The Rewarding Value of Gentle Touch in Mice

by

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Abstract
There exists strong evidence that both human and non-human primates have a specialized “affective touch” system for transmitting socioemotionally relevant touch. There is increasing evidence that rodents possess an analogous system, but the neural mechanisms remain largely unknown. One challenge for studying affective touch in rodents is the lack of a reliable and valid behavioural assay. The current project describes a novel tactile conditioned place preference (CPP) paradigm, the results from which suggest that gentle touch is rewarding for young male C57Bl/6 mice. In order to address whether tactile reward is driven by or otherwise related to innate sociality, the classic three-chambered test of social approach was used, but no significant association was found. Lastly, the brain regions associated in human affective touch (i.e. the insular and somatosensory cortices) and in reward processing (i.e. the nucleus accumbens) were analyzed using immunohistochemistry probing for c-Fos expression in response to gentle touch and tactile CPP, but no significant insights can be reported.
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Table of Contents

Table of Contents........................................................................................................ iv
List of Tables .................................................................................................................. vi
List of Figures ................................................................................................................ vii
Chapter 1: Background & Literature Review ................................................................. 1
  1.1 Mechanisms of Touch ......................................................................................... 1
    1.1.1 Peripheral Mechanisms of Touch: Aβ & C Fibers .......................................... 2
    1.1.2 Central Mechanisms of Touch ..................................................................... 3
    1.1.3 CTs & Pleasure: The Affective Touch Hypothesis....................................... 6
    1.1.4 Top-Down Mechanisms of Affective Touch .............................................. 10
  1.2 The Social Functions of Touch ........................................................................... 12
    1.2.1 Parental Care ............................................................................................. 13
    1.2.2 Tactile Interactions between Peers ............................................................ 16
      1.2.2.1 Social Play Behaviour in Young Rats ................................................... 18
      1.2.2.2 Tickling in Juvenile Rats: Experimenter-Driven Tactile Reward........... 20
    1.2.3 Autism Spectrum Disorders: When Touch Isn’t Pleasant ......................... 21
  1.3 Conclusion .......................................................................................................... 24
Chapter 2: Introduction to Current Project ..................................................................... 25
  2.1 Statement of the Problem ................................................................................... 25
  2.2 Purpose of the Study ........................................................................................... 25
  2.3 Research Question & Hypotheses ..................................................................... 26
      2.3.1 Investigating the Rewarding Value of Gentle Touch in a Mouse Model .... 26
      2.3.2 Investigating the Link between Social Approach & Tactile Preference .... 27
      2.3.3 Investigating the Brain Regions involved in Gentle Touch ..................... 27
Chapter 3: Materials & Methods ............................................................................... 29
  3.1 Animals .............................................................................................................. 29
  3.2 Handling ............................................................................................................. 29
  3.3 Three-Chambered Test of Social Approach ....................................................... 30
      3.3.1 Apparatus ............................................................................................... 30
      3.3.2 Protocol .................................................................................................. 31
  3.4 Tactile Conditioned Place Preference ................................................................ 32
      3.4.1 Apparatus ............................................................................................... 32
      3.4.2 Protocol .................................................................................................. 33
  3.5 Immunohistochemistry ...................................................................................... 34
  3.6 Data Analysis ..................................................................................................... 35
      3.6.1 Three-Chambered Test ............................................................................ 35
      3.6.2 Tactile Conditioned Place Preference ..................................................... 36
      3.6.3 The Relationship between Social Approach & Tactile Preference ........... 36
      3.6.1 Fos-Immunoreactive Cell Counting ....................................................... 36
Chapter 4: Results ....................................................................................................... 38
  4.1 Three-Chambered Test ....................................................................................... 38
  4.2 Tactile Conditioned Place Preference ................................................................ 39
  4.3 The Relationship between Social Approach & Tactile Preference ................. 40
  4.4 Immunohistochemistry ...................................................................................... 41
List of Tables

Table 4.1 Unpaired t-tests of Fos-I cell counts of stimulated vs. unstimulated mice

Table 4.2 Pearson correlations between tactile preference and Fos-I cell counts
List of Figures

Figure 3.1 Three-chambered test set-up

Figure 3.2 Tactile conditioned place preference schedule and set-up

Figure 4.1 Three-chambered test results

Figure 4.2 Tactile conditioned place preference results

Figure 4.3 Relationship between social approach and tactile preference

Figure 4.4 Fos-I cell counts graphs for (a) anterior insular cortex, (b) posterior insular cortex, (c) nucleus accumbens, and (d) somatosensory cortex

Figure 4.5 Relationships between Fos-I cell counts and tactile preference for (a) anterior insular cortex, (b) posterior insular cortex, (c) nucleus accumbens, and (d) somatosensory cortex

Figure 4.6 Representative images of immunohistochemistry staining for (a) anterior insular cortex, (b) posterior insular cortex, (c) nucleus accumbens, and (d) somatosensory cortex
Chapter 1
Background and Literature Review

The sense of touch is essential for navigating the physical world, allowing for the manipulation and location of objects in space. However, touch also guides us through the social world, facilitating inter-individual contact, affiliative behaviours, and the formation and maintenance of social bonds (Morrison, Löken, & Olausson, 2010; Olausson, Wessberg, Morrison, McGlone, & Vallbo, 2010). As such, the functions of touch can be dichotomously characterized as discriminative (sensory) touch and affective touch (Morrison et al., 2010).

The term “affective touch” can be used to describe any tactile processing with a hedonic or motivational value (Morrison, 2016). While the neurobiology of discriminative touch is very well established, there is still much to uncover regarding the mechanisms of affective touch. This review aims to elucidate the neural pathways that underlie affective touch by discussing the current knowledge of peripheral and central mechanisms and how they relate to the social functions of touch in both humans and rodents.

1.1 Mechanisms of Touch

In order to understand affective touch and how it compares to discriminative touch, a basic understanding of the afferent pathways underlying both is useful. In this first section, I outline the journey that tactile stimulation travels from the
surface of the skin to the brain, as well as how these signals are encoded and interpreted by both the peripheral and central nervous systems.

1.1.1 Peripheral Mechanisms of Touch: Aβ Fibers & C Fibers

Two separate classes of sensory afferent nerve fibers support the peripheral encoding of touch: thick, myelinated A-beta (Aβ) fibers and thin, unmyelinated C fibers. The myelination of Aβ fibers allows for rapid conduction to support quick central processing of discriminative information such as pressure, vibration, and texture (Prechtl & Powley, 1990). This fast-acting system demonstrates clear sensory benefits, enabling the ability to detect, discriminate, and identify external stimuli to guide behaviour (McGlone, Wessberg, & Olausson, 2014). Unmyelinated C fibers, on the other hand, conduct at a velocity that is approximately 50 times slower than myelinated afferents and have been linked to sensations such as pain, temperature, and itch (McGlone et al., 2014). Therefore, it seems we have evolved a fast touch system with clear discriminative and sensorimotor functions, as well as a slower “secondary” touch system.

There are two different types of skin: glabrous skin, found only on the palms and soles of the feet, and hairy skin, which is much more abundant. Most primate research into skin sensory processing has focused on the glabrous palm, (Mountcastle, 2005), with relatively few studies of tactile sensitivity in hairy skin. One subclass of C fibers, known as C-tactile (CT) fibers, is found exclusively in
hairy skin and is apparently absent in glabrous regions (Löken, Wessberg, Morrison, McGlone, & Olausson, 2009). CT fibers respond specifically to light and slow stroking at indentation forces of 0.3-2.5 mM and a rate of 1-10 cm per second, similar to a gentle caress (Cole et al., 2006; Löken et al., 2009; Vallbo et al., 1999). CT fibers demonstrate high-frequency responses to innocuous stimuli, such as gentle stroking movements delivered from a soft brush (Vallbo et al., 1999). In addition to being tuned to a specific velocity, CT fibers are also tuned to a specific temperature: they appear to discharge preferentially to slowly moving stimuli at a neutral (typical skin) temperature, rather than cooler or warmer ones (Ackerley, Saar, McGlone, & Backlund Wasling, 2014).

