TACTILE MODULATION OF THE SENSORY AND CORTICAL RESPONSES ELICITED BY FOCAL COOLING IN HUMANS AND MICE

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I, Ivan Ezquerra-Romano, declare that:

- The work presented herein is my own except where explicitly stated.
- The text and figures in this document were produced by me unless explicitly stated.
- This thesis has not been submitted for any other degree or professional qualification.
- Two AI tools, ChatGPT (https://chat.openai.com/) and ProWritingAid (https://prowritingaid.com/), were used as writing assistants, in conformity with UCL’s plagiarism and AI guidelines (https://www.ucl.ac.uk/students/exams-and-assessments/assessment-success-guide/engaging-ai-your-education-and-assessment). Specifically, these tools were used for reviewing and improving planning documents for the introduction and discussion that had been originally generated by me. They were also used for grammar checking, style improvements and rephrasing suggestions. The following are two examples template prompts used during writeup:
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  - “Please help me write the following sentence in more simple terms: [sentence]"
Abstract

Distinct sensory receptors transduce thermal and mechanical energies, but we have unified, coherent thermotactile experiences of the objects we touch. These experiences must emerge from the interaction of thermal and tactile signals within the nervous system. How do thermal and mechanical signals modify each other as they interact along the pathway from skin to conscious experience? In this thesis, we study how mechanical touch modulates cooling responses by combining psychophysics in humans and neural recordings in rodents. For this, we developed a novel stimulator to deliver focal, temperature-controlled cooling without touch. First, we used this method to study in humans the sensitivity to focal cooling with and without touch. We found that touch reduces the sensitivity to near-threshold cooling, which is perhaps analogous to the well-established ‘gating’ of pain by touch. Second, we studied the perceived intensity of cooling with and without touch. We found that tactile input enhances the perceived intensity of cooling. Third, we measured the responses of the mouse primary somatosensory cortex to cooling and mechanical stimuli using imaging and electrophysiological methods. We found multisensory stimuli elicited non-linear cortical responses at both the population and cellular level. Altogether, in this thesis, we show perceptual and cortical responses to non-tactile cooling for the first time. Based on our observations, we propose a new model to explain the interactions between cooling and mechanical signals in the nervous system. This thesis advances our understanding of how touch modulates cold sensations during thermotactile stimulation.
Impact Statement

The work presented in this thesis contributes to the field of sensory perception in three ways. First, it describes the development and application of a novel non-tactile cooling stimulator for studying thermotactile interactions in both humans and animals. Second, it reveals for the first time in humans a gate control mechanism of cooling inputs by touch. Third, it presents new data of the cellular and population responses of the mouse’s primary somatosensory cortex to cooling and mechanical stimulation.

These contributions can impact scientific research in three ways. First, the novel stimulator enables sensory researchers to study the responses to non-tactile cooling in new ways. In Chapter 2, three different stimulation scenarios are described, but the rest of the thesis applies only one of these scenarios to study the thermotactile system. Future studies can use the stimulator to answer other scientific questions about thermotactile perception with different experimental strategies. Second, the finding of the gate control of cooling inputs by touch raises new questions about the underlying neural mechanism of this interaction. Neurophysiologists should use anatomical and electrophysiological techniques to identify the cellular system mediating this gate mechanism. Third, the neural data of the responses in the mouse’s cortex advances our understanding of how thermal and mechanical signals are processed in the nervous system. This data could inform the development of computational models of thermotactile processing.

Professionals outside fundamental research on perception can also benefit from the work presented here in three ways. First, the novel non-tactile cooling stimulator could inspire further innovations in the development of cooling devices. These innovations could be applied both in the medical sector and the consumer product industry. In the medical sector, this work could inform the design of cooling therapy products for the management of pain and inflammation such as cooling gels, pads, and wraps. Additionally, it could also be used to develop more advanced temperature-sensitive medical devices to improve patient outcomes and safety such as catheters and surgical instruments. In the consumer product industry, the methodological advancements presented in this thesis could inspire innovations for the design of thermal control systems for buildings and the development of wearable thermal technology for personal use. Second, the discovery of a new interaction between cooling and mechanical signals could contribute to a more profound understanding of conditions that affect sensory processing such as chronic pain and neuropathies. Together with the methodological innovation, these advancements
could be applied to the developments of new diagnostic tools and treatments. Third, the understanding gained about thermotactile integration in both humans and mice could improve the development of neuroprosthetic devices and other products that require thermotactile feedback such as haptic tools for virtual reality.
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I can now combine random materials with hardware and software to build stuff for measuring things. This is partly thanks to Martin and all those content creators on the internet. Toni was always there to give me the coldest material of all. If AI has helped me, who should I thank? It’s unclear to me, and it’s dangerous to anthropomorphise these tools, but it’s difficult not to. We are trapped by language.

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Thanks to my funders for giving me the chance to meet new people and enjoy time with them both inside and outside academia. In the website of my main funder, it says they “co-invest in training the next generation of skilled people for the research base and wider bioeconomy.” To invest means “to put money, effort, time, etc. into something to make a profit or get an advantage”. I accept the challenge to create value for society both in conventional and unconventional ways.
Research Paper Declaration Form

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g Was the work peer reviewed?

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h Have you retained the copyright?

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i Was an earlier form of the manuscript uploaded to a preprint server?

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• Chapter 7

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Outline

**Chapter 1** introduces the existing understanding of the thermotactile system from the skin to the perception of objects.

**Chapter 2** presents a novel stimulator, which delivers non-tactile, focal, temperature-controlled cooling stimuli.

**Chapter 3** investigates whether tactile inputs modulate focal cooling detection in humans with the novel cooling stimulator.

**Chapter 4** further characterises in humans the tactile modulation of cooling detection by studying the spatiotemporal features of this interaction.

**Chapter 5** conducts a study on whether tactile inputs modulate intensity ratings to focal cooling in humans with the non-tactile cooling stimulator.

**Chapter 6** combines the novel cooling stimulator with widefield imaging in anaesthetised mice to study the responses of forepaw primary somatosensory cortex (fS1) to mechanical and non-contact thermal stimulation.

**Chapter 7** describes an electrophysiological study in mouse forepaw primary somatosensory cortex (fS1) during mechanical and contact thermal stimulation.

**Chapter 8** concludes with the proposal of a new model to describe interactions between cooling and mechanical signals in the nervous system.
1 Separated in the Skin, Unified in the Mind
1.1 Summary

This chapter reviews relevant literature to understand how the perception of thermal objects emerges in the mind. We will follow the structure of a somatosensory pathway from the receptors at the skin to the perceived objects. Along this pathway, we identify gaps in our knowledge such as the role of polymodal afferents and of cortex in thermotactile perception. We then turn to the theory of sensory encoding. There are two theoretical frameworks that attempt to explain how sensory signals are encoded and result in perception: the labelled-line and population coding theory. Neither of these theories seem to unify existing experimental observations. One reason might be that most studies on somatosensation use stimulators which fail to selectively activate one pathway. Therefore, we end the review by motivating the need for non-tactile stimulators to study thermotactile interactions.
1.2 From Contact to Percept

When a cooled coin lies on your hand, you feel its shape as well as its temperature. You feel these features because your nervous system transforms external energy into neural activity. Specifically, the experience of the shape results from processing mechanical energy, whereas the experience of temperature emerges from transforming thermal energy. In the skin, there are dedicated receptors for transforming each type of energy (Abraira & Ginty, 2013; Ezquerra-Romano & Ezquerra, 2017). These receptors are the doors for thermal and mechanical information to enter the nervous system. Although the energies are transduced separately, we distinctively perceive the coin as a single, unified, thermotactile object.

How do unified perceptual objects emerge from different external energies? To approach this question, we need to understand the series of transformations that are needed to go from contact to percept. During this process, mechanical and thermal signals interact with each other. In the periphery, mechano- and thermo-sensitive receptors can be expressed in different primary afferents, but there are polymodal neurons which express receptors sensitive to both mechanical and thermal energy (Belmonte & Viana, 2008; Paricio-Montesinos et al., 2020). Then, primary afferents synapse in the dorsal horn where somatosensory channels experience excitatory and inhibitory interactions (Boyle et al., 2019; Horwitz et al., 2022; Melzack & Wall, 1965; Wang et al., 2022; Zheng et al., 2010). Then, mechanical and thermal signals further interact in subcortical and cortical regions. For instance, it is now known that their representations either co-localise or are adjacent to each other in the cortex, which suggests these signals interact at these sites (Milenkovic et al., 2014; Vestergaard et al., 2023). Finally, tactile sensations are modulated by temperature and vice versa. For instance, cooled or warmed objects feel heavier than neutral ones (Kuhtz-Buschbeck & Hagenkamp, 2020; Stevens & Green, 1978). The combination of thermal and tactile signals results in the experience of complex thermotactile features of objects.

What is the significance of understanding the interactions between thermal and tactile signals? First, the thermotactile system is an ideal model for studying multisensory integration, which is a primary role of the nervous system. Second, the thermotactile system is involved in drawing the boundary between the experience of ‘self’ and the external world (Craig, 2002). Third, the market for virtual reality is growing, but immersive somatosensory experiences are still poor. Therefore, studying the
interactions between thermal and tactile signals would help us understanding multisensory integration and consciousness as well as developing new technologies.

In this literature review, research is introduced to understand how thermotactile objects emerge in the mind. The structure of the review is based on the concept of a somatosensory pathway. Therefore, we will follow the pathway to understand the successive steps to transform external mechanical and thermal energies into the percept of a thermotactile object. Along the pathway, we will focus on the interactions that occur between mechanical and thermal channels. We will identify gaps in our knowledge about the thermotactile system.

1.3 From External Energy to Neural Activity

1.3.1 Skin

Thermal and mechanical energies are first transformed in the skin. The skin is the largest sensory interface, covering a surface area of 1-1.5 m² in a healthy human adult (Gehan & George, 1970). This organ is divided in three layers: the epidermis, dermis, and hypodermis. The epidermis is the outermost layer and mainly consists of two cells: keratinocytes, which produce the structural protein keratin, and melanocytes, which generate the pigment melanin. At the innermost layer of the epidermis, we find Merkel cells, a type of mechanosensory end organ (Handler & Ginty, 2021). Beneath the epidermis, we find the dermis. This layer is mainly made up of fibroblast cells, which provide structure to the skin with collagen and elastin fibres. In this layer, we find most mechanosensory end organs and thermosensitive nerve endings (Handler & Ginty, 2021) as well as immune cells and blood vessels. Finally, the innermost layer of the skin is the hypodermis, which is primarily made up of adipocytes (fat cells), so it has an insulating and energy-storing role. Together, these cells shape the thermomechanical properties of the skin, which define the travelling speed and distance of thermal and mechanical energies within this tissue.

When we touch an object, the skin is subject to five main forces: normal, friction, shear, tensile and compressive forces (Pawluk & Howe, 1999; Serina et al., 1998; Srinivasan et al., 1992). Crucially, the mechanical response of the skin to these forces endows mechanosensitive fibres with specific response properties (Saal et al., 2017). For example, the way the skin reacts to the normal and compressive forces results in tactile fibres showing edge enhancement and surround suppression as well as responding to vibration at a distance from the source (Manfredi et al., 2012; Phillips & Johnson, 1981; Sripati et al., 2006). In other words, the skin transforms mechanical
energy and ‘performs computations’ that are then directly transferred to mechanosensitive fibres.

Moreover, heat transduction in the skin is modulated by the state of the skin. Specifically, experimental and modelling work has shown the speed and spread of heat flux is modulated by the shape of the skin as well as the level of blood perfusion, contact pressure and hydration (Ho & Jones, 2008; Hristov, 2019; Pennes, 1998; Rykaczewski, 2019). For instance, changes to the skin temperature are localised to the area of contact and the relationship between contact pressure and temperature change is described by a logarithmic function (Ho & Jones, 2008). Crucially, this shows how touch modulates thermal signals already at the skin level. Temperature also influences the mechanical properties of the skin. For instance, a recent study found that at lower temperatures frictional forces are decreased (Filingeri et al., 2022). Altogether, thermal and mechanical energies interact at the skin by influencing the thermomechanical responses of the tissue, which modulates mechano- and thermosensitive fibre activity and influences thermotactile perception.

Interestingly, the skin also shapes thermotactile signalling chemically. For instance, cellular studies have suggested that keratinocyte activity may be required for innocuous cold sensation (Sadler et al., 2020). Specifically, keratinocytes seem to release ATP upon cooling and this ATP increases likelihood of neural responses to cooling by binding to purinergic P2X4 receptors in cooling-sensitive neurons. This suggests two intriguing possibilities. First, thermotactile sensations might be modulated by the biochemical conditions of the skin. Second, thermo- and mechanosensitive fibres might directly interact at the skin through chemical signals. Rather than a passive interface, future research should consider the active role of the skin in thermotactile perception.

1.3.2 Receptors

Thermosensitive receptors transform thermal changes in the skin to ion movements, which results in neural activity. These receptors are embedded in the membrane of free nerve endings. They belong to the Transient Receptor Potential (TRP) ion channel superfamily. Thermosensitive TRP channels depolarise fibres by influx of cations such as calcium (Ca$^{2+}$) upon cooling or warming. Rather than a single receptor, animals express a family of thermosensitive receptors (McKemy, 2007). These are traditionally categorised into 4 groups based on the specific temperature ranges that they are sensitive to. First, the TRPA1 channel is activated by noxious cooling, approximately below 17°C (Story et al., 2003). Second, the TRPM8 channel
is activated by innocuous cooling (~17°C-32°C) and menthol (Yin et al., 2018; Dhaka et al., 2008; Zhao et al., 2022). Third, the TRPV3 and TRPV4 channels are activated by innocuous warming from 32°C to 42°C (Güler et al., 2002; Liedtke et al., 2000; Xu et al., 2002). Fourth, the TRPV1 and TRPV2 are activated by noxious warming over approximately 42°C (Caterina et al., 1997; Caterina et al., 1999). Together these channels allow us to detect temperature changes from noxious cooling to noxious heat.

Mechanical energy is transformed by specialised structures called mechanosensory end organs. There are four types of end organs: Merkel's discs, Ruffini endings, Meissner corpuscles and Pacinian corpuscles (Abraira & Ginty, 2013). These structures are associated with fibres to transform mechanical energy into neural activity. They express the mechanosensitive ion channel, Piezo2, which depolarises cells by allowing influx of cations such as Ca²⁺ (Coste et al., 2010; Ranade et al., 2015). Together with thermosensitive receptors, these receptors are the doors into our nervous system for thermal and mechanical energies.

Interestingly, receptors seem to play an active role in multimodal perception. For instance, TRPA1 is activated by brief and focal mechanical forces (Kwan et al., 2006). Another thermosensitive channel, TRPV4, has been found to be sensitive to mechanical stress, which seems to involve cells expressing this channel in anti-inflammatory signalling (Fu et al., 2021). Interestingly, a recent study found that low temperatures increase the peak and amplitude of mechanically activated currents neurons expressing Piezo2 (Zheng et al., 2019). Altogether, there seems to be thermotactile integration already at the level of cutaneous receptors.

### 1.3.3 Primary Afferents

Thermosensitive primary afferents transmit thermal information from cutaneous receptors to the central nervous system. The classic view is that cooling-sensitive fibres are both Aδ- and C-fibres, whereas warming-sensitive fibres are only C-fibres. Aδ-fibres are thinly myelinated and have faster conduction velocities than C-fibres, which are smaller in diameter and unmyelinated (Campero et al., 2001; Mackenzie et al., 1975; McKemy et al., 2002; LaMotte and Campbell, 1978) (Figure 1.1A). Furthermore, the nerve endings of cooling-sensitive fibres are found in the epidermis at a depth of 0.2 mm, whereas the endings of warming-sensitive fibres are at approximately 0.5 mm (Dhaka et al., 2008; Hensel & Zotterman, 1951; Lv & Liu, 2007). Therefore, cooling and warming signals are transmitted at different speeds in the periphery.
Mechanosensitive primary afferents convey tactile information from mechanosensory end organs to the central nervous system. These fibres are classified into four major types based on the ending type, location and response pattern: rapidly adapting type I (RAI), rapidly adapting type II (RAII), slowly adapting type I (SAI), and slowly adapting type II (SAII) fibres (Abraira & Ginty, 2013; Vallbo et al., 1995; Koltzenburg et al., 1997) (Figure 1.1B). RAI and RAll fibres are associated with Meissner corpuscles and Pacinian corpuscles, respectively, whereas SAI and SAII fibres are associated with Merkel's discs and Ruffini endings, respectively (Abraira & Ginty, 2013). Each fibre type is sensitive to different features of mechanical stress in the skin such as vibration, pressure, and skin stretch (Johansson & Flanagan, 2009; Saal & Bensmaia, 2014; Saal et al., 2017).

Primary afferents show integration of thermal and mechanical energies due to the polymodal sensitivity of some receptors and to the co-expression of unimodal receptors (Belmonte & Viana, 2008). First, slowly adapting mechanosensitive fibres are known to respond to cooling (Burton et al., 1972; Duclaux & Kenshalo, 1972). Second, across species, many cooling- and warming-responsive C-fibres are polymodal and respond to mechanical inputs (Beitel & Dubner, 1976; Campero & Bostock, 2010; Paricio-Montesinos et al., 2020; Shea & Perl, 1985). The functional role of polymodal primary afferents is unclear and is not explained by some classic theories of perception such as the labelled-line theory (Fando et al., 2020; Ma, 2012).

