

Running Head: SEX DIFFERENCES IN EMPATHIC APPROACH

Sex Dependent Affiliation Behavior and Empathic Approach

in *Mus musculus*

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Contents

Abstract	3
Introduction	4
Empathy	4
Affiliation	24
Present Study	32
Experiment 1: Sex differences in approach behavior; a continuation of the pilot	36
Experiment 2: Pharmacological manipulation of oxytocin in female mice	39
Results	40
Discussion	43
References	54
Appendix	60

## Abstract

The purpose of this study was to examine the effects of sex and oxytocin on approach behavior in mice elicited by empathy for the pain of another mouse. The approach behavior of a free mouse towards an enclosed mouse was measured during a 30 minute period. Sex differences in approach behavior were observed during the first experiment. Results showed that females approached mice in pain more often than mice not in pain, whereas this distinction between pain and no pain conditions was not present in males. We hypothesized that this effect may be mediated by a “tend and befriend” response to stress produced by oxytocin. Using the same experimental setup to measure approach behavior, oxytocin was administered by subcutaneous injection to female mice. We expected that female mice with increased oxytocin levels would approach a mouse in pain more often than a mouse not in pain. We also predicted that they would approach a mouse in pain more frequently than mice not injected with oxytocin. Results did not demonstrate effects of pain manipulation in the predicted direction. These results most likely stem from a number of unexpected side effects of high doses of oxytocin including its sedative effects. Studies such as these contribute to the larger goal of researchers to establish animal models of social relationships and emotion as a means of better understanding and treating social disorders in humans.

Sex Dependent Affiliation Behavior and the Presence of  
Empathic Approach in *Mus musculus*

Human and animal models of social relationships allow researchers to define associated behaviors and study variables that modulate the expression of these relationships in a controlled setting. The characterization of animal models specifically provides a simple representation of complex emotional and social behaviors in humans. The study of empathy and its behavioral correlates has been a recent focus for researchers in determining animal models of social behavior. Brain activation studies have revealed empathy-specific patterns of activation in humans, although such brain activation studies have not been performed using animal subjects. However, behavioral observation has suggested the presence of low-level empathy in animals, primarily for the pain of another animal. With these data, our understanding of empathy and its manifestation in animals has improved. The focus of studies of empathy now lie in utilizing animal models of empathy to determine its effect on social behaviors such as affiliation, and the effect of physiological factors such as sex and sex specific hormones on the production of these empathic affiliation behaviors.

Empathy

*Operationalizing Human Empathy*

The Oxford English Dictionary defines empathy as, “The power of projecting one's personality into (and so fully comprehending) the object of contemplation”.

Empathy is regarded by some as similar to the concept of emotional contagion, whereby the subject's state is altered simply from perceiving another's emotional state (Preston & deWaal, 2002). This is a simple transference of emotional states, positive or negative, between individuals, and does not involve an understanding of the other's emotional state. Empathy, on the other hand, can only be applied in situations involving negative emotional states. Empathy and sympathy are likewise falsely regarded as analogous, although the distinction between sympathy and empathy is less clear. Sympathy is situationally focused, and can be described as recognition of the situation, but no understanding of the emotional state of the other individual (Preston & de Waal, 2002). Sympathy, therefore, can occur in the absence of the understanding that underlies empathy (Preston & de Waal, 2002). Empathy therefore involves a higher degree of cognitive understanding of the other's emotional state than either sympathy or emotional contagion.

While researchers studying empathy might agree that empathy in humans involves the ability to understand and react to another individual's emotional state, operationalizing empathy in the laboratory is more problematic. Operationalizing empathy is dependent on the ability to observe behaviors which indicate empathy. The Perception-Action Model of empathy proposed by Preston and de Waal (2002) places emphasis on the behavioral responses involved in the experience of empathy. The Perception-Action Model states that perception of a target animal's state activates the observer's mental representation of that state, which will generate associated physical responses in the observer (Preston & de Waal, 2002). This model enables researchers to regard empathy as an emotional experience that stimulates directly observable associated

behaviors which can be measured in the laboratory. Preston and de Waal further identify two types of behavioral responses to the target in the experience of empathy; responses *to* the target and responses *with* the target. Responses to the target do not necessarily match the target's emotional state as in consolation in response to distress, whereas responses with the target bring the emotional state of the observer closer to that of the emotional state of the target. In both classes of response, empathy produces overt behaviors in the observer in response to the emotional state of the target, allowing researchers to directly observe behaviors which indicate empathy.

Empathy in humans is often associated with behaviors which indicate a cognitive understanding of the target's situation. Singer defines empathy in humans as a negative emotional state which is produced in reaction to the observation of the emotional state of another (Singer, 2006). Singer distinguishes empathic behaviors from isomorphism, which is simply the act of mimicking a target's emotional state. Producing tears at the sight of another person crying is an example of isomorphism, and would not be regarded as an indication of empathy in humans by Singer (Singer, 2006). Singer also states that empathy for another must be produced by direct observation or knowledge of that individual's emotional state (Singer, 2006). In addition, empathy according to Singer is present only when the empathic observer is aware that their own altered emotional state is a reaction to the affective state of the observed. This definition requires empirical support, but it is a reasonable example of one way empathy can be defined in human laboratory studies of empathy. Definitions of empathy such as these allow researchers to begin to construct a basic set of behavioral correlates involved in experiencing empathy defined in this manner.

Animals, like humans, are expected to produce behaviors in reaction to the perception of another animal's state as the Perception-Action Model predicts. However, establishing an operational definition for empathy in animals provides an entirely new set of problems because of the limited repertoire of observable social and emotional behaviors in animals compared to humans. Research in humans describing physiological and behavioral correlates of empathy have therefore been used to establish parallel behaviors in mice and other mammals as indicators of empathic-like emotional states. The study of empathy in the laboratory therefore must utilize a method that can reliably induce these behaviors that signify low level empathy in animals.

*Empathic modulation of pain: Gate control theory*

One way in which researchers have consistently evoked empathy in both humans and animals in the laboratory is through the use of pain manipulations. Pain is defined by the International Association for the Study of Pain (IASP) as an "unpleasant sensory and emotional experience related to the threat of actual or potential tissue damage, or defined in terms of that damage". Pain, unlike other emotions which may evoke empathy such as sadness, produces overt observable behaviors in the target across a wide variety of species. Pain therefore provides a stimulus for empathy in the observer that can be directly measured in both humans and animals. The ability to measure pain behavior allows researchers to control for variations in the stimulus (pain) when studying the effects of other factors on empathy. Additionally, because the Perception-Action model states that empathic behaviors are induced by observation of another individual, a stimulus must be used that produces obvious behaviors which are identifiable among both

humans and animals. By using pain manipulations, researchers in the field have been able to study the bidirectional effects of empathy on the pain experience of the observer and of empathy induced by observing a target in pain.

Studies of empathy in humans demonstrate that observers experience an increase in pain sensitivity when participants experience empathy for the pain of another individual. These studies provide evidence for an effect of empathy on pain sensitivity in the empathic individual. A study performed by Loggia et al. (2007) asked participants to watch an actor experiencing painful or non-painful stimuli after the subject had established a negative or positive attitude toward that actor. To induce character judgments of the actors, participants were asked to watch one of two videos of the actor telling a story, one that induced feelings of empathy (such as a story about how the man's girlfriend had died), and another in which the actor told a story that lead the participant to believe that he was an unpleasant person (Loggia et al., 2007). Both anecdotes were designed to induce a negative mood state, but different opinions about the character of the actor (Loggia et al., 2007). Subjects were then allowed to watch the same actor undergoing a pain manipulation (Loggia et al., 2007). After this phase, the subjects themselves were tested for pain sensitivity to a series of painful and non-painful heat stimuli (Loggia et al., 2007).

Results of this study showed that participants who felt a high level of empathy for the actor had higher pain ratings themselves than those who felt low empathy for the actor (Loggia et al., 2007). Because the motivation for the subject's empathy towards the actor was not pain itself, but the anecdote that the actor gave, researchers were able to control for imitation of the actor by the observer (Loggia et al., 2007). By creating both

anecdotes with the purpose of provoking a negative mood state, researchers were additionally able to control for mood of the subject (Loggia et al., 2007). This study therefore provides support for the modulation of pain sensitivity by empathy for an individual's emotional state, regardless of whether the empathy is induced by the physical pain state of the observed individual.

One possible explanation for the modulation of pain experience by empathy is that psychological factors such as empathy work along with peripheral sensation in pain processing centers to modulate pain experience. The modern theory of pain perception and processing allows for the modulation of pain by factors other than peripheral sensation, such as time of day, emotion and attention. This theory proposes that the experience of pain is produced by the interaction of a set of "pain centers" in the peripheral and central nervous systems that is able to modulate the peripheral pain signal (Melzack & Wall, 1965).

A variety of fibers are present in the periphery which carry afferent information to the spinal cord. These fibers vary in their conductance velocity, which depends on fiber myelination and diameter. Thickly myelinated fibers have faster conductance velocity, as do larger diameter fibers. Two fibers involved in the experience of pain are A $\delta$  fibers, which are small and thickly myelinated fibers that conduct information quickly, and small diameter, un-myelinated C fibers, which carry information from the periphery more slowly than A $\delta$  fibers (Melzack & Wall, 2006). Both fibers have high activation thresholds, meaning they will be activated only in the presence of a high intensity stimulus such as very hot temperatures. A $\delta$  and C fibers are both involved in the pain experience, although C fibers respond to a broader spectrum of pain sensations, including

chemical, thermal and mechanical stimuli, whereas A $\delta$  fibers respond only to mechanical or thermal stimuli (Melzack & Wall, 2006). Somatosensory pain information enters the system activating A $\delta$  and C fibers which carry information to the dorsal horn of the spinal cord (Melzack & Wall, 1965).

