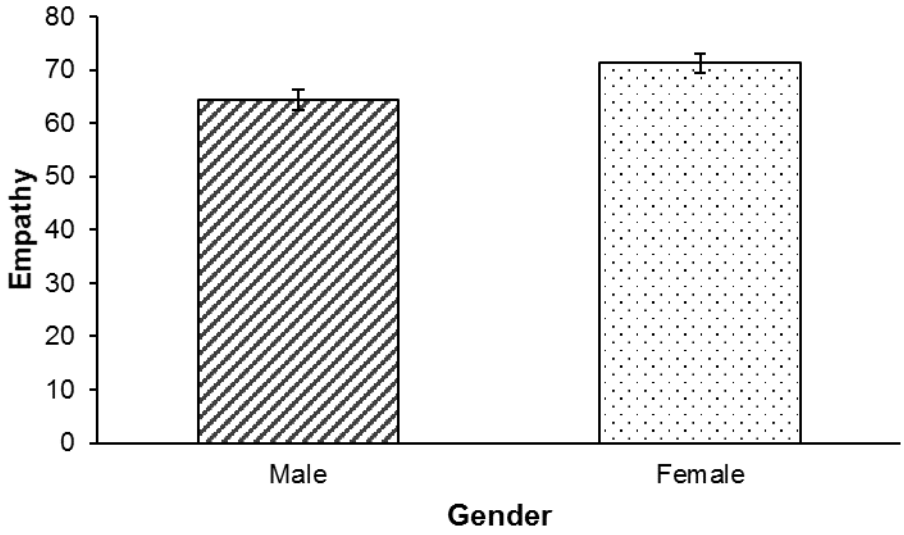

Figure 9 Mean gender differences in empathy

Note. Significant at $p<0.05$

3.5.4. Chronic Stress

An independent samples T-test was conducted to determine whether there were gender differences in the amount of stress experienced over the previous month. Females had higher levels of chronic stress (5.3 ± 0.606) than males (5.1 ± 0.426), a statistically significant difference [$t(87) = -2.1$, $p<0.05$; Figure 10].

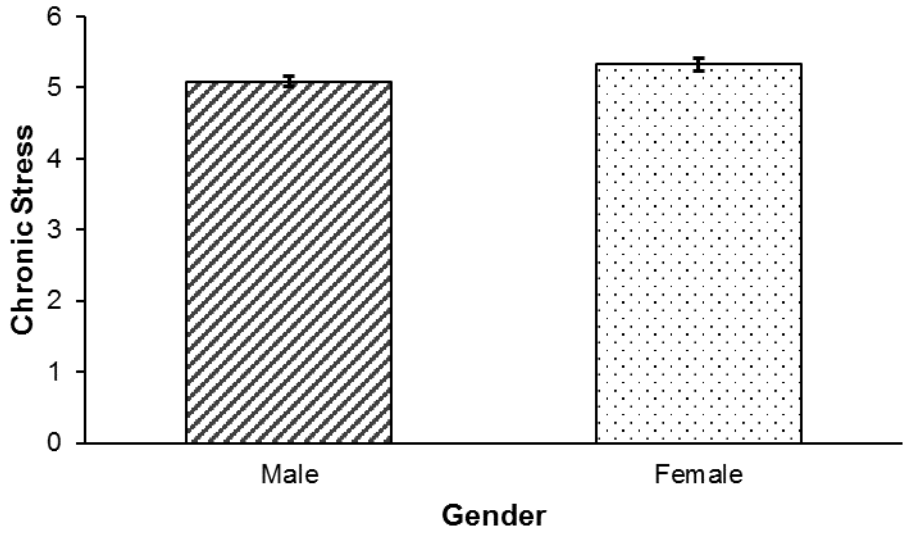

Figure 10 Mean gender differences in chronic stress

Note. Significant at $p<0.05$

3.5.5. Self-Esteem

An independent samples T-test was conducted to determine whether there were differences in self-esteem between males and females. There were no statistically significant differences between males (19.4 ± 4.192) and females (18.8 ± 5.329) in self-esteem [t (91) =0.6, p=0.532].

3.6. Baseline Hormones and Pain

A series of hierarchical multiple regression analyses were performed to determine the effect of baseline cortisol and testosterone concentrations on post-manipulation pain threshold and tolerance. Each analysis was separated by condition, such that baseline hormonal profiles were examined separately for the acceptance and rejection conditions. In examining both pain threshold and tolerance, centered baseline cortisol and testosterone were entered in the first step. The interaction between baseline cortisol and testosterone was then entered in the second step.

3.6.1. Pain Threshold

The full model of baseline cortisol, testosterone, and the CxT interaction was not significant for either the rejection (R^2=0.006, F (3, 36) =0.1, p=0.973; adj R^2=-0.077) or acceptance conditions (R^2=0.153, F (3, 46) =2.8, p=0.052; adj R^2=0.098), although the acceptance condition approached significance. Baseline testosterone was found to be a significant predictor of post-manipulation pain threshold in the acceptance (β=0.389, p<0.05) but not rejection (β=-0.054, p=0.769) condition.