The spatial distribution of fibre endings influences perception (Green, 1982; Longo & Haggard, 2011). In humans, we lack reliable data about the thermotactile innervation density over the body, but we can use psychophysical testing to develop sensitivity maps, which presumably reflect innervation. Thermal and tactile sensitivity vary across the body (Corniani & Saal, 2020; Stevens & Choo, 1998). Tactile thresholds decrease towards distal regions, particularly towards the fingertips (Corniani & Saal, 2020). Thermosensitivity increases distally within the forearm, whereas it decreases distally within the hand (Ezquerra-Romano et al., 2023; Filingeri et al., 2018; Luo et al., 2020), which is opposite to what is observed for touch sensitivity. Crucially, there are small areas (~1 mm²) of skin within a body region that are particularly sensitive to temperature. These are called thermal spots (Blix, 1882; Dallenbach, 1927; Ezquerra-Romano et al., 2023; Green & Cruz, 1998). In this sense, thermal sensitivity follows a spot pattern, whereas tactile sensitivity changes in homogenous gradients within body regions. Altogether, data suggests thermo- and mechano-sensitive fibre density differs across the body. Then, the relative number of endings of each modality that
become activated when a region is stimulated will vary across the body. Thus, it is a possibility that thermotactile integration is different between skin regions.

**Figure 1.1. Thermotactile pathways from the skin to the brain.** A) Diagram of the thermosensory pathway in the mouse model from the skin to the brain. The thermosensory pathway is shown schematically from skin to cortex through the spinal cord and the thalamus. Abbreviations: insular cortex (IC), secondary somatosensory cortex (S2) and primary somatosensory cortex (S1). The right panel shows schematic cross-sections of the mouse nervous system at the levels indicated in the left panel. Thermosensitive primary afferents (Aδ- and C-fibres) synapse primarily at laminae I-II and occasionally at deeper layers. Secondary afferents decussate and send projections via the spinothalamic tract to the thalamus, where
they synapse with ascending projections to the cortex. Abbreviations: posterior triangular nucleus (PoT), posterior medial (Pom), ventral posterolateral (VPL). Adapted from Bokiniec et al., 2018. B) Diagrams of the mechanosensory pathway from the skin to the brain. The left panel represents the skin layers and the organisation of mechanoreceptors in the glabrous skin. The legend labels the corresponding mechanosensitive end organs. Abbreviations: stratum corneum (SC); stratum granulosum (SG); stratum spinosum (SS) stratum basalis (SB). Figure copied from Abraira & Ginty, 2013. The right panel represents the pathway from skin to brain. Mechanical forces are transduced by mechanosensitive end organs, which are associated with primary afferents. Then, primary afferents carry mechanical information to the spinal cord. Their afferent fibres travel through the dorsal columns (gracile fasciculus and cuneate fasciculus) to the dorsal column nuclei (the gracile nucleus and the cuneate nucleus) in the lower medulla, where they synapse with secondary afferents. These fibres decussate and send ascending projections to the ventral posterior lateral nucleus of the thalamus. Mechanosensitive fibres in the thalamus then send projections to the primary somatosensory cortex. Figure copied from Saal & Bensmaia, 2014.

1.4 From the Periphery to the Cortex

1.4.1 Spinal Cord

Thermosensitive fibres enter the spinal cord and synapse with neurons in laminae I and II (Figure 1.1A). In mice, cooling-sensitive spinal neurons encode relative temperature changes, whereas warming-sensitive spinal neurons encode absolute changes (Ran et al., 2016). Crucially, primary afferents do not seem to encode cooling and warming signals differently (Yarmolinsky et al., 2016). In the spinal cord, thermal signals are shaped by excitatory and inhibitory interneurons. Thus, cooling and warming signals might be processed by different spinal circuits (Horwitz et al., 2022; Wang et al., 2022). The Thermal Grill Illusion suggests this might be the case in humans. In this illusion, interlaced warming and cooling stimuli create a burning sensation. This illusion is thought to result from the disinhibition of nociceptive fibres. Cooling-sensitive fibres inhibit nociceptive fibres during resting state. During the Thermal Grill Illusion, warming-sensitive fibres inhibit cooling-sensitive fibres, which unmask nociceptive fibres (Craig & Bushnell, 1994; Fardo et al., 2018). This shows that thermal signals undergo interactions in the spinal cord.

Mechanosensitive fibres also synapse in specific laminae within the spinal cord (Figure 1.1B). There are terminations of mechanosensitive fibres from laminae I to IV (Abraira & Ginty, 2013). Here, Ginty and colleagues are characterising the cellular and synaptic architecture of mechanosensory circuits in the spinal cord. Importantly, mechanosensitive fibres extend their terminations rostrocaudally interacting with signals at adjacent spinal segments (Li et al., 2011). Additionally, there are multiple interneurons that modulate tactile signals (Abraira et al., 2017; Bourane et al., 2015).

Therefore, the spinal cord is a site of multimodal interactions and integrations. For instance, many studies have identified neurons along the rostrocaudal axis of the
spinal cord, which respond to different modalities over a broad stimulus amplitude range (Andrew et al., 2001; Chirila et al., 2022; Craig et al., 2001; Turecek et al., 2022). Many of these neurons send ascending projections to the midbrain and thalamus through the spinothalamic tract, contributing to perception. A well-known spinal interaction between different modalities is described by the Gate Control Theory (Figure 1.2A), which states that mechanoreceptive fibres inhibits nociceptive transmission at the spinal cord level, thereby attenuating pain (Melzack and Wall, 1965, Mancini et al., 2014, 2015). Additionally, itch is inhibited by pain (Ward et al., 1996). Altogether, innocuous thermal and mechanical incoming signals likely interact between each other at the spinal cord, but there is not yet any direct evidence that describes their interaction.

Descending projections from the midbrain and cortex are also known to modify incoming somatosensory signals. Motor control is known to suppress sensory afferent signals through several mechanisms (Blakemore et al., 1998; Seki & Fetz, 2012; Voss et al., 2006). For instance, recordings in monkeys have suggested that descending projections presynaptically inhibit tactile signals in the spinal cord (Seki et al., 2003). Other cognitive processes also modulate incoming sensory signals at the spinal cord. For example, areas associated with affective states can enhance or suppress pain via descending projections in the periaqueductal gray in the midbrain (Bushnell et al., 2013). Therefore, thermotactile-encoding brain regions may modulate incoming thermotactile signals through descending pathways.

1.4.2 Thalamus

In the thalamus, there are temperature- and mechano-sensitive neurons predominantly in the ventral posterior medial (VPM) and ventral posterior lateral (VPL) nuclei (Figure 1.1A), which receive ascending inputs from the spinal cord and the trigeminal system (Dovstrovsky & Craig, 1996; Davis et al., 1998; Davis et al., 1999; Lindstedt et al., 2011; Leung et al., 2014; Hsiao et al., 2008; Hellon & Misra, 1973; Verhagen et al., 2003). Neurons in these thalamic nuclei project to the primary somatosensory cortex (SI) and posterior insular cortex (pIC), where these thermal signals are further processed (Bokiniec et al., 2022; Craig, 2002; Hsiao et al., 2008; Milenkovic et al., 2014; Vestergaard et al., 2023; Romo & Salinas, 2001; Zhang et al., 2001).

In the thalamus, there are multimodal neurons that respond to both thermal and mechanical stimuli (Hillon & Misra, 1973; Lenz & Dougherty, 1998; Verhagen et al., 2003). Many thalamic neurons likely receive inputs from secondary afferents in the
spinal cord that are responsive to both thermal and mechanical stimuli. It is unknown whether integration of unimodal channels happens in the thalamus. The function of the thalamus in sensory processing is still unclear and a matter of debate in the research literature.

The classic view is that this brain structure simply relays incoming and outgoing signals, which implies thermotactile signals do not interact and integrate at this stage. However, there is evidence that suggests sensory signals are actively processed in the thalamus. First, anatomical studies are revealing the cellular organisation of bidirectional circuits between the thalamus and the cortex, known as cortico-thalamo-cortical loops, which are thought to be involved in higher order functions such as perception and attention (Halassa & Michael, 2017; Shepard & Yamawaki, 2021). Second, there are populations of excitatory and inhibitory interneurons, forming circuits within the thalamus that shape incoming signals (Cox et al., 1998; Wang et al., 2011). Altogether, it is likely that thermotactile interactions occur within the thalamus. Future studies should characterise the activity of thermo- and mechano-sensitive neurons in the thalamic nuclei and compare their responses during unimodal and multimodal stimulation.

### 1.4.3 Cortex

Thalamic projections transmit thermal signals to other brain areas (Bokiniec et al., 2022). In humans, imaging and clinical evidence have implicated the orbitofrontal cortex and the cingulate cortex in the representation of thermal information in the brain (Boivie et al., 1989; Craig et al., 2000) (Figure 1.1A). It was then accepted that the primary and secondary somatosensory cortices were not involved in temperature perception. However, recent rodent and human studies have challenged this view. Specifically, a study in mice showed robust responses upon cooling of the paw in the primary somatosensory area (SI) (Milenkovic et al., 2014). A recent study revealed features of neuronal responses to temperature changes both in SI and the insular cortex (IC), highlighting the central role of IC in thermal perception (Vestergaard et al., 2023). Interestingly, this study identified a small number of neurons responsive to warming stimuli in SI. These findings are supported by brain-wide mapping of thermosensory cortices (Bokiniec et al., 2022). In humans, an fMRI study showed the activity of SI could be used to decode with machine learning the temperature of a thermal stimulus applied to the hand (Jung & Walther, 2021). Although this study does include an fMRI experiment in which people are exposed to stones at different temperatures without particular task, the focus of the experiment was the neural representation of scene categories and attributes. However, the researchers used a
subset of the BOLD activity in SI while people held the stone to train a classifier and predict the temperature of the stone from brain activity in the untrained dataset. This suggests SI contains thermal information in humans, but future studies should focus on thermal encoding in SI in humans to confirm this.

The processing of mechanical signals in the cortex is thought to follow a hierarchical organisation (Delhaye et al., 2018). First, the primary somatosensory cortex receives direct thalamic projections carrying tactile information (Figure 1.1B). Here, tactile information is organised somatotopically, and neurons represent simple features of the tactile world such as the orientation of a stimulus on the skin (Bensmaia et al., 2008; Lieber & Bensmaia, 2019). Then, mechanical signals travel via cortico-cortical connections to the secondary somatosensory cortex, where more complex pattern representations emerge (Felleman & Van Essen, 1991; Reed et al., 2004; Vogt & Pandya, 1978; Young, 1993). Lastly, tactile signals are further processed and integrated with other modalities (e.g. vision) in the parietal and frontal areas (Avillac et al., 2007; Stoeckel et al., 2003).

Although thermal and mechanical signals are integrated seamlessly during haptic exploration, no studies have specifically investigated thermotactile processing in the cortex. This is probably due to the lack of robust, well-defined and co-localised cortical responses to both stimulus types. Nonetheless, recent findings of thermal and tactile responses in SI point to this area as a potential candidate for thermotactile integration (Milenkovic et al., 2014; Vestergaard et al., 2023).

1.5 Thermotactile Perception

In the previous two sections, we have reviewed the literature on the neurobiology of thermal and tactile sensations from the skin to the cortex. At each step of the pathway, we have considered how both channels might interact with each other. In this section, we review perceptual studies in humans on thermotactile perception.

1.5.1 Thermal and Tactile Sensations

Sensations are the mental representation of simple features in our environment. In this sense, sensations are unimodal and serve as the basic building blocks of perception. For example, the pressure that you feel when you hold an object is a sensation and is the direct result of stimulating mechanosensitive fibres. When thermal and mechanical stimuli are presented together, the resulting sensations from each modality are modulated by the input of the other.
Thermotactile illusions reveal the profound interactions between thermal and mechanical stimuli. When an object is cooled or warmed, it feels heavier than at neutral temperatures. This is known as the Weber’s Thaler illusion (Kuhtz-Buschbeck & Hagenkamp, 2020; Stevens & Green, 1978). This illusion is presumably explained by the responsiveness of mechanosensitive fibres to cooling (Cahusac & Noyce, 2007). Crucially, the temperature of the skin modulates this illusion. Specifically, a study found the increased sensation of heaviness to warmed objects was dampened when the skin was warmed or cooled, whereas these changes in skin temperature had a weaker effect on the intensification of weight by cooled objects (Stevens & Hooper, 1982). Consistent with the Weber’s Thaler illusion, people are better discriminating between two closely spaced mechanical inputs when they are both cooled and warmed (Stevens, 1982).

Intriguingly, we lack dedicated hygroreceptors for sensing humidity in our primary afferents, but we have a clear sensation of wetness. Studies by Filingeri and colleagues suggest that this sensation is a result of integrating mechanical and thermal signals (Filingeri et al., 2014). Specifically, cooling seems to play a key role because wetness sensations can be elicited with dry-cold stimuli and warming suppresses sensations of wetness (Filingeri et al., 2013; Filingeri et al., 2015). Additionally, factors such as skin-object friction and low mechanical pressure also contribute to the perception of wetness (Adams et al., 2013; Filingeri & Havenith, 2015). Therefore, the sensation of wetness is seemingly a combination of specific thermal and mechanical features. Similarly, affective touch (e.g. caresses) is elicited most optimally by thermally-neutral strokes at 3 cm s$^{-1}$ (Ackerley et al., 2014).

Touch can also modulate the spatial extent and intensity of thermal sensations. When two objects at neutral temperatures are placed close to each other and one of them is cooled or warmed, the other also feels to be cold or warm. This is known as ‘thermal referral’ (Green, 1977; Ho et al., 2011; Stevens & Green, 1978). However, a recent study showed that thermal referral can occur without touch (Cataldo et al., 2016). This suggests that thermal referral might be explained by low-level organisation of the thermosensory pathway, rather than by thermotactile interactions. Additionally, Green and colleagues found that touch attenuates both warm and cold sensations in humans (Green & Schoen, 2005; Green, 2009). Specifically, they found that dynamic tactile stimulation reduced the perceived intensity of contact thermal stimulation compared to static tactile stimulation. Altogether, thermal and mechanical inputs modulate the intensity and spatial characteristics of the sensations elicited by the
other modality. Importantly, qualitatively distinct sensations such as the sensation of wetness can emerge for specific energy combinations.

We are familiar with the thermal sensations elicited by changes in body temperature. In this literature review, we are focussing on the neurobiological and perceptual mechanisms underlying thermotactile sensations that support the perception of objects. We are therefore not addressing a key function of the thermosensory system: body thermoregulation. Crucially, changes in body temperature of a few degrees can lead to tissue damage or even death. The mechanisms that regulate body temperature are shared with those that support perception of thermotactile objects. For instance, the receptor TRPM8 is expressed in the thermoregulatory circuits of the rodent brain and is involved in the regulation of body temperature (Reimúndez et al., 2022; Ordás et al., 2019). On the other hand, the hypothalamus is implicated in thermoregulation but presumably not in thermosensation (Boulant, 1980; Boulant, 1998; Nagashima et al., 2000).

However, there are projections from the hypothalamus to the spinal cord and stimulation of the hypothalamus regulates thermal sensitivity to focal stimuli (Holstege, 1988; Jürgens et al., 2009). Although the mechanisms of thermotactile perception and thermoregulation largely overlap, we still do not understand their relationship. The standard practice in studies on thermotactile perception is to test healthy participants in temperature-controlled rooms to control for the potential modulation of thermoregulatory processes.

1.5.2 Object-Level Perception in Thermal Perception

Object-level perception involves integrating and organising sensations to form representations of events in the external world (Marr, 1982). This process often involves integrating information across multiple sensory channels such as combining visual and tactile information to recognise an object's shape, texture, or material (Lederman & Klatzky, 1993; Ernst & Bülthoff, 2004). For instance, when you hold an object in your hand, you not only feel the pressure on your skin (sensation) but also perceive the object's size, shape, and texture (object-level perception) by integrating tactile inputs with your prior knowledge and visual information.

The mental representation of objects is used during ongoing behaviour to make decisions and perform goal-directed actions. Interestingly, the temperature of objects influences the grip and lift forces exerted by the fingers. Specifically, a recent study found that grip force was 10% higher for lifts of cooled objects than for lifts of identical
objects at a neutral temperature (Kuhtz-Buschbeck & Hagenkamp, 2020). This finding is consistent with and might be related to Weber's Thaler illusion. Although a recent study suggested ‘thermal referral’ is explained by low-level organisation of the nervous system (Cataldo et al., 2016), the traditional view is that this illusion reflects object-level mechanisms. Namely, we have the prior that the temperature of most objects is homogenous and tactile information is weighted more highly over thermal information during object recognition. According to this, when we touch a thermally heterogenous object, it feels thermally homogenous due to these top-down object-level perceptual processes (Green, 1977; Ho et al., 2011; Stevens & Green, 1978).

Interestingly, thermal information seems to contribute to material recognition (Ho, 2017). In a series of experiments, Ho and colleagues have shown that when visual and tactile information is limited, thermal sensations can be used to successfully discriminate between materials (Ho & Jones, 2006). Strikingly, people can differentiate between different materials based on simulations of the thermal transients associated with skin-object contact (Ho & Jones, 2004; Ho & Jones, 2007).

Our own body can be considered an object and temperature has been found to play a role in body ownership perception. For instance, losing perceptual ownership of a limb leads to a decrease of temperature of the given limb (Moseley et al., 2008). Consistently, decreasing the temperature of a limb facilitates manipulations to body ownership (Kammer et al., 2011). Intriguingly, the thermotactile mechanisms involved in discriminating between external (objects) and internal (body) signals could be involved in the perception of ‘self’ (Craig, 2002).

1.6 Theoretical Frameworks of Somatosensation

In the previous three sections, we have reviewed experimental results from studies on the thermodactile system. Researchers have developed several theoretical frameworks to unify these results. There is still no clear consensus yet on what theory best describes how sensations emerge from sensory neural activity. Here, we focus on two models which place the focus on how the central nervous system receives and interprets incoming sensory signals: the labelled-line theory and the population coding theory (Figure 1.2B).