Once the afferent pain stimulus has reached the dorsal horn, the signal can be amplified or reduced through the use of interneurons (Melzack & Wall, 1965). These neurons, when activated, inhibit transmission cells that carry the pain signal to the brain (Melzack & Wall, 1965). Inhibitory interneurons are activated by large diameter low intensity threshold fibers, called A $\beta$  fibers, which perceive low intensity sensations such as light touch in the periphery. When these fibers are activated, no pain signal will be transmitted to the brain due to the simultaneous activation of the inhibitory interneurons which prevent transmission of the pain signal to the brain (Melzack & Wall, 1965). Nociceptive, high intensity threshold fibers inhibit these interneurons and allow the activation of transmission cells and a strong nociceptive signal to the brain, effectively “opening” the pain gate (Melzack & Wall, 1965). The classic example of the interplay between low threshold fibers and nociceptors in gate control theory is in the act of rubbing the site of an injury. Rubbing the site stimulates low threshold fibers and activates interneurons that effectively “close” the pain gate, resulting in decreased pain at the injury site in response to this rubbing action.

Researchers suggest that the same effect might be achieved by psychological factors that act in the same way as low intensity threshold fibers and nociceptors in “closing” or “opening” this pain gate (Melzack & Wall, 1965). Efferent signals, originating in the brain and traveling to the spinal cord, could conceivably alter the

function of the transmission cell, just as low intensity threshold fibers can, thereby mediating pain perception (Melzack & Wall, 1965). Evidence for this hypothesis was given by a study performed by Bandura et al. (1987) in which participants were taught methods of cognitive pain control, and then given an injection of saline or nalaxone, an opiate antagonist. After administration participants were tested for pain tolerance while holding their arm in a bucket of ice water, called a cold pressor test (Bandura, 1987). Results of the study showed that cognitive control increased the pain tolerance of participants in both the nalaxone and saline injection condition, suggesting that cognitive methods were able to modulate pain perception (Bandura et al., 1987).

The same modulatory effects have been observed in research involving catastrophizing, which has been shown to increase sensitivity to pain in humans (Sullivan et al., 2001). Catastrophizing is a negative mental state in which the individual exaggerates the threats or consequences of a painful experience (Sullivan et al., 2001). People who are categorized as catastrophizers tend to report greater painful experiences than their non-catastrophizing counterparts, and catastrophizing has been reported to account for approximately 7 to 31% of pain ratings in adults (Sullivan et al., 2001).

A study by Sullivan et al. (1997) examined the relationship between differences in pain ratings of undergraduate students and their score on the Pain Catastrophizing Scale (Sullivan et al., 1997). Experimenters asked participants to immerse their hands in cold water as a pain manipulation. Researchers included a thought suppression dimension to the study as well, asking some participants to actively suppress thoughts about the upcoming pain manipulation for nine minutes before the test (Sullivan et al., 1997). After completion of the experiment, subjects were given a measure that determined the

amount of thought intrusions that had occurred during the pain manipulation (Sullivan et al. 1997). Results of the study showed that thought intrusions were significantly higher in subjects with high scores on the Pain Catastrophizing scale, and that further these people reported greater amounts of pain than those who were not catastrophizers or who were in the “no suppression” condition. (Sullivan et al., 1997) These results further support the modulation of pain and pain experience through psychological factors and mental states such as catastrophizing and empathy.

#### *Affective dimensions of pain and empathy*

Empathy for the pain state of a target is therefore capable of modulating the pain state of the observer. Pain, according to the IASP definition, is not simply a perceptual experience and involves a negative affective component in addition to the physical experience. Research has demonstrated that the mental processes which occur in understanding the affective dimension of the observer’s own pain is involved in the experience of empathy for another’s pain. Support for the presence of an affective dimension of pain comes from brain imaging studies that have shown that pain sensation branches as it reaches the brain, activating numerous areas involved in a multitude of emotional functions. These regions include areas of the limbic system such as the anterior cingulate (ACC) and insular cortex (IC), as well as the ventroposterior lateral nucleus (VPL) and the ventroposterior inferior (VPI) nucleus in the thalamus, the somatosensory cortices, amygdala, perirhinal cortex, and hippocampus (Price, 2000). Many of these areas, such as the amygdala, are well known centers for emotional

regulation, and could therefore be involved in the production of an affective dimension of pain.

Empirical studies using neural imaging have provided further evidence for an affective dimension of pain by demonstrating separate patterns of brain activation for the emotional and sensory components of pain. A study performed using positron emission tomography to map activation of the brain displayed specialized affective pain regions of the brain (Rainville, 1997). Researchers used hypnotic suggestion to vary the degree of unpleasantness to a consistent pain stimulus (Rainville, 1997). In this study, subjects in the high unpleasantness conditions showed much greater activity in the posterior sector of the ACC, implicating this region as an important center for the emotional dimension of pain (Rainville, 1997). These results provide evidence that a separate pattern of brain activation exists for the affective dimensions of pain.

Further research demonstrates that this affective dimension of processing self-related pain could be related to the ability to understand another individual's pain. Studies on empathy for pain have demonstrated that affective pain areas are involved in the comprehension of pain in others and empathy for that pain. A study conducted by Singer et al. (2004) used functional magnetic resonance imaging to assess the difference in brain activation between perceiving a relationship partner in pain and experiencing pain on the self (Singer, 2004). Female participants were observed while a painful shock was administered to either herself or her relationship partner. Brain activation during the "self" and "other" pain conditions were compared using fMRI imaging, allowing researchers to distinguish between activation pathways for pain in another and pain in the self (Singer, 2004). Brain activation in the "other" condition was hypothesized to be

representative of empathy for pain in the subject's partner. Results showed that self pain was characterized by activation in the posterior insula, the sensorimotor cortex, and the caudal anterior cingulate cortex. This pattern of activation was also observed when the subject was told that her male partner was receiving a painful shock, instead of directly observing her partner in pain (Singer, 2004). Activation in the bilateral anterior insula, rostral anterior cingulate cortex, brainstem and cerebellum was present in both the self and other pain conditions (Singer, 2004). Singer hypothesized that the experience of empathy for another in pain involves the affective dimension of pain processing in the self, identified by the overlapping brain areas activated in the self and other condition (Singer, 2004). This study supported the presence of separate activation for sensory and affective dimensions of pain, and demonstrated that the affective pain pathway is involved in empathy towards another in pain.

#### *Evolutionary adaptiveness of empathy*

Empathy for pain in humans is capable of modulating the pain state of the observer, and has been shown to include the same mental processes involved in affective pain in the self. These studies have indicated the presence of a relationship between pain and empathic behaviors in human models. Empathy, however, has yet to be studied at length in animal models. Mechanisms of pain perception are highly conserved within animals and humans, and therefore the study of such behaviors as empathy in response to pain could conceivably be performed in lower mammals. The study of empathy in humans is susceptible to a number of confounds, including social and environmental factors, as well as complex biological interactions present in the study of higher

organisms such as humans. Because researchers are able to control the environment and social interactions of laboratory animals, establishing a model of empathy for pain in animals such as mice could allow researchers to study empathy in a simple system and in a more controlled setting.

Studies of animal empathy use separate operational definitions for empathic behaviors, termed low level empathy, than studies in humans that involve a degree of cognitive perspective taking considered not present in animals. Rather than an absolute, experimenters treat empathy as a continuum, with behaviors considered to be empathic ranging from low to high levels. Empathy in humans is the consequence of years of ontological development, and can vary along this spectrum, ranging from the low level empathy that toddlers and infants exhibit, to the higher form of empathy that adults experience. Researchers hypothesize that the phylogenically conserved form of empathy in animals is located on the low end of the empathy spectrum, and involves empathy like behaviors such as distress at the pain of another, much like that of a human infant.

A study of infants performed by Sagi and Hoffman demonstrated low level empathy in humans by presenting infants with both a computer generated and natural audio tape of an infant crying (Sagi & Hoffman, 1976). The behaviors of these infants were compared to control infants who had not been exposed to audio tapes (Sagi & Hoffman, 1976). Researchers found that infants exposed to either crying stimulus were more likely to cry than control infants (Sagi & Hoffman, 1976). These responses were not due to imitation, as the crying displayed by the subject was at a high level of intensity, leading researchers to conclude that these infants were in a distressed state and were not only mimicking the audiotapes (Sagi & Hoffman, 1976). These results

demonstrate an inborn low-level empathic distress reaction (Sagi & Hoffman, 1976). Studies of lower mammals such as rodents and primates that look for these types of distress behaviors are thought to display a low level empathy like those described in infant studies. The fact that these behaviors are the result of more primitive mechanisms does not undermine their classification as empathic behaviors, and provides insight into the evolutionary history of these mechanisms.

The type of low level empathic behaviors that researchers predict to find in studies of animal empathy are hypothesized to be present in animals because of the inherent adaptiveness both empathy and of the pain experience. The unpleasantness of the pain experience motivates animals that experience pain to avoid potentially harmful stimuli. Humans who have a congenital insensitivity to pain due to a genetic mutation effecting their nociceptive response provides a modern day example of the benefits of the ability to feel pain (Drenth & Waxman, 2007). People afflicted with this abnormality are subject to consistent injury due to a failure to avoid harmful stimuli such as burning coffee or a sharp object, and generally suffer a shortened life span (Drenth & Waxman, 2007).

Evolutionarily, animals that were able to learn from previous experiences of pain were subsequently able to avoid the noxious stimulus that produced that pain in the future, therefore increasing their chances of survival. The evolutionary adaptiveness of empathy is likewise involved in the avoidance of pain. The ability to understand the pain of another animal might aid in an animal's ability to avoid objects and situations that might place them in harm's way. An animal that witnesses a conspecific eat a poison berry and later writhe in pain will most likely avoid that berry in future. This is

predicated upon the observer's ability to recognize the negative emotional state of the animal in pain and alter their behavior by avoiding that stimulus. Empathic behaviors are similarly based on the ability to perceive and understand the physical and mental states of others, and for this reason, it is conceivable that empathy is an adaptive behavioral ability.