1.1.2 Central Mechanisms of Touch

Laboratory animal work has provided insight into the spinal projections of C fibers. Central terminals of mouse C fibers project to inner lamina II of the spinal dorsal horn (Sugiura, 1996) and there is a population of spinal neurons that respond exclusively to slow-moving brush stimuli with inputs from unmyelinated afferents and cell bodies in inner lamina II (Light & Perl, 1979). In rats, CT-specific stimulation appears to show projection neurons in lamina I of the spinal cord, where low-velocity brush strokes (9.2 cm/s) elicits the most activation (Sewards & Sowards, 2002). The supraspinal circuits activated by CT activity in experimental animals are currently unknown, but a route via the ventral posterior
thalamic nuclei to the insula has been proposed based on neuroanatomical tracing work (Andrew, 2010). In some early human studies wherein the anterolateral spinothalamic tract was surgically removed as a treatment for chronic pain, it was reported that these patients experienced a loss of pain, temperature, itch, and pleasure, suggesting CT fibers ascend along the same tract as other C fibers such as C-nociceptors (Foerster et al., 1932).

Cortical processing of CT fibers is not yet known in rodents, but human imaging studies have repeatedly described CT projections to a network of regions that are also implicated in social reward processing. In healthy subjects, soft brush stroking of hairy skin activates the classical somatosensory areas S1 and S2, as well as the posterior portion of the contralateral insular cortex (Olausson et al., 2002). The roles of S1 and S2 in discriminative touch are well documented and they are known to receive Aβ projections. The insula, on the other hand, has been reported to produce an emotionally relevant context for sensory experiences (Augustine, 1996; Craig, 2009). When brushing stimuli were applied to neuropathy patients lacking in Aβ fibers, no activation in S1 or S2 was observed while insular activity remained, suggesting CT afferents act independently to innervate the insula (Olausson et al., 2010). In healthy subjects, CT-optimal stroking speeds show more activation of the insula than slower or faster rates, as determined by analyzing MRI-obtained voxel counts (Morrison et al., 2011; Perini,
Morrison, & Olausson, 2015). Insular activity has also been widely observed in other affective states such as social pain and reward processing (Cristofori, Harquel, Isnard, Mauguière, & Sirigu, 2015), thereby making it a likely candidate for supporting the hedonic value of affective touch. As such, much of the work investigating central mechanisms of affective touch has focused on the insula as a primary region of interest.

In addition to the insula, Gordon and colleagues (2013) have also identified several other regions to create a so-called “social touch” network. These regions include the right posterior superior temporal sulcus (pSTS), right medial prefrontal cortex (mPFC) and dorsal anterior cingulate cortex (dACC) in affective touch. These areas, along with the insula, showed fMRI activation in response to CT-optimal touch of the hairy forearm but not when the same stimulus was applied to the glabrous skin of the palm. Voos, Pelphrey, & Kaiser (2013) also demonstrated that the orbitofrontal cortex (OFC) shows activation in response to slow but not fast velocities of tactile stimulation, suggesting it may also contribute to the affective touch network. Importantly, this “social touch” network has also been implicated in other socially-relevant processes, such as detecting biological motion (Grossman et al., 2000; Saygin, 2007), theory of mind, and mentalizing (Frith & Frith, 2003; Gallagher et al., 2000).
The contribution of tactile Aβ fibers to emotional processing alongside CT fibers has not been widely explored. Aβ fibers are likely still involved for pleasant sensations because touch stimuli to the glabrous palm can be perceived as pleasant (Krämer et al., 2007) and elicit fMRI responses in a target area for insular efferents in the OFC that are involved in emotional evaluations (Francis et al., 1999; Rolls, 2004). However, while pleasant touch from hairy skin is represented in the firing frequency of CT fibers (Löken et al., 2009) and processed in the limbic (Björnsdotter, Morrison, & Olausson, 2010), there is no correlation between firing of Aβ fibers and pleasant touch perception (Löken et al., 2009). Thus, it remains unclear what role of Aβ fibers play in affective touch sensation, but it has been hypothesized that pleasant glabrous stimulation is a learned or secondary reinforcement mechanism, while pleasant hairy skin stimulation represents an innate process (McGlone et al., 2012).

1.1.3 CTs and Pleasure: The Affective Touch Hypothesis

Before the discovery of CTs, C fibers were classically associated with pain, temperature, and itch. Now, we know that a specialized population of C fibers exist in human hairy skin and tuned to gentle stroking, a stimulus outside of nociceptive range. This begs the question: what is the function of CT fibers?

Evolutionarily speaking, a stimulus associated with reward or punishment is linked to survival. For instance, it is generally accepted that two types of nerves
signal cutaneous pain: a fast-acting A-delta (Aδ) fiber system that encodes sensory properties of the stimulus and provides discriminative spatial information to identify threats of tissue damage on the body, and a slow system of C fibers that transmits information about the affective or motivational consequences of skin damage (McGlone, 2014). A similar “duality” has been proposed for touch (McGlone, 2014): first, touch transmitted by myelinated Aβ nerves that signal the presence of an object on the body surface and provides discriminative spatial information to identify where on the body the stimulus is located. The second system, mediated by CT afferents, is activated if the stimulus has the appropriate properties, i.e. is a low-force and low-velocity dynamic touch, the response to which would be an affective or motivational one. Recall that CT fibers are also tuned to respond to tactile stimuli with the thermal properties of normal skin temperature (Ackerley et al., 2014), which implies a role as a peripheral mechanism for signaling pleasant tactile interaction.

The notion that affective touch involves a hedonic or motivational component is made explicit with the “affective touch hypothesis”, which postulates that the role of CT fibers is to provide or support emotional and behavioural responses in skin-to-skin contact (Olausson, Wessberg, Morrison, McGlone, & Vallbo, 2010). Specifically, proponents of the affective touch hypothesis suggest that the CT system evolved alongside the discriminatory Aβ system to ensure the
maintenance of physical and social well-being (Björnsdotter et al., 2010; Cascio et al., 2012; Marco et al., 2012). In support of this hypothesis, many human behavioural studies have shown that activation of CT afferents is consistently accompanied by reported feelings of pleasantness. For instance, Löken and colleagues (2009) asked participants to fill out visual analog scales of pleasantness while receiving gentle stroking across the forearm at six velocities (0.1, 0.3, 1, 3, 10, and 30 cm/s). Using microneurography, the researchers discovered that stroking at 1, 3, and 10 cm/s yielded the highest firing rates and pleasantness ratings (Löken et al., 2009). Slower and faster velocities were rated as less pleasant, thus creating an inverted U-shaped relationship of CT firing rates. Importantly, the same tactile stimulation applied to the glabrous palm, which lacks CT afferents, showed no relationship between velocity and pleasantness ratings (Löken, Evert, & Wessberg, 2011). Morrison et al. (2011) replicated this finding in a healthy control group that was used in comparison to a neuronopathy patient group that showed reduced C-fiber density. The patients rated stroking as significantly less pleasant than the controls, for whom the inverted U relationship was once again observed. Alternatively, two patients with neuronopathy affecting myelinated fibers (and presumed to lack Aβ fibers) reported experiencing slightly to moderately pleasant sensations in response to gentle brush stroking, despite
being unable to identify the spatial location of the stimulation (Cole et al., 2006; Olausson et al., 2008; Olausson et al., 2002).

Perini, Olausson, and Morrison (2015) implemented a feedback-based paradigm in which the arm skin was stroked at five different velocities (0.3, 1, 3, 10, and 30 cm/s). Following each stimulation, participants were asked to choose whether the caress they would receive in the next trial would be the same speed or a different speed. In other words, this paradigm provided a measure of pleasantness in the form of an active choice, which might be loosely compared to a rodent place preference paradigm. The researchers report that a preference for CT-optimal stroking was reflected in the behavioural choices observed, such that velocities of 1-10 cm/s were requested more frequently than slower or faster ones.