First, the labelled-line theory suggests that sensations emerge directly from unimodal, dedicated channels that go from the skin to the brain (Müller, 1843; Ma, 2010). Each channel is dedicated to a specific type of sensory information such as touch and temperature (Figure 1.2B). Thus, the quality of a sensation (e.g. cold) emerges from
reading the activity from a specific channel (e.g. cold-sensitive neurons). This theory is supported by the presence of receptors like TRPV1 and TRPM8, which are selectively activated by thermal stimuli (Caterina et al., 1997; Peier et al., 2002). However, the labelled lines theory cannot directly explain three observations. First, some receptors such as TRPA1 are activated by more than one stimulus type (Kwan et al., 2006). Second, many primary afferents are polymodal because they either have polymodal receptors or express multiple receptor types. Third, we do not have dedicated receptors or afferents for each type of perceptual experience. For instance, we lack hygroreceptors, but we have a clear experience of wetness. Finally, this theory has a profound philosophical flaw. If the sensory lines are labelled, who is the agent that reads them? Despite its limitations, this theory is a valuable framework to understand certain aspects of somatosensation such as the specificity of certain receptors and afferents.

Figure 1.2 Gate Control Theory and theories of sensory encoding. A) Illustration of the gate control theory of pain. Nociceptive fibres (red) carry signals to the spinal cord where they synapse with ascending fibres (yellow). These ascending fibres are inhibited by interneurons (black), which are activated by mechanosensitive fibres (green). Mechanosensitive fibres also send signals to supraspinal areas directly or through other ascending fibres. B) Left panel: illustration of the labelled-line theory. Dedicated channels carry thermal (blue) and mechanical (green) information from the periphery to the brain. The quality of a sensation is the result of a
central reader processing activity from either of the channels. Right panel: illustration of population coding theory. Unimodal (blue and green) and polymodal fibres (yellow) carry thermal and mechanical information. A central reader integrates the activity across channels, which results in the quality of a sensation.

Second, the population coding theory states that sensations emerge from the specific patterns of activity across unimodal and polymodal channels (Georgopoulos et al., 1986; Nafe, 1929; Ma, 2012; Fardo et al., 2020). The results from a recent rodent study on thermal perception support this theory. The authors found that cooling-sensitive fibres are required for warm sensations (Paricio-Montesinos et al., 2020). On the other hand, recent rodent work has also shown distinct cortical responses to touch, warming and cooling in the mouse SI and IC (Vestergaard et al., 2023). These results suggest warming and cooling responses are encoded in a labelled-line-like fashion, mirroring responses observed in primary afferents and the spinal cord (Ran et al., 2016; Wang et al., 2018; Yarmolinsky et al., 2016). Nevertheless, modern versions of this theory are gaining support thanks to methods that allow measuring the activity, morphology and genetic makeup of large populations of neurons (Belmonte & Viana, 2008; Paricio-Montesinos et al., 2020; Fardo et al., 2020; Meltzer et al., 2021). However, it still does not explain how perception emerges from neural activity.

1.7 Methods to Study the Thermotactile System

In the thermotactile system, primary afferent activity is elicited by thermal and mechanical energies. Therefore, we must manipulate these energies to study thermotactile sensations. Researchers have engineered stimulators to generate stimuli with specific properties for their studies. Crucially, the characteristics of the stimulus such as its size and precision must be suitable to the research aim and can limit the conclusions that can be drawn from the results. Here, we briefly review some of the most common stimulators used in thermotactile research.

Thermal sensations are commonly manipulated in the lab with Peltier devices (De Keyser et al., 2018; Jones & Ho, 2008; Leone et al., 2019; Milenkovic et al., 2014; Vestergaard et al., 2023). These devices deliver controlled temperature stimuli to the skin by heating or cooling a metal plate. Peltier elements can provide rapid and precise temperature changes. Another strategy involves submerging body parts in cooled or warmed water baths (Cataldo et al., 2016; Vabba et al., 2022). This method allows for changing the temperature over large regions in a homogenous way, but it alters the hydration of the skin. A common method used in thermal comfort research is controlling the room temperature with heating or air conditioning system (Gagge et
al., 1967). This approach is well suited for experiments focussed on thermoregulation but cannot be used to study focal thermal sensations. Some studies have used menthol to investigate cold sensations because this molecule binds to TRPM8, activating TRPM8-expressing fibres (Typolt & Filingeri, 2020; Xu et al., 2020). Several studies have employed stimulators that minimise contact such as blowing cooled or warmed air (Parra et al., 2010; Murphy et al., 2001; Xu et al., 2019; Xu et al., 2022) and combining ultrasound waves with cooled or warmed vapour (Nakajima et al., 2021). However, these methods involve a small degree of mechanical stimulation. Lasers overcome this limitation and heat the skin without any mechanical stimulation (Iannetti et al., 2004; Qiu et al., 2003; Truini et al., 2010). However, they can only warm the skin up and seem to be most optimal for noxious warm stimulation.

<table>
<thead>
<tr>
<th>Stimulator type</th>
<th>Advantages</th>
<th>Limitations</th>
<th>Afferent selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peltier devices</td>
<td>Rapid, precise temperature changes</td>
<td>Involves contact</td>
<td>Low</td>
</tr>
<tr>
<td>Water baths</td>
<td>Large, homogenous temperature changes</td>
<td>Alters skin hydration, non-focal</td>
<td>Medium</td>
</tr>
<tr>
<td>Room temperature control</td>
<td>Non-invasive, suitable for thermoregulation studies</td>
<td>Not suitable for focal sensations</td>
<td>High</td>
</tr>
<tr>
<td>Menthol application</td>
<td>Selectively activates TRPM8 fibres</td>
<td>Poor-temporal control, only for cold sensation</td>
<td>High</td>
</tr>
<tr>
<td>Airflow</td>
<td>Non-contact</td>
<td>Poor-temporal control, low range of intensities</td>
<td>Medium</td>
</tr>
<tr>
<td>Ultrasound waves</td>
<td>Non-contact</td>
<td>Under development</td>
<td>Medium</td>
</tr>
<tr>
<td>Lasers</td>
<td>Non-contact</td>
<td>Limited to heating</td>
<td>High</td>
</tr>
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*Table 1. Summary of thermal stimulators.*
<table>
<thead>
<tr>
<th>Stimulator type</th>
<th>Advantages</th>
<th>Limitations</th>
<th>Afferent selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>von Frey filaments</td>
<td>Standardised, calibrated forces, widely used</td>
<td>Single-point stimulation, poor-spatial control</td>
<td>Medium</td>
</tr>
<tr>
<td>Vibration motors</td>
<td>Variable frequencies and amplitudes, high spatiotemporal control</td>
<td>Not standardised or calibrated</td>
<td>Low</td>
</tr>
<tr>
<td>Linear actuators</td>
<td>Precise control of movement, high spatiotemporal control</td>
<td>Not standardised or calibrated</td>
<td>Low</td>
</tr>
<tr>
<td>Airflow</td>
<td>Non-contact</td>
<td>Low range of intensities</td>
<td>Medium</td>
</tr>
<tr>
<td>Sanshool</td>
<td>Non-contact</td>
<td>Low temporal control</td>
<td>High</td>
</tr>
<tr>
<td>Ultrasound waves</td>
<td>Non-contact</td>
<td>Still under development</td>
<td>High</td>
</tr>
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Table 2. Summary of tactile stimulators.

Tactile sensations are commonly elicited in the lab with mechanical stimuli. Von Frey filaments are among the most common tools used in tactile research. These calibrated monofilaments exert different forces when applied to the skin (Deuis et al., 2017; Johansson et al., 1980; Rolke et al., 2006). Moreover, vibration motors are used to study the perception of vibrotactile stimuli (Kooijman et al., 2022), while linear actuators are employed to investigate the spatial aspects of touch (Arslanova et al., 2020; Arslanova et al., 2022; Cataldo et al., 2022; Dupin & Haggard et al., 2019). Additionally, tactile sensations can also be manipulated with air puffs and currents in the lab (Kooijman et al., 2022). Interestingly, vibration-like tingling sensations can be elicited chemically with sanshool, which selectively activates RA fibres (Bautista et al., 2008; Cataldo et al., 2021). This allows the interesting possibility of studying touch without physical contact. Similarly, mid-air devices can elicit tactile sensations by emitting ultrasonic acoustic waves, which do not activate SA fibres (Cataldo et al., 2023; Moore et al., 2021).

1.8 Summary and Thesis Overview

In this chapter, we have provided an overview of the thermotactile system from the skin to the conscious experience of objects. We have considered the interactions between the thermal and mechanical pathways. Initially, thermal and mechanical energies are transformed within the skin. At this early stage, there are already
interactions between thermal and mechanical signals, mainly through the changes in mechanical and thermodynamic properties of the skin. Next, thermo- and mechano-sensitive receptors transduce thermal and mechanical energies into neural activity. Intriguingly, the main mechanosensitive receptor, Piezo2, is modulated by cooling. This confers mechanosensitive primary afferents with sensitivity to cooling stimuli. Additionally, there are polymodal primary afferents, which express thermo- and mechano-sensitive receptors. Primary afferents synapse in the spinal cord. Here, afferent signals interact between each other and with descending signals directly or through excitatory and inhibitory interneurons. Ascending signals travel to subcortical and cortical areas, where they are further processed and integrated with ongoing neural activity.

Thermal and tactile sensations emerge from neural activity in the thermotactile pathways. Therefore, they reflect the characteristics of these pathways and the interactions between them. For instance, cooled objects feel heavier than neutral ones, which is perhaps explained by the responsiveness of Piezo2 and mechanosensitive fibres to cooling. Moreover, specific combinations of thermal and tactile signals can generate qualitatively different sensations such as the sensation of wetness. Finally, complex characteristics of objects such as their material can be inferred both with thermal and mechanical sensations, showing the intricate relationship between thermal and mechanical signals even at object-level perception.

Theories of perception fail to accommodate all the complexities of the thermotactile system. For instance, the labelled-line theory cannot explain the function of polymodal fibres and emergence of sensations without a dedicated receptor such as the sensation of wetness. On the other hand, the population coding theory cannot directly explain the observation of labelled-line-like encoding properties in the cortex. Thus, a clear challenge in thermotactile perception is to reconcile the existence of unimodal and polymodal receptors and afferents. To this end, we need to selectively stimulate each receptor and afferent type. By following this strategy, we can compare the neural and behavioural responses between unimodal and multimodal conditions to elucidate the principles of thermotactile integration. However, most thermal stimulators fail to selectively manipulate a single stimulus dimension. Specifically, current cooling stimulators involve a degree of mechanical stimulation that presumably stimulates mechanosensitive fibres.

In this thesis, we therefore study non-tactile cooling responses. In Chapter 2, we show the development of novel cooling stimulator, which delivers non-tactile, focal,
temperature-controlled cooling stimuli (Ezquerra-Romano et al., 2022). We demonstrate that the level of mechanical stimulation of our cooling stimulus is unlikely to directly trigger mechanosensitive fibres.

In Chapter 3, we use this novel stimulator to investigate the interaction between cooling and mechanical signals in humans. Specifically, we compare the detection to cooling stimuli with and without mechanical input in a signal detection paradigm. In this experiment, the mechanical input is a pair of von Frey filaments bracketing the cooling input. We find that touch reduces detection of cooling inputs.

In Chapter 4, we adapt our stimulator to study the spatiotemporal features of the interactions between cooling and tactile signals. In one experiment, we stimulate with cooling and touch different spinal segments while keeping the distance between the stimuli constant. In another experiment, we manipulate the distance between the stimuli within the same spinal segment. Finally, we manipulate the time onset between cooling and touch. Overall, we find further evidence for the reduction of cooling detection by touch.

In Chapter 5, we study the interaction between cooling and tactile signals beyond detection. Specifically, we use a scaling paradigm to measure whether tactile inputs modulate intensity ratings to focal non-tactile cooling. Over the tested cooling amplitudes, we find that touch enhances the perceived intensity of focal, non-tactile cooling. However, touch slows down the rate at which the intensity of cold sensations increases as a function of stimulus amplitude.

In Chapter 6, we move on to study thermotactile integration in the mouse model. We adapt our non-tactile cooling stimulator to study the responses of forepaw primary somatosensory cortex (fSI) to mechanical and non-contact cooling with widefield imaging. We performed our recordings in mice anaesthetised with isoflurane to minimise attentional and motion confounds. We find that thermotactile stimulation drives cortical activity more effectively than either cooling or mechanical stimulation alone. However, the sum of the arithmetic responses to unimodal stimuli, cooling and touch, is smaller than the response to bimodal, thermotactile stimulation.

In Chapter 7, we perform single-unit recordings in fSI with dense multi-electrode arrays during mechanical and contact cooling (Schneipel et al., 2023). Consistent with our widefield imaging, we find that thermotactile stimulation boosts firing rate responses compared to the unimodal conditions. In line with previous studies in the cortex, we observed units with supra-additive, additive or sub-additive responses to
bimodal stimulation. Finally, thermotactile stimulation prolonged the response window of supra-additive cortical neurons.

In Chapter 8, we put forward a model of cooling-mechanical signal interactions to explain our results. We propose mechanosensitive fibres inhibit cooling-sensitive fibres in the spinal cord. Then, there are two distinct cortical pathways. One pathway is unimodal and is involved in detecting cooling inputs. The other pathway is bimodal, integrates cooling and mechanical inputs and is involved in object-level perception such as estimating the magnitude of a stimulus.
2 A Novel Method to Selectively Elicit Cold Sensations Without Touch
2.1 Summary

In the previous chapter, we identified the need for a method that precisely controls non-mechanical cooling. This method would allow to study cooling responses without the confound of mechanical input. In this chapter, we describe a non-tactile, focal, temperature-controlled, multi-purpose cooling stimulator. This method controls the exposure of a target skin region to a dry-ice source by monitoring skin temperature with a thermal camera and adjusting the source-skin distance. We can now deliver non-tactile cooling stimuli with customisable profiles for studying different aspects of cold sensation. We used this stimulator to measure detection thresholds for non-tactile cooling in 13 human volunteer participants using the method of limits. The absolute cooling detection threshold was 32.71°C ± 0.88°C. The threshold relative to each participant’s baseline skin temperature was -1.08°C ± 0.37°C. This method allows cooling stimulation without the confound of mechanical contact, in a controllable and focal manner.
2.2 Introduction

The first step of thermoception is the activation of free nerve endings in the epidermis. However, contact thermal stimuli unavoidably coactivate deeper, touch-related afferents. These tactile signals interfere with thermonociceptive input both at spinal and supraspinal levels (Cahusac & Noyce, 2007; Ho et al., 2011; Mancini et al., 2015). As a result, both physiological and psychophysical responses to Aδ cooling-responsive units are confounded with tactile inputs, and quite possibly modulated by them. Therefore, cooling-mechanical co-stimulation precludes the study of non-tactile cooling responses. Yet, most research on cooling responses has used mechanical contact stimulators (e.g. Duclaux et al., 1974; Green, 2009). A logical way to study these interactions would involve comparing the effects of a cooling stimulus with the effects of a combined cooling and mechanical stimulus.

Therefore, studies of cold sensation would benefit from a cooling stimulation technique, which does not involve skin contact and mechanoreceptor activation such as laser stimulation in research on noxious warm sensations (Iannetti et al., 2004). This technique would in turn allow interactions between cold and other sensations to be studied. Previous studies have attempted non-tactile cooling with stimulators that use ultrasound, chemicals, air flow or dry ice (CO₂ solid form). These approaches have some advantages, but importantly have limitations for studying interactions between mechanical and cooling signals in the context of sensory binding and object perception.

A recent study presented a new method to cool the skin with a non-contact tactile display driven by ultrasound waves (Nakajima et al., 2021). While this method allows precise spatial and temporal control of the cooling stimulation, it does not selectively elicit cold sensations because ultrasounds generate vibrotactile sensations. In contrast, chemical approaches (e.g. menthol) specifically elicit cold sensations without mechanical pressure, but controlling the duration and intensity of chemically-induced sensations is limited (Typolt & Filingeri, 2020). Non-contact methods based on blowing chilled air at tissues allow precise control of the duration, area and intensity of stimuli, but involve a certain level of air pressure that presumably stimulate mechanosensitive afferents (Murphy et al., 2001; Bujas, 1937). Radiation or convection methods might achieve cooling without activation of tactile afferents. However, studies which have achieved temperature decrease of the skin using radiation and/or passive convection transfer between dry ice and the skin lacked precise spatial and temporal control (Cataldo et al. 2016; Ferrè et al., 2018; Hardy &
Oppel, 1938). Thus, they could not produce point-estimates of cold perceptual sensitivity of the kind used in psychophysical perceptual testing.

We have therefore developed a non-tactile, focal, temperature-controlled, multi-purpose cooling stimulator suitable for psychophysical testing on the perceptual aspect of cold sensation. Here, we describe three potential stimulation scenarios using this system. We then measured in humans thresholds for detection of non-tactile, focal cooling in the absence of touch.

2.3 Methods

Dry ice (CO$_2$) was used to deliver non-tactile cooling stimulation. The dry ice was held in a container, which varied in shape and dimension (10-2000 mL) according to the experimental application. The container was secured on a wooden support (30x2x2 cm), which could be moved in three axes using motorised linear stages (Figure 2.1) (A-LSQ150B and X-LSQ150B series, Zaber Technologies Inc.). To control the time of exposure of the skin to the dry ice, a polystyrene shutter was placed below the syringe tip, and controlled the shutter with a servo motor (SG90 Micro Servo Motor KY66-5, Longruner), driven by an Arduino Mega 2560 (microcontroller board). The shutter was closed between trials. A thermal camera (Lepton 3.5, Teledyne FLIR), which interfaced with a computer through a I/O module (PureThermal 2 - FLIR Lepton I/O Smart module, Teledyn FLIR), was used to measure the temperature of the skin immediately below the syringe tip. The thermal camera had the following characteristics: module temporal resolution, 8.7 Hz; Field of View, 57° & camera resolution, 60 x 120.
Figure 2.1. Non-tactile cooling stimulator. A) Picture of the set-up. This picture includes a third set of three motorised linear stages that control the position of a tactile stimulus used and described in Chapters 3, 4 & 5. B) A schematic of the set-up with the main components for illustration purposes. The dry ice was contained in a syringe and exposure to the skin was controlled by a shutter. The position of the shutter was controlled by a Servo motor. A weight ensured there was constant pressure on the syringe’s plunger. The position of the syringe was controlled by three motorised linear stages. People’s left hand rested on a platform and their position throughout the experiment was monitored with 3 LED lasers. The temperature of the skin was monitored by a thermal camera, which position was controlled by 3 motorised linear stages. At each trial, the position of the thermal camera ensured the field of view of the camera contained the point at which the skin was exposed to the dry ice and the temperature of this point was the mean of defined, circular region of interest (ROI).