The evolutionary adaptiveness of empathic behaviors is additionally related to the theory of inclusive fitness, whereby individuals are motivated to aid in the survival of their close relatives, in so doing increasing the probability of the survival of their own genetic material. Parental care, which involves the protection of offspring from harm, is an example of how low level empathic behavior contributes to the concept of inclusive fitness. According to Preston and de Waal, the ability to recognize and react to another's emotional state inherent in empathy is necessary in the success of the parent-offspring bond (Preston & de Waal, 2002). With respect to maternal care, the ability of a mother to adequately recognize and respond to her offspring's emotional state is needed for the successful rearing of those offspring. Studies of various species have demonstrated that mothers are affected by the emotional states of their offspring, expressed through a variety of social cues, and will consequently attend to those offspring. Crying in human babies has been shown to motivate maternal responsiveness, namely physical contact (Bell & Ainsworth, 1972). In addition, female rats demonstrate maternal retrieval and grooming behaviors in response to rat pup distress cues such as ultra-sonic vocalizations and odors (Smotherman et al, 1978). Evidence of maternal response to distress in offspring across various species supports the adaptiveness of low-level empathy in the mother-infant relationship.

Evidence of how maternal care can affect the survival and well-being of her offspring come from studies of rats that demonstrate the physiological effects of inadequate maternal care. Rat pups taken from their mother at age 10 days for a period of 1 to 4 hours demonstrate significantly decreased serum growth hormone concentration compared with control pups not separated from their mother (Kuhn et al., 1978). In addition, orphenine decarboxylase (ODC) activity, involved in organ growth and differentiation, was decreased in deprived rat pups. Researchers conclude from this information that maternal neglect for prolonged periods could feasibly negatively affect the normal rate of growth of deprived rat pups (Kuhn et al., 1978). Studies of human infants also suggest that depressed mothers, who are more likely to exhibit inadequate parental care, could effect the growth of their infants. A study that identified 196 children below the 2nd percentile on infant growth charts found that the mothers of these infants were significantly more likely to suffer from postpartum depression than the mothers of control infants (O'Brien et al., 2004). These studies demonstrate that maternal care elicited by recognition of offspring's emotional state affects the physical health of these offspring, illustrating the adaptiveness of empathy across species.

The motivation to protect or care for offspring could also extend to family or group members who are likely to be related (Masserman et al., 1964). A relative or group member in pain will evoke behaviors from other group-members that indicate low level empathy because of this vested interest. Preston and de Waal propose that interrelatedness influences the Perception-Action Model of empathy (Preston & de Waal, 2002). They posit that an observer will perceive and form a mental representation of the target's state more readily if the observer and target are interrelated (Preston & de Waal,

2002). The concept of interrelated, however, could be interpreted as genetically interrelated or simply residing in the same social group.

If animals do alter their empathic responses based on the genetic relatedness of the target, one would expect that this differentiation would be facilitated by kin recognition. Studies have demonstrated that kin recognition can occur in humans by olfactory cues alone (Weisfield et al., 2003). Primate studies that show equal treatment of adopted and biological offspring by mothers demonstrate that complex levels of kin recognition are could be absent in primates (Bernstein et al., 1991). Pigtail macaques, however, display “aiding” behavior, defined as supporting one animal against an attacker, in increasing amounts dependent on the genetic relatedness of the animal it aids (Massey, 1977). It is therefore plausible that primates are able to distinguish between related family members and unrelated group members, and therefore we might expect to see preferential empathic behavior towards family members versus familiar group members in primates. However, familial recognition has not been demonstrated in lower mammals such as mice, who most likely regard prolonged and early life association as indications of kin. It is possible that mice therefore would not distinguish between group members and genetically related conspecifics in their altruistic behaviors, and therefore using familiar conspecifics as models for empathy in interrelated pairs in mice would be justified.

The presence of empathic behaviors in animals is based on the perceived ability of animals to communicate their own emotions and understand the emotions of others. Studies performed by Engh et al. (2006) on dominance relationships in female baboons supported primate’s ability to understand the emotions of others and appropriately alter

their behaviors. In this experiment audio playback of the threat-grunt of a dominant female was played to the subject either when the subject and the female giving the threat grunt had recently groomed (a sign of friendly relations), or had not (Engh et al., 2006). Subjects who had recently groomed with the dominant female were less likely to move away from the origination of the sound and were more likely to approach that female later (Engh et al., 2006). These results demonstrate that the subjects were able to integrate past relationship experience with the dominant female (had groomed recently or had not groomed recently) and infer whether aggression was likely to be aimed at themselves at that time (Engh et al., 2006).

Research dating back to the early 20<sup>th</sup> century has provided support for the presence of low level empathy in animals. These studies have demonstrated that animals will consistently perform empathic behaviors that express distress over another animal's emotional state, similar to the behaviors of infants described by Sagi and Hoffman. Anecdotal observations of primates have illustrated numerous incidences of these distress behaviors. One primatologist described the behaviors of a juvenile chimpanzee that she had reared, when she pretended to be sad and feigned weeping. "He hastily runs around me, as if looking for the offender; looking at my face he takes my chin in his palm [and] lightly touches my face with his finger." (Ladygina-Kohts, 1935, p.121).

Experimental studies of non-human primates have also demonstrated low level empathic behaviors. Rats in one study ceased pressing a lever that they had learned would deliver a food reward if pressing the lever caused a shock to a conspecific (Church, 1959). Studies in monkeys have demonstrated similar results, and will pull a lever to prevent a painful shock to a conspecific, even if the shock is not delivered to

themselves (Miller et al., 1963). Another study revealed that rhesus monkeys will avoid pulling a chain that delivers a food reward if pulling that chain will induce a painful shock on a companion animal (Masserman et al., 1964). One monkey even resisted pulling the chain to receive food for a full 12 days (Masserman et al., 1964). These effects were present in greater degrees in monkeys than in similar studies of rats and pigeons, indicating the possibility of higher levels of empathy in primates (de Waal, 2001). This behavior was more pronounced in familiar versus non-familiar companions, demonstrating that the tendency to avoid harming another may be related to an element of familiarity (Masserman et al., 1964). These studies supply evidence for the presence of a rudimentary recognition of the pain states of others in animals, as well as an alteration of behavior in response to this knowledge. These studies, however, do not imply full understanding of the pain state of another animal, as they do not rule out simple aversion to the distress of others.

A study performed on rhesus monkeys demonstrated a further ability to understand and communicate emotions involved with pain experience in primates. Researchers designed a cooperative experimental setup, in which one monkey was able to hear a clicker warning sound immediately preceding a painful shock. Another monkey who was able to see the first monkey was able to press a lever to stop the painful shock to both animals, but was unable to hear the warning sound (Miller et al., 1959). Results showed that in this setup the two monkeys were able to effectively avoid the shock with the same proficiency as an isolated monkey who was both able to hear the sound and press the lever (Miller et al., 1959). The monkey with the lever was therefore able to understand through the use of visual or behavioral cues when an impending painful shock

was about to occur, and respond in an appropriate manner by pressing the lever to avoid the shock (Miller et al., 1959).

These studies reveal the ability of animals to understand the emotions of others, and highlight the role of empathy in predicting the behaviors of others. Studies also demonstrate the ability of animals to use these predictions to respond in a way that may increase survival. The adaptive value of empathy within this context is two-fold. Primarily, the emotional states of others can govern and shape our own behavioral responses. The ability to realize and respond to another's emotional state can have important consequences when reacting to possible threats or opportunities for affiliation. Attempting to affiliate with a threatening animal could feasibly lead to aggression and injury, and would therefore be deleterious to the survival of the animal. In addition, the ability to empathize enables animals who observe another animal in pain to understand and avoid the source of that other's pain, without actually experiencing such harmful consequences themselves.

### *Empathy in Animals*

Evidence in animals of distress behavior in response to the pain of others as well as data on primate emotional understanding supports the adaptiveness of empathy for pain. Empirical studies of animal empathy in the laboratory, however, have yet to be performed extensively. Though these types of behaviors point to an ability in animals to empathize, further empirical support involving the relationship between empathy and pain is needed to fully characterize and describe the behaviors associated with low level empathy in animals. Specifically, studies on the modulation of pain through empathy,

like those performed on humans by Loggia et al. (2007), can create a link between human and animal empathy that allows us to characterize empathic behaviors in animals in the laboratory.

A recent study performed by Langford et al. (2006) produced an animal model of empathy to explore the modulation of pain by empathy in mice. In this study, mice were placed in transparent plastic enclosures and injected with a dilute solution of acetic acid into the peritoneal cavity (Langford et al., 2006). This stimulus, called the “writhing test” causes symptoms much like that of a stomach ache, producing an overt pain behavior in which the mouse periodically extends and contracts its abdomen (Malmberg & Bannon, 1999). Mice were placed in the cylinder either alone or in a paired condition with a conspecific (Langford et al., 2006). In the paired condition, the researchers performed either a “one writhing” condition, in which one mouse was injected as the other observed, or a “both writhing” condition in which both mice were simultaneously injected and able to observe each other in pain (Langford et al., 2006).

Langford hypothesized that, in accordance with the Perception-Action Model of empathy, an experience of empathy would provoke a response in the observer with the target (Preston & de Waal, 2002). Mice who empathized with the target would therefore demonstrate a movement of their own state closer to the state of the target, indicated by an increase in writhing behavior. Results of the study showed that when both mice were injected, the paired mice exhibited significantly greater writhing behavior with more incidences of co-writhing than in the single mouse condition (Langford et al., 2006). This effect, however, was present only when the mouse dyads were cage mates. Like primate studies that demonstrated altruistic behaviors present in greater degrees between

familiar animals, this shows a connection between motivation for empathy and familiarity (Langford et al., 2006). This effect was blocked only by obstructing the view of the animals, demonstrating that the empathic behaviors for the pain of the other mouse were motivated by visual cues primarily, as opposed to olfactory or auditory cues. (Langford et al., 2006). These results are similar to those found by Loggia et al. (2007) in humans, in which pain sensitivity was increased when subjects viewed the pain of a target with which they empathized. This study therefore supports the presence of empathic behaviors in mice as described by the Perception-Action Model as an empathic response with the pain state of the target.