While we know that the stimulation of CT fibers in human work is accompanied by self-reported feelings of pleasantness, very little is known about a congruent pathway in an animal model. A class of C fibers have been identified in rodents that express the G-protein-coupled receptor MRGPRB4 and respond to massage-like stroking of hairy skin, but their relationship to human CT fibers is currently not known (Dong, Han, Zylka, Simon, & Anderson, 2001; Zylka, Dong, Southwell, & Anderson, 2003). While subjective pleasantness reports cannot be obtained during the stimulation of rodent MRGPRB4 afferents, pharmacogenetic activation of MRGPRB4+ neurons in freely behaving mice was sufficient for
producing conditioned place preference (CPP), implying their activation is positively reinforcing and/or anxiolytic (Vrontou, Wong, Rau, Koerber, & Anderson, 2013).

1.1.4 Top-Down Mechanisms of Affective Touch

As can be imagined, contextual factors also influence our perception of touch as “pleasant”; one might differentially register gentle strokes on the arm from a romantic partner, a parent, and a stranger on a quiet public bus, for example. The identity, physical characteristics, and intentions of the toucher can dramatically shape the hedonic experience (e.g. pleasant or disgust) and the behavioural response (e.g. approach or withdraw) (Ellingsen, Leknes, Løseth, Wessberg, & Olausson, 2016). Thus, an important contextual element is the relationship between the “touchee” and the toucher. Most of the current research on affective touch has looked at the touch stimulus itself, and much less is known about the neurobiological processes whereby top-down factors – cross-sensory, cognitive, and affective information – shape touch signals.

In two similar studies, a recipient’s beliefs about the sex of the toucher affected the pleasantness of a gentle caress (Gazzola et al., 2012; Scheele et al., 2014). Heterosexual male subjects rated a gentle caress as pleasant while watching a video clip of a female experimenter delivering the touch, and rated the same
touch as unpleasant when the clip starred a male experimenter. In reality, a female experimenter, hidden from view, delivered all of the stimulation.

Ellingsen and colleagues (2014) also showed that the perceived emotional state of the toucher also seems to play a role in how touch is interpreted. Their study’s participants rated touch as most pleasant when delivered with a photograph of a smiling face, and least pleasant when delivered with a frowning face. The effect was seen even though participants were aware that the person in the photograph was not the person touching them, suggesting cross-sensory modulation. Similarly, gentle brush stroking to the forearm can be rated as unpleasant when accompanied by a disgusting odor, even with the knowledge that the odor and touch are unrelated (Croy, Angelo, & Olausson, 2014). Ellingsen (2016) suggests that these effects may appear as a result in a “shift in affective or motivational state”, which in turn change the hedonic value of touch.

Touch also has the ability to modulate social perceptions. It was recently reported that gentle touch from another human could differentially shape social impressions of visually presented faces, depending on the emotional expression of the face (Ellingsen et al., 2014). When photographs of innocuous neutral and smiling faces were accompanied by gentle touch, the faces were rated as more attractive and friendly. When angry faces were presented alongside gentle touch, the faces were rated as less attractive and friendly than when presented alone. The
effect was also modulated by intranasal administration of an oxytocin receptor agonist, i.e. gentle touch improved social evaluations to an even larger degree following administration.

Thus, it appears that socially relevant cues perceived by the recipient of touch can influence how the brain interprets tactile stimulation. Even if a stimulus satisfies the physical requirements for CT optimal touch (slow and gentle), social context can modulate the perception of pleasantness or reward. This underscores the relationship between CT touch and sociality, which I will turn to next.

1.2 The Social Functions of Touch

Touch is the first method of communication that humans learn. Given that our sense of touch is fully developed at birth, before the ability to communicate verbally, it holds immense potential to shape social development and consequently, later social outcomes. Physical touch has been suggested to express more emotion and to convey more genuine intention or meaning than speech (Dunbar, 2010). Where verbal language barriers exist, touch compensates, even enabling communication between different species. For example, humans stroke and scratch domesticated dogs to convey approval and affection, and dogs (appear to) have learned to understand and seek out these interactions. The significance of tactile communication has not gone unnoticed by scientists; there exists plenty of research
on the topic. The following section will highlight some of these findings to illustrate the importance of touch as a function of sociality.

1.2.1 Parental Care

In research, mother-infant social interactions are often thought of as the classic model of affiliative bonding behaviour. The famous work of Harlow and Zimmerman (1959) was among the first to popularize mother-infant touch. In their study, baby monkeys who had been removed from their biological mothers showed a preference for a “surrogate” that was made of soft terrycloth and provided physical comfort over a wire one that provided food. The authors concluded that the absence of comforting touch led to psychological stress in the infants, even when food, which holds more obvious survival advantages, is offered as the alternative. In a more recent study, infant rhesus monkeys were separated from their mothers by a transparent screen. While auditory, visual, and olfactory contact were still possible, the loss of tactile contact resulted in chronic activation of the infants’ hypothalamic pituitary stress axis, which highlights the importance of mother-infant touch for healthy development (Suomi, 2011).

This early work on non-human primates was mirrored in humans by the introduction of Attachment Theory, about a decade later (Ainsworth, 1969; Bowlby, 1970). According to Ainsworth and Bowlby, infants that become distressed upon separation from their caregiver or parent are motivated by an
“attachment-behavioural system” to re-establish physical proximity, which then allows the infant to feel safe and secure. The authors suggested that the caregiver’s ability and willingness to respond would predict the infant’s personality and social tendencies later in life. In an investigation of actual interactions between mothers and infants, Stack and Muir (1990) found that touch occurred approximately 65% of the time during face-to-face interactions, which reduced stress and increased positive affect. However, this was not driven by tactile contact alone – the type of contact that mattered. Specifically, the authors report that it was slow stroking, but not passive touch, that led to facial signs of reward, i.e. the infant smiling (Stack & Muir, 1992).

More recently, kangaroo care, the practice of skin-to-skin contact between human infants and parents, has gained popularity for its ability to improve both parent-infant bonds and health outcomes in pre-term infants. Kangaroo care for low birth weight infants has been shown to reduce mortality, illness, infection, and length of hospital stay (Barros et al., 2010; Chan, Valsangkar, Kajeepeta, Boundy, & Wall, 2016; Conde-Agudelo & Díaz-Rossello, 2014; Lawn, Mwansa-Kambafwile, Horta, Barros, & Cousens, 2010). Preterm infants receiving kangaroo care exhibit more stable cardio-respiratory and temperature profiles during skin-to-skin contact, compared to infants that do not receive the same care (Ludington-Hoe, 2006; Messmer et al., 1997). Sharp and colleagues (2015) also found a
significant interaction between prenatal depression and maternal stroking, where increased maternal depression is associated with more negative emotionality of offspring, but only when maternal stroking was low. Thus, early CT stimulation could represent a socially protective role for offspring of depressed mothers. Kangaroo care also has benefits for the “toucher”, the participating mothers, who report feelings of being needed and heightened confidence in their role as a parent (Johnson, 2007). Mothers that practiced skin-to-skin contact with their infants also spent more time looking at and touching their infants (in addition to kangaroo care itself), showed more positive affect, and adapted faster to their infant’s signals than mothers that did not practice the same contact (Feldman, Eidelman, Sirota, & Weller, 2002). An abundance of work implicates the neuropeptide oxytocin as the basis for infant attachment. The stimulation that promotes oxytocin release during parental nurturing behaviour is exactly the type of gentle, stroking touch that is also required for CT stimulation (Uvnäs-Moberg, Arn, & Magnusson, 2005).