To stimulate different skin regions during the same experiment, we had to control the position of the thermal camera. We used a second set of 3 motorised linear systems (2 stepper motor controllers LSM100B-T4 and 1 stepper motor controller LSM200B-T4, Zaber Technologies Inc.), which interfaced with the computer through controllers (2 stepper motor controllers X-MCB2 and 1 stepper motor controller X-MCB1, Zaber Technologies Inc.). These moved the thermal camera under computer control.
Custom Python and Arduino code was written to control the hardware, build the experiment and analyse the data (see software repository: https://github.com/iezgrom/publication-cold-sensation-without-touch).

The motorised linear systems were positioned relative to the participant’s left hand. Three red lasers (5V 650nm 5mW, HiLetgo®) fixed to the wall pointed at the hand dorsum. The participant’s skin was marked with ink at the beam locations, so the experimenter could visually monitor the position of the participant’s hand throughout (Figure 2.1). Importantly, room temperature fluctuations and air currents were minimised by closing windows and doors. Room temperature was monitored with a high accuracy analog wall thermometer (BUDGET, Thermometer World)

Using these general principles, three separate stimulation scenarios were realised suitable for psychophysical and neurophysiological experiments. Here, we do not report combining these scenarios, but in principle they could be combined to realise other scenarios. Firstly, we developed a focal cooling stimulus to measure cooling detection thresholds relative to baseline. Secondly, we developed a temperature feedback-controlled Proportional-Integral-Derivative (PID) solution for delivering prolonged customised cooling profiles. Thirdly, we produced a wide area rapid-cooling thermal pulse, designed to investigate cooling-evoked EEG potential. Finally, to validate this method, we collected psychophysical data for scenario 1 and stimulation data from scenarios 2 and 3.

2.3.1 Participants and Ethics

A total of 13 human participants took part in Experiment 1 (mean age: 24.1 SD = 3.5). The individuals included in the study did not have any underlying skin conditions or conditions that would affect skin sensations. Furthermore, there was no current evidence or history of neurological or psychiatric disease present in any of the participants, nor were they taking psychoactive medication. They had not previously participated in experiments using this method, and prior to participation, written informed consent was obtained from all participants.

Measures were selected and implemented to treat potential risks around handling dry ice and having mobile parts in the setup. Appropriate risk management procedures, notably around handling dry ice, were implemented. The research protocol was approved by the UCL Research Ethics Committee (ID number: ICN-PH-PWB-0847/010).
2.3.2 Scenario 1: Focal Cooling for Detection Threshold Estimation

In this scenario the dry ice was contained in a 10-ml syringe with a 4-cm blunt needle (BD Emerald Hypodermic Syringe - Luer Slip Concentric, BD). The syringe was wrapped in aluminium foil to reduce thermal loss. To obtain a constant pressure on the dry ice powder throughout, a weight of 1600 g was placed on the syringe plunger and a continuous rotation servo (FS5106R, Feetech). The circular motion of the plunger was transformed to linear motion for pushing the plunger by a 3D printed linear servo actuator (design available at: https://www.thingiverse.com/thing:3170748) (Figure 2.1).

Thermal image recording started 2 s before the shutter opened. After shutter opening, the skin immediately below the syringe tip was exposed to convection of air cooled by the dry ice. Further, the skin lost heat to the cooled syringe by radiation. As a result, the skin temperature gradually decreased, as recorded by the thermal camera. A Region Of Interest (ROI, Figures 2.1B & 2.3D) under the syringe tip was selected for online image analysis. Because the thermal camera was located slightly to the side of the syringe, and therefore had an oblique view, the circular ROI in the image had an elliptical projection (3.4x3.3 mm; 11.2 mm²) on the skin. The pixel values in degrees Kelvin (K) were transformed into degrees Celsius (°C). Although the temperature was not homogenous across the ROI, the temperature of the ROI was obtained by calculating the mean across all the pixels within the ROI. Alternative analyses were considered but they were too computationally expensive for online calculation, slowing down the software during online analysis.

The capacity of the device to cool the skin decreases as the distance from the tip of the needle to the skin increases. We constructed a linear regression model (Figure 2.2) for selecting an appropriate distance to achieve the desired temperature range. For this scenario, we used a 5-cm distance.
Figure 2.2 Linear regression model for obtaining feedforward stimulus parameters. A) Traces showing mean temperature of the skin ROI for 3 different distances between the needle’s tip and the skin. Each trace is the mean of 5 recordings at the given distance. The shaded grey area indicates the duration of cooling (shutter is open). The black open boxes indicate the data used to calculate the model represented in Figure B. B) The datapoints show the final temperature at each distance and the black line is the linear fit. A total of 5 recordings were made during cooling at 6 difference distances. After performing the mean of the traces at each distance, the mean temperature during the final 1 s of stimulation was extracted (black box in Figure A). The error bars represent the standard deviation during this final second. A linear regression was used to model the relation between distance and temperature. The R value is the Pearson correlation coefficient of the linear regression fit. The model could then be used to position the syringe to either allow a desired temperature to be reached using feedforward control, or as an initial estimate of distance for a feedback-controlled stimulation.

Airflow evaluation

To evaluate whether the cooling produced by the dry ice stimulated mechanosensitive fibres and elicited tactile sensations, we calculated the airflow exerted by the airflow produced by the sublimated dry ice and estimated the detectability of an equivalent airflow in an informal psychophysics experiment with humans.

Dry ice is solidified CO\textsubscript{2}. It sublimates directly from its solid state below -80°C to a gaseous state at standard temperature and pressure. These characteristics make this material suitable for non-tactile cooling. The sublimation of dry ice produces airflow from the needle’s tip of the syringe. To obtain the force that this airflow exerts on the skin, we used the following formula for fluid dynamics:

$$F = P \times A \quad \text{(Formula 1)}$$

To calculate the force, we need to obtain the pressure (P) and the area (A) of the airflow when it collides with the skin.

In a fluid, the dynamic pressure is the kinetic energy per unit volume. To calculate the dynamic pressure, we can use the following formula:
\[ p_D = \frac{1}{2} \rho v^2 \]  
(Formula 2),

where \( \rho \) is the density of the fluid and \( v \) is the speed of the fluid. We used the density of CO\(_2\) at standard room temperature (25 °C) and pressure (1 atm) - \( \rho = 1.84 \text{ kg/m}^3 \). The velocity of the jet of air was measured with a Pitot tube (MPXV7002DP pressure sensor, NXP). The Pitot tube was placed at 5 cm below the tip of the needle, which is the distance at which the skin was during the experiment described in Figure 2.3E. The mean velocity of the jet of air sampled at 10 Hz and averaged over a 4 s period was 4.06 m/s ± 0.30 m/s. Therefore, following from Formula 1, the dynamic pressure that the jet of air exerts on the skin is:

\[ p_D = \frac{1}{2} \times 1.84 \times 4.06^2 = 15.16 \text{ N/m}^2. \]

To obtain the area of the airflow when it collides with the skin, we can use the ellipse drawn on the skin by the circular ROI taken from thermal camera measurements in scenario 1. The cooled area of the skin was measured as an ellipse with axis lengths 3.37 mm and 3.32 mm. The area of an ellipse is:

\[ A = \pi \times a \times b \]  
(Formula 3).

Therefore, the area cooled in scenario 1 was,

\[ A = \pi \times 3.32 \text{ mm} \times 3.37 \text{ mm} = 3.52 \times 10^{-5} \text{ m}^2. \]

Following from Formula 1, the force that the jet of air exerts on the skin is:

\[ F = P \times A = 0.53 \text{ mN}, \]

The estimated threshold for exciting a single mechanoreceptor afferent by punctate stimulation of glabrous skin has been estimated using microneurography (Johansson et al., 1980). They found that RA units had a median threshold of 0.58 mN. PC units had a median threshold of 0.54 mN. Slowly adapting SAI and SAII units had median values of 1.3 mN and 7.5 mN, respectively. In this setup, convection currents from the cooling source may be assumed constant. Therefore, the mechanoreceptor afferents most likely to be stimulated are the SAI and SAII units, which are sensitive to sustained pressure (Saal & Bensmaia, 2014; Saal et al., 2017). Therefore, the mechanical element of the cooling is less than half the force level suggested to trigger a single mechanoreceptor afferent action potential. We therefore conclude that convection currents from this cooling stimulator were unlikely to produce any effective mechanical stimulation.
To further assess whether the mechanical stimulation generated by the minimal airflow during the main experiment was perceptually detectable, we performed a pilot signal detection paradigm.

The experimenter blew through the syringe on a participant’s forearm. In 10 trials, the syringe was perpendicular to the skin and at distance of 5 cm (stimulus present). In another 10 trials, the syringe was moved away so that the participant’s arm was not stimulated (stimulus absent). The participant was asked to detect the jet of air. For this experiment, 4 naïve, blindfolded participants were tested.

Before performing the experiment, the experimenter was trained to blow through the syringe to generate a jet of air with a velocity of 5.09 m/s as recorded by the Pitot tube. Therefore, the airflow of atmospheric air (density: 1.204 kg/m$^3$) produced a force of 0.55 mN.

On average, the hit rate was 26% and the false alarm rate was 15%. Across 4 participants, $D’$ was 0.53 and the criterion response ($c$) was -0.94. This pilot psychophysical test suggests that the level of airflow (speed and area) generated by the syringe with dry ice was below perceptual detection threshold for mechanical sensations.

### 2.3.3 Experiment 1: Threshold to Detect Non-tactile Cooling

The method of limits was used to measure cooling detection thresholds. At the start of each trial, a tone (frequency: 500 Hz) alerted the participant, and the shutter opened at the same time, exposing the participant’s skin to the dry ice, and leading to a progressive decrease of the measured temperature in the ROI. The participant was instructed to press a foot pedal only when they first felt a cold sensation on the stimulated skin region. When the pedal was pressed, the stimulator shutter closed, the tone terminated, and the final skin temperature was stored (Figure 2.3D). The reaction time was measured only in response to cooling stimuli. Therefore, the reaction time values include motor delays that are unrelated to the afferent cold signal, yet introduce a bias in estimates of cold detection threshold measured by this method. However, this bias is assumed to be constant across all trials. To allow skin temperature to return to baseline, 4 locations were randomly stimulated. The locations were arranged in a square grid with a spacing of 2 cm. The same location was restimulated only after 2 other locations had been visited, ensuring a minimum of 30 s for thermal recovery between stimulations at each site. A total of 40 estimates were obtained per participant. The intertrial interval was 6 s.
The data from each trial was also used to calculate the relative threshold, i.e., the smallest drop in temperature from baseline that the participant could detect (ΔT) (Hafner et al., 2015). Unlike contact thermal stimulators, our stimulator does not set the initial temperature of the skin before each stimulation like the lasers used in the study of heat sensation (Iannetti et al., 2004). We observed variability (mean: 33.8°C ± 0.9) in the baseline skin temperature across participants and grid locations. This variability is probably unrelated to the experimental procedure because we only restimulated one location after at least 30 s. Thus, ΔT is arguably a more ecologically valid threshold measurement of cooling sensitivity than an absolute measurement.

To measure ΔT, an average of the baseline skin temperature within the ROI was taken across 26 frames in the 300 ms before shutter opening. Then, the temperature of the ROI upon pedal press was subtracted from the previous averaged baseline value to obtain ΔT (Figure 2.3A).

2.3.4 Scenario 2: Feedback Temperature-Controlled Cooling

This scenario allowed delivery of temperature-controlled non-tactile cooling stimulation for psychophysical paradigms which require long periods of constant low temperature. Raising the cooling source resulted in less cooling, while lowering it towards the skin produced more cooling. Therefore, the distance between dry ice and skin was continuously adjusted to achieve the desired constant temperature reading from the thermal camera (Figure 2.3B).

A PID algorithm closed the feedback loop between thermal image and the cooling source height above the skin. First, a desired temperature is set. Then, the thermal camera detects the temperature of a skin ROI, and a simple PID feedback controller sends position commands to the motorised linear system, adjusting the height of the container until the desired temperature is reached. This allows precise temporal and spatial control of skin temperature for psychophysical experiments. For instance, in combination with a non-tactile warm stimulator, a temperature-controlled radiant Thermal Grill Illusion (TGI) could be elicited for the first time.

In this set-up, the dry ice was held in a cardboard container. The cardboard container had dimensions 10.2x10.2x21.8 cm with a total volume of 1600 ml. It was filled with 300 g of dry ice. The base was perforated with a 6-mm diameter copper tube. For these studies, the ROI had a projected elliptical shape on the skin of 5x4 mm. The
interior of the container was covered with foil and its exterior with polystyrene foam in order to limit thermal loss and convection to the copper tube.

2.3.5 Scenario 3: Rapid, Wide-area, High-Intensity Cooling

This scenario allowed delivery of fast non-tactile cooling of a large skin area, designed to produce a strong afferent volley and an evoked brain response. Event-related EEG potentials require such strong stimuli with rapid onsets. Steep cooling ramps cause synchronous activation of many cooling-sensitive afferents, and therefore improve the signal-to-noise ratio of event-related EEG potentials, thus paralleling what has been demonstrated for steep radiant heating ramps (Iannetti et al., 2004).

In this set-up, the dry ice container for scenario 2 was used, but the base was perforated with three outlet tubes to increase the stimulation area. Therefore, the exposed skin area was larger, and the skin ROI was a 10x8 mm ellipse. Stimulation led to a rapid temperature decrease at 13 ± 3°C in the first 200 ms of cooling (Figure 2.3C). Previous studies have shown that a cooling ramp of 10-17°C/s delivered to an 1444 mm² skin area is sufficient to detect a reproducible evoked potential (Duclaux et al., 1974). Thus, the stimulation method permits rapidly cooling a large patch of skin without touch, and potentially measuring the EEG responses elicited by cooling stimuli without mechanical input.

2.4 Results

The stimulator successfully delivered cooling stimulation without touch. We determined absolute and relative temperature threshold of cooling detection (Figure 2.3A & E). We also achieved repeatable, sustained, temperature-controlled cooling stimulation suitable for psychophysical studies of cold perception with stimuli varying in intensity, location, and duration (Figure 2.3B). Finally, we demonstrated a rapid decrease in skin temperature without touch, which is suitable for electrophysiological recordings (Figure 2.3C).
Figure 2.3. Experiment 1: scenarios and cooling detection thresholds in. A) Example of focal cooling for determining thresholds by method of limits (scenario 1). The trace shows mean skin temperature in the skin ROI. The threshold level at which the participant first reported detecting focal cooling is expressed relative to baseline (ΔT). The grey zone indicates the duration of cooling (shutter is open). B) Example traces (blue lines, n = 4 repetitions) of feedback-controlled stimulation (scenario 2). The horizontal, dashed red line indicates the set-point for the PID controller. The height of the stimulator above the skin is adjusted by PID control to achieve the desired temperature (one illustrative trace is shown by the black line, referring to right-hand ordinate scale). C) Examples of rapid, large-area cooling ramps (thin blue lines, n = 5 normalised repetitions; scenario 3). The thick blue line shows the mean. D) The panel to the left is a schematic displaying the temporal sequence of events in a Method of Limits trial. The panel to the right shows a thermal image during dry ice stimulation. The orange circle represents the ROI. E) Cooling detection thresholds relative to baseline skin temperature (ΔT) of 13 participants. Each datapoint represents the mean of 40 threshold estimates based on the method of limits (figure B). The error bars are the standard deviation for each participant. The horizontal yellow line represents the mean.

2.4.1 Experiment 1: Cooling Detection Thresholds

Baseline temperature before cooling stimulation was 33.8 °C ± 0.9) across 13 participants. The absolute threshold for reporting cooling stimulation was 32.7°C ± 0.9) across participants. Thus, the relative threshold for cooling detection (ΔT) was -1.1°C ± 0.4) across participants (Figure 2.3E).
2.5 Discussion

In this chapter, we report a novel method to generate controlled and focal cooling stimulations without the confounds of mechanical input. We show how the system can be used to perform psychophysical experiments of cold sensation. We have focussed on the methodological issues around non-contact cooling stimulation. Importantly, this approach overcomes some of the limitations of previous non-contact cooling approaches. Firstly, non-contact methods using ultrasound and air blow do involve mechanical stimulation (Bujas, 1937; Nakajima et al., 2021; Murphy et al., 2001). Secondly, previous studies using chemicals and dry ice to selectively elicit cold sensations had poor spatio-temporal controllability (Cataldo et al. 2016; Ferrè et al., 2018; Hardy & Oppel, 1938; Typolt & Filingeri, 2020).