## Affiliation

### *Affiliation and the “Affiliation Hormones”*

Langford et al. (2006) defined low level empathic behaviors as increased pain and co-writhing behavior in mouse pairs. Langford’s study inferred empathy in mice because of the movement of the observer’s state closer to the state of the target, illustrated by an increase in writhing behavior. This is described by the Perception-Action Model as an empathic response *with* the target, matching the target’s state. In the present study, measures of empathic behavior will instead focus on a response *to*, and not with the target. In this situation, the state of the observer does not match that of the target, and therefore increased pain behavior in the observer is not a measure of such an empathic response. Alternative behaviors must therefore be characterized to indicate the presence of empathy in response to the pain of a target when the observer mouse itself is not in pain. We hypothesize that empathy under the tend and befriend model of stress response,

because it is a motivator of conciliatory behaviors towards the target, will motivate affiliative behavior in the observer that experiences empathy. Affiliation, and more specifically approach behaviors, can therefore be used as a measure of low level empathy for the pain of another.

Affiliation can be described as a motivation to seek out and associate with certain individuals, sometimes creating a persevering bond that outlasts the initial meeting and withstands separation from that individual. This bond can occur between a variety of relationship pairs depending on the species of the subjects. Affiliation behavior can be described as a three phase process (Lim & Young, 2006). The first phase of this process is approach, in which one animal is motivated to increase proximity to another animal (Lim & Young, 2006). Social recognition follows, wherein the animal is able to recognize and distinguish a familiar animal from other animals to which it has not been exposed (Lim & Young, 2006). Finally a bond between the two animals may form, marked by preference for that animal, desire for proximity, and anxiety under separation (Lim & Young, 2006). The most basic level of affiliative behavior, social approach, will be the focus of the present study.

Affiliative behavior can be motivated by circumstances such as in pair bonding during mating. However, affiliative responses are also present when motivated by specific events, such as dangerous or stressful situations. Affiliative behavior induced by distress is described as a “tend and befriend” response to stress (Taylor, 2006). Unlike the “flight or fight” response to stress, which states that animals respond to stress by aggression or fleeing behaviors, the tend and befriend model posits that animals will be motivated instead to affiliate and attend to conspecifics during stressful events (Taylor,

2006). The application of the tend and befriend model in studies of empathy for pain conjecture that pain in another is a sufficient stressor for the observer to activate affiliation behavior in response to the target's pain. This behavior suggests that these animals are both able to recognize the pain state of the target and react to it accordingly, thereby demonstrating empathy.

Evidence for affiliation in response to the pain or distress of a target typically involves maternal response to infant distress. Nurturing behaviors exhibited by rat mothers when a rat pup emits distress signals include affiliation behaviors such as licking and crouching that involve increased approach and attention (Lim & Young, 2006). Affiliation in response to the distress of a target can be extended beyond the mother-infant relationship. Observations of captive primate groups demonstrate the presence of affiliation behaviors, like those shown in maternal care in mice, between adult conspecifics. Toque macaques in Sri Lanka have been observed licking the wounds of their injured cohorts (de Waal, 1996). The antiviral and antibacterial properties of the saliva of the macaques contributes to faster healing and less infection of the wounds and therefore provides an important service for their companions, increasing the probability of survival of the group as a whole (de Waal, 1996). These behaviors, according to de Waal, demonstrate the macaque's awareness of both the pain of another and of what might remedy that animal's pain (de Waal, 1996). Studies such as these support the hypothesis that the observation of another's pain will elicit approach, and that approach behavior demonstrates the presence of low level empathy.

These observations provide evidence for affiliation behavior in response to the pain of another across a variety of species and relationship dyads. The expression and

release of the “affiliation” hormones oxytocin and vasopressin have been implicated in the presence of these types of affiliation behaviors across numerous species, and have specifically been implicated in the production of tend and befriend behaviors. This discussion will focus specifically on oxytocin and its effect on affiliation behavior in response to stress (Keverne & Curley, 2004). Originating from vasotocin, oxytocin has been linked to formation of social bonds and in the production of approach behavior (Keverne & Curley, 2004). Studies involving manipulation of oxytocin through administration of exogenous hormone or hormone antagonists, as well as using genetic tools such as transgenic knockout mice support the contribution of these hormones to affiliative behavior. Like studies of empathy, animal models of affiliation behavior allow researchers to examine the interactions and effects of affiliation hormones in the production of these behaviors in a simple system under controlled settings. Results of these studies can then be applied to the social interactions of more complex organisms such as humans.

### *Oxytocin*

The mediation of affiliation behavior across a wide variety of species in many cases involves the study of the affiliation hormones and their role in the production of these behaviors. Oxytocin is associated primarily with affiliation behavior in female mice, and is implicated in a number of bonding aspects in females, including pair-bonding and the onset of nurturing behavior after parturition. Oxytocin, a nonapeptide consisting of a ring connected by a disulfide bond, is synthesized in the paraventricular and supraoptic regions of the hypothalamus, projecting into the pituitary gland (Lim &

Young, 2006; Young, 2002). These structures also have links to regions of the limbic system, normally specified in pathways of emotion, such as the hippocampus, amygdala, and nucleus accumbens (Lim & Young, 2006).

Studies of affiliation behavior have pointed to oxytocin's role in the formation of the mother-infant bond (Lim & Young, 2006). Rats with lesions of the periventricular nucleus (a major seat for the synthesis of oxytocin), or who have been given an oxytocin receptor antagonist, show decreased maternal care for their infants (Insel & Harbaugh, 1989; Fahrbach et al., 1985). Additionally, maternal behavior can be induced in female rats without pups by intracerebroventricular (i.c.v.) injection of oxytocin (Pederson et al., 1982). However, oxytocin's role in affiliative behavior extends beyond the initiation of maternal care. The endogenous release of oxytocin is normally triggered by events that expand the birth canal, for example parturition and copulation. Such events are vital in the development of pair bonding after mating. Young et al. (2001) demonstrated this link by focusing on the effects of oxytocin on partner preference. After mating, attachment in adults is generally characterized by partner preference, or the inclination to maintain proximity to a preferred partner rather than another random individual. Because of the high density of oxytocin in the nucleus accumbens (NAcc) and the pre-limbic cortex (PLC), both important relays in the dopamine reward pathway, Young hypothesized that partner preference and pair bonding in general may be facilitated through oxytocin release, and because of its connection to areas of reward, be reinforced through the dopamine pathway (Young et al., 2001). Results showed that injection of an oxytocin antagonist into the NAcc and PLC inhibited partner preference in female prairie voles (Young et al., 2001).

Young's conclusion that oxytocin is involved in pair bonding is supported by studies involving the chronic i.c.v. injection of oxytocin into the brains of gonadally intact males and their interactions with ovariectomized or estrous females (Witt et al., 1992). These oxytocin infused males spent more time in physical contact with the females even without the possibility of sexual interaction (Witt et al., 1992). Oxytocin infused males also displayed significantly higher levels of anogenital sniffing of females than control males (Witt et al., 1992). Primate studies have likewise lent support to the role of oxytocin in pair bonding behavior, as studies show that the brains of more social species such as the bonnet monkey have higher levels of endogenous oxytocin than those that prefer isolation such as the pigtail macaque (Rosenblum et al., 2002). Oxytocin, therefore, might be involved in affiliation behavior among both infants and mothers and mating pairs in a variety of species.

Oxytocin is also involved in social recognition in both male and female mice. Social recognition is significant in the formation of both pair and mother-infant bonds, but particularly within the context of empathic behaviors towards familiar animals (Ferguson et al., 2001). Social recognition in mice is generally measured by olfactory investigation, a typical cue for mice of familiarity (Lim & Young, 2006). It is hypothesized that oxytocin plays a role in the ability of mice to use odor to recognize familiar conspecifics (Lim & Young, 2006). Ferguson et al. (2001) used transgenic oxytocin knockout mice to study the role of oxytocin in social recognition during social encounters. Brain activation in wild type and oxytocin knockout mice was examined using neural activity markers (Ferguson et al., 2001).

During a social encounter, wild type mice showed activation of the olfactory bulbs, piriform cortex, and medial amygdala areas of the brain. The activation of the medial amygdala, involved in emotional regulation, indicated a connection between the olfactory cues and recognition of a familiar. Transgenic mice showed activation only in the olfactory bulbs and piriform cortex, demonstrating the absence of a link between these odors and any social recognition (Ferguson et al., 2001). This effect was not caused by a decrease in olfactory function, and normal social recognition capabilities could be restored with one dose of oxytocin directly into the brain (Lim & Young, 2006; Ferguson et al., 2001; Young, 2002). This effect was also not caused by learning or memory dysfunctions, as these mice perform normally on tests of these skills (Young, 2002). The results obtained by Langford et al. (2006) that increased writhing behavior indicative of low level empathy was present only between cage mates demonstrated that empathic behaviors are modulated by social recognition in mice. Oxytocin's role in an animal's ability to recognize a familiar individual may indicate that oxytocin is indirectly involved in displays of low level empathy between familiar animals.

#### *Sex differences in affiliative response to stress*

In addition to previously described dimensions of affiliation and social recognition, researchers suggest that oxytocin is involved in the affiliation response to stressful stimuli, described by the tend and befriend model. Evidence, including studies described earlier which demonstrate oxytocin's role in maternal care behaviors and pair bonding, suggest that oxytocin could be involved in the activation of the tend and befriend model of stress response. Virgin female rats, when exposed to a noise stressor,

increase overall activity and ambulation in a non-directed way (Windle et al., 1997).