Parental stimulation has also been shown to influence the development and expression of social behaviour in rodents. For instance, maternal licking and grooming (LG) of rat pups can permanently alter how the offspring respond to stressful events later in life (Champagne & Meaney, 2007). Rats that receive more LG during infancy display lower levels of plasma corticosterone in response to acute stress in adulthood, compared with adult offspring of low LG mothers.
(Meaney, 2001). Behaviourally, the rats that received more LG show less fear reactivity, as evidenced by decreased acoustic startle responses, increased exploration of a novel open-field, and decreased latency to feed in a novel chamber, compared to offspring that received less LG (Caldji et al., 1998; Francis, Diorio, Liu, & Meaney, 1999; Liu, 1997). Interestingly, mother rats target their LG behaviours to specific body sites of the pup, i.e. dorsal back, head, and ears, where it is known that C fibers are most densely represented (Li et al., 2011; Liu et al., 2007; Vrontou et al., 2013).

1.2.2 Tactile Interactions between Peers

Touch also appears to serve important social functions between conspecific peers. In non-human primates, studies have found that reciprocal grooming, or ‘allogrooming’, provides a means for groups to form social bonds, particularly amongst kin (Silk, 2002). Monkeys spend much more time allogrooming than is needed for hygiene alone, suggesting it represents a rewarding or enjoyable activity between conspecifics (Dunbar, 2010). In support of this idea, large increases of endorphins in the cerebrospinal fluid of monkeys have been observed following allogrooming (Keverne, Martensz, & Tuite, 1989). This grooming-induced opioid activation is thought to facilitate formation and maintenance of long-term relationships that provide social support and protection (Dunbar, 1980, 2010). In addition, pharmacological administration of opioids in primates reduces
grooming requests and peer grooming, while blockade leads to increased solicitation and receipt of grooming (Fabre-Nys, Meller, & Keverne, 1982; Graves, Wallen, & Maestripieri, 2002; Keverne et al., 1989; Martel, Nevison, Simpson, & Keverne, 1995; Meller, Keverne, & Herbert, 1980; Schino & Troisi, 1992). Thus, it appears that primates have evolved a neurochemical system that promotes social touch.

In rats, social reward appears heavily dependent on tactile interactions between conspecifics. Fritz and colleagues (2011) demonstrated that four pairings of social interaction (between a subject rat and a “target” stimulus rat) with a context was sufficient to produce CPP. This effect was strong enough to reverse cocaine-conditioned place preference and its associated mesocorticolimbic effects when the opposite chamber was associated with just 15 minutes of direct social interaction per day. Interestingly, when tactile access between the subject rat and the target rat was obstructed with a perforated Plexiglass divider, conditioned place aversion (CPA) was instead observed, despite the availability of all other sensory cues, i.e. olfactory, visual, and auditory (Kummer et al., 2011). Thus, it appears that tactile stimulation is necessary to produce social reward in rats. Despite the repeatedly observed “social touch” network in the human brain, nothing is currently known about the regions to which CT afferents project in rodents.
1.2.2.1 Social Play Behaviour in Young Rats

Rodent play behaviour is highly pleasurable and rewarding (Trezza, Baarendse, & Vanderschuren, 2010; Trezza, Campolongo, & Vanderschuren, 2011), but like CT stimulation, the evolutionary benefits of play are not immediately obvious. Social play behaviour is energetic and vigorous in nature and sometimes even referred to as “rough-and-tumble” play (Panksepp, 1981; Vanderschuren, 1997); so one might suggest play behaviour actually depletes energy resources that could otherwise be allocated towards alimental and reproductive efforts. Thus, the rewarding nature of play may serve to establish and maintain social bonds, again, similar to CT stimulation. Although we know relatively little about the CT system, especially in laboratory animals, researchers have been studying the social functions and neurobiology of rodent play for the over three decades. It certainly helps that play behaviours are easy to recognize and quantify, especially in rats, where these behaviours are accompanied by explicit physical, facial, and vocal signals (Vanderschuren, Achterberg, & Trezza, 2016). Pouncing and pinning are considered the main indicators for social play behaviour in rats (Panksepp & Beatty, 1980; Vanderschuren et al., 2008). Pouncing refers to the initiation of play, wherein a rat touches its snout to a conspecific’s neck, while pinning is a response to pouncing, wherein the recipient rat rolls onto its back.
The rewarding properties of social play are reflected by its ability to produce conditioned place preference. Calcagnotti and Schecter (1992) were the first to demonstrate place preference using social play as a reinforcer. Juvenile rats (29 to 33 days old) were conditioned with a partner that had been rendered non-playful by pharmacological treatment in one compartment, and with a non-treated playful partner in the other compartment. At test, the rats showed a significant preference for the compartment paired with a playful social partner. This preference was observed after only two pairings of each chamber, suggesting play is a salient reinforcer. Douglas and colleagues (2004) showed that isolated adolescent and adult rats of both sexes acquired a preference for a compartment paired with play, with adolescent males showing the strongest preference. Importantly, rats conditioned with a partner that had been treated with methylphenidate, a drug that reduces play-related behaviours without affecting general social interest, did not develop CPP (Achterberg, van Kerkhof, Damsteegt, Trezza, & Vanderschuren, 2015; Vanderschuren et al., 2008), isolating the rewarding value of play itself. Further, the total amount of physical contact (pouncing and pinning) during the conditioning sessions positively correlated with the magnitude of CPP (Vanderschuren et al., 2008).
1.2.2.2 Tickling in Juvenile Rats: Experimenter-Driven Tactile Reward

More recently, a fascinating technique related to rat play has emerged: rat tickling. Rat tickling consists of whole-body playful stimulation delivered through rapid finger and hand movements similar to those used in human tickling (Burgdorf & Panksepp, 2001). Panksepp and Burgdorf (2000) were the first to suggest that manual tickling by an experimenter elicits positive affect in young rats. They reported that young rats (30 to 44 days of age) produced ultrasonic vocalizations (USVs; 50-kHz chirping) in response to tickling, which they hypothesized to be functionally or evolutionarily analogous to human laughter. Socially isolated animals vocalized more and were quicker to perform tasks to receive tickling, compared to socially housed animals. The researchers then bred the rats for high and low vocalization rates in response to tickling and reported increased play behaviour in the high USV line compared to the low USV line. Subsequent work has shown that 50-kHz USVs are also produced during play, mating, exploration, amphetamine administration, and in anticipation of food rewards, further suggesting they are good indicators of positive affect (Knutson, Burgdorf, & Panksepp, 2002; Wöhr & Schwarting, 2013; Wright, Gourdon, & Clarke, 2010). It is noteworthy that the tickling stimulation mimics the type of bodily contact the animals receive during rough-and-tumble-play (Panksepp, 1981; Vanderschuren, 1997).
Like play behaviour, tickling is positively reinforcing for young male rats, as demonstrated through operant learning and CPP (Burgdorf & Panksepp, 2001). In the operant learning task, socially isolated rats were successfully trained to press a bar to receive tickling over nine trials, and stopped after six days when pressing on the same bar no longer yielded tickling. For the CPP experiment, the researchers used a modified protocol wherein rats were given the choice to spend time near an immobile hand that had tickled them or a conspecific with opioid-induced catatonia. Animals that received tickling for five days prior to testing spent significantly more time with the experimenter’s hand than with the conspecific, compared to controls that had not received tickling. The tickled rats also vocalized more near the experimenter’s hand than near the conspecific.

1.2.3 Autism Spectrum Disorders: When Touch Isn’t Pleasant

To further underscore the importance of gentle touch to sociality, and to provide some clinical context, touch perception appears to be dysfunctional in autism spectrum disorders (ASD). Autism spectrum disorders are a highly prevalent class of neurodevelopmental disorders characterized by social and communicative impairments, as well as restricted patterns of behaviour (American Psychological Association, 2000). In addition to the core social deficits, a growing body of work also implicates abnormal responses to sensory stimuli as a critical symptom of the disorder (Baker, Lane, Angley, & Young, 2008; Brandwein et al.,
While sensory abnormalities in autism occur in multiple forms and across modalities (Kern et al., 2007), tactile hypersensitivity appears to be especially common, appearing in up to 95% of cases (Rogers & Ozonoff, 2005). Tactile hypersensitivity often presents itself as defensiveness or avoidance and interferes with social behaviour that involves interpersonal touch (Baranek, David, Poe, Stone, & Watson, 2006).