Furthermore, we show that this method can be used for perceptual psychophysics. The most widespread cold perception test is the calculation of a cooling detection threshold using a method of limits. This is the basis of the detection threshold test performed in Quantitative Sensory Testing (QST) studies that are frequently used in clinical studies. The data from the device (Figure 2.3E) showed cooling detection thresholds close to the published normative values for QST using methods of limits (Rolke et al., 2006: 30.9°C from 32°C baseline; Hafner et al., 2015: -1.0°C degrees). However, all these studies used contact thermodes, thus introducing a tactile element. Here, we have shown that our method could reliably estimate focal cooling detection thresholds without mechanical stimulation, which are comparable to existing normative values. This validates the use of this method for perceptual psychophysical experiments. Crucially, the duration and size of the cooling stimuli is unlikely to trigger homeostatic responses. Thus, the method is suitable for studying the perceptual aspect of cold sensation, but not its regulatory aspect. However, we did not perform equivalent studies with contact thermodes, matched for properties such as stimulus size. Comparing cooling detection thresholds and other aspects of cold perception between our stimulator and other, conventional stimulators, could more fully characterise how touch modulates cold perception. Future studies could potentially measure psychophysical detection curves for cold perception with standard contact thermodes and our new non-tactile cooling stimulator.

In this method, two modes of heat transfer contribute to skin cooling: convection and radiation. Convection cooling takes place as sublimated CO₂ and cooled air flows down from the container to the skin because they are more dense than ambient air. Radiative cooling also transfers thermal energy from warmer objects (the skin) to
cooler ones (the stimulator). The design cannot distinguish the respective contributions of convection and radiation to skin cooling. The very focal cooling achieved in scenario 1 suggests that convection dominates. One might object that cooling air currents flowing downwards to the skin constitute a mechanical stimulus, and that this method is not therefore completely non-tactile. We addressed this limitation by measuring the convection airflow in the exposure Scenario 1 with a Pitot tube, and calculating the resulting mechanical forces at the skin. Calculations confirmed that the resulting forces on the skin were below published mechanoreceptor threshold values. Finally, in informal pilot testing, we gently blew air through the syringe at this velocity, and found that this level of airflow was not perceptually detectable. Therefore, the cooling stimulator could be considered having both high sensitivity (effectively stimulating cooling-sensitive afferents) and high specificity (not stimulating non-thermal afferents, notably mechanoreceptor afferents). However, further psychophysical and electrophysiological studies should investigate the sensitivity and specificity of this method.

The measurements of cooling detection thresholds have some limitations. First, the method of limits does not distinguish between two key components of sensory detection: sensitivity and bias. Further, we did not include ‘catch’ trials, in which the auditory tone would occur without any cooling stimulation. The absence of catch trials could potentially induce a response bias, with participants responding based on the expectation that a cold sensation would occur. Thus, the measures of cooling detection thresholds should not be taken as perfect estimates of sensitivity. However, this does not detract from the scientific value of the stimulator apparatus.

The classic method to study cold-touch interactions would involve comparing the responses to thermal stimuli both with and without concomitant mechanoreceptor stimulation. However, the mechanisms of thermotactile interactions are poorly understood because most methods for delivering cooling stimulation involve mechanical stimulation. In future studies, the stimulator will be used to address the scientific question of how mechanical stimulation interacts with cooling stimulation. For example, cooling detection thresholds could be measured in the presence or absence of concomitant touch. If an interaction between touch and thermal sensitivity is established, EEG studies could investigate the neurophysiological mechanisms underlying this interaction. The rapid, large-scale cooling stimulator could potentially allow future electrophysiological recordings of cooling-evoked potentials, and of how they might be modulated by tactile input. Furthermore, future studies could combine scenarios to deliver more sophisticated thermal profiles such as PID-controlled ramps.
(scenarios 2 & 3). This combined scenario could be used to study cold perception for different temperature gradients without tactile input. Altogether, the non-tactile thermal cooling stimulator opens the possibility of investigating cold-touch interactions.
3 Mechanical Input Reduces the Sensitivity to Non-Tactile Focal Cooling
3.1 Summary

In the previous chapter, we described a new method to decrease the temperature of the skin without touch. We used this method to estimate detection thresholds during non-tactile cooling. In this chapter, we use this to study the sensitivity to non-tactile focal cooling with and without touch in a signal detection paradigm. We found sensitivity to non-tactile focal cooling was reduced by concomitant mechanical stimulation (reduction in sensitivity in ‘Cold & touch’ condition compared to ‘Cold’ condition = 0.72 ± 0.52; one-tailed paired-sample t-test; \( t_{11} = 4.51; p = 0.00004; d = 1.05 \)). Our mechanical stimuli were two von Frey filaments, which touched the skin 1 cm on either side of the cooling point. This finding was replicated in three different experiments (n = 12 x 3). In a control experiment, we showed that the reduction of sensitivity to cooling inputs is specific to touch (reduction in sensitivity = 0.25 ± 0.39; one-tailed paired-sample t-test; \( t_{11} = 2.09; p = 0.03; d = 0.36 \)), and did not occur for a non-tactile sensory input, sound (difference in sensitivity = 0.13 ± 0.62; one-tailed paired-sample t-test; \( t_{11} = 0.70; p = 0.25; d = 0.18 \)). This rules out explanations based on general factors such as distraction. Our finding suggests that touch inhibits cold perception, which recalls the reduction of pain by touch described by the Gate Control Theory.
3.2 Introduction

Somatosensory channels interact with each other. For instance, touch reduces pain, but pain relieves itch (Bautista et al., 2014; Feng et al., 2018; Mancini et al., 2014, 2015; Melzack & Wall, 1965; Yosipovitch et al., 2007). Researchers have studied these interactions by selectively stimulating one sensory channel and comparing the responses when another somatosensory input is or is not present. In combination with other methods, it has been shown that inhibitory interneurons in the spinal cord underlie these interactions (Abraira & Ginty, 2013; Abraira et al., 2017; Mancini et al., 2014, 2015; Melzack & Wall, 1965). Therefore, methods to selectively stimulate one sensory channel allow us to study multisensory perception. This research strategy has remained elusive for cold sensation due to the lack of controllable non-tactile stimulators (Ezquerra-Romano et al., 2022).

The neurophysiology of cold sensation shares similarities with that of pain. Specifically, pain is mediated both by A\(\delta\)- and C-fibres (Campero et al., 1996; Mancini et al., 2014; Mouraux et al., 2003, 2010). Cold sensations are also mediated by these types of fibres (Campero et al., 2001; Paricio-Montesinos et al., 2020; Yarnitsky and Ochoa, 1991). Additionally, recent studies have found robust and overlapping responses to both mechanical and cooling inputs in the mouse primary somatosensory cortex (SI) (Milenkovic et al., 2014; Vestergaard et al., 2022). Moreover, the direction of a thermal stimulus applied to the hand could be decoded with machine learning from the BOLD activity of SI in humans (Jung & Walther, 2021). Although their focus of their study was not thermotactile perception, their data suggests there is thermal information in the human SI. Altogether, it is a possibility that tactile input modulates cooling responses in a similar way to how it inhibits nociceptive input.

In fact, Green and colleagues have found evidence that touch attenuates cold sensations in humans (Green & Schoen, 2005; Green, 2009). Specifically, they found that the intensity of a cold sensation is higher when touching a cooled object as opposed to touching a thermally-neutral object that then decreases of temperature. Therefore, dynamic touch attenuates the perceived intensity of cooling as opposed to static touch. A limitation of these studies is that in both stimulations there was some degree of mechanical input. In other words, skin cooling was not isolated from tactile input, so the cold sensations were not selectively elicited in either of the conditions. Thus, somatosensory research would benefit from studies on cold sensation that
selectively stimulate the thermosensory system to understand how touch modulates cold sensations.

We have therefore studied detection to non-tactile cooling with and without tactile stimulation with our novel non-tactile cooling stimulator (Ezquerra-Romano et al., 2022). Specifically, we measured sensitivity (d’) and response bias (C) to non-tactile cooling in humans with a signal detection paradigm. Our results show that touch decreases sensitivity to focal, non-tactile cooling. We replicated our finding three times and show with a control condition that this effect is modality-specific.

3.3 Methods

3.3.1 Participants and Ethics

A total of 12 human participants took part in Experiment 2 (mean age: 25.92 ± 5.57 SD; 9 females). A total of 12 human participants took part in Experiment 3 (mean age: 28.33 ± 6.74 SD; 9 females). A total of 12 human participants took part in Experiment 4 (mean age: 25.5 ± 5.88 SD; 11 females). We aimed to obtain a power of 0.9. Therefore, we determined the sample size with a power analysis using a one-tailed t-test, an effect size of 0.857 and a significance level of 0.050. In this case, the effect size is the magnitude of the difference between the mean sensitivity to cooling with and without touch across participants. These values were obtained from a previous study investigating the reduction in pain sensitivity by touch with an experimental strategy similar to the one followed here (Mancini et al., 2015). The same sample size was then used for all human psychophysical experiments presented in Chapters 4, and 5. To minimise contamination of our results by floor and ceiling effects, we established a priori criteria. After testing, we excluded participants who had an average proportion of correct responses greater than 0.95 or less than 0.50 in any of the two conditions in the signal detection procedure. The average proportion of correct responses was the mean between the ratio of correct responses in both the stimulus present (hits) and absent conditions (correct rejections) (Figure 3.1). None of the participants were excluded in the analysis of Experiments 2, 3 and 4.

The individuals included in these experiments did not have any underlying skin conditions or conditions that would affect skin sensations. Furthermore, there was no current evidence or history of neurological or psychiatric disease present in any of the participants, nor were they taking psychoactive medication. They had not previously participated in experiments using this method, and prior to participation, written informed consent was obtained from all participants.
We implemented measures to manage potential risks around handling dry ice, having mobile parts in the setup and warming up the hand with an infrared lamp. The UCL Research Ethics Committee approved this research (ID number: ICN-PH-PWB-0847/010).

### 3.3.2 Experimental Set-up

The experimental set-up for Experiments 2, 3 and 4 was similar to the one used in scenario 1 (Chapter 2) with a few modifications (Figure 2.1A) (Ezquerra-Romano et al., 2022).

Both cooling and tactile stimuli were delivered to the back of the left hand. A tactile stimulus was delivered by two von Frey monofilaments (bending force: 1 gram-force (gf, force equivalent to one gram acting under the acceleration of gravity at the Earth's surface), diameter: 0.4 mm, length: 15 mm), which were aligned in the mediolateral axis and were 1 cm apart either side the cooling point in the proximodistal axis (Figure 3.1A & B). The position of the monofilaments was controlled by a third set of 3 motorised linear systems (2 stepper motor controllers LSM100B-T4 and 1 stepper motor controller LSM200B-T4, Zaber Technologies Inc.), which interfaced with the computer through controllers (2 stepper motor controllers X-MCB2 and 1 stepper motor controller X-MCB1, Zaber Technologies Inc.) (Figure 3.1). To prevent participants from associating visual cues with a stimulus type, a black curtain was placed in front of the participant to block the view of the hand.

To finely control the orientation of the thermal camera, a pan/tilt bracket (ROB-14391, SparkFun Electronics) was integrated onto the set of motorised linear systems controlling the position of the thermal camera. The pan/tilt bracket rotated in three planes thanks to three servo motors (SG90 Micro Servo Motor KY66-5, Longruner), which were controlled by an Arduino Mega 2560 (Figure 3.1).

To maintain the temperature of the skin stable within participants and minimise temperature baseline differences across participants, an infrared light lamp (Infrasec IR2 250W bulb, Tungsram) was used to warm up participants' hands during breaks. The light was controlled by a dimmer, which was driven by an Arduino Mega 2560 independent to the Arduino boards controlling the servo motors and the pan/tilt brackets. Additionally, windows and doors were closed to minimise airflows and thermal fluctuations in the room. As described in Experiment 1, the stimulation locations in the skin were arranged in a square grid with a spacing of 1 cm. A total of 9 locations were randomly stimulated to allow skin temperature to return to baseline.
(Figure 3.1B). The same location was restimulated with cooling only after at least 3 other locations had been visited, ensuring a minimum of 30 s for thermal recovery between cooling at each site. The distance between the nozzle and the skin was chosen based on the target temperature and the linear regression model obtained in Chapter 2 (Figure 2.2B).

In Experiments 2 and 3, a speech recognition algorithm was used to transform the participants’ responses (either ‘Yes’ or ‘No’) from voice to text (IBM Watson, IBM). This was implemented to minimise the surfaces that participants had to touch given the existing social restrictions and hygienic requirements at the time.

In Experiment 4, participants responded either ‘Yes’ or ‘No’ by pressing labelled keys on a keypad (Pauk10, Targus International LLC). In the trials of this experiment, a red LED (red LEDs, VCC) was used to indicate the duration of the cooling stimulation. The LED was placed in front of the black curtain between the participant’s head and the stimulated hand. The light was controlled by an Arduino Uno. In this section, one of the stimuli was a tone with a frequency of 500 Hz and a loudness of 50 db at the position of the participant. The aim of this experiment was to show whether the reduction in sensitivity is specific to touch or due to general factors such as distraction. The parameters of this tone were chosen based on two criteria. First, the tone was distinguishable to the tone used to indicate the duration of the cooling stimulation in Experiments 2 and 3. Second, the tone’s intensity matched the subjective intensity of the tactile stimulation in Experiments 2 and 3. Briefly, the detection threshold at the back of the hand is around 0.2 gf when the tactile stimuli are von Frey filaments (Bowden & McNulty, 2013). The von Frey filaments used in the ‘Cold & touch’ condition were 1 gf- five times stronger than the detection threshold. The hearing threshold for young people at 500 Hz is around 10 db. Then, the intensity of the tone was set at 50 db- five times more than the hearing detection threshold. The tone was played with two speakers (Sonic Mobil 185, Hama (UK) Ltd.). To mirror the position of the tactile stimulation in the ‘Cold & touch’ condition (Figure 3.1B), the speakers were bracketing the stimulated hand in the proximodistal axis.
Figure 3.1. Experiment 2: experimental set-up, trial structure and design. A) An illustration of the set-up with the main components including the mechanical stimulator. B) Illustration of the experimental conditions in the signal detection paradigm. In both conditions, there were trials with cooling and without cooling as shown in the following table. C) Table showing how hits, misses, false alarms, and correct rejections are defined for each trial type and condition based on response data. D) Schematic displaying the temporal sequence of events in a signal detection paradigm trial. People responded either ‘Yes’ or ‘No’ to the question: ‘Was there any temperature change during the tone?’.

3.3.3 Experimental Design and Task

Experiments 2, 3 and 4 had two psychophysical procedures: a staircase and a signal detection paradigm. The staircase procedure was used to obtain an estimation of the relative temperature decrease without touch which participants could detect at a fixed probability. This value was then used at the signal detection paradigm to obtain the sensitivities (d') and response biases (c) with and without touch.

In all experiments, the staircase procedure followed a 3-down/1-up rule. This rule was only applied after the first negative response (‘No’). The step sizes were fixed at +0.1°C for the down step and -0.14°C for the up step. The boundaries of the staircase were established at -0.2°C and -2°C. The performance of the stimulator was most optimal within this range. The staircase algorithm followed the carry-on rule when the staircase value surpassed the established boundaries. When boundaries were crossed, the value tracked by the staircase algorithm and the value used for the
stimulator differed: the value tracked by the staircase algorithm followed the 3-down/1-up rule with the fixed step sizes based on the participant's responses, whereas the value used for the stimulator was clamped at either -0.2°C or -2°C. These values coincided again once the value tracked by the staircase algorithm crossed back into the established range. The design of this staircase yielded a temperature change which participants could detect with a probability of approximately 0.80. This value is called percent-correct point herein (García-Pérez, 1998; Prins & Kingdom, 2016).

The structure of a trial in the staircase procedure was identical in the three experiments. At the start of each trial, the thermal camera started recording to obtain baseline measurements of skin temperature. After 1 s, a tone alerted the participant, and the shutter opened at the same time, exposing the participant's skin to the dry ice. When the temperature of the skin in the ROI reached the value provided by the staircase algorithm, the stimulator shutter closed, and the tone terminated. If the temperature was reached, a short tone (duration: 0.2 s; frequency: 100 Hz) indicated participants to respond. At the beginning of the experiment, they had been instructed to focus on the thermal stimulus and respond after the short tone to the question: ‘Was there a temperature change during the tone?’ In Experiments 2 and 3, people responded by either saying out aloud ‘Yes’ or ‘No’, whereas in Experiment 4, they responded by pressing the corresponding key on a keypad (Figure 3.2B). If the temperature was not reached after a timeout period of 10 s, the trial was considered failed and immediately repeated in another position of the stimulation grid. To refill the stimulator with dry ice and maintain participants' engagement, there were 2-min breaks every 6-8 min.

In all experiments, there were 2 parallel, interleaved staircases: one started ascending from -0.2°C and the other started descending from -1.2°C. The ascending staircase started at a low temperature change and participants usually responded ‘No’ in the initial trials, so the temperature change increased (i.e. stronger cooling). The descending staircase started at a high temperature change and participants usually responses ‘Yes’ in the initial trials, so the temperature change decreased at the beginning of the staircase procedure (i.e. weaker cooling). The starting value of the descending staircase was established based on the results from Experiment 1 in Chapter 2. Both staircases were stopped after 12 reversals (Figure 3.2B).
In Experiments 2 and 3, there was one signal detection paradigm with two conditions: ‘Cold’ and ‘Cold & touch’ (Figures 3.1B & C). In Experiment 4, there was one signal detection with three conditions: ‘Cold’, ‘Cold & touch’ and ‘Cold & sound’.

In the three experiments, each condition of the signal detection procedure had stimulus present (‘Cooling’) and stimulus absent (‘No cooling’) trials, following the classic design in Signal Detection Theory (Green & Swets, 1966) (Figure 3.1C). In both experiments, there were 54 trials per condition, which were always randomly interleaved. In Experiments 2 and 3, there were a total of 108 trials. In Experiment 4, there were a total of 162 trials.