Including only baseline cortisol and testosterone in the model was also not significant for the rejection condition (R^2=0.005, F (2, 37) =0.1, p=0.905; adj R^2=-0.048), but was significant for the acceptance condition (R^2=0.153, F (2, 47) =4.2, p<0.05; adj R^2=0.117). Again, baseline testosterone was found to be a significant predictor of post-manipulation pain threshold in the acceptance (β=0.386, p<0.05) but not the rejection condition (β=-0.046, p=0.793).

3.6.2. Pain Tolerance

The full model of baseline cortisol, testosterone, and the CxT interaction for predicting pain tolerance was not significant for the rejection condition (R^2=0.019, F (3, 36) =0.2, p=0.870; adj R^2=-0.062) but was significant for the acceptance condition (R^2=0.167, F (3, 48) =3.2, p<0.05; adj R^2=0.115). Again, baseline testosterone was a significant predictor for the socially accepted group (β=0.425, p<0.05) but not the rejected group (β=-0.104, p=0.570).

The model for the rejection condition containing only cortisol and testosterone was not significant (R^2=0.014, F (2, 37) =0.3, p=0.769; adj R^2=-0.039), but the model for the acceptance condition was significant (R^2=0.165, F (2, 49) =4.9, p<0.05; adj R^2=0.131). Baseline testosterone was again a significant predictor in the acceptance condition (β=0.433, p<0.05) but not the rejection condition (β=-0.126, p=0.475).

3.7. Hormone Fluctuations and Pain

Another series of hierarchical regression analyses were conducted to determine whether hormonal fluctuations resulting from the socially accepting or rejecting manipulation would affect pain threshold and tolerance. As above, each analysis was separated by condition. Centered post-manipulation cortisol and testosterone were entered into the first step of the model. Next, the CxT interaction was entered into the next step.

3.7.1. Pain Threshold

The full model of post-manipulation cortisol, testosterone and the CxT interaction in predicting pain threshold was not significant for the rejection condition (R^2=0.071, F (3, 36) =0.9, p=0.442; adj R^2=-0.006) but was significant for the acceptance condition (R^2=0.178, F (3, 47) =3.4, p<0.05; adj R^2=0.125). Both post-manipulation testosterone (β=0.418, p<0.05) and cortisol (β=-0.288, p<0.05) were significant predictors of pain threshold in the acceptance condition, however the CxT interaction was not significant (β=-0.025, p=0.856). Neither testosterone (β=-0.157, p=0.388), cortisol (β=0.269, p=0.163), nor the CxT interaction (β=0.207, p=0.232) were significant predictors in the rejection condition.

The model containing only cortisol and testosterone was also not significant in the rejection condition (R^2=0.033, F (2, 37) =0.6, p=0.539; adj R^2=-0.019) but was in the acceptance condition (R^2=0.177, F (2, 48) =5.2, p<0.05; adj R^2=0.143). Again, in the acceptance condition, testosterone (β=0.425, p<0.05) and cortisol (β=-0.290, p<0.05) were significant predictors. Neither testosterone (β=-0.132, p=0.468) nor cortisol (β=0.195, p=0.286) were significant predictors in the rejection condition.

3.7.2. Pain Tolerance

The full model of post-manipulation cortisol, testosterone, and the CxT interaction in predicting pain tolerance was not significant for the rejection condition (R^2=0.112, F (3, 36) = 1.5, p=0.229; adj R^2=0.038), but was for the acceptance condition (R^2=0.174, F (3,

49) =3.4, p<0.05, adj R^2=0.124). Testosterone was a significant predictor of pain tolerance in the acceptance condition (β=0.410, p<0.05) but not in the rejection condition (β=-0.294, p=0.104).

The model containing only cortisol and testosterone was also not significant in the rejection condition (R^2=0.109, F (2, 37) =2.3, p=0.119; adj R^2=0.060) but was in the acceptance condition (R^2=0.167, F (2, 50) =5.0, p<0.05; adj R^2=0.134). In the acceptance condition, testosterone was a significant predictor (β=0.432, p<0.05) but not in the rejection condition (β=-0.294, p=0.104).

3.8. Mediation Analysis

As part of an exploratory analysis to further understand the mechanisms underlying some of the relationships found in this study, several mediation analyses were conducted. Several psychological variables were of interest in these analyses, including positive and negative affect, empathy and chronic stress. Following Baron & Kenny (1986) and MacKinnon, Fairchild, & Fritz (2007), mediation was determined using the causal steps approach in which all of the paths of the mediation model were tested by a series of regression analyses. First the dependent variable (DV) was regressed on the independent variable (IV), the mediator was regressed on the IV, and finally the DV was regressed on both the IV and the mediator. Complete mediation of the relationship between the IV and DV is indicated when the effect of the IV on the DV is zero when controlling for the mediator. A decrease in the relationship between the IV and the DV with the inclusion of the proposed mediator indicates partial mediation (Baron & Kenny, 1986; MacKinnon et al., 2007).