Given that touch serves as the primary method of communication in the first years of life, early signs of tactile defensiveness can represent a very early indicator of ASD. Indeed, aversion to social touch is among several of the atypical behaviours seen in infants that are later diagnosed with autism (Baranek, Foster, & Berkson, 1997). Specifically, parents of children with ASD report that their child will stiffen when touched, try to avoid touch, and prefer to be touched on their own terms (Kern et al., 2007; Siegel, 1998). Autistic children also report significantly lower pleasantness ratings in response to tactile stimuli than typically developing (TD) children (Cascio, Lorenzi, & Baranek, 2016). Hypersensitivity to touch has also been documented in adults with ASD. For example, adults with Asperger’s display a significantly lower detection threshold for vibro-tactile stimuli and have described a mild sensation applied to their hand as being more “tickly” and intense than did control subjects (Blakemore et al., 2006). In autobiographical accounts, patients with high-functioning autism have described touch as “an intense feeling”
that can be “overwhelming and confusing” (Cesaroni & Garber, 1991) and serve as impetus for social withdrawal (Grandin, 1992). Tactile defensiveness has even been documented in animal literature. He and colleagues (2017) reported that an autistic mouse model was unable to habituate to continuous whisker stimulation, and in response displayed defensive behaviours that were not observed in wildtype (WT) controls.

Recall that several imaging studies describe a “social touch network” that responds to CT-optimal stimulation, consisting primarily of the insula, right pSTS, right mPFC, dACC, and OFC (Gordon et al., 2013; Olausson et al., 2008; Olausson et al., 2002; Voos et al., 2013). In a study meant to address the neural processing of social stimuli in ASD, individuals were shown biological motion stimuli while undergoing fMRI scanning (Kaiser et al., 2010). In the same regions that were later implicated in the social touch network, neurotypical controls demonstrated BOLD responses to biological movement, while ASD individuals showed hypoactivity in these areas, begging the question of whether this circuitry is fundamentally abnormal in ASD populations. Thus, among somatosensory submodalities that may contribute to tactile hypersensitivity in ASD, a dysfunction of the CT system presents as a possible candidate (Cascio et al., 2008).
1.3 Conclusion

This literature provides strong evidence that both human and non-human primates have a specialized system for transmitting socio-emotionally relevant touch. There is increasing evidence that rodents possess an analogous system, but the neural mechanisms remain largely unknown. Understanding the affective touch system at a mechanistic level could provide insights for autism spectrum disorders, but also social neuroscience on a general level. Thus, in our endeavors to better understand affective touch, it is an important next step to bridge the translational gap between laboratory rodents and primates.
Chapter 2
Introduction to Current Project

2.1 Statement of the Problem

In an intuitive sense, the social and emotional functions of touch are well known. We know that an embrace from a loved one is a source of comfort and that a gentle pat on an infant’s back is a soothing gesture. Empirically, there are still many questions that need to be asked and answered. As aforementioned, there is a strong foundation of literature outlining the affective touch system in humans. The translational work on rodents is emerging and highly intriguing, but largely incomplete. One barrier in our understanding of affective touch in laboratory animals is the lack of a standardized behavioural method for studying gentle touch in rodents. In rats, the tickling method is proving itself to be a reliable technique for measuring tactile reward (Burgdorf & Panksepp, 2001), but it is not known whether this behaviour is related to affective touch, per se, or if it applies beyond rats, which are uniquely playful in their youth. Therefore, the research may benefit from a novel behavioural method using a different rodent model: the mouse.

2.2 Purpose of the Study

The purpose of this study was to investigate the rewarding value of gentle touch in a mouse model and to elucidate the associated neural mechanisms. Past mouse
work has shown that the selective stimulation of fibers associated with gentle stroking of hairy skin was sufficient for establishing conditioned place preference (Vrontou et al., 2013). Ecologically speaking, affective touch involves the stimulation of all skin afferents, including those involved in discriminative touch. Further, human studies suggest that gentle touch is only rewarding in the presence of specific social cues. Thus, the current study used a form of tactile stimulation that addressed those points.

2.3 Research Question and Hypotheses

The primary objectives of this project were: (1) to investigate the rewarding value of gentle touch in a mouse model and (2) to elucidate the associated neural correlates. To answer these questions, I established the following aims.

2.3.1 Investigating the Rewarding Value of Gentle Touch in a Mouse Model

First, I used a tactile conditioned place paradigm in order to investigate the rewarding value of gentle touch. Following a period of handling, gentle stroking touch was repeatedly paired with one of two contextually distinct chambers. In the other chamber, the mice were left untouched. I predicted mice would develop a preference for the context paired with gentle touch, based on: (1) human work that characterizes gentle stroking touch as pleasant, and (2) previous mouse work by
Vrontou and colleagues (2013) that established CPP by stimulating MrgB4⁺ afferents, which respond selectively to gentle stroking touch.

### 2.3.2 Investigating the link between Social Approach and Tactile Preference

Given the socio-affective basis of gentle touch, I wanted to know whether any observed tactile preference might be driven by innate sociability. To do so, I implemented the three-chambered test of social approach, in which mice were given the choice to spend time with either a novel conspecific or a novel object. I predicted that mice that showed a stronger preference for the conspecific over the object would also show a stronger preference for tactile stimulation, based on previous work by Kummer et al. (2011) that demonstrated the necessity of tactile interaction for observing social reward in rats.

### 2.3.3 Identifying the Brain Regions involved in Gentle Touch

Lastly, I aimed to identify the activational network of affective touch in the mouse brain. Immunohistochemistry (IHC) was performed on fixed mouse brains in order to measure activated regions. On the day of perfusion, all mice were returned to their stroke-paired context and either did or did not receive one last stroking session (‘stimulated’ and ‘unstimulated’ groups). Based on the human “social touch” network, I predicted that stimulated mice would show higher levels of neural activity in the anterior/posterior insular cortices and S1, relative to
unstimulated mice. I also predicted that mice that showed a stronger preference for the stroke-paired context would show more neural activity in the nucleus accumbens, an area associated with reward processing.
Chapter 3  
Materials & Methods

3.1 Animals

I used male C57Bl/6 mice ordered from Jackson Laboratories (n=12) and bred our own colony (n=13), which is maintained in the University of Toronto Mississauga’s Animal Care Facility. Mice were housed in cages containing 3-5 animals. Each plastic cage included a wire top, corncob bedding, a plastic dome, and free access to food (Harlan Teklad 8604) and water. The housing room was temperature-controlled (20 ± 4° C) with a 12/12h light/dark cycle; experiments were conducted only during the light period. Similar to the work by Vrontou and colleagues (2013) that established CPP via selective stimulation of MrgB4⁺ afferents, animals were aged postnatal day (PD) 23-35 (approx. 3-5 weeks) at the time of use. All procedures adhered to Canadian Council on Animal Care and institutional guidelines and were approved by the University of Toronto Mississauga Local Animal Care Committee.

3.2 Handling

Prior to any testing, the animals (n=25) were handled by the experimenter in order to habituate them to the gentle stroking stimulation. From PD 23 to PD 27, the animals were handled for 5 minutes per day. Each mouse was supported in the
experimenter’s palm and gentle strokes from a gloved finger were applied to the dorsal side of the animal at an interval of 1 every 15 seconds, at a velocity of approximately 1-5 cm per second, in accordance with CT-optimal stroking. Each stroke reached from the nape of the neck to the base of the tail. This served to familiarize the mouse to both the experimenter and the tactile stimulation. Gloves were disposed and replaced after each mouse. Handling was performed in the same room and at the same time of day as the behavioural testing.