The structure of a trial in the signal detection procedure was identical in all experiments (Figure 3.1D). At the start of each trial, the thermal camera started recording to obtain baseline measurements of skin temperature. After 0.5 s, if it was a trial from the ‘Cold & touch’ condition, the motorised linear system moved the von Frey filaments to a predefined position in which the filaments touched the skin while bracketing the cooling stimulation point (Figure 3.1B). In stimulus present trials (Figure 3.1C), the shutter opened after 2.5 s of the onset of the thermal camera recording and at the same time a tone started to alert the participant during cooling. In Experiment 4, an LED light turned on to alert participants during cooling instead of a tone. When the temperature of the skin in the ROI reached the percent-correct point estimated by the staircase algorithm, the stimulator shutter closed, and the tone terminated or the LED light turned off. The duration of the cooling stimulation was saved to establish the duration of stimulus absent trials. If the temperature was reached, a short tone indicated participants to respond. At the beginning of the experiment, they had been instructed to focus solely on the cold sensations and to respond after the short tone to the question ‘Was there a temperature change during the tone?’ by either saying out aloud ‘Yes’ or ‘No’ in Experiments 2 and 3 or by pressing the corresponding key in Experiment 4 (Figure 3.2B). If the temperature was not reached after a timeout period of 10 s, the trial was considered failed and repeated later at a random time and position. In stimulus absent trials (Figure 3.1C), the shutter moved after 2.5 s of the onset of the thermal camera recording, but it did not expose the syringe to the skin. In these trials, the tone also started at the same time the shutter moved position. The duration of the tone was established based on a random selection from a list containing the duration of the stimulus present trials. If the first trials were stimulus absent ones, the duration was set based on a random selection of a number between 1 and 6. At the end of the stimulus absent trial, the shutter moved back to the close position and the tone terminated. The intertrial
interval was 8 s. To refill the stimulator with dry ice and maintain the participant’s engagement, there were 2-min breaks every 6-8 min.

3.3.4 Data Analysis and Statistics

In the three experiments, the staircase procedure was used to calculate a relative temperature decrease which each participant could detect with a probability of approximately 0.80 (percent-correct point). The percent-correct point was calculated by performing the mean of the relative temperatures at each reversal. A reversal was a trial in which the response of the participant was different to the response in the previous trial (Figures 3.2B & 3.4B). The first 3 reversals were discarded. The final percent-correct point was calculated by first calculating the percent-correct point for the ascending and descending staircases separately and then computing the average of these two values (Figure 3.2B).

In the three experiments, the signal detection paradigm was used to calculate the percent correct responses, the hit and false alarm rates, the sensitivity (d’) and the response bias (c). The percent correct responses were calculated for each trial type (stimulus present and absent) for each condition as the percentage of trials which the participant answered correctly. The hit rate was obtained by adding the number of trials the participant answered correctly and dividing by the total number of trials. The false alarm rate was obtained by adding the number of trials the participant answered incorrectly and dividing by the total number of trials.

Sensitivity and bias were calculated with the hit and false alarm rates. Sensitivity (d’) is a measure of the ability to detect the presence or absence of a specific stimulus. Response bias (C) is thought to measure a systematic tendency to favour one response over another, which is independent to the presence of the stimulus. (Green & Swets, 1966; Macmillan & Creelman, 2004). Sensitivity (d’) was calculated using the following formula:

\[ d' = Z(\text{hitrate}) - Z(\text{falsealarmrate}) \] (Formula 4),

where \( Z(p) \), \( p \in [0,1] \), is the inverse of the cumulative distribution function of a Gaussian distribution.

The response criterion was calculated using the following formula:

\[ C = \frac{Z(\text{hitrate}) + Z(\text{falsealarmrate})}{2} \] (Formula 5).
When the hit or false alarm rate are equal to 0 or 1, the z scores (Formulas 4 & 5) are equal to infinity. To calculate \( d' \) and \( c \) even when the rates were equal to 0 or 1, we followed the loglinear approach (Hautus, 1995; Stanislaw & Todorov, 1999). This approach involves adding 0.5 to both the number of hits and false alarms and adding 1 to both the total number of stimulus present and absent trials.

The sensitivities obtained in Experiments 2, 3 and 4 were submitted to a one-tailed paired t-test as we hypothesised that the sensitivity in the ‘Cold’ condition would be greater than the sensitivity in the ‘Cold & touch’ condition within participants. This hypothesis was based on the Gate Control Theory (Mancini et al., 2014, 2015; Melzack & Wall, 1965). The biases were submitted to a two-tailed paired t-test because we did not expect a difference in any direction between the biases in the two conditions within participants. The sensitivities and biases obtained in Experiment 4 were submitted to a repeated ANOVA and one-tailed t-tests were used for pairwise comparisons.

### 3.4 Results

#### 3.4.1 Experiment 2: Detection of Focal Cooling with and without Touch

![Figure 3.2. Experiment 2: staircase procedure. A) Schematic of the temporal sequence of events in a trial during the staircase procedure. People responded either ‘Yes’ or ‘No’ to the question: ‘Was there any temperature change during the tone?’ B) An example percent-correct point estimation with a staircase procedure from one participant. The red line follows the value tracked by the staircase algorithm for the descending branch, whereas the green line follows the value tracked for the ascending branch. The black line follows the relative temperature decrease that participants were exposed to at each trial and it is overlaid with the green and red lines for most of the procedure. The black dots indicate the trials in which the participant said ‘Yes’. The light red dots indicate the first three trials in which the participant said ‘No’. The red dots indicate the trials in which the participant said ‘No’. The temperature at these trials was used to calculate the final percent-correct value. The red horizontal dashed lines are the percent-correct points for the descending and ascending staircases and the black one is the mean of these values. C) Each datapoint represents the percent-correct point of one participant (blue dots) based on a staircase procedure (figure b). The horizontal blue line is the mean.](image-url)
Percent-correct point estimation

The relative temperature decrease that participants could detect 80% of the time was 
-0.80°C ± 0.25 (Figures 3.2B & C).

Sensitivity (d’)

Sensitivity was significantly reduced when non-tactile cooling was accompanied by 
concurrent tactile stimuli (‘Cold & touch’) as compared to the unimodal, cooling 
condition (‘Cold’) (Cold d’: 1.97 ± 0.66; Cold & touch d’: 1.25 ± 0.69; difference in 
sensitivity: 0.72 ± 0.52; one-tailed paired-sample t-test; t_{11} = 4.51; p = 0.00004; d = 
1.05) (Figures 3.3A & C).

Figure 3.3. Experiment 2: signal detection paradigm of ‘Cold’ and ‘Cold & touch’ 
conditions. A) The sensitivity (d’) at each stimulation condition. Each datapoint (coloured dot) 
is the sensitivity of each participant during the signal detection paradigm. The light grey lines 
join the datapoints belonging to the same participant. The horizontal, coloured lines represent 
the mean of the sensitivities. B) The response bias (C) at each stimulation condition. Each 
datapoint (coloured dot) is the response bias of each participant during the signal detection 
paradigm. The light grey lines join the datapoints belonging to the same participant. The 
horizontal, coloured lines represent the mean of the response biases. The dashed, horizontal, 
grey line follows y = 0. A negative value indicates a tendency to say ‘No’, whereas a positive 
value indicates a tendency to say ‘Yes’. C) The difference between the sensitivity of the ‘Cold’ 
stimulation condition and the sensitivity of the ‘Cold & touch’ condition. Each datapoint (black 
dot) is the difference between the sensitivities for each participant. The light grey lines join the 
datapoints belonging to the same participant. The horizontal, black lines are the means of the
differences across participants. D) The difference between the response bias of the ‘Cold’ stimulation condition and the response bias of the ‘Cold & touch’ condition. Each datapoint (black dot) is the difference between the response biases for each participant. The light grey lines join the datapoints belonging to the same participant.

Response bias (C)

Participants had a tendency to say ‘No’ in both conditions as shown by the negative response bias (Cold C: -0.42 ± 0.45; Cold & touch: -0.46 ± 0.47). There was no significant difference between the two conditions (difference in response bias: 0.04 ± 0.34; two-tailed paired-sample t-test, t_{11} = 0.43; p = 0.67; d = 0.10) (Figure 3.3B & D).

3.4.2 Experiment 3: Replication

Figure 3.4. Experiment 3: staircase procedure. A) An example percent-correct point estimation with a staircase procedure from one participant. The red line follows the value tracked by the staircase algorithm for the descending branch, whereas the green line follows the value tracked for the ascending branch. The black line follows the relative temperature decrease that participants were exposed to at each trial and it is overlaid with the green and red lines for most of the procedure. The black dots indicate the trials in which the participant said ‘Yes’ to ‘Was there a temperature change during the tone?’’. The light red dots indicate the first three trials in which the participant said ‘No’. The red dots indicate the trials in which the participant said ‘No’. The temperature at these trials was used to calculate the final percent-correct value. The horizontal dashed red lines are the percent-correct points for the descending and ascending staircases and the black one is the mean of these values. B) Each datapoint represents the percent-correct point of one participant (blue dots) based on a staircase procedure (figure b). The horizontal blue line is the mean.

Percent-correct point estimation

The relative temperature decrease that participants could detect 80% of the time was -1.12°C ± 0.54 (Figures 3.4A & B).
Figure 3.5. Experiment 3: signal detection paradigm of ‘Cold’ and ‘Cold & touch’ conditions. A) The sensitivity (d’) at each stimulation condition. Each datapoint (coloured dot) is the sensitivity of each participant during the signal detection paradigm. The light grey lines join the datapoints belonging to the same participant. The horizontal, coloured lines represent the mean of the sensitivities. B) The response bias (C) at each stimulation condition. Each datapoint (coloured dot) is the response bias of each participant during the signal detection paradigm. The light grey lines join the datapoints belonging to the same participant. The horizontal, coloured lines represent the mean of the response biases. The dashed, horizontal, grey line follows y = 0. A negative value indicates a tendency to say ‘No’, whereas a positive value indicates a tendency to say ‘Yes’. C) The difference between the sensitivity of the ‘Cold’ stimulation condition and the sensitivity of the ‘Cold & touch’ condition. Each datapoint (black dot) is the difference between the sensitivities for each participant. The light grey lines join the datapoints belonging to the same participant. The horizontal, black lines are the means of the differences across participants. D) The difference between the response bias of the ‘Cold’ stimulation condition and the response bias of the ‘Cold & touch’ condition. Each datapoint (black dot) is the difference between the response biases for each participant. The light grey lines join the datapoints belonging to the same participant.

Sensitivity (d’)

Sensitivity was significantly reduced when non-tactile cooling was accompanied by concurrent tactile stimuli (‘Cold & touch’) as compared to the unimodal, cooling condition (‘Cold’) (Cold d’: 1.90 ± 0.64; Cold & touch d’: 1.63 ± 0.85; difference d’s: 0.27 ± 0.43; one-tailed paired-sample t-test; t_{11} = 2.09; p = 0.03; d = 0.36) (Figures 3.5A & C).
Response bias (C)

Participants had a small tendency to say ‘No’ in both conditions as shown by the negative response bias (Cold C: -0.05 ± 0.56; Cold & touch: -0.14 ± 0.59). There was no significant difference between the two conditions (difference in response bias: 0.09 ± 0.3; two-tailed paired-sample t-test; t₁₁ = 0.99; p = 0.34; d = 0.16) (Figures 3.5B & D).

3.4.3 Experiment 4: Control

![Figure 3.6. Experiment 4: staircase procedure. A) An example percent-correct point estimation with a staircase procedure from one participant. The red line follows the value tracked by the staircase algorithm for the descending branch, whereas the green line follows the value tracked for the ascending branch. The black line follows the relative temperature decrease that participants were exposed to at each trial and it is overlaid with the green and red lines for most of the procedure. The black dots indicate the trials in which the participant said ‘Yes’. The light red dots indicate the first three trials in which the participant said ‘No’. The red dots indicate the trials in which the participant said ‘No’. The temperature at these trials was used to calculate the final percent-correct value. The horizontal dashed red lines are the percent-correct points for the descending and ascending staircases and the black one is the mean of these values. B) Each datapoint represents the percent-correct point of one participant (blue dots) based on a staircase procedure (figure b). The horizontal blue line is the mean.

Percent-correct point estimation

The relative temperature decrease that participants could detect 80% of the time was -1.27°C ± 0.37 (Figures 3.6B & C).
Figure 3.7. Experiment 4: signal detection paradigm of ‘Cold’, ‘Cold & touch’ and ‘Cold & sound’ conditions. A) The sensitivity (d') at each stimulation condition. Each datapoint (coloured dot) is the sensitivity of each participant during the signal detection paradigm. The light grey lines join the datapoints belonging to the same participant. The horizontal, coloured lines represent the mean of the sensitivities. B) The response bias (C) at each stimulation condition. Each datapoint (coloured dot) is the response bias of each participant during the signal detection paradigm. The light grey lines join the datapoints belonging to the same participant. The horizontal, coloured lines represent the mean of the response biases. The dashed, horizontal, grey line follows y = 0. A negative value indicates a tendency to say ‘No’, whereas a positive value indicates a tendency to say ‘Yes’. C) The difference between the sensitivity of the ‘Cold’ stimulation condition and the sensitivity of the ‘Cold & touch’ and ‘Cold & sound’ conditions. Each datapoint (black dot) is the difference between the sensitivities for each participant. The light grey lines join the datapoints belonging to the same participant. The horizontal, black lines are the means of the differences across participants. D) The difference between the response bias of the ‘Cold’ stimulation condition and the response bias of the ‘Cold & touch’ and the ‘Cold & sound’ conditions. Each datapoint (black dot) is the difference between the response biases for each participant. The light grey lines join the datapoints belonging to the same participant.

Sensitivity (d')

Sensitivity was calculated for the three conditions (Cold d': 1.88 ± 0.61; Cold & touch d': 1.64 ± 0.74; Cold & sound d': 1.75 ± 0.80) (Figure 3.7A). Overall, sensitivity was not significantly different across the three conditions (one-way ANOVA; F2, 22 = 1.23; p = 0.31; η² = 0.02). However, sensitivity was significantly reduced when non-tactile cooling was accompanied by concurrent tactile stimuli (‘Cold & touch’) as compared to the unimodal cooling condition (‘Cold’) (difference d's: 0.25 ± 0.39; one-tailed paired-sample t-test; t11 = 2.09; p = 0.03; d = 0.36). Sensitivity was not significantly reduced when non-tactile cooling was accompanied by a sound (‘Cold & sound’).
compared to the unimodal cooling condition (‘Cold’) (difference d’s: 0.13 ± 0.62; one-tailed paired-sample t-test; \( t_{11} = 0.70; p = 0.25; d = 0.18 \) (Figure 3.7C).

Response bias (C)

Participants had a tendency to say ‘No’ in the three conditions as shown by the negative response bias (Cold C: -0.42 ± 0.48; Cold & touch C: -0.26 ± 0.49; Cold & sound: -0.17 ± 0.60) (Figure 3.7B). Overall, response bias was not significantly different across the three conditions (one-way ANOVA; \( F_{2, 22} = 2.93; p = 0.07; \eta^2 = 0.03 \)). Response bias was not significantly different when non-tactile cooling was accompanied by concurrent tactile stimuli (‘Cold & touch’) as compared to the unimodal cooling condition (‘Cold’) (difference C’s: -0.16 ± 0.33; one-tailed paired-sample t-test; \( t_{11} = -1.57; p = 0.15; d = -0.32 \)). However, response bias was significantly different when non-tactile cooling was accompanied by a sound as compared to the unimodal cooling condition (‘Cold’) (difference C’s: -0.24 ± 0.24; one-tailed paired-sample t-test; \( t_{11} = -3.33; p = 0.007; d = -0.45 \) (Figure 3.7D). This means that people were less likely to say ‘No’ with a sound than without it.

3.5 Discussion

We investigated the effect of touch on the detection of focal, non-tactile cooling. We used a staircase procedure to find the stimulus intensity that each participant could detect 80% of the time. Then, we measured the sensitivity to focal, non-tactile cooling with and without touch. We found that sensitivity to non-tactile cooling was significantly reduced when there was touch. Crucially, this effect is modality-specific because detection of cooling was not decreased when there was a concomitant auditory stimulus. We have found a novel interaction between cooling and tactile inputs, which is analogous to the well-known Gate Control Theory.

Green and colleagues found that dynamic touch attenuates cold sensations compared to static touch (Green & Schoen, 2005; Green, 2009). Crucially, both static and dynamic touch involved contact with the thermal stimulator. In our case, we found that touch reduces detection of adjacent non-contact cooling. Our stimuli are therefore fundamentally different from theirs, but our observations are in line. Nevertheless, our results are more specific because we stimulated the cooling-sensitive pathway in isolation to the tactile one. Crucially, our mechanical stimulus was effectively static during cold perception, which preferentially activates slowly adapting type I (SAI) fibres. On the other hand, the dynamic touch in Green’s experiments presumably activates different mechanosensitive fibres (Johansson &
Together with previous experiments, our results suggest that the interaction between cooling and tactile inputs might be specific to a type of mechanical force. Future research should compare the sensitivity to non-tactile cooling across different tactile stimuli.

The Gate Control Theory states that non-painful tactile input can suppress the perception of pain through spinal cord mechanisms (Mancini et al., 2014, 2015; Melzack & Wall, 1965). This theory suggests that the activation of tactile Aβ fibres can inhibit the transmission of pain signals carried by Aδ- and C-fibres, thus reducing the perceived intensity of the painful stimulus. Cold sensations are also mediated both by Aδ- and C-fibres (Campero et al., 2001; Paricio-Montesinos et al., 2020; Yamitsky and Ochoa, 1991). It is thus possible that a similar gating mechanism explains the reduction in sensitivity to non-tactile cooling in the presence of touch. Specifically, SA1 Aβ fibres activated by static touch may activate inhibitory interneurons, which in turn decrease the activity of cooling-sensitive Aδ- and C-fibres. Although the airflow generated by our cooling stimulus is below published mechanoreceptor threshold values, it is a possibility that mechanosensitive fibres were activated by cooling (Cahusac & Noyce, 2007, Zheng et al., 2019). However, this activity was presumably present with and without touch because it is originated by cooling. Additionally, people were asked to report thermal sensations, making any tactile sensations irrelevant to the task. Microneurography is needed to confirm what type of fibres are activated upon non-tactile cooling with our stimulator.