The effect of chronic stress on pain sensitivity was of interest because stress experienced over long periods of time alters concentrations of glucocorticoids to which the body is exposed (McEwen, 2000). If pain sensitivity is responsive to varying levels of glucocorticoids, the relationship between chronic stress and pain sensitivity may be mediated by cortisol. However, baseline cortisol was found to not mediate the relationship between chronic stress and pain sensitivity. Other psychological variables, such as empathy, were of interest in terms of the mediation of the chronic stress and pain

sensitivity association. Empathy, however, was found to not mediate this relationship either.

Mood was of particular interest in this analysis because many studies report no differences in mood after social rejection, suggesting that changes in pain sensitivity may not be the result of changes in mood (DeWall & Baumeister, 2006; Bernstein & Claypool, 2012; Blackhart, Nelson, Knowles & Baumeister, 2009). There were, however, mood differences found in this study between participants who were rejected and accepted, prompting interest in whether it may be able to account for the relationship between hormones and pain. In keeping with the regression analyses conducted earlier, each mediation analysis in this section was separated by condition, such that each relationship was analyzed separately for accepted and rejected conditions. Neither positive nor negative affect mediated the relationship between baseline testosterone and pain sensitivity in the acceptance or rejection conditions. Mood was also not a mediator of the relationship between post-manipulation testosterone and pain sensitivity. Finally, neither positive nor negative affect mediated the association between post-manipulation cortisol and pain threshold in either condition.

Chapter 4.

Discussion

None of the a priori hypotheses for this study were directly supported by the data. However, there were some unexpected results that require discussion and interpretation. This next section will summarize the results found in this study as well as provide explanations for why or why not significant results were found. In conclusion, the implications of these results for social pain theory as well as future research will be discussed.

4.1. Hormones

4.1.1. Cortisol

Contrary to prediction, cortisol did not increase as a result of being placed in a socially rejecting situation. In addition, there were no gender differences in cortisol secretion. This surprising lack of effect on cortisol may indicate that the manipulation was not strong enough to induce feelings of rejection that may be associated with stress related secretion of cortisol. However, significant effects on mood, such that included individuals had higher levels of positive affect and rejected individuals had higher negative affect, were found. Therefore, while this manipulation was strong enough to result in significant psychological effects, it did not result in concurrent effects on cortisol. Although this result was contrary to predicted hypotheses, it is not entirely unprecedented. Several researchers have failed to find any effect of social exclusion on cortisol secretion and some have even found opposite results, such that increases were associated with inclusion and decreases associated with exclusion (Bass, Stendnitz, Simonson, Shen, & Gahtan, 2014; Seidel et al., 2013; Zoller, Maroof, Weik, & Deinzer, 2010).

In addition, previous research has shown that oral contraceptive use and menstrual cycle phase can alter the HPA response to stress (Kudielka, Hellhamar, & Wust, 2009; Lustyk, Olsen, Gerrish, Holder, & Widman, 2010; Zwolinski, 2012; Bass et al.,

2014). For example, cortisol reactivity to stressors is higher in the luteal rather than the follicular phase of the menstrual cycle. Furthermore, cortisol reactivity has been shown to be blunted in women taking oral contraceptives (Bass et al., 2014). Neither contraceptive use nor menstrual phase was controlled for in this study, possibly accounting for the lack of a cortisol response in women.

4.1.2. Testosterone

Similar to previous studies, males and females were found to differ in baseline testosterone levels, with males having higher concentrations than females before the experimental manipulation (Mazur & Booth, 1998). Interestingly post-manipulation testosterone was higher in males who were socially accepted than those who were socially rejected. In females, post-manipulation testosterone was equivalent between conditions. This result may be interpreted using the *biosocial status theory* described earlier (Seidel et al., 2013). Higher levels of testosterone amongst socially accepted males may reflect feelings of dominance and increased status that males experience in response to being in a situation in which another person acts friendly and accepting towards them. On the other hand, lower testosterone concentrations amongst socially rejected males may reflect feelings of lost social status associated with threats to dominance in the form of a rejecting interpersonal experience. The fact that females did not differ in post-manipulation testosterone may suggest that dominance and social status motives are less prevalent in females or that this type of social interaction did not relate to these motives in females.

4.2. Pain Sensitivity

Unlike previous research that has found gender differences in pain sensitivity (Giles & Walker, 2000), no differences in baseline pain threshold and tolerance were found between males and females in this study. However, pain threshold and tolerance decreased from baseline to post-manipulation measurements. In other words, participants were becoming more sensitive to pain after both acceptance and rejection manipulations. Pain sensitivity also demonstrated an interesting two-way interaction between gender and condition across pre- and post-manipulation pain measurements. Males experienced significantly higher thresholds and tolerances than females during social acceptance.

However, during social rejection, males and females had equivalent levels of pain sensitivity.