3.3 Three-Chambered Test of Social Approach

3.3.1 Apparatus

Social approach was tested using a three-chambered Plexiglas apparatus. Each chamber measured 20 cm x 40.5 cm x 22 cm. The dividing walls were made of clear Plexiglas, with small openings (10 cm x 5 cm) that allowed access into each chamber when a pair of sliding doors was removed. An empty overturned wire cup served as a novel object. An identical overturned cup with an unfamiliar mouse inside served as a novel social stimulus. The bars of each wire cup were approximately 1 cm apart and allowed for direct tactile, olfactory, and visual interactions between the target and stimulus mouse. Each stimulus mouse was matched to the subject mouse by age and sex and spent 30 minutes habituating to the wire cups the day before testing.
3.3.2 Protocol

On PD 28, prior to any tactile testing, each mouse’s tendency for social approach was measured by the three-chambered test, following the original protocol outlined by Yang, Silverman, & Crawley (2011) (n=12). This test is comprised of three consecutive 10-minute phases. In phase 1, each mouse was confined to the center chamber for habituation; in phase 2, the sliding doors were removed and the mouse was allowed to explore all three chambers. Live tracking software (Ethovision, Noldus) automatically calculated the time the mouse spent in each of the side chambers, as a percentage of the total 10 minutes. The chamber in which the animal spent less time was labeled as the animal’s Social zone, while the opposite was labeled as the Object zone. Immediately following phase 2, the mouse was gently guided back to the center chamber and the sliding doors were replaced. An empty wire cup was then placed in the Object zone, followed by the novel mouse in the Social zone. For phase 3, the sliding doors were once again removed and the subject was given free access to both the novel object and the novel mouse (see Figure 3.1). Time spent in proximity to the novel object or mouse was calculated based on time spent inside the interaction zone (i.e. 5 cm radius from wire cup), which was expressed as a percentage of the total 10 minutes.
3.4 Tactile Conditioned Place Preference

3.4.1 Apparatus

The tactile conditioned place preference was performed using a three-chambered Plexiglas apparatus. The two side chambers (18 cm x 18 cm x 18 cm) served as contextually distinct conditioning zones. Zone 1 was composed of black walls and a metal bar floor, while Zone 2 was composed of white walls and a metal grid floor (Zone 2). Zone 3, a narrower center zone (10.5 cm x 18 cm x 18 cm), was composed entirely of green Plexiglas allowed free passage between Zones 1 and 2 when two sliding doors were removed. A clear Plexiglass lid with small air holes (approx. 2 mm in diameter and 2 cm apart) covered each of the zones during testing.
3.4.2 Protocol

On PD 29 (‘Baseline’), animals (n=25) were tested for pre-conditioning (innate) place preference. Each mouse was placed into the center (Zone 3) of the CPP apparatus and given 30 minutes to move freely between Zones 1-3. The amount of time the animal spent in each zone was automatically calculated as a percentage of the total 30-minute trial (% of cumulative duration) by live tracking software (Ethovision, Noldus). The zone in which the animal spent more time was identified as the animal’s Initial Preferred (IP) zone, while the other was labeled as the Initial Non-Preferred (INP) zone. A biased CPP design was used, i.e. the INP zone became the stroke-paired chamber, while the IP zone became the unpaired chamber. On alternating and counterbalanced days from PD 30-37, gentle strokes were applied via a gloved finger to the animal in their INP zone for 30 minutes. On the days the animal did not receive tactile stimulation, they remained undisturbed in their IP zone for 30 minutes. Overall, the animals spent four sessions in their stroked chamber and four sessions in their unpaired chamber, e.g. Day 1: stroked (30 min.); Day 2: unpaired (30 min.); Day 3: stroked (30 min.), etc. Strokes were performed using the same velocity, frequency, and approximate pressure as during the handling period. On PD 38 (‘Test’), post-conditioning preferences were quantified, again as a percentage of the total 30 minutes (% of cumulative duration).
The following day, on PD 39, a portion of the animals that received conditioning was put back into their stroke-paired chamber for 30 minutes (n=17). To account for potential differences in the effects of tactile stimulation vs. conditioning, half of the animals received stroking during this time (‘stimulated’ group, n=9), while the other half remained undisturbed in the chamber (‘unstimulated’ group, n=8). 60 minutes following, all animals were deeply anesthetized with sodium pentobarbital and transcardially perfused with phosphate buffered saline (PBS) followed by 4% paraformaldehyde (PFA).

3.5 Immunohistochemistry

Immediately following transcardial perfusion, whole brains were isolated and post-fixed in 4% PFA for 12h at 4°C, followed by 20% sucrose/PBS and 30% sucrose/PBS for 24h each, and stored in PBS containing 0.2% sodium azide until
processing. Free-floating coronal sections were obtained at a thickness of 40 µm using a cryostat (−20°C to −22°C) (Thermo Fisher Scientific, Waltham, MA, USA). Sections were washed in PBS and endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide. Non-specific sites were blocked with normal goat serum (Vector Laboratories, Burlingame, CA, USA). Sections were incubated in primary antibody against c-Fos protein (1:1000 dilution; Millipore, Burlington, MA, USA) at 4°C for 24-48h with slow agitation. After the primary immunoreaction, sections were incubated with biotinylated secondary antibody (1:1500 dilution; Vector Laboratories) in PBS containing 2.5% NGS, followed by avidin-biotin horseradish peroxidase complex (1:200 dilution; Vectastain ABC Peroxidase Kit, MJS BioLynx, Brockville, ON, CAN) for signal amplification. The immunoreaction was visualized with peroxidase substrate (DAB Peroxidase Substrate Kit, Vector Laboratories). After staining, sections were mounted onto SuperFrost Plus Gold slides (Thermo Fisher Scientific), air-dried, dehydrated in a graded series of alcohols, cleared in xylene, and coverslipped with permount.

3.6 Data Analyses

Prism 7 (GraphPad, San Diego, CA, USA) was used to perform all analyses. A conventional two-tailed level of significance at 0.05 was required.

3.6.1 Three-Chambered Test
For the three-chambered test, two difference scores were calculated for each mouse (n=12), one for each chamber (Object and Social): (\% cumulative time at test) – (\% cumulative time at baseline). The difference scores were presented as mean±SEM and compared using Student’s paired t-test.

3.6.2 Tactile Conditioned Place Preference

For the tactile conditioned place preference test, two differences scores were again calculated for each mouse (n=25), one for each chamber (stroke-paired and unpaired): (\% cumulative time at test) – (\% cumulative time at baseline). These scores were also presented as mean±SEM and compared using Student’s paired t-test.

3.6.3 Relationship between Social Approach and Tactile Preference

To investigate the relationship between social approach and tactile preference, the differences scores from the relevant chambers of both tests (i.e. Social chamber and stroked-paired chamber, respectively) were used to perform a Pearson correlation.

3.6.4 Fos-immunoreactive (Fos-I) Cell Counting

Images of brain sections were acquired using the Cytation 5 Cell Imaging Multi-Mode Reader (BioTek, Winooski, VT, USA) at 4x objective. The brain regions analyzed were chosen on the basis of the human “social touch network” and those
implicated in reward processing: anterior and posterior insular cortices, nucleus accumbens, and the somatosensory cortex. ImageJ (National Institute of Health, Bethesda, MA, USA) software was used to “threshold” the images and the number of Fos-I cells characterized by clearly labeled cells was counted bilaterally in three serial sections per region. The three serial sections were taken as close to the center of the region as possible to ensure the region was properly captured, with the Allen Mouse Brain Atlas as a reference. For example, the entire nucleus accumbens spans from coronal plate #36 to plate #50, so plates #43, #44, and #45 were quantified. The number of Fos-I cells from bilateral sites was summed to obtain a count for each slice. The slice counts were then averaged between the three serial slices to obtain a regional count. For a comparison of regional counts in animals that either did or did not receive stroking on perfusion day (i.e. ‘stimulated’ vs. ‘unstimulated’ mice, respectively), an unpaired t-test was performed. Regional counts for each brain region were then compared with tactile preference scores for each mouse (n=17), using Pearson’s correlation.