Lactating female rats, however, who have higher levels of oxytocin, when exposed to the same noise stressor will direct their behaviors exclusively towards their pups, and do not display the random activity increase present in virgin females. These findings demonstrate relationship between high oxytocin levels and production of tending behaviors (Windle et al., 1997).

Stress activates similar neural mechanisms in both males and females, namely the sympathetic adrenal medullary (SAM) area and hypothalamic-pituitary-adrenal (HPA) axis, however, oxytocin is proposed to mediate this stress response (Taylor et al., 2000). Oxytocin is shown to have stress reducing effects in both males and females. Studies of breast-feeding women who have high levels of oxytocin demonstrate decreased stress response, as measured by blood pressure, after undergoing a stress test (speech preparation and delivery), indicating that oxytocin could have anti-stress properties (Light et al., 2000). Men receiving intranasal administration of oxytocin showed decreased stress response, as measured by score on the State-Trait Anxiety Inventory, during a stress test consisting of a public speaking task and mental arithmetic (Heinrichs et al., 2003). Researchers suggest that the anti-stress properties of oxytocin could facilitate tending and affiliation behaviors in stressful situations in females, allowing mothers to be calm and attend to their offspring instead of responding in an aggressive manner or fleeing (Taylor, 2006).

Although an anti-stress effect of oxytocin has been shown in males, there is more evidence supporting the effect of oxytocin on stress in females than in males (Taylor, 2006). Taylor suggests that selection pressures which favored mothers who could

respond to stressful situations by attending first to their offspring could have influenced the differential development of stress responses in males and females (Taylor, 2006). It is therefore possible that in response to stress, females are more likely to exhibit tend and befriend behaviors than males. Sex differences in endogenous levels of estrogen, which amplifies the effects of oxytocin between males and females, could also be involved in affiliation behaviors in response to stress seen primarily in females and not males (Taylor, 2006).

### *Present Study*

The tend and befriend model of stress response exemplifies one possible reaction to a stressful situation. With respect to the Perception-Action Model of empathy, it is possible that tend and befriend behaviors could be elicited by the recognition of distress in a target animal. Perception of pain in the target could be a stressor, and according to this model, motivate affiliation behavior in the observer towards the target. In the Perception-Action Model of empathy, this affiliation behavior in reaction to the pain of a target animal exemplifies one category of action; a response *to*, and not *with* the target. Affiliation could therefore be a measure of empathic response to a target in pain which does not require the observer to be in pain as well.

The adaptiveness of empathic behaviors validates the study of empathy in non-human animals. As Langford et al. (2006) recently demonstrated, social modulation of pain sensitivity in mice provides evidence for the presence of empathy in lower mammals. Langford's study demonstrated the presence of a response in the observer *with* the target animal. In the present study, an animal model of affiliation behavior,

specifically social approach, will be used as a measure of empathy for the pain of target in male and female mice. The tend and befriend model will be used as a guideline for the affiliation behavior elicited by the target mouse in pain. Because tend and befriend responses to stressful stimuli are sex dependent, we will investigate sex differences in affiliation behavior in response to the pain of a target, as well as oxytocin's role in the production of this behavior. Using these models, we would expect that if low level empathy exists in the observer, they will approach a target in pain more frequently than a target not in pain. We also propose that because of sex differences in affiliation and response to stress, females will approach a target in pain more than a male. Sex differences in these behaviors could propose a hormonal mechanism for these empathic approach behaviors.

Sex differences in affiliation behaviors in males and females across species lead us to hypothesize that females will be more likely than males overall to display empathic approach behaviors towards mice in pain. We propose that the mechanism in the female responsible for maternal care can be extended beyond treatment of her offspring to treatment of other familiar mice. Females will therefore be motivated by a tend and befriend response to stress to affiliate with a familiar target in pain. In addition, because females have higher levels of estrogen, which amplifies the effects of oxytocin, females could conceivably display more approach behavior towards a mouse in pain than males. We predict therefore that females will have a differential approach pattern towards a mouse in pain and a mouse not in pain.

A pilot study was performed using male and female Sprague-Dawley CD-1 strain mice to determine if a baseline difference in approach behavior existed between males

and females in response to a target in pain and a target not in pain. ANOVA tests showed a significant main effect of sex on time spent near the target mouse,  $F(1,15) = 7.6$ ,  $p = .01$ , with females spending a significantly greater time on the mouse enclosure than males (see figure 1). These results show trends in the predicted directions, with females spending more time near a cage mate in pain than males. Because these results were obtained using small N values, more subjects are needed for these findings to reach significance. We further hypothesize that these behaviors can be amplified or diminished with manipulation of oxytocin because of its proposed role in tend and befriend response to stressful stimuli.

The value of studies such as these is in the increased knowledge researchers can gain about their animal subjects from studies of social relationships and behaviors in these laboratory animals. Mechanisms of social attachment in male and female laboratory mice have relevant implications for future studies on these animals from the way in which experiments are carried out to the housing conditions of these animals. Increasing knowledge about the social relationships of animals will therefore allow experimenters to reduce sources of variability in their research.

Ultimately, studies of social and emotional behaviors such as empathy and affiliation have broader implications in the examination of human social disorders. The roles of affiliation hormones in the development of disorders of social deficiency such as Autism Spectrum Disorder (ASD) and social phobia have been a focal point for recent investigation. Studies have demonstrated that subjects with ASD do not have the same pattern of brain activation in the amygdala when looking at human faces as control subjects. Animal subjects of social recognition in transgenic oxytocin knockout mice

show similar results, lacking activation of the medial amygdala with olfactory investigation (Pierce et al., 2001). This may indicate the involvement of oxytocin or perhaps oxytocin deficiency in ASD. Children diagnosed with ASD have also been shown to have particularly low levels of endogenous oxytocin, and do not show the normal increase in oxytocin that occurs in adolescent years (Modhal, 1998). Deficiencies in ability to empathize have also been indicated in patients with ASD (Blair, 2005). Autistic individuals are unable to recognize emotions from photographs, implying that their ability to recognize and empathize with the emotional states of others could be impaired (Bormann-Kischkel et al., 1995). Animal models of empathy could elucidate the relationship between affiliation hormones and deficiencies in empathy and affiliation present in social disorders.

Investigation of the relationship between affiliative hormones and social disorders such as ASD in humans has proven challenging. Human models of social disorders such as these cannot account for all variables such as environment, genetic variation, and social upbringing (Bartz & Hollander, 2006). The complexity of humans both biologically and socially makes it difficult to draw overarching conclusions with respect to the progression and treatment of these diseases in human populations. Studies of animals provide a simplistic model of social relationships and interactions that allows researchers to control for variables such as environment or genetics by standardizing housing conditions or strain. Producing a viable animal model of social relationships and social disorders has therefore become the goal of research in the field. Researchers could ultimately apply the results obtained by studies of social interactions in animals to human social disorders. This enables researchers to employ neurobiological tools to determine

biological correlates to social disorders, as well as test proposed treatments in simple systems before studying these dimensions in humans (Depue & Morrone-Strupinsky, 2005).

In the present study we will focus on affiliation behaviors of mice which indicate empathy in an observer mouse not in pain. Male and female Sprague-Dawley CD-1 strain mice will be used in this experiment to determine sex differences in empathic approach behavior as motivated by the pain of a target mouse. A target mouse in pain or not in pain will be isolated in a small enclosure. The proximity and approach behavior of an observer able to freely move toward or away from the target mouse will be observed during a 30 minute period. In the first experiment, sex differences in empathic approach behavior toward a mouse in pain will be explored, measured by the difference in maintenance of proximity to a mouse in pain versus a mouse not in pain. In a second experiment, oxytocin will be administered subcutaneously to female mice to determine the role of oxytocin in the production of affiliation behaviors in response to a target mouse in pain.

*Experiment 1: Sex differences in approach behavior*

Methods

*Participants*

Male and female Sprague-Dawley CD-1 strain mice were used for all studies. Mice were housed in cages of 5-6 mice each, kept on a 12 hour to 12 hour light to dark cycle, with the light cycle beginning at 8AM. Mice were fed with Harlan Teklad 8604

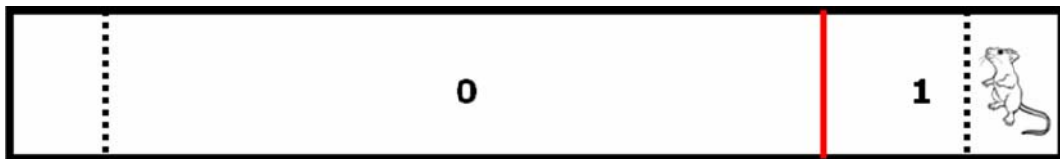
diet and provided with tap water ad lib. Mice were chosen at random to be in pain or no pain condition, free or enclosed mouse. One mouse from each cage was set aside as no pain condition enclosed mouse, and used as free mouse in the pain condition in the final run of each cage.

### *Materials*

A modified elevated plus maze was used to analyze approach behavior in female mice in this experiment. Open arms were removed from the maze and remaining open spaces were covered with clear plastic to allow for video observation, creating a narrow alley. At each end of this closed alley a small piece of wire mesh was placed to isolate the enclosed mouse. The wire mesh allowed the free mouse to perceive olfactory, auditory and visual pain cues of the enclosed mouse. A video camera (recorded using MEDIACRUISE computer software) with ultraviolet light filter was used with an ultraviolet light source to record behavior during experimental period in dark conditions.

Empty Enclosure

Mouse Enclosure



*Figure 1.* Diagram of modified elevated plus maze. The narrow alley was created by removing two arms of the maze and placing wire mesh enclosures at opposite ends of the alley. A dichotomous variable was used to measure proximity to the target mouse. A 10 inch proximity bin was demarcated adjacent to the mouse enclosure.