In addition, it was found that baseline testosterone was predictive of post-manipulation threshold and tolerance in socially accepted participants. In other words, individuals with higher baseline testosterone also had lower pain sensitivity after being socially accepted. This relationship did not hold for rejected participants. In addition, post-manipulation testosterone and cortisol were predictive of pain threshold in accepted participants. Increased testosterone and decreased cortisol were associated with higher threshold pain sensitivity, although their interaction was non-significant. For pain tolerance, however, only increased testosterone was associated with increased tolerance after social acceptance. Again, these relationships were non-significant for individuals that were rejected.

It is unknown why these hormones displayed significant relationships with pain sensitivity after social acceptance but not social rejection. It seems unlikely that testosterone would have no relationship to pain sensitivity after rejection considering its clear role during acceptance. Therefore, perhaps methodological issues precluded the identification of the role of hormones in pain sensitivity after rejection. For example, due to uneven distribution into experimental groups and attrition due to suspicions regarding the authenticity of the social interaction, the number of participants in the rejection group was lower than in the acceptance group. Therefore, nonsignificant results for the rejection condition may be a result of a lack of power rather than there truly being no relationship between hormones and pain sensitivity after social rejection. It is interesting to note however, that while nonsignificant, examination of the β coefficients in the rejection condition revealed relationships in the opposite direction to those in the acceptance condition. Clearly further research is needed to determine whether hormones such as testosterone and cortisol can affect pain sensitivity after rejection and, if so, what that relationship might be.

The finding that baseline and post-manipulation testosterone can affect pain sensitivity after social acceptance requires interpretation. As discussed previously, the *biosocial status theory* suggests that social acceptance is associated with increased

feelings of dominance and social status that is related to increased concentrations of testosterone (Seidel et al., 2013). In addition, increased testosterone after winning a competition was associated with the desire to compete again, presumably in an attempt to gain more status (Mehta & Josephs, 2006). If acceptance during a social interaction results in feelings of dominance, perhaps increased testosterone concentrations resulting from such feelings can be interpreted as a desire or intention to gain more status. Intentions to compete in order to gain status may benefit from lower pain sensitivity that would make potentially aggressive interactions less likely to result in competition loss or injury. Therefore, the positive relationship between testosterone and pain sensitivity may be a function of dominance and increased status seeking resulting from acceptance in a social interaction. The finding that men, but not women, had increased testosterone after being accepted is in line with findings that testosterone is related to dominance seeking in men (Mazur & Booth, 1998). This, in turn, may explain the interaction found between gender and condition. If men, but not women, experienced increased feelings of dominance after being socially accepted and these feelings were related to pain sensitivity it would make sense that males would have much lower pain sensitivities after acceptance than females. Perhaps equivalent pain sensitivity between males and females after rejection indicates feelings of lost status in males that result in increased pain sensitivity designed to discourage further status seeking. However, methodological limitations preclude such interpretations for the rejection condition. Further research that examines feelings of dominance in relation to testosterone and pain sensitivity after social interactions would shed light on this subject.

4.3. Psychological Variables

Several interesting results were found with regard to the psychological variables examined in this study. For example, participants who were rejected rated the social competence of the confederate much lower than those who were accepted. Rejected participants, therefore, believed that the confederate was less friendly, had fewer social skills that would allow them to successfully engage in relationships, and were less interested in getting to know the person further. This is, perhaps, not surprising as individuals who are rejected may be more likely to rationalize this rejection as a result of

some characteristic of the other person rather than stemming from their own personality (Baumeister & Leary, 1995). Individuals who were accepted, on the other hand, may be eager to validate the social abilities of the confederate as it justifies their own acceptance by that person.

In addition, females reported experiencing more stress over the previous month than males did. The reason for this gender difference in chronic stress is unclear, however, it may have some interesting implications. Given the effects of stress on concentrations of cortisol, if cortisol was to affect pain sensitivity, it seems logical that higher levels of stress may be related to altered levels of pain sensitivity. Stress did indeed predict baseline pain sensitivity; such that higher levels of stress were associated with lower pain threshold and tolerance (i.e. higher pain sensitivity). This result may help explain differing levels of pain sensitivity reported by males and females in many studies (Giles & Walker, 2000). Cortisol, however, did not mediate the relationship between stress and pain sensitivity, suggesting that cortisol concentrations resulting from varying levels of stress cannot account for this relationship.