For one mouse in the stimulated group, the IHC failed to produce a quantifiable section containing the posterior insular cortex, so this region was not analyzed for this animal.
Chapter 4
Results

4.1 Three-Chambered Test

For the three-chambered test of sociality (n=12), the Student paired t-test revealed no significant difference between mean difference scores from the Object chamber and Social chamber \([t(11)=0.116, p=0.91]\), i.e. the change in time spent at baseline and test did not differ between the chambers (Figure 4.1).

![Three-Chambered Test](image)

**Figure 4.1.** Three-chambered test for sociality (n=12). Difference scores are presented (test-BL) for each chamber (Object: grey, Social: red). There was no significant difference found between mean difference scores.
4.2 Tactile Conditioned Place Preference

For the tactile conditioned place preference test (n=25), the Student paired t-test revealed a significant difference between mean difference scores \[ t(24)=4.152, \ p=0.0004 \], i.e. the change in time spent at baseline and test differed between the chambers (unpaired vs. stroke-paired) (Figure 4.2).

Figure 4.2. Tactile conditioned place preference (n=25). Difference scores are presented (test-BL) for each chamber (unpaired: grey, stroke-paired: yellow). There is a significant difference between mean difference scores, ***\(p<.01\).
4.3 Relationship between Social Approach and Tactile Preference

Addressing the question of whether social approach is predictive of tactile preference (n=12), a Pearson’s correlation was performed but did not reveal a significant relationship between three-chambered scores and tactile CPP difference scores from the relevant chambers, i.e. Social chamber and stroke-paired chamber, respectively (R=-0.2993, p=0.34) (Figure 4.3).

![Figure 4.3. The relationship between social approach and tactile preference (n=12). Difference scores from the relevant chamber of both tests (Social and stroke-paired, respectively) were correlated. No significant relationship was found.](image)

4.4 Immunohistochemistry

4.4.1 Stimulated vs. Unstimulated Groups

Unpaired t-tests revealed no significant differences in Fos-I cell counts between stimulated and unstimulated animals, for any of the regions analyzed (Fig. 4.4).

<table>
<thead>
<tr>
<th>Region</th>
<th>n</th>
<th>t (df)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior insular cortex</td>
<td>17</td>
<td>0.4867 (15)</td>
<td>0.63</td>
</tr>
<tr>
<td>Posterior insular cortex</td>
<td>16</td>
<td>0.103 (14)</td>
<td>0.92</td>
</tr>
<tr>
<td>Nucleus accumbens</td>
<td>17</td>
<td>1.869 (15)</td>
<td>0.08†</td>
</tr>
<tr>
<td>Somatosensory cortex</td>
<td>17</td>
<td>0.6514 (15)</td>
<td>0.52</td>
</tr>
</tbody>
</table>

Table 4.1. Unpaired t-test of Fos-I cell counts in unstimulated (n=8) vs. stimulated (n=9) animals. t and p-values displayed, †p<.1.

Figure 4.4. Fos-I cell counts of unstimulated and stimulated animals compared using unpaired t-tests: (a) anterior insular cortex, (b) posterior insular cortex, (c) nucleus accumbens, and (d) somatosensory cortex. No significant differences were found, but the nucleus accumbens showed a trending difference, †p<.1.
4.4.2 Fos-I Cell Counts and Tactile Preference

Pearson correlations between Fos-I cell counts and tactile preference (stroke-paired difference scores) revealed no significant relationship for the anterior/posterior insular cortices (Fig. 4.5a-b), nucleus accumbens (Fig. 4.5c), for stimulated or unstimulated groups. Somatosensory cortex Fos-I counts correlated with tactile preference for unstimulated ($p=0.03$), but not stimulated mice (Fig. 4.5d).

<table>
<thead>
<tr>
<th>Region</th>
<th>Unstimulated</th>
<th>Stimulated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Pearson’s r</td>
</tr>
<tr>
<td>Anterior insular cortex</td>
<td>8</td>
<td>0.2003</td>
</tr>
<tr>
<td>Posterior insular cortex</td>
<td>8</td>
<td>-0.0554</td>
</tr>
<tr>
<td>Nucleus accumbens</td>
<td>8</td>
<td>-0.3375</td>
</tr>
<tr>
<td>Somatosensory cortex</td>
<td>8</td>
<td>0.7689</td>
</tr>
</tbody>
</table>

Table 4.2. Pearson correlations between tactile preference and Fos-I cell counts. Pearson’s r and p-values displayed.

Figure 4.5. Fos-I cell counts correlated with tactile preference revealed no significant relationship for (a) anterior insular cortex, (b) posterior insular cortex, (c) nucleus accumbens, for stimulated or unstimulated groups; (d) somatosensory cortex Fos-I counts correlated with tactile preference for unstimulated ($p=0.03$), but not stimulated animals.
Figure 4.6. Representative images of the ROIs for the (a) anterior insular cortex, (b) posterior insular cortex, (c) nucleus accumbens, and (d) somatosensory cortex, captured from ImageJ software.
5.1 Summary of Results

This study used a novel tactile conditioned place preference paradigm to assess tactile reward in twenty-five young male mice. Overall, four pairings of tactile stimulation with a chamber increased the amount of time the animals spent in the chamber, from baseline to test. Prior to tactile conditioning, the three-chambered test of social approach was performed with twelve mice to assess whether an innate tendency for sociability was predictive of or related to tactile preference. The finding here did not suggest that social approach is related to tactile preference. The brains of seventeen mice were assessed using immunohistochemistry, looking at c-Fos expression as a marker of neural activation. Fos-I cell counts from the anterior/poster insula, nucleus accumbens, and somatosensory cortex were obtained. There was no difference in Fos-I cell counts of stimulated and unstimulated animals, for any region. The correlational analyses revealed only one relationship between the Fos-I cell count and tactile preference score, for the somatosensory cortex of unstimulated mice.

5.1.1 Three-Chambered Test of Social Approach
The three-chambered test of social approach was used to assess innate sociability of twelve male mice, prior to tactile preference testing. This test is a reliable method of testing for sociability, and is widely used for identifying differences in social approach between wildtype mice and those with autistic phenotypes (Moy et al., 2007; Moy et al., 2004). I therefore predicted that the difference scores (increase in time spent from baseline to test) would be greater for the chamber in which a novel mouse was placed, compared to the chamber in which an empty wire cup was placed. The current result does not support the hypothesis. In other words, the mice in the current study did not show a significant preference for the novel mouse, relative to the empty wire cup. This is unusual given the reliability of the three-chambered test to show a significant preference for a novel mouse over an empty wire cup. One possible explanation for this is that the mice used in the current study were younger than the mice typically used for the three-chambered test. These mice were 4 weeks old at the time of testing, while other studies, including Moy et al. (2007), use mice between 5-6 weeks of age. The behavioural testing lasted a total of 17 days; so younger mice were chosen here to account for the long study duration and in accordance with the work by Vrontou et al. (2013). However, the younger age of the mice may not necessarily account for the unusual result seen here, given that the age of our mice coincides with the age at which mice display the most social play behaviours (4-5 weeks of age) (Hol, Van den
Berg, Van Ree, & Spruijt, 1999). Given that approaching a conspecific is a required first step for play behaviour, social approach behaviour should be increased (or at the very least, present) during this developmental time period.

5.1.2 Tactile Conditioned Place Preference

I predicted that gentle stroking of hairy skin would be rewarding for young male mice. A novel tactile conditioned place preference was used to assess tactile reward and the result supports our hypothesis: the difference scores (T – BL) were significantly larger for the stroke-paired chamber than for the unpaired chamber. In other words, the mice showed a larger increase in the time spent in a chamber associated with touch than one that was not. This finding is consistent with work by Vrontou and colleagues (2013), in which selective stimulation of MRGPRB4 afferents established place preference in young mice. While we cannot state that MRGPRB4 afferents were stimulated here, the type of stimulation used in the current study (slow, massage-like stroking of hairy skin) has been reported to activate these afferents in young mice (Vrontou et al., 2013). This finding also relates to those wherein play behaviour and tickling have been shown to establish CPP in adolescent male rats (Burgdorf & Panksepp, 2001; Calcagnetti & Schechter, 1992; Douglas et al., 2004), both of which involve a high degree of tactile stimulation. Thus, the current finding contributes to the existing literature.
that states tactile stimulation is rewarding in young rodents, while introducing a novel behavioural paradigm that may be used to study this phenomenon.