*General Procedure*

During the experiment the free mouse was placed in the alley and allowed to roam throughout the entire space for a habituation period of twenty minutes. A same sex cage mate was then placed behind a wire mesh at one of the ends of the alley. Only same sex dyads were used in this experiment, as mixed sex dyads could not be housed in the same cage without the risk of copulation and pregnancy. The mesh enclosure at the opposite end was left empty as a control. These enclosures were created with the purpose of keeping the stimulus mouse in a fixed area so that we could examine proximity dictated by the free mouse. A small piece of clear Plexiglas was placed above the enclosure at each end to discourage the mice from escaping.

In each trial the pain state of the enclosed mouse was modified. For all pain manipulations the acetic acid writhing test was used. The writhing test involves intra-peritoneal injection of 10mL/kg dose of .9% acetic acid. This chemical produces stomach-ache like symptoms in the mouse, consequently causing the mouse to display “writhing behavior”, in which the mouse overtly stretches its abdomen. In the “pain” condition, the enclosed mouse was injected with dilute acetic acid solution 3 minutes prior to completion of the free mouse habituation period. In the non-pain condition, the enclosed mouse’s pain state was not altered. The free mouse’s approach behavior was observed for 30 minutes.

Behavior was indirectly observed by videotape and each run was scored for physical proximity to the enclosed animal. To measure this proximity variable, one “close proximity” bin was demarcated, approximately 10 inches from the enclosed mouse. Using this dichotomous variable, the location of the free mouse was noted at 20

second intervals. Amount of time spent in contact with or climbing on the mouse mesh and the empty mesh was also measured. By measuring these variables we sought to determine whether the incentive to be near a conspecific in pain was greater in females than in males. Runs in which either the free mouse or the enclosed mouse escaped from the alley were dismissed.

*Experiment 2: Pharmacological manipulation of oxytocin in female mice*

Methods

*Participants*

Sprague-Dawley CD-1 strain mice were used in this study. Mice were housed in cages of 5-6 mice each, kept on a 12 hour to 12 hour light to dark cycle, with the light cycle beginning at 8AM and fed with Harlan Teklad 8604 diet and provided with tap water ad lib.

*Drugs*

Administration of oxytocin by subcutaneous injection has been supported as an effective means of oxytocin increase in one study involving the effects of oxytocin administration on memory storage. (Boccia et al., 1998) In this study a .3 $\mu$ g/kg dosage of oxytocin was given diluted in an acetic acid solution. The dosage for the present study was increased to 2mg/kg (Mogil, unpublished research).

### *Specific Procedure*

To increase oxytocin in the system for this study, a 10ml/kg dose of oxytocin was injected subcutaneously into the skin covering the scapula of the free mouse before habituation. The enclosed mouse was not injected with oxytocin. Those mice used as the free mouse were allowed a period of 2 days from injection to clear the system of oxytocin before being used as enclosed mice for their remaining cage mates. Behavior was recorded by video and precautions were made to limit the amount of exposure of the mice to human experimenters.

## Results

### *Sex differences in approach behavior*

Proximity was measured as the total sum of bin values throughout the 30 minute period, called the “proximity score”. An independent samples t-test was performed on 40 female mice (those 20 used in the pilot study with the addition of 20 new female subjects) using proximity score as the dependent variable. Results of the t-test revealed a significant difference in proximity score between pain and no pain conditions in females,  $t(37) = -2.13, p=.040$ , with a high proximity score signifying a larger portion of time spent near the enclosed mouse (see figure 2). Mice in the pain condition exhibited significantly higher proximity scores ( $M=39.85, SEM=3.14$ ) than those in the no pain condition ( $M= 30.84, SEM=2.81$ ). An independent samples t-test on male subjects using proximity score as a dependent variable was also performed. Males did not display a

significant difference in proximity score across conditions, with  $t(16)=-.503$ ,  $p=.622$ , not significant (see figure 2).

A “time difference” measure was produced by subtracting the time spent on or in contact with the empty enclosure from the time spent on or in contact with the mouse enclosure. An independent samples t-test using time difference as a dependent variable revealed no significant difference between pain condition in females,  $t(77)=-.855$ ,  $p=.395$ , not significant. An independent samples t-test using “time difference” as a grouping variable likewise showed no significant difference between males in the pain or no pain conditions,  $t(16)=.392$ ,  $p=.701$ , not significant. Further statistical analysis did not use time difference as a dependent variable.

#### *Pharmacological manipulation of oxytocin in female mice*

A 2x2 factorial ANOVA was performed to determine any significant effects of drug condition or pain condition in female mice using proximity score as the dependent variable. All ANOVA results were analyzed with an alpha criteria level for significance of .05. Results of the factorial ANOVA showed no significant main effects of drug condition or pain condition on proximity score and no interaction effects.

A 2x2 mixed factorial ANOVA using a within-subjects dependent variable of “time spent on enclosure” (time spent in contact with the mesh of the empty enclosure and mouse enclosure) was performed to determine any main effects of drug condition or pain condition or any interaction effects. Results demonstrated a main effect of side on time spent on both enclosures,  $F(1,75)=64.34$ ,  $p=.000$ , with more time spent across conditions on the mouse enclosure ( $M=367.84$ ,  $SEM=21.47$ ) than on the empty enclosure

( $M=168.31$ ,  $SEM=10.28$ ). A main effect of drug was also observed,  $F(1,75) = 17.34$ ,  $p=.000$ , with mice injected with oxytocin spending significantly less time in contact with the mesh ( $M=220.65$ ,  $SEM=16.01$ ) than those not given an injection ( $M=315.53$ ,  $SEM=16.22$ ). Results likewise showed a significant condition by drug interaction,  $F(1,75)=7.22$ ,  $p=.009$ . Un-injected mice in the pain condition spent significantly more time climbing on the wire mesh at either end of the alley, ( $M=346.2$ ,  $SEM=22.63$ ), than oxytocin injected mice ( $M=190.1$ ,  $SEM=22.63$ ).

In order to analyze the effect of drug condition, pain condition and side (mouse enclosure or empty enclosure) on time spent on the mesh enclosures, this measure was split into two variables; time spent on mouse enclosure and time spent on empty enclosure. A 2x2 factorial ANOVA was performed using time spent on mouse enclosure only as the dependent variable. This allowed us to determine whether the significant difference in time spent on enclosure between drug and pain conditions was due to time spent on the mouse enclosure, the empty enclosure, or both. Results of the ANOVA revealed a significant main effect of drug,  $F(1,75)=9.542$ ,  $p=.003$ , with mice not injected with oxytocin spending significantly more time on the mouse enclosure ( $M=434.20$ ,  $SEM=30.56$ ) than those injected with oxytocin ( $M=301.55$ ,  $SEM=30.17$ ). Analysis also revealed a significant pain condition by drug interaction, with  $F(1,75)=4.12$ ,  $p=.046$ , with animals in the pain condition spending more time on the mouse enclosure when not injected with oxytocin ( $M=488.45$ ,  $SEM=45.18$ ) than those injected with oxytocin ( $M=268.65$ ,  $SEM=34.51$ ) (see figure 3)

The ANOVA test was repeated using time spent on empty enclosure as the dependent variable, and again a main effect of drug was observed,  $F(1,75)=7.72$ ,  $p=.007$ ,

with mice in the no drug condition spending significantly more time on the empty enclosure ( $M=184.09$ ,  $SEM=12.53$ ) than those injected with oxytocin ( $M=139.75$ ,  $SEM=14.35$ ). Mice injected with oxytocin therefore spent less time in contact with the wire mesh than un-injected mice at both ends of the alley.

## Discussion

The purpose of these studies was to establish a model of empathy using social approach towards familiar conspecifics in pain as a measure of empathic affiliation behavior. We hypothesized that if empathy is present in mice, mice that experience empathy should approach a target mouse in pain more than a mouse not in pain. We further examined sex differences in empathic approach behavior as modulated by the pain condition of another mouse, as well as the role oxytocin plays in this behavior. We predicted that females would display more approach behavior overall and specifically when the mouse was in pain, than male mice, and that this effect was primarily regulated by oxytocin's role in tend and befriend behavior in female mice. We further predicted that increase of oxytocin in female mice would result in significantly more approach behavior toward the mouse in pain.

### *Sex differences in approach behavior*

Male and female mice not injected with oxytocin reacted to the stimulus mouse as predicted by our hypotheses in our approach paradigm. Results of statistical analysis performed on female mice revealed that female mice in the pain condition had higher

proximity scores than those in the no pain condition, indicating that female mice were spending more time near a mouse in pain than a mouse not in pain (see figure 2). These results are consistent with our hypothesis that females will differentiate their approach behavior and approach a mouse in pain with more frequency than a mouse not in pain. As predicted, male mice did not distinguish between pain conditions in their approach behavior.

The differential pattern of behavior of female mice towards mice in pain suggests that females are able to perceive and respond to the enclosed mouse's pain state and alter their behavior in accordance with that pain state. These results could, as predicted by our hypotheses, be a consequence of the tend and befriend model for stress response present in female mice. It is supposed that the motivation of female mice to care for infants could predispose females to also exhibit tending behavior towards a familiar cage mate, and therefore provoke approach behavior towards a mouse in pain (who may evoke an empathic nurturing response in females). This result suggests that oxytocin could play a role in this empathic approach behavior, as the mechanism involved in the onset of maternal behaviors and tend and befriend responses are primarily generated by elevated levels of oxytocin in the system. It is also possible that estrogen could be related to the production of affiliation behavior in response to stress by amplifying the effects of oxytocin (Taylor, 2006). Future researchers may choose to vary the levels of both oxytocin and estrogen in the system to determine their role and interaction effects in tend and befriend behaviors.