Mood was also found to vary between rejection and acceptance conditions. Participants who were accepted generally felt more positive emotions than those who were rejected. Rejected participants, however, experienced greater levels of negative affect than those who were accepted. Given the fundamental nature of the need to belong, changes in mood after social rejection seems to make logical sense; however, it is not always what is found in the literature. In fact, many studies report no differences in mood or if differences are found, they do not mediate relationships between social exclusion and behavior (DeWall & Baumeister, 2006; Bernstein & Claypool, 2012; Blackhart et al., 2009). One possible explanation for this unexpected result may be that social rejection results in emotional numbness, the purpose of which may be to temporarily separate oneself from the pain that results from threats to the need to belong (DeWall & Baumeister, 2006). Such emotional numbness has been postulated to result in the physical insensitivity to pain that is often found in response to social rejection (DeWall & Baumeister, 2006; Bernstein & Claypool, 2012). The fact that variation in mood was found in this study may, similarly, explain the fact that pain sensitivity was also found to increase in response to a social manipulation. Mood, however, did not mediate the relationships between any of the

hormones and pain sensitivity, suggesting that emotional affect in response to social rejection does not mediate either its behavioral or biological consequences.

It has been hypothesized that the inconsistent results for both mood and pain sensitivity after social rejection may reflect the severity of the social manipulation itself (Bernstein & Claypool, 2012). As with physical pain, in which increased injury severity is associated with increased pain up to a point after which numbing occurs, pain after rejection may also be a function of the severity of the social stimuli. The *severity hypothesis,* postulated by Bernstein & Claypool (2012), suggests that social manipulations associated with minor social injuries should result in hypersensitivity, while major social injuries, in which the need to belong is seriously threatened, result in hyposensitivity. Furthermore, hyposensitivity to pain should also be associated with an emotional numbing associated with a defensive reaction to severe social injury. Indeed, there is a division in the literature in which manipulations leading participants to believe they will have a lonely future are associated with hyposensitivity and emotional numbness (Bernstein & Claypool, 2012; DeWall & Baumeister, 2006; Macdonald & Leary, 2005). On the other hand, Cyberball, a manipulation in which participants are left out of a virtual ball tossing game is associated with hypersensitivity and increased distress (Bernstein & Claypool, 2012; Eisenberger et al., 2003; Eisenberger & Lieberman, 2004; Eisenberger et al., 2006). It is not hard to imagine that being told that your future will be filled with loneliness is considered a more severe threat to belonging than not being tossed a virtual ball by strangers. Given these considerations, perhaps the manipulation used in this study was not severe enough to result in emotional numbing and hyposensitivity. It is possible that participants were not invested enough in their interaction with a stranger to see their rejection as a serious threat to belonging.

4.4. Conclusions

Evolution has seemingly expanded on a pre-existing neural pain structure to equip the social attachment system with a mechanism with which to mitigate the fitness reducing consequences of social isolation (Panksepp, 1998; Nelson & Panksepp, 1998). The need for such a system, in which the dissolution of relationships is met with pain, is reinforced by humanity's social nature in which interpersonal connection is a prerequisite to the

fitness benefits characteristic of group living. Our extended period of vulnerability as infants and the benefits of continued social relatedness into adulthood has instilled a fundamental need to belong which can only be satisfied by deep, meaningful connections with others (Baumeister & Leary, 1995). The loss of such relationships cause painful reactions that go beyond mere metaphorical use of the term. Instead it reveals a relationship between physical and emotional pain that highlights the importance of social relationships to humans, the lack of which has physically and psychologically distressing consequences (Baumeister & Leary, 1995; Seeman, 1996).

This research has attempted to further clarify the connections underlying pain sensitivity and rejecting experiences, which result in feelings of threat to interpersonal relationships. These goals were based on the theory that emotionally distressing experiences, such as exclusion from relationships, causes pain that parallels that resulting from physical injury. The bidirectional relationship between hormonal fluctuations and social processes make hormones an intriguing avenue of inquiry with regard to the physiological and behavioral effects of social rejection. The intriguing relationship found between testosterone and pain sensitivity may suggest that dominance plays more of a role in everyday social interactions than previously thought, particularly with regards to males. If so, this may have implications for social pain theory, as it may explain some of the behavioral consequences of social interactions, such as increased social withdrawal after rejection (Baumeister & Leary, 1995). Furthermore, this study found similar results as many others with regards to the effect of social rejection on mood and how altered affect may influence subsequent behavioral and physical reactions to such situations (DeWall & Baumeister, 2006; Bernstein & Claypool, 2012; Blackhart et al., 2009). Further research will be needed to determine mediators of the relationship between rejection and pain sensitivity, as mood does not appear to play a role despite its seeming intuitiveness. Previous experimental evidence that cortisol is reactive to stressful stimuli makes it an obvious candidate for studying social pain associated with interpersonal rejection. This is due to the fact that rejection from important relationships threatens the fundamental need to belong, a presumably stressful experience. Although nonsignificant results were found for cortisol, the idea that a female adaptive strategy encouraging interpersonal bonding in response to stress that may result in altered pain sensitivity in the face of rejection from such relationships cannot be ruled out by this experiment. Stronger manipulations in which

the need to belong is truly threatened and which utilize proper controls for salivary hormones may yet reveal the hypothesized interactions between gender and cortisol in response to rejection.

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Appendix A.