The brain has limited resources for processing sensory information. Therefore, it could be that touch is simply a distraction for detecting cooling and the effect we observe is due to attentional shift rather than to a gating mechanism. In our study, we minimised attentional effects in four ways. First, the tactile stimulus was never relevant to the task. Second, in all trials there was either a tone or a light that alerted the participant there might be a temperature change, so temporal expectancy for the cold sensation was balanced across conditions and was independent of the presence of touch. Third, the tactile stimulus was designed to not to divert spatial attention. It was composed of two filaments, which were symmetrically bracketing the focal cooling point. Finally, the filaments always touched the skin 2 s before the onset of cooling and remained static. New events attract attention transiently ("exogenous attention") for around 200 ms (Posner, 1980), but sustained stimuli may not attract attention (e.g., we tend to ignore tactile input from our clothes). Therefore, it is
unlikely the onset of touch distracted participants from their task of detecting cooling inputs.

In our control experiment (Experiment 4), we had a condition with an auditory stimulus to control for attention effects. In this experiment, the cue that alerted participants there might be a cold sensation was a light rather than a tone like in the other two experiments. In the control experiment, we found no difference between the sensitivity to cooling with and without a sound. Interestingly, we found that the probability of saying “No” was higher for the non-tactile cooling condition than the condition with a sound. We did not find differences in the response bias between any other conditions in any of the experiments. At the end of the control experiment, some participants told us informally that they found difficult to stay engaged during the trials without a tone. Therefore, it seems the cue tone may have had attentional effects, but attentional effects were probably counterbalanced and independent to touch in the first two experiments because there was a tone in all trials. Nevertheless, we found a difference in the sensitivity to cooling between the touch present and absent conditions in all three experiments. Altogether, it is unlikely the inhibitory interaction we found is due to attentional mechanisms.

In conclusion, our study found a novel interaction in the thermotactile system. Specifically, touch reduces detection of focal, non-tactile cooling. The thermotactile system is part of the somatosensory system, which is composed of many modalities. The classic view in somatosensation is that stimuli are detected in the periphery and the signals are relayed forward to secondary cortical regions where information is integrated with other modalities and cognitive processes. However, each step of the pathway from the skin to perception should be considered as a point where interactions between modalities can occur and contribute to the final output: perception. Our finding contributes to our understanding of somatosensation, but further perceptual and neurophysiological studies are required to confirm the nature of the interaction and describe its neural mechanism.
4 Spatiotemporal Characterisation of the Interaction between Cold and Tactile Sensations
4.1 Summary

In the previous chapter, we found that mechanical stimulation reduced the sensitivity to non-tactile focal cooling in humans. This suggests that touch inhibits cold perception, which is like the reduction of pain by touch described by the Gate Control Theory. In this chapter, we further characterise the modulation of cold sensations by touch. In a series of 3 experiments in humans, we investigated the spatiotemporal characteristics of this interaction by manipulating the distance and time onsets between the cooling and tactile stimuli. First, we targeted both stimuli to either the same spinal segment, or to adjacent or non-adjacent ones by stimulating different dermatomes on the hand. We found that the reduction in sensitivity to cooling decreased as we increased the segmental distance between cooling and tactile stimuli. Second, we changed the distance between the stimuli within the same spinal segment by stimulating different points on the forearm with the same dermatome. We found that the reduction in sensitivity by touch increased as we increased the distance between the stimuli. Third, we varied the relative time onset from 0 s to 3 s between the thermal and tactile stimuli. We found no clear trend as we varied the time onset between the stimuli. Together with the previous chapter, we conclude that touch inhibits cooling detection and show initial evidence on the spatiotemporal properties of this interaction.
4.2 Introduction

Multimodal integration is most efficient when unimodal stimuli occur at approximately the same location and close in time. This is known as the principle of spatiotemporal congruency. This has been studied primarily in audiovisual perception, where perception is enhanced by simultaneous auditory and visual stimuli (Meredith & Stein, 1986; Meredith & Stein, 1983; Wallace et al., 1998). Behavioural and neurophysiological evidence suggests somatosensation follows this principle, but the integration is suppressive rather than facilitatory. Specifically, a study found that as the distance between laser and tactile stimulation increased, the relief of pain by touch decreased linearly (Mancini et al., 2014). In other words, the further away touch is from pain, the less it inhibits the nociceptive signal. We do not know whether the same principle applies other multimodal interactions in the somatosensory system.

The somatosensory system has known spatial organisations, which can influence how sensory channels interact in space. First, the innervation of the skin is divided in regions called dermatomes, which are areas of the skin innervated by a single spinal nerve (Keegan & Garrett, 1948). Many studies have shown that different somatosensory interactions are partly or completely decreased when the stimuli are not targeted within the same dermatome (Douglas et al., 1992; Fardo et al., 2018; Lee et al., 1996; Marks & Stevens, 1973). Second, the spatial representation of the body surface in the cortex is somatotopic. In other words, the organisation of the cortex reflects the topographical arrangement of both thermal and tactile inputs from different body parts (Berman et al., 1998; Penfield & Boldrey, 1937; Penfield & Rasmussen, 1950; Sanchez-Panchuelo et al., 2010; Vestergaard et al., 2022). Altogether, we expect thermotactile interactions to depend on the spatial location of the stimuli on the skin. Specifically, if the site of interaction is the spinal cord, we expect to find a strong decrease of the effect as we increase the dermatomal distance between the stimuli. Additionally, whether the interaction is mainly spinal or cortical we expect to see a decrease in the effect as we increase the distance between the stimuli within the same dermatome.

The neurophysiology of the thermotactile system dictates the speed at which signals travel, which can influence how thermal and tactile signals interact. First, the transfer of mechanical and heat energies in the skin has different temporal scales (Pawluk & Howe, 1999; Okabe et al., 2018; Veronda & Westmann, 1970). Second, the endings of mechanosensitive and thermosensitive fibres are found at different depths (Abraira & Ginty, 2013; Ezquerra-Romano & Ezquerra, 2017). Third, mechanosensitive (Aβ-
and Aδ-fibres) fibres have faster conduction velocities than thermosensitive fibres (Aδ- and C-fibres for cold) (Campero et al., 1996; Campero et al., 2001; Mancini et al., 2014; Mouraux et al., 2003, 2010; Paricio-Montesinos et al., 2020; Yarnitsky and Ochoa, 1991). Altogether, tactile and thermal signals are transmitted at different speeds within the nervous system. Nevertheless, our thermotactile perception feels stable and temporally congruent, so there must be perceptual mechanisms to compensate for the differences in the speed of the signals. Therefore, we expect thermotactile integration to depend on the relative time onset of the stimuli.

In a previous study, we found that touch reduces detection performance of focal cooling (Chapter 3), but we do not understand the spatiotemporal properties of this interaction. We have therefore characterised the spatiotemporal properties of this interaction with our novel non-tactile cooling stimulator (Ezquerra-Romano et al., 2022). Specifically, we investigated how the interaction between cooling and tactile inputs varies as a function of the position and temporal onset of the stimuli in three experiments. In the first experiment, the distance between the stimuli in the skin was kept constant, but stimuli were delivered to different dermatomes. In the second experiment, the distance between the stimuli in the skin was manipulated within the same dermatome. In the third experiment, detection of cooling inputs was probed while varying the time between the onset of cooling and tactile stimuli.

4.3 Methods

4.3.1 Participants and Ethics

A total of 16 human participants took part in Experiment 5 (mean age: 24.75 ± 5.57; 14 females) and 4 were excluded from the analysis. A total of 16 human participants took part in Experiment 6 (mean age: 23.19 ± 4.60; 11 females) and 4 were excluded from the analysis. A total of 20 human participants took part in Experiment 7 (mean age: 23.55 ± 4.85; 15 females) and 8 were excluded from the analysis. The exclusion criteria were the same as the ones described in Chapter 3 for Experiments 2, 3 and 4. The individuals included in these experiments did not have any underlying skin conditions or conditions that would affect skin sensations. Furthermore, there was no current evidence or history of neurological or psychiatric disease present in any of the participants, nor were they taking psychoactive medication. They had not previously participated in experiments using this method, and prior to participation, written informed consent was obtained from all participants.
We implemented measures to treat potential risks around handling dry ice, having mobile parts in the setup and warming up the hand with an infrared lamp. UCL Research Ethics Committee approved this research (ID number: ICN-PH-PWB-0847/010).

4.3.2 Experimental Set-up

The experimental set-up in the three experiments was similar to the one used in Experiments 2, 3 and 4 (Chapter 3) with only a few modifications.

In Experiments 5 and 6, the tactile stimulation was only one von Frey filament, whereas it was two von Frey filaments in Experiment 7. In Experiment 6, the tactile and cooling stimuli were delivered to the volar forearm. Thus, the infrared lamp was used to warm up participant’s volar forearm during each break. In this experiment, the three red lasers used to monitor the position of the participant were pointing at the volar forearm and their skin was marked with ink at those locations. Similar to Experiments 2, 3, and 4, the area of stimulation was the back of the hand in Experiments 5 and 7.

4.3.3 Experimental Design and Task

The three experiments described in this chapter were each composed of two psychophysical procedures similar to those used in Experiments 2, 3 and 4 (Chapter 3): a staircase and a signal detection paradigm.

In the three experiments, the staircase procedure was similar to the one described in Experiments 2, 3 and 4 (Chapter 3), but there was one single staircase instead of two parallel, interleaved staircases. This single staircase started at -1.2°C and stopped after 25 reversals. Using a single, long staircase has been proposed to be more accurate and efficient than using two short staircases (García-Pérez, 2000). The boundaries were -0.2°C and -2°C for Experiments 5 and 6, and -0.2°C and -3°C for Experiment 7.

In the three experiments, the signal detection paradigm had a 2x3 design. The first factor was always the presence or absence of the tactile stimulus as described for Experiments 2, 3 and 4 (Figures 3.1B & C), whereas the second factor differed across experiments. In Experiment 5, the second factor was whether the cooling and tactile stimuli were delivered within the same dermatome (C7 & C7), in adjacent ones (C6 & C7) or in non-adjacent ones (C6 & C8) (Figure 4.2C). In Experiment 6, the second factor was the distance between the cooling and tactile stimuli: 1, 5 or 9 cm (Figure
In Experiment 7, the second factor was the delay of the cooling onset with respect to the onset of touch: 0, 1, 2 or 3 s (Figure 4.6C).

In Experiments 5 and 6, there were 2 stimulation sites at each condition. To control for differences in sensitivity between these two sites, the same number of cooling and tactile stimulations were delivered to each site (Figure 4.2C & Figure 4.4C). Importantly, the same location was restimulated with cooling only after at least 3 other locations had been visited, ensuring a minimum of 30s for thermal recovery between cooling at each site. In Experiment 7, the stimulation locations in the skin were arranged in a square grid with a spacing of 1 cm as described in Experiments 2, 3 and 4.

In these experiments, there were 44 trials per condition for a total of 264 trials in Experiment 5, a total of 264 trials in Experiment 6 and a total of 220 trials in Experiment 7. We reduced the number of trials per condition compared to Experiments 2, 3 and 4 to keep the experimental time under 2 hours for maintaining participants’ engagement throughout the entire experiment. Specifically, the number of trials per condition was reduced from 54 per condition to 44 per condition. This reduction was based on a simulation performed with the data collected in Experiment 2 (Figure 4.1). Briefly, the effect size was calculated after removing a number $N$ of trials. $N$ took values from 0 to 35. At each $N$ value, the sensitivity was calculated 1000 times for both ‘Cold’ and ‘Cold & touch’ conditions per participant. Then, the effect size of the difference between the sensitivities was calculated at each $N$ value. The mean and spread of the effect sizes across the 1000 simulations at each $N$ value was used to decide how many trials to do per condition. The effect size when 10 trials were removed was $1.03 \pm 0.08$. Thus, we decided to remove a total of 10 trials per condition to ensure we did not lose more than 0.1 effect size overall.
4.3.4 Data Analysis and Statistics

The staircase percent-correct point was calculated as described in Chapter 3, but the final percent-correct point was obtained directly from the single staircase used in the staircase section.

The signal detection paradigms were used to calculate the percent correct response, the hit and false alarm rates, the sensitivity (d’) and the response bias (c) as described in Chapter 3. The sensitivities and biases obtained in the three experiments were submitted to a repeated ANOVA with factors: presence of touch and dermatome configuration for Experiment 5, presence of touch and distance for Experiment 6, and cooling onset delay for Experiment 7. We used a Dunnett’s test to compare the condition without touch with each cooling onset delay.
4.4 Results

4.4.1 Experiment 5: Dermatomal Modulation

Figure 4.2. Experiment 5: staircase procedure and experimental conditions for signal detection paradigm. A) An example percent-correct point estimation with a staircase procedure from one participant. The black dots indicate the trials in which the participant said ‘Yes’. The light red dots indicate the first three trials in which the participant said ‘No’. The red dots indicate the trials in which the participant said ‘No’. The value of these trials was used to calculate the final percent-correct point for one participant. The horizontal dashed line is the final percent-correct point for one participant. The horizontal solid black lines represent the clamp boundaries (-0.2, -2) of the staircase algorithm. B) Each datapoint represents the percent-correct point of one participant (blue dots) based on a staircase procedure (figure A). The horizontal blue line is the mean across participants (n=12). C) Illustration of the experimental conditions. In the Within condition, stimuli were delivered within the same dermatome (C7). In the Adjacent condition, stimuli were delivered to adjacent dermatomes (C7 & C8). In the non-adjacent condition, stimuli were delivered to non-adjacent dermatomes (C6 & C8). The half circles indicate the positions at which cooling (blue) and mechanical (gold) stimuli were delivered.

Percent-correct point estimation

The relative temperature decrease that participants could detect 80% of the time was -1.20°C ± 0.45 (Figures 4.2A & B).
Figure 4.3. Experiment 5: sensitivity and bias. A) The sensitivity (d') at each dermatome configuration (within, adjacent and non-adjacent) and stimulation (Cold and Cold & Touch) condition. Each datapoint (coloured dot) is the sensitivity of each participant during the signal detection paradigm. The light grey lines join the datapoints belonging to the same participant. The horizontal, coloured lines represent the mean of the sensitivities. B) The response bias (C) at each dermatome configuration and stimulation condition. Each datapoint (coloured dot) is the response bias of each participant during the signal detection paradigm. The light grey lines join the datapoints belonging to the same participant. The horizontal, coloured lines represent the mean of the response biases. The dashed, horizontal, grey line follows y = 0. A negative value indicates a tendency to say ‘No’, whereas a positive value indicates a tendency to say ‘Yes’. C) The difference between the sensitivity of the ‘Cold’ stimulation condition and the sensitivity of the ‘Cold & touch’ condition. Each datapoint (black dot) is the difference between the sensitivities for each participant. The light grey lines join the datapoints belonging to the same participant. The horizontal, black lines are the means of the differences across participants. D) The difference between the response bias of the ‘Cold’ stimulation condition and the response bias of the ‘Cold & touch’ condition. Each datapoint (black dot) is the difference between the response biases for each participant. The light grey lines join the datapoints belonging to the same participant.

Sensitivity (d’)

Sensitivity was calculated across the factors of presence of touch and dermatome configuration (Within Cold d’: 2.14 ± 0.50; Within Cold & touch d’: 1.77 ± 0.52; Adjacent Cold d’: 2.02 ± 0.56; Adjacent Cold & touch d’: 1.73 ± 0.60; Non-adjacent Cold d’: 1.91 ± 0.55; Non-adjacent Cold & touch d’: 1.83 ± 0.63). Sensitivity was significantly reduced when non-tactile cooling was accompanied by concurrent tactile stimuli (repeated measures ANOVA; F1, 11 = 4.97; p = 0.048; η² = 0.04). This is consistent with the results from Chapter 3. We then calculated the difference between the sensitivity to focal cooling with and without touch at each dermatome.
configuration (Within difference $d'$: 0.36 ± 0.68; Adjacent difference $d'$: 0.29 ± 0.49; Non-adjacent difference $d'$: 0.08 ± 0.45). We observed a monotonic decrease in the difference of the sensitivities as the dermatomal distance increased from the within to the non-adjacent configuration (Figure 4.3C). However, sensitivity did not vary significantly as a function of the dermatome configuration (repeated measures ANOVA; $F_{2, 22} = 0.114; p = 0.893; \eta^2 = 0.004$). In other words, there was no interaction between the presence of touch and the dermatome configuration (repeated measures ANOVA; $F_{2, 22} = 0.927; p = 0.37; \eta^2 = 0.01$) (Figures 4.3A & C).

Response bias (C)

Response bias was calculated across the factors of presence of touch and dermatome configuration (Within Cold C: -0.08 ± 0.36; Within Cold & touch C: 0.03 ± 0.37; Adjacent Cold C: -0.10 ± 0.49; Adjacent Cold & touch C: -0.04 ± 0.47; Non-adjacent Cold C: -0.10 ± 0.45; Non-adjacent Cold & touch C: -0.25 ± 0.44). The difference between the response bias to focal cooling with and without touch was calculated for each dermatome configuration (Within difference C: -0.11 ± 0.34; Adjacent difference C: -0.07 ± 0.29; Non-adjacent difference C: 0.14 ± 0.34). Response bias did not vary significantly as a function of the presence of touch (repeated measures ANOVA; $F_{1, 11} = 0.05; p = 0.83; \eta^2 = 0.0002$) or of the dermatome configuration (repeated measures ANOVA; $F_{2, 22} = 1.71; p = 0.20; \eta^2 = 0.02$). There was no interaction between the presence of touch and the dermatome configuration (repeated measures ANOVA; $F_{2, 22} = 2.25; p = 0.13; \eta^2 = 0.016$) (Figures 4.3B & D).