*Pharmacological manipulation of oxytocin in female mice*

Studies have demonstrated that increase of endogenous oxytocin levels induce maternal care behavior. In this study we hypothesized that the increased nurturing behavior associated with higher levels of oxytocin could be applied to a more general treatment of adult mice, encouraging pro-social empathic behaviors in these female mice. In addition, oxytocin's role in tend and befriend behaviors could indicate that manipulation of oxytocin may affect the production of approach in response to an animal in pain. Oxytocin has been implicated as having anti-stress properties, and therefore it is possible that exogenous oxytocin administration would calm the observer mouse and allow it to exhibit tending behaviors towards the target mouse. In association with our results from the previous study, we hypothesized that a boost in oxytocin levels in female mice by subcutaneous injection would increase approach behavior towards a mouse in pain.

The pharmacological manipulation of oxytocin in female mice did not produce results consistent with our predictions. Statistical tests using time spent on either enclosure as a within-subjects variable showed that mice injected with oxytocin spent significantly less time on either enclosure than those not injected with oxytocin, a result that directly contradicted our hypothesis that increased oxytocin would result in increased approach behavior towards the enclosed mouse.

It is possible that these results can be attributed to a number of unexpected side effects of high doses of oxytocin. Studies of oxytocin administration have demonstrated that high doses of oxytocin can yield overall decreased locomotion in male rats. (Uvnas-Moberg et al., 1996) In this study doses of 250-1000  $\mu\text{g}$  administered subcutaneously to

male rats were shown to have clear sedative effects, including reduced overall ambulation and reduced rearing effective over a one hour period. (Uvnas-Moberg et al., 1996) These sedative effects have also more recently been shown to be present under administration of 1000  $\mu\text{g}$  in female rats. (Peterson et al., 1998) The proposed anti-stress and calming effects of oxytocin could have been overly effective in these mice, causing overall decrease in mobility. It is therefore possible that the significant difference between in oxytocin injected mice and un-injected mice in time spent on either of the enclosures could be accounted for by overall decreased mobility due to high doses of oxytocin.

These results could also be explained by an increase in aggression and maternal defense by increased levels of oxytocin in females. After parturition, increases in oxytocin serve to enhance the mother's natural defense of her offspring, thereby increasing aggression towards intruders, normally characterized by increased attack behavior towards novel mice. (Campbell, 2008) Studies have demonstrated that administration of 10 to 20 ng of oxytocin into the central amygdala of lactating female rats inhibits aggression, most likely a carryover from the inhibition of aggression towards her pups. (Consiglio et al., 2005) However, in studies performed on virgin rats, increase of oxytocin receptors in the same brain region by i.c.v. injection caused increased aggression towards female intruders, indicated by an increase in attacks. (Bosch et al., 2006) It is possible that our theoretical interpretation of the effect of increased oxytocin was faulty, and that instead of the free mouse treating the enclosed mouse as she would her own offspring, the enclosed mouse was instead interpreted as a threat and therefore a source of danger to be avoided.

Although this increase in aggression may have been involved in the decreased social approach towards the enclosed mouse in oxytocin injected mice, it is presumably not the primary cause for such an effect. Overtly aggressive behavior was not observed in any of the subjects injected with oxytocin, and furthermore even if an aggressive disposition was induced by the oxytocin, this would not necessarily equate to lower levels of approach, and may in fact increase approach in order to make defensive attack possible.

Results of the effect of drug condition and pain condition on time spent on either enclosure also revealed an interaction effect of drug condition by pain condition, with female mice in the pain condition spending less time on the enclosures if they were injected with oxytocin than those who were not; an effect not present in the no pain condition. To determine whether the drug's effect was present in both the time spent on the mouse enclosure and the time spent on the empty enclosure, statistical tests were performed using each of these measures separately. Results showed that mice not injected with oxytocin spent more time on both the mouse enclosure and the empty enclosure than those injected with oxytocin, in accordance with previously explained results. The most compelling finding however was that un-injected mice in the pain condition spent more time near the mouse in pain than mice injected with oxytocin, while both un-injected and injected mice spent equal amounts of time near the mouse not in pain (see figure 3).

Taken together these results imply that the drug's effect cannot be attributed to an overall decrease in mobility and locomotion in mice across pain conditions, but rather point to a specific aversion of oxytocin injected female mice to mice in pain. This effect

could be due to a self protective mechanism involved in preventing the spread of contagious illness. Female mice, when looking for a mate or during gestation, both times in which oxytocin is increased in the system, are particularly vulnerable to infection by harmful viruses or parasites. Recent studies have demonstrated that female mice are able to distinguish by behavioral cue, namely odor, between male mice with and without intestinal parasites, and further that oxytocin plays a key role in this skill. (Kavaliers et al., 2005) In this study wild type female mice and oxytocin knockout mice were allowed to roam in a clear plastic tube containing a piece of filter paper at one end that possessed the urinary odors of a male mouse with or without a parasite. (Kavaliers et al., 2005) Results of the study showed that wild type mice displayed avoidance responses to the urinary odors of parasitized male mice, while oxytocin knockouts were not able to make this distinction. (Kavaliers et al., 2005)

Because the pain manipulation used in this study was one that involves overt pain behaviors associated with the abdomen, it is possible that the free mice were responding to the enclosed mice in pain much as they would to a mouse with a parasite. The parasite study demonstrated that female mice are able to use social cues as a means of predicting the danger associated with approaching another mouse. We can therefore conjecture that the oxytocin-injected free mouse avoided the mouse in pain as part of an evolutionarily adaptive mechanism to avoid contracting a contagious illness. This may have resulted in the observed decrease in approach behavior towards the enclosed mouse in pain.

*Limitations and future directions*

Aside from the unexpected side effects of high doses of oxytocin, it is conceivable that the manipulation was unsuccessful because peripheral injection of oxytocin did not affect levels of oxytocin in the brain, and therefore did not stimulate pro-social behaviors. While the time constraints of this study did not allow for the use of i.c.v. injections, in future studies it would be preferable to avoid subcutaneous injections to increase oxytocin because of the uncertain nature of their effectiveness on hormone levels in the brain. Although research has been performed using peripherally administered oxytocin, none so far has studied its effect on social behaviors, and therefore future researchers might choose to utilize this procedure.

In addition to i.c.v. administration of oxytocin, the peripheral administration of drugs that block oxytocin could prove useful to the continued study of oxytocin's role in social motivation of females. Research in the field of social motivation and female affiliation hormones has recently concentrated on the use of an oxytocin antagonist by subcutaneous injection to study social correlates of oxytocin. Researchers used a non-peptide oxytocin agonist, L368,899 administered intravenously in non-human primates to determine whether this drug blocked oxytocin receptors in regions of the brain that might control female sexual and maternal behaviors. (Boccia et al., 2007) Results of the study displayed selective accumulation of the drug in the hypothalamus, septum, orbitofrontal cortex, amygdala and hippocampus; all brain areas that are implicated in regulation of primate social behavior. (Boccia et al., 2007) In addition, the intravenous administration of this drug incited disinterest in infants as well as decreased sexual behavior female non-human primates, implying that this drug could also inhibit social approach and

motivation to nurture in the present study (Boccia et al., 2007). The continuation of studies involving oxytocin and affiliation behavior may therefore include the use of this antagonist.

The use of males instead of females in the pharmacological manipulation of affiliative behaviors could also be a direction in which future researchers could take this investigation. Central administration of arginine vasopressin in male mice has been shown to increase paternal and pro-social behavior towards offspring. (Parker & Lee, 2001) It is therefore conceivable that male mice with increased levels of vasopressin might be more motivated to associate with the enclosed mouse. More specifically, because of the nature of paternalistic care, which necessarily involves the protection of one's offspring and relatives to promote the proliferation of one's own gene pool, one might predict that males would approach a mouse in pain more frequently than a mouse not in pain, essentially making male mice behave like female mice in the first experiment.

Within the present study paradigm, there are manipulations and measures which could add to our understanding of the reason for affiliation behavior in response to a mouse in pain. The approach behavior elicited by female mice towards mice in pain may indicate the use of social cues by the mouse in pain to solicit approach and aid. In Langford's study, increased writhing behavior indicating empathy towards a mouse in pain was not present when visual observation was obstructed. Auditory and olfactory obstruction had no effect on the increased writhing behavior of the observer mouse. This indicates that visual cues are the primary stimulus for empathy in this study. Future researchers may investigate the role of visual, auditory and olfactory cues in the present experiment by measuring ultra-sonic vocalizations of the target mouse, or by blocking

auditory, olfactory, and visual senses in the observer mouse to determine whether and in what ways the target mouse may be soliciting approach.

Additionally, because the approach behavior observed in this study is hypothesized to be a result of a tend and befriend mechanism of response to stress, the stress state of the observer animal should be measured in the future to ascertain a more concrete connection between the observed affiliation behaviors and the tend and befriend model. If the observer mouse approaches the mouse in pain because the pain of the target is a stimulus for stress, we might expect the cortisol levels to increase in both males and females when exposed to a mouse in pain. However, because of the anti-stress effects of oxytocin, the cortisol levels of female mice may be lower than those of the male mice. The low cortisol levels in females compared to males might suggest a stronger connection between affiliative empathic behavior and the tend and befriend model.

The next step of the current study was not completed before this report due to time constraints, but will be continued for the duration of the semester. Oxytocin gene knockout mice with B-6 strain background obtained from Dr. Jeffrey Mogil's laboratory at McGill University will be used in the next stage as a comparison to wild type female mice. These mice will be run using the same social approach paradigm, with wild type cage mates as their enclosed compatriots in order to preserve the knockout mice for future studies. We predict that oxytocin knockout mice will have similar approach behavior to male mice in our first stage of the current study, and will not differentiate their approach behavior with respect to the pain state of the enclosed mouse.

The most significant finding in our study was the difference in approach behavior of female mice towards mice in pain and mice not in pain that was not present in males.