Consent Form

Behavioral Neuroendocrinology Laboratory
Department of Psychology

Hormones, Mood, and Social Interaction

CONSENT FORM

You are being invited to participate in a Behavioral Neuroscience study conducted by Lindsay Cooper, a researcher from the Psychology Department at Simon Fraser University (BNEL), as part of her MA degree (in collaboration with [redacted] and [redacted]).

There are no known risks if you decide to participate in this research study. There are no costs to you for participating in the study. The information you provide will help us understand the effect of mild physical discomfort on mood and social interactions. Hormone levels will be assessed through two saliva samples which you will provide. You will also answer a series of paper questionnaires, including a demographics questionnaire that will include questions about your age, education, health, eating and sleeping habits, etc. These measures are necessary when we conduct our statistical analyses as they represent useful covariates for some of our outcome measures (e.g., hormone levels). Providing answers to these questions is completely voluntary. Finally, you will engage in a task with another participant. You may stop participating at any time without penalty. There are no known risks if you decide to participate in this study. There are also no costs to you for participating. An identification number will be used to identify the results of your tests. In case the data is published, no individual information will be disclosed.

All paper-format data will be kept and locked in a filing cabinet in our research laboratory (RCB 5209) at Simon Fraser University's Burnaby campus. All electronic data will be stored using a confidential ID number on a password protected SFU Psychology Department data server. Only researchers in the Behavioral Neuroendocrinology Laboratory will have access to the cabinet and files on the server. Each participant will be assigned an ID number. We are going to retain the data for a period of 3 years at which time it will be destroyed.

Your participation is voluntary, and you may withdraw from the study at any time without penalty. You are still entitled to be entered into the draw regardless if you choose to withdraw from the study. Saliva samples will be collected using passive drool in a plastic tube (approximately 2-3 ml each sample, 4-6 ml in total) and won't be used for future studies. If you choose to enter the study and then decide to withdraw at a later time, all data collected about you, including the saliva samples, during your enrollment in the study will be destroyed.

If you choose to participate, please sign below and carefully follow the research assistant's instructions during the experiment. When the tasks are done, if you have any questions about the study, please contact Lindsay Cooper from the Behavioral Neuroendocrinology Laboratory, Department of Psychology at Simon Fraser University (mailing address: 8888 University Drive, Burnaby, BC V5A 1S6; phone number: [redacted]; email: [redacted]).

If you have any concerns about your rights as a research participant and/or your experiences while participating in this study, you may contact Dr. Jeffrey Toward, Director, Office of Research Ethics at [redacted] or [redacted].

Your signature indicates that you consent to participate in this study.

Participant Signature: **Date:**

Printed Name of Participant:

Appendix B.

Debriefing Form

Behavioral Neuro-Endocrinology Laboratory (BNEL)

Cortisol Release as a Means for Reducing Pain Sensitivity after Social Rejection

Debriefing Form

Thank you for participating in this study for the Behavioral Neuroendocrinology Laboratory (BNEL). The general purpose of this study was to explore how cortisol affects pain sensitivity after a social rejection. Previous research has shown that increases in cortisol can have a numbing effect on pain. It has also been found social interactions that involve an element of rejection results in the release of cortisol. Therefore, we expect that cortisol should lower pain sensitivity after a social rejection.

In this study, you interacted with another individual in which you engaged in asking each other questions. You were also asked to view a questionnaire about you that was completed by this individual. In actual fact, this individual was a confederate. The way the confederate acted and the information in this questionnaire was dependent upon which condition you were placed into. If you were placed in the rejection condition, please note that the interaction and questionnaire did not reflect on you or your personality in any way. However, this deception was necessary to create a stressful environment in order to stimulate the release of cortisol.

This study should add to the psychology literature and help determine the effects of hormones like cortisol in pain sensitivity and social interactions.

Please remember that your participation in this study is completely voluntary and all data pertaining to this study will be kept confidential. The data from this study are stored using a confidential ID number and cannot be traced back to you. If your concerns are such that you would now like to have your data withdrawn, we will do so.

Please indicate whether you consent to allow your data to be used in this research:

YES **NO**

Participant Signature: **Date:**

_____

Printed Name of Participant:

_____

If you have any further questions about the study or would like to obtain research results please contact Lindsay Cooper from the Behavioral Neuroendocrinology Laboratory of the Department of Psychology at Simon Fraser University (mailing address: 8888 University Drive, Burnaby, BC V5A 1S6; telephone number: [redacted]; [redacted]).

If you have any questions or concerns about being in this study, you may contact Dr. Neil Watson, Chair, Department of Psychology at [redacted] or [redacted]. Alternatively, you may contact Dr. Jeffery Toward, Director, Office of Research Ethics at [redacted] or [redacted].

Please again accept our appreciation for your participation in this study.

Appendix C

PANAS

Date:_____ Participant ID:_____

The PANAS

This scale consists of a number of words that describe different feelings and emotions. Read each item and then mark the appropriate answer in the space next to that word. Indicate to what extent you feel this way right now, that is, at the present moment. Use the following scale to record your answers.