5.1.3 The Relationship between Social Approach and Tactile Preference

I hypothesized that mice that were more likely to approach a novel conspecific would also be more likely to perceive gentle touch as rewarding, given the social basis of affective touch. Difference scores obtained from the three-chambered test and the tactile CPP test were compared but did not reveal a significant correlation. Taken at face value, this finding shows no relationship between innate sociality and tactile preference, which may be the case. However, this may also be partly due to the unusual result seen in the three-chambered test itself. The hypothesis that social approach and tactile preference would be related was based on the assumption that the three-chambered test would produce results similar to those reported in the literature, i.e. that wildtype mice show a preference for a novel conspecific over an empty cup. While degrees of preference for the novel mouse were expected, i.e. some subjects spending more time with the novel conspecific than others, it was not expected that a preference would not emerge at all in some cases. Thus, the basic criterion upon which this relationship was considered was not met.

5.1.4 The Brain Regions Involved in Gentle Touch
Seventeen mouse brains were fixed and dissected after tactile conditioning. Brain regions implicated in the human affective touch network and reward processing were assessed using immunohistochemistry, where c-Fos expression was used as a reflection of neuronal activity. Fos-I cell counts from the anterior/posterior insular cortices, nucleus accumbens, and somatosensory cortex from stimulated and unstimulated mice were obtained.

5.1.4.1 Fos-I Cell Counts of Stimulated vs. Unstimulated Mice

Based on the human “social touch” network, I predicted that stimulated mice would show higher levels of neural activity in the anterior/posterior insular cortices and S1, relative to unstimulated mice. The comparison of Fos-I cell counts between stimulated and unstimulated mice revealed no significant differences, for any of the brain regions assessed. The nucleus accumbens showed a trending difference between stimulated and unstimulated animals, with stimulated animals showing more c-Fos activity. Unfortunately and unexpectedly, the somatosensory Fos-I cell counts did not differ significantly between stimulated and unstimulated animals. Given the widely reported and reliable activation of S1 and S2 in response to tactile stimulation, this result calls into question the validity of the immunohistochemistry findings altogether. It is difficult to know what accounts for the lack of difference seen between somatosensory Fos-I cell counts of stimulated and unstimulated animals without replicating these analyses. An issue could have
arisen during any of the processes that led up to the quantification of cell counts: transcardial perfusion, post-fixation, slicing, or staining. Regardless of the reason, without a proper positive control region, it is not possible to responsibly interpret anything from the current Fos-I cell counts.

5.1.4.2 Fos-I Cell Counts Compared to Tactile Preference

I predicted that mice that showed a stronger preference for the tactile-paired context would show more neural activity in the nucleus accumbens, an area associated with reward processing. Tactile preference (stroke-paired difference scores) and Fos-I cell counts did not show any significant correlations for the anterior/posterior insular cortices or nucleus accumbens, for the stimulated and unstimulated animals. Tactile preference correlated positively with Fos-I cell counts for the somatosensory cortex in unstimulated, but not in stimulated animals. In other words, these mice showed somatosensory c-Fos expression after simply being exposed to the stroke-paired chamber, and the magnitude of this expression related to the magnitude of their tactile preference. However, due to the aforementioned fact that I lack a proper positive control, it is not responsible to assume this is a true significant correlation.

5.2 Limitations and Future Directions

There are several ways to improve upon and expand the current study. With regards to the novel tactile conditioned place preference paradigm, the
experimenter (myself) serves as the rewarding stimulus, and rodents have been shown to behave differently depending on whether the experimenter is male or female. For example, one study reported that exposure of both mice and rats to male, but not female, experimenters produce pain inhibition via robust physiological stress response that resulted in stress-induced analgesia (Sorge et al., 2014). I was very close to the mice throughout the duration of the study: holding them in the palm of my hand during the handling and sitting within arms reach during conditioning sessions. Given this proximity, this paradigm should be replicated with other female experimenters, as well as with male experimenters, to investigate the role of experimenter characteristics in establishing tactile CPP.

Secondly, while the overall finding was that our mice showed a propensity for the stroke-paired chamber, there was some variation observed. Some animals demonstrated a very robust preference for the stroke-paired chamber, while others showed aversion. There are many possible factors (and interactions of factors) that could be responsible for this variability, including differences in paternal care, i.e. varying amounts of maternal grooming during early development; dominance relationships between cagemates, i.e. aggression displayed through over-grooming; methodological issues, i.e. 30 minutes of continuous tactile stimulation might be an ecologically invalid way to study affective touch in mice; or sociability, which has
been addressed (but unfortunately, not answered) in the current project using the three-chambered task.

Third, the unusual results of the three-chambered test seen here make it challenging to ascertain whether the rewarding value of gentle touch is related to sociality. Given the relatively young age of the mice compared to animals typically used for this assay, the tactile preference test can be attempted with older mice, which would be better suited for the three-chambered test. In a similar vein, it would be fascinating to investigate: a) whether social play or tickling can be rewarding in young male mice, and b) whether the reward established by social play and tickling in rats is dependent on CT and/or MrgB4⁺ afferents. Answering both of these questions would certainly provide some insight into the relationship between sociality and rodent affective touch. In addition, the tactile CPP could be repeated after social isolation, as was done in the rat tickling studies, where they reported that isolated rats vocalized more and were quicker to perform tasks to receive tickling, compared to socially housed animals (Burgdorf & Panksepp, 2001).

Finally, it is obvious that the immunohistochemistry results need to be replicated. Without a proper positive control, there is no possibility of interpreting anything. It
could very well be that the biological samples were not properly prepared, i.e. perfusions, slicing, and staining. To address this, the process should be repeated. However, there is also a chance that c-Fos is not a good marker for gentle tactile stimulation. Firstly, prior to perfusion, all animals are given access to the entire stroke-paired chamber for 30 minutes. During this time, they are performing numerous behaviours i.e. sniffing, rearing, sleeping, jumping, exploring, and grooming, all of which may elicit c-Fos expression throughout the brain that dilutes any neural activity specific to gentle touch (or lack thereof). Several ways to address this would be to a) change the conditioning protocol to isolate the animal to a platform during stimulation to prevent non-specific behaviours and/or b) to decrease the duration of the session to a more discrete window that would not afford the animal time to perform non-specific behaviours. In addition, the animals spend 60 minutes in their home cage before perfusion, during which time they are able to perform non-specific behaviours and further, are exposed to their cagemates and bedding, which may serve as social stimuli. This could easily be remedied by isolating the animal to an empty cage with fresh bedding prior to perfusion. There is also some evidence that immediate early genes (IEG) like c-Fos are not a good marker for familiar sensory stimuli. For example, taste and smell do not elicit c-Fos expression in limbic areas when familiar, but do so when experienced for the first time (Montag-Sallaz, Welzl, Kuhl, Montag, & Schachner,
The animals in the current study are exposed to the tactile stimulation on 10 separate occasions (five times during handling, four times during conditioning, and one time before perfusion). Thus, the stimulation may no longer be salient enough to reach the threshold required for selective c-Fos expression.

5.3 Conclusion

Overall, the current study demonstrates that gentle stroking touch delivered from a human experimenter can be rewarding in young male C57Bl/6 mice. Whether or not this relates to sociality, and to other socially related tactile behaviours such as social play and tickling in rats, should be explored to build upon the growing body of literature on rodent affective touch. Unfortunately, the current study did not reveal any insights on the involvement of the central nervous system, but this novel behavioural paradigm may help in future studies that wish to elucidate the underlying mechanisms of rodent affective touch.
References