These results clarify in some respect a theoretical paradox in the connection between approach behavior and empathy. An animal can exhibit two opposing actions in response to a mouse in pain; it can either increase proximity to the mouse in pain, or it can avoid the mouse in pain. Results of the present study, which demonstrate that females spent more time near a mouse in pain than a mouse not in pain, support the concept that a mouse experiencing empathy will be motivated to approach the target mouse in pain. Previous studies of empathic approach have demonstrated this type of behavior in higher mammals such as primates, but less extensively in lower mammals such as mice and rats. This study therefore shows that the mechanisms involved in tending behavior in primates, such as in captive primate groups described by de Waal who demonstrate care behaviors toward their compatriots such as wound cleaning, could also be present in lower mammals such as mice (de Waal, 1996). Past research by Langford et al. (2006) has supported the ability of mice to alter their own pain behavior in response to another mouse's pain state. Until recently however, empathic behaviors have not been examined in situations in which both mice are not in pain. This type of research could lead to better and more realistic animal models of empathic behavior that could then be used to extrapolate more generally to human social relationships.

In the greater scope of research on the effects of hormones on social behavior, these results demonstrate that continued research on animal models of affiliation hormones and their involvement in social interactions is justified. Using these animal models of social behavior aids researchers in the larger goal of determining factors involved in social dysfunction, and studies such as the present study involving empathy in animals could contribute to a better understanding of social disorders that involve a

lack of empathizing and other social skills, such as in Autism Spectrum Disorder. The continuation and perfection of research practices that allow for reliable and reproducible ways of manipulating affiliation hormones and social interactions in animals is vital to the development of better and more effective treatments for these disorders.

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Appendix

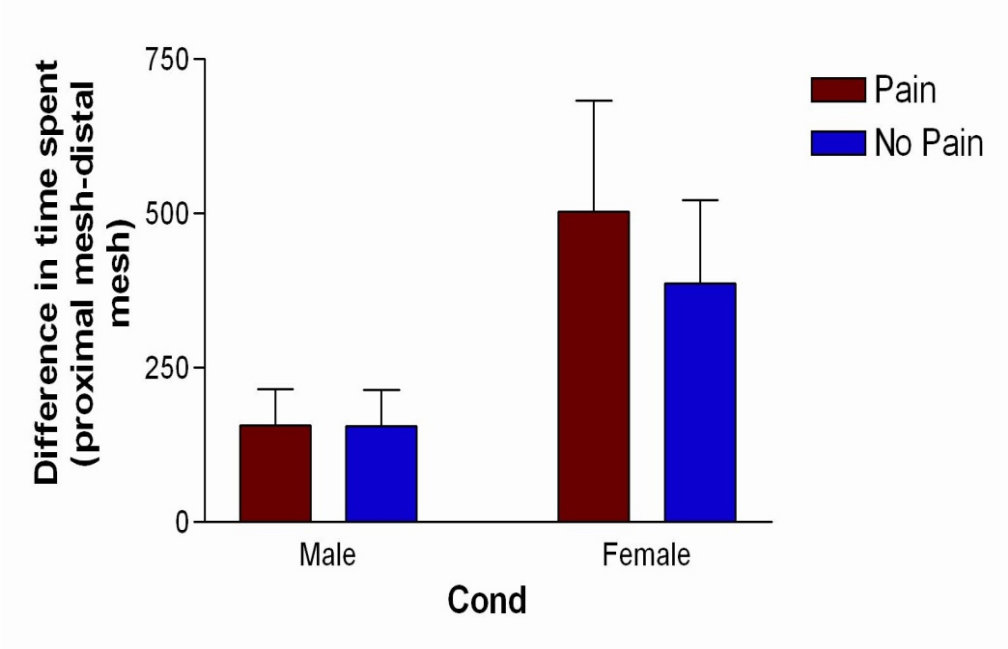


Figure 1. Results of the pilot study demonstrated a trend in female mice approaching mice in pain more than mice not in pain. Males did not demonstrate this differential approach behavior.

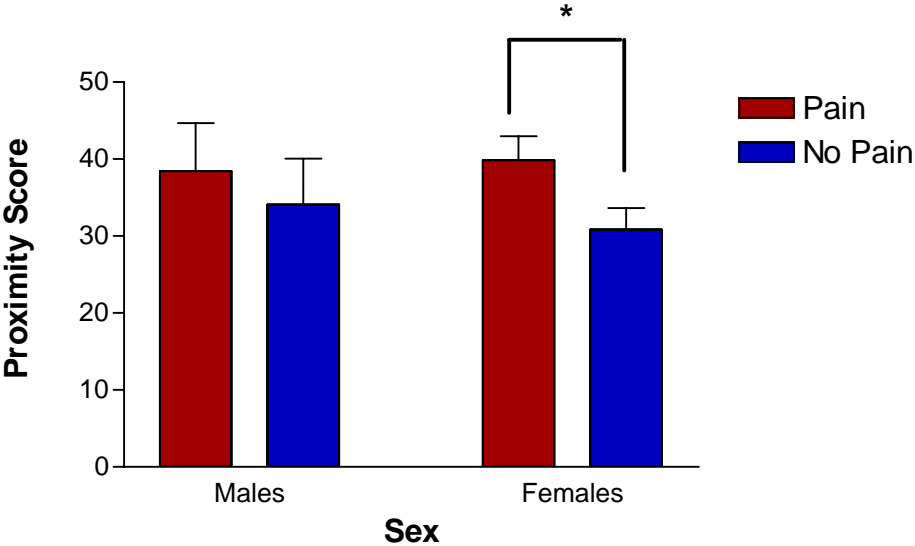
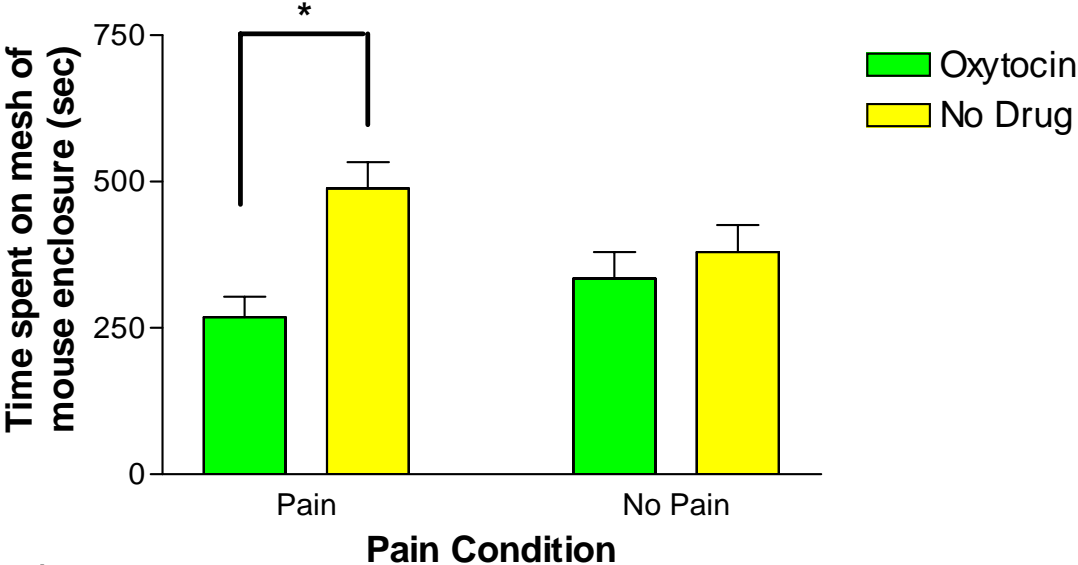
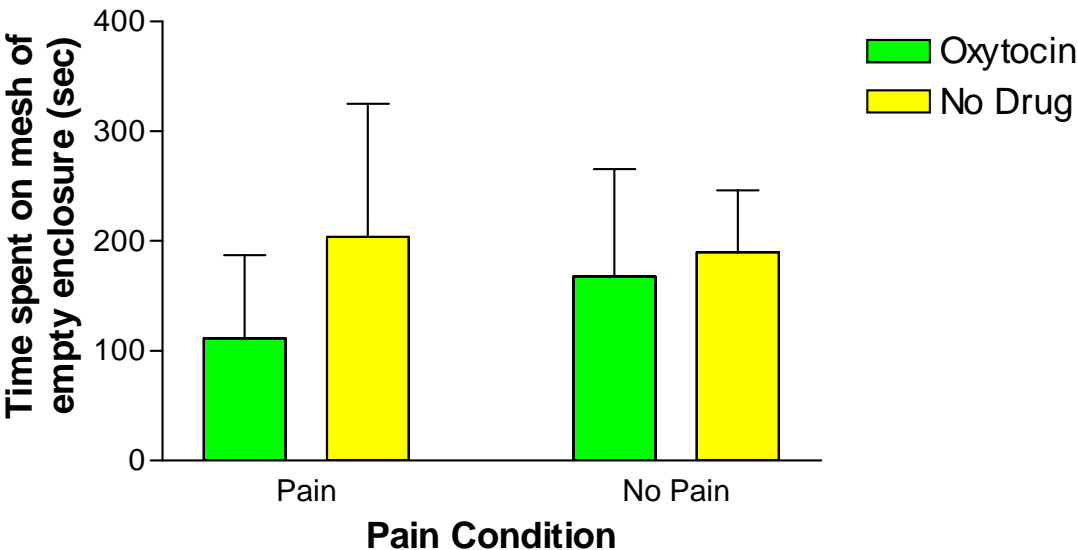


Figure 2. Results demonstrated that a female mouse will approach a mouse in pain more than a mouse not in pain. Males did not distinguish significantly between a mouse in pain and a mouse not in pain, and displayed similar approach behavior towards both.



A



B

Figure 3. Female mice injected with oxytocin spent less time in contact with the mesh on either the mouse or empty enclosure. A) In the pain condition, female mice injected with oxytocin spent significantly less time in contact with the mesh of the mouse enclosure than female mice not injected with oxytocin. B) This difference was not present between non-injected and oxytocin injected mice in the pain condition in time spent in contact with the mesh of the empty cage.