1	2	3	4	5
Very Slightly or not at all	A little	Moderately	Quite a Bit	Extremely

_____ Interested
_____ Distressed
_____ Excited
_____Upset
_____ Strong
_____ Guilty
_____Scared
_____ Hostile
_____Enthusiastic
_____ Proud

_____ Irritable
_____ Alert
_____ Ashamed
_____ Inspired
_____ Nervous
_____ Determined
_____ Attentive
_____ Jittery
_____ Active
_____ Afraid

Appendix D.

Rosenberg Self-Esteem Scale

Date:_____ **Participant ID:**_____

Rosenberg Self-Esteem Scale

Instructions: Below is a list of statements dealing with your general feelings about yourself. If you strongly agree, circle **SA**. If you agree with the statement, circle **A**. If you disagree, circle **D**. If you strongly disagree, circle **SD**.

1.	On the whole, I am satisfied with myself.	SA	A	D	SD
2.	At times, I think I am no good at all.	SA	A	D	SD
3.	I feel that I have a number of good qualities.	SA	A	D	SD
4.	I am able to do things as well as most other people.	SA	A	D	SD
5.	I feel I do not have much to be proud of.	SA	A	D	SD
6.	I certainly feel useless at times.	SA	A	D	SD
7.	I feel that I'm a person of worth, at least on an equal plane with others	SA	A	D	SD
8.	I wish I could have more respect for myself	SA	A	D	SD
9.	All in all, I am more inclined to feel that I am a failure	SA	A	D	SD
10.	I take a positive attitude towards myself	SA	A	D	SD

Appendix E.

Perceived Stress Scale-14 Item

Date:_____ Participant ID: _____

PSS-14

The questions in this scale ask you about your feelings and thoughts during the last month. In each case, you will be asked to indicate *how often* you felt or thought a certain way. Although some of the questions are similar, there are differences between them and you should treat each one as a separate question. The best approach is to answer each question fairly quickly. That is, don't try to count up the number of times you felt a particular way, but rather indicate the alternative that seems like a reasonable estimate.

For each question choose one of the following alternatives:

0	1	2	3	4
Never	**Almost Never**	**Sometimes**	**Fairly Often**	**Very Often**

1. In the last month, how often have you been upset because of something that happened unexpectedly? _____
2. In the last month, how often have you felt that you were unable to control the important things in your life? _____
3. In the last month, how often have you felt nervous and "stressed"? _____
4. In the last month, how often have you dealt successfully with irritating life hassles? _____
5. In the last month, how often have you felt that you were effectively coping with important changes that were occurring in your life? _____
6. In the last month, how often have you felt confident about your ability to handle your personal problems? _____
7. In the last month, how often have you felt that things were going your way? _____
8. In the last month, how often have you found that you could not cope with all the things that you had to do? _____

9. In the last month, how often were you able to control irritations in your life? _____

10. In the last month, how often have you felt that you were on top of things? _____

11. In the last month, how often have you been angered because of things that happened that were outside of your control? _____

12. In the last month, how often have you found yourself thinking about things that you have to accomplish? _____

13. In the last month, how often have you been able to control the way you spend your time? _____

14. In the last month, how often have you felt difficulties were piling up so high that you that you could not overcome them? _____

Appendix F.

Relationship Closeness Induction Task

RCIT

1. What is your first name?
2. How old are you?
3. Where are you from?
4. What year are you at Simon Fraser University?
5. What do you think you might major in? Why?
6. What made you come to Simon Fraser University?
7. What is your favorite class at Simon Fraser University? Why?

8. What are your hobbies?
9. What would you like to do after graduating from Simon Fraser University?
10. What would be the perfect lifestyle for you?
11. What is something you have always wanted to do, but probably will never be able to do?
12. If you could travel anywhere in the world, where would you go and why?
13. What is one strange thing that has happened to you since you've been at Simon Fraser University?
14. What is one embarrassing thing that has happened to you since arriving at Simon Fraser University?
15. What is one thing happening in your life that makes you stressed out?
16. If you could change anything that happened to you in high school, what would that be?
17. If you could change one thing about yourself, what would that be?
18. Do you miss your family?
19. What is one habit you would like to break?

20. If you could have one wish granted, what would that be?
21. Is it difficult or easy for you to meet people? Why?
22. Describe the last time you felt lonely?
23. What is one emotional experience you've had with a good friend?
24. What is one of your biggest fears?
25. What is one of your most frightening early memory?
26. What is your happiest early childhood memory?
27. What is one thing about yourself that most people would consider surprising?
28. What is one recent accomplishment that you are proud of?
29. Tell me one thing about yourself that most people who already know you don't know

Appendix G.

Attribution Questionnaire

Date:_____ Participant ID:_____

Consider a situation in which you are given the chance to work with this person in the future, would you like to work with them based on your compatibility and what you know about them?

YES NO

Please write two-three sentences where you give us feedback about the experiment so far.

_____

_____

_____

_____

_____

_____

Appendix H.

Demographics Questionnaire

Date: _____ Participant ID: _____

Demographics Questionnaire

Please answer the following questions to the best of your ability. Your responses are strictly confidential and will be used for research purposes only. Your responses will be identified only by a confidential participation number.

1. Age: _____
2. Sex: M F
3. When was the last time you ate? _____
4. When was the last time you had caffeine? _____
5. Have you experienced any gum bleeding over the past few days?

   YES NO
6. Have you experienced any other oral infections and/or oral lacerations over the past few days?

   YES NO
7. Do you have a diagnosed with an endocrine disorder?

   YES NO

   5a. If Yes, which? _____ (Please be specific)
8. Do you smoke? YES NO

   7a. If Yes how many cigarettes in a day? _____ (Please be specific)
9. Do you take anabolic steroids? YES NO
10. What is your occupation? _____
11. Are you currently taking any prescription or non-prescription medications, oral contraceptives, or other hormone supplements? (Please circle one)

NO, I am not taking any medication.

YES (please list the medications you are taking): _____

_____

12. What is the highest level of education you have completed?
(Please circle one)

_____High school graduate
_____At least one year of college, university, or specialized training
_____College or university graduate

13. What is your weight (please indicate kg or lbs):
_____?

14. What is your height (please indicate, cm, inches, feet):
_____?

15. What is your ethnicity (e.g. caucasian)?
_____

Sleep/Week Cycle (please indicate am/pm)

1. What time do you normally wake up on weekdays? _____
2. What time do you normally wake up on weekends? _____
3. What time do you normally go to sleep on weekdays? _____
4. What time do you normally go to sleep on weekends? _____
5. What time did you go to sleep last night? _____
6. What time did you get up this morning? _____
7. If you did not have to wake up because of external circumstances like school or work, when would you most prefer to wake up? (please check one)

_____ Before 6:30 am
_____6:35 am-7:30 am
_____7:35 am-9:00 am
_____9:05 am-10:30 am
_____10:35 am-12:00 pm

_____12:05 am-1:30 pm
_____after 1:35 pm

Appendix I.

Interpersonal Reactivity Index

Date:_____ **Participant ID:**_____

Interpersonal Reactivity Index

For each item, indicate how well it describes you by indicating the appropriate letter on the scale bellow: A, B, C, D, or E. When you have decided on your answer, fill in the letter next to the item number. READ EACH ITEM CAREFULLY BEFORE RESPONDING. Answer as honestly as you can.

Answer Scale:

A	B	C	D	E
Does not describe me well				Describes me very well

1. I daydream and fantasize, with some regularity, about things that might happen to me.
2. I often have tender, concerned feelings for people less fortunate than me.
3. I sometimes find it difficult to see things from the “other guy’s” point of view.
4. Sometimes I don’t feel very sorry for other people when they are having problems.
5. I really get involved with the feelings of the characters in a novel
6. In emergency situations, I feel apprehensive and ill-at-ease.
7. I am usually objective when I watch a movie or play, and I don’t often get completely caught up in it.
8. I try to look at everybody’s side of a disagreement before I make a decision.

9. When I see someone being taken advantage of I feel kind of protective towards them.

10. I sometimes feel helpless when I am in the middle of a very emotional situation.

11. I sometimes try to understand my friends better by imagining how things look from their perspective.

12. Becoming extremely involved in a good book or movie is somewhat rare for me

13. When I see someone get hurt, I tend to remain calm.

14. Other people's misfortunes do not usually disturb me a great deal.

15. If I'm sure I'm right about something, I don't waste much time listening to other people's arguments.

16. After seeing a play or a movie, I have felt as though I was one of the characters.

17. Being in a tense emotional situation scares me.

18. When I see someone being treated unfairly, I sometimes don't feel very much pity for them.

19. I am usually pretty effective in dealing with emergencies.

20. I am often quite touched by things that I see happen.

21. I believe there are two sides to every question and try to look at them both.

22. I would describe myself as a pretty soft-hearted person.

23. When I watch a good movie, I can very easily put myself in the place of a leading character.

24. I tend to lose control during emergencies.

25. When I'm upset at someone, I usually try to "put myself in his shoes" for a while.

26. When I am reading an interesting story or novel, I imagine how I would feel if the events in the story were happening to me.

27. When I see someone who badly needs help in an emergency, I go to pieces.

28. Before criticizing somebody, I try to imagine how I would feel if I were in their place.

Appendix J.

Social Competence Scale

Participant ID:_____

Social Competence Scale

The questions on this scale ask you about your feelings and impressions about the person you just met. Please answer as accurately as possible.

For each question choose one of the following alternatives:

1	2	3	4	5
Disagree	**Somewhat Disagree**	**Neither Agree nor Disagree**	**Somewhat Agree**	**Agree**

1. This person is able to contribute interesting points to the discussion _____
2. This person appears to be friendly and open to communicating with new people _____
3. This person probably has an easy time making and keeping new friends _____
4. I would consider getting to know this person better _____