4.4.2 Experiment 6: Distance Modulation

Figure 4.4. Experiment 6: staircase procedure and experimental conditions for signal detection paradigm. A) An example percent-correct point estimation with a staircase procedure from one participant. The black dots indicate the trials in which the participant said 'Yes'. The light red dots indicate the first three trials in which the participant said 'No'. The red dots indicate the trials in which the participant said 'No'. The temperature at these trials was
used to calculate the final percent-correct value. The horizontal dashed black line is the final percent-correct point for one participant. The horizontal solid black lines represent the clamp boundaries (-0.2, -2) of the staircase algorithm. **B)** Each datapoint represents the percent-correct point of one participant (blue dots) based on a staircase procedure (figure A). The horizontal blue line is the mean. **C)** Illustration of the experimental conditions. The dotted lines delineate the dermatomes of the dorsal hand and forearm. All stimuli were delivered within the same dermatome (T1). The half circles indicate the positions at which cooling (blue) and mechanical (gold) stimuli were delivered.

**Percent-correct point estimation**

The relative temperature decrease that participants could detect 80% of the time was -1.56°C ± 0.33 (Figures 4.4A & B).

**Sensitivity (d')**

Sensitivity was calculated across the factors of presence of touch and distance between the stimuli (1-cm Cold d’: 2.02 ± 0.53; 1-cm Cold & touch d’: 2.11 ± 0.46; 5-cm Cold d’: 1.95 ± 0.59; 5-cm Cold & touch d’: 1.76 ± 0.63; 9-cm Cold d’: 2.10 ± 0.42; 9-cm Cold & touch d’: 1.62 ± 0.45). We found that sensitivity did not change significantly as a function of the presence of touch (repeated measures ANOVA; F1, 11 = 4.38; p = 0.06; η² = 0.03). We then calculated the difference between the sensitivity to focal cooling with and without touch for each distance condition (1-cm difference d’: -0.10 ± 0.63; 5-cm difference d’: 0.19 ± 0.34; 9-cm difference d’: 0.48 ± 0.47). We observed a monotonic increase in the difference of the sensitivities as the distance between the stimuli increased from 1-cm to 9-cm (Figure 4.5C). However, we did not find sensitivity changed significantly as we varied distance between the stimuli (repeated-measures ANOVA; F2, 22 = 1.59; p = 0.23; η² = 0.03). In this case, there was a significant interaction between the presence of touch and the distance between the stimuli (repeated measures ANOVA; p = 0.032; F2, 22 = 4.03) (Figures 4.5A & C). A pairwise comparison revealed that in the presence of touch the sensitivity was significantly reduced between the 1-cm and the 5-cm (two-tailed paired test; t11 = 2.91; p = 0.019; η² = 0.05). However, when Bonferroni correction was applied, this effect was not significant (p = 0.085).

**Response bias (C)**

Response bias was calculated across the factors of presence of touch and distance between the stimuli (1-cm Cold C: -0.32 ± 0.49; 1-cm Cold & touch C: -0.37 ± 0.45; 5-cm Cold C: -0.39 ± 0.41; 5-cm Cold & touch C: -0.54 ± 0.39; 9-cm Cold C: -0.48 ± 0.43; 9-cm Cold & touch C: -0.64 ± 0.51). The difference between the response bias to focal cooling with and without touch was calculated for each distance between the
stimuli (1-cm difference C: 0.02 ± 0.33; 5-cm difference C: 0.16 ± 0.41; 9-cm difference C: 0.12 ± 0.36). Response bias did not differ significantly as a function of the presence of touch (repeated measures ANOVA; F\(_{1, 11}\) = 1.84; p = 0.20; \(\eta^2 = 0.02\)), but it differed significantly as a function of the distance between the stimuli (repeated measures ANOVA; F\(_{2, 22}\) = 3.59; p = 0.044; \(\eta^2 = 0.04\)). A pairwise comparison revealed that the difference between response bias with and without touch was significantly different between the 1-cm and the 9-cm conditions (two-tailed paired test; t\(_{11}\) = 2.46; p = 0.03; d = 0.48). However, when Bonferroni correction was applied, this effect was not significant (p = 0.10). There was no interaction between the presence of touch and distance between the stimuli (repeated measures ANOVA; F\(_{2, 22}\) = 0.34; p = 0.71; \(\eta^2 = 0.0020\)) (Figures 4.5B & D).

**Figure 4.5. Experiment 6: sensitivity and bias.**

A) The sensitivity (d') at each distance (1-cm, 5-cm and 9-cm) and stimulation (Cold and Cold & Touch) condition. Each datapoint (coloured dot) is the sensitivity of each participant during the signal detection paradigm. The light grey lines join the datapoints belonging to the same participant. The horizontal, coloured lines represent the mean of the sensitivities.

B) The response bias (C) at each distance and stimulation condition. Each datapoint (coloured dot) is the response bias of each participant during the signal detection paradigm. The light grey lines join the datapoints belonging to the same participant. The horizontal, coloured lines represent the mean of the response biases. The dashed, horizontal, grey line follows y = 0. A negative value indicates a tendency to say ‘No’, whereas a positive value indicates a tendency to say ‘Yes’.

C) The difference between the sensitivity of the ‘Cold’ stimulation condition and the sensitivity of the ‘Cold & touch’ condition at each distance condition. Each datapoint (black dot) is the difference between the sensitivities for each participant. The light grey lines join the datapoints belonging to the same participant. The horizontal, black lines are the means of the differences across participants.
D) The difference between the response bias of the ‘Cold’ stimulation condition and the response bias of the ‘Cold & touch’ condition at each distance condition. Each datapoint (black dot) is the difference between the response biases for each participant. The light grey lines join the datapoints belonging to the same participant.

### 4.4.3 Experiment 7: Temporal Modulation

![Graph A](image1.png)

![Graph B](image2.png)

![Graph C](image3.png)

**Figure 4.6. Experiment 7: staircase procedure and trial structure of signal detection paradigm showing the experimental conditions.**

- **A)** An example percent-correct point estimation with a staircase procedure from one participant. The black dots indicate the trials in which the participant said ‘Yes’. The light red dots indicate the first three trials in which the participant said ‘No’. The red dots indicate the trials in which the participant said ‘No’. The temperature at these trials was used to calculate the final percent-correct value. The horizontal dashed line is the final percent-correct point for one participant. The horizontal solid black lines represent the clamp boundaries (-0.2, -3) of the staircase algorithm.
- **B)** Each datapoint represents the percent-correct point of one participant (blue dots) based on a staircase procedure (figure B). The horizontal blue line is the mean.
- **C)** Schematic of the temporal sequence of events in a signal detection paradigm trial. The conditions are represented by the numbers above the green dotted line, which represents the tactile event. The numbers indicate the seconds between the onset of touch and cooling. People responded either ‘Yes’ or ‘No’ to the question: ‘Was there any temperature change during the tone?’.

**Percent-correct point estimation**

The relative temperature decrease that participants could detect 80% of the time was $-1.76^\circ C \pm 0.91$ (Figures 4.6A & B).
Figure 4.7. Experiment 7: sensitivity and bias. A) The sensitivity (d') at each cooling onset delay (0-s, 1-s, 2-s and 3-s delay) including when there was no tactile stimulation. Each datapoint (coloured dot) is the sensitivity of each participant during the signal detection paradigm. The light grey lines join the datapoints belonging to the same participant. The horizontal, coloured lines represent the mean of the sensitivities. B) The response bias (C) at each cooling onset and when there was no tactile stimulation. Each datapoint (coloured dot) is the response bias of each participant during the signal detection paradigm. The light grey lines join the datapoints belonging to the same participant. The horizontal, coloured lines represent the mean of the response biases. The dashed, horizontal, grey line follows y = 0. A negative value indicates a tendency to say 'No', whereas a positive value indicates a tendency to say 'Yes'. C) The difference between the sensitivity of the ‘no touch’ condition and the sensitivities of the conditions with touch (0-s, 1-s, 2-s and 3-s onset delay). Each datapoint (black dot) is the difference between the sensitivities for each participant. The light grey lines join the datapoints belonging to the same participant. The horizontal, black lines are the means of the differences across participants. D) The difference between the response bias of the condition without touch and the response biases of the conditions with touch (0-s, 1-s, 2-s and 3-s onset delay). Each datapoint (black dot) is the difference between the response biases for each participant. The light grey lines join the datapoints belonging to the same participant.

Sensitivity (d’)

Sensitivity was calculated when touch was not present and at each cooling onset delay when touch was present (0-s d': 1.55 ± 0.77; 1-s d': 2.07 ± 0.78; 2-s d': 2.22 ± 0.69; 3-s d': 1.96 ± 0.74; no touch d': 2.13 ± 0.61). When touch was present, we found that sensitivity differed significantly as a function of the cooling onset delay (repeated measures ANOVA; F_3, 33 = 3.31; p = 0.03; η^2 = 0.10). A pairwise comparison revealed that in the presence of touch the sensitivity was significantly
reduced between the cooling onset delays of 0 s and 1 s (two-tailed paired test; \( t_{11} = -3.86; p = 0.003; d = -0.64 \)) and 0 s and 2 s (two-tailed paired test; \( t_{11} = -2.93; p = 0.014; d = -0.88 \)). However, after applying Bonferroni correction, only the difference between the sensitivities for the 0-s and 1-s cooling onset delays remained significant \((p = 0.016)\). We then calculated the difference between the sensitivity to focal cooling without touch and with touch for each cooling onset delay (0-s difference \( d' \): 0.58 ± 0.80; 1-s difference \( d' \): 0.06 ± 0.99; 2-s difference \( d' \): -0.09 ± 0.67; 3-s difference \( d' \): 0.16 ± 0.71). Sensitivity to cooling did not differ with or without touch regardless of the onset delay (Dunnett’s test) (Figures 4.7A & C).

Response bias (C)

Response bias was calculated when touch was not present and at each cooling onset delay when touch was present (0-s C: 0.03 ± 0.39; 1-s C: -0.01 ± 0.35; 2-s C: 0.015 ± 0.39; 3-s C: -0.03 ± 0.41; no touch C: -0.32 ± 0.38). When touch was present, we found that response bias did not change significantly as a function of the cooling onset delay (repeated measures ANOVA; \( F_{3, 33} = 0.09; p = 0.97; \eta^2 = 0.004 \)). We then calculated the difference between the response bias to focal cooling without touch and with touch for each cooling onset delay (0-s difference C: -0.35 ± 0.34; 1-s difference C: -0.31 ± 0.39; 2-s difference C: -0.34 ± 0.31; 3-s difference C: -0.29 ± 0.40). Response bias to cooling only differed between the condition without touch and the 2-s onset delay condition (Dunnett’s test; \( p = 0.048 \)) (Figures 4.7B & D).

4.5 Discussion

We investigated the spatiotemporal characteristics of the interaction between cooling and tactile inputs. We used a staircase procedure to find the stimulus intensity that each participant could detect 80% of the time. Then, we measured the sensitivity to focal, non-tactile cooling with and without touch in three different scenarios. First, we measured the sensitivity to focal, non-tactile cooling with and without touch at three segmental distances. In other words, we applied our stimuli within, adjacent and non-adjacent dermatomes while we kept constant the distance between the stimuli on the skin. Second, we measured the sensitivity to focal, non-tactile cooling with and without touch at three different distances within the same dermatome. Third, we measured the sensitivity to focal, non-tactile cooling with and without touch at four onset delays. Specifically, we manipulated the delay between the onset of the cooling and mechanical stimulations, keeping the distance between them constant. In a previous experiment, we found that touch reduces detection of focal, non-tactile cooling (Chapter 3). Here, we partly replicated this finding across all experiments.
In Experiment 5, we found that sensitivity to cooling decreased with and without touch regardless of the dermatome configuration (Figure 4.3C). Although we found a monotonic decrease of this effect as the segmental distance increased, we did not find a significant effect of the factor dermatome configuration. We advance three possible explanations for this observation. First, the site of interaction might not be the spinal cord, but rather higher brain regions like the primary somatosensory cortex. We think this is unlikely given that we found a monotonic decrease as we increased segmental distance. Interactions between these signals likely occur at various levels along the thermotactile pathway, as found for pain and touch (Mancini et al., 2015). Second, our dermatome configurations might not have been suitable for the effect of interest. Spinal segments are interconnected by short-range intersegmental connection known as Lissauer tract (Defrin et al., 2008; Fardo et al., 2018; Kerr, 1975; Lamotte, 1977). Thus, a non-adjacent dermatome configuration with a distance of more than two spinal segments may have revealed a further decrease in the interaction between cooling and mechanical signals. Additionally, the dermatome map we used in this study is widely accepted in the medical community, but its accuracy was recently challenged (Downs & Laporte, 2011; Keegan & Garrett, 1948). Third, the sample size may not have been sufficient for the effect of interest. Our sample size was based on previous experiments on the same interaction (Chapter 3), but the experimental design and set-up in Experiment 5 was considerably different. Specifically, our tactile stimulus was a single filament and was approximately 5 cm away from the cooling point, whereas, in previous experiments, we had two filaments bracketing the cooling point 1 cm apart. Altogether, further psychophysical and neurophysiological experiments with bigger sample sizes and alternative stimulation configurations are required to confirm or rule out whether cooling and tactile signals interact in the spinal cord.

In Experiment 6, we found an improvement in cooling detection in the presence, compared to absence, of touch when there was a separation of 1 cm between the cooling and mechanical inputs. This may seem inconsistent with what we found in previous studies and Experiment 5. On the other hand, when the separation was 5 or 9 cm, we found a decrease of cooling detection. The 5 and 9 cm findings align with previous studies (Figure 4.5). However, the spatial modulation in the present study became increased monotonically as we increased the distance between cold and touch stimuli. Therefore, the results of this experiment are inconsistent with previous experiments (Chapter 3) and with our predictions based on the neurophysiological literature. We advance two possible explanations for this discrepancy. First, although the distance between the stimuli in the 1-cm condition was the same as that in
Experiments 2, 3 and 4 (Chapter 3), in the current experiment we used a single filament instead of two as in previous experiments. The configuration in the current experiment could have promoted binding of the sensory inputs (Usher & Donnelly, 1998), to produce a single thermotactile percept, and recruit a different neural mechanism. Binding is more likely to occur when there is coherence across the features of the stimuli: 1 filament is coherent with 1 cooling focal point. Second, we stimulated the volar surface of the forearm, whereas in the other experiments we stimulated the back of the hand. Crucially, each condition had only 2 stimulation sites in the current experiment (i.e. each of the 2 proximodistal combinations), whereas the previous experiments had 9 stimulation sites for each condition. Although both regions are hairy skin, innervation densities might differ (Corniani & Saal, 2000; Filingeri et al., 2018; Luo et al., 2020). We control for differences in skin sensitivity in all experiments by stimulating the same sites with both cooling and touch, but the current experiment differs from the previous ones in the site of stimulation and the total of stimulation sites in each condition. Thus, the 1-cm condition in Experiment 6 is fundamentally different to the conditions in the experiments in Chapter 3 in a number of respects, so the current results do not invalidate the observations from Experiments 2, 3 and 4.

In Experiment 7, we found sensitivity decreased when touch was present compared to when it was not for all onset delays except for the 2-s condition (Figure 4.7). The 2-s condition in this experiment mirrors the design in previous experiments in which we found the opposite effect- touch decreases the sensitivity of non-tactile, focal cooling. Therefore, these results conflict with each other. In the current experiment, trials for each condition were presented in blocks, as opposed to randomly interleaved like in Experiments 2, 3, and 4. Block designs encourage building expectations and may enhance the influence of top-down processes on the resulting percept. On the other hand, randomly interleaved designs minimise the development of expectations and may enhance the influence of bottom-up processes on the resulting percept. Thus, Experiment 7 had a different experimental design to the previous ones, which makes challenging interpreting the inconsistent results.

Altogether, the results of these experiments do not weaken the validity of previous results and call for revisiting how the spatial and temporal distance between the thermal and tactile stimuli impact the interaction of their evoked signals in future experiments. The apparent inconsistency across experiments can be explained by three factors. First, stimuli were delivered on different skin sites. Second, the spatial relation between the cooling and tactile inputs was not the same across experiments.
Third, the experimental designs did not completely match each other and may have focussed on different levels of thermotactile perception.

In conclusion, our series of experiments revealed that the interaction between non-tactile cooling and mechanical signals depends on the spatiotemporal relationship between the inputs. We observed trends that follow our predictions based on previous studies. However, we cannot draw strong conclusions about the exact spatiotemporal characteristics of the interaction due to the inconsistent results across experiments and the lack of significance in our statistical tests. Nevertheless, our work here shows a psychophysical approach to study the spatiotemporal characteristics of multimodal interactions in the somatosensory system. Future studies should repeat these experiments on the thermotactile system with larger sample sizes and control for factors such as sensitivity to the unimodal inputs, the presence of hair and the structure of the skin. Our psychophysical approach can be applied to study the spatiotemporal characteristics of the interaction between other somatosensory channels to inform neurophysiological studies and product design.
5 Touch Enhances the Intensity of Cold Sensations
5.1 Summary

In the previous chapters, we studied the sensitivity to non-tactile focal cooling with and without touch in a series of experiments in humans. The main finding was that mechanical input reduces the sensitivity to non-tactile focal cooling. In this chapter, we study the interaction between cooling and mechanical signals beyond detection. Specifically, we investigate in humans the perceived intensity of non-tactile focal cooling with and without touch. We measured intensity ratings at 6 relative decreases of temperature from 0°C to -3.5°C in 0.7°C steps (0°C, -0.7°C, -1.4°C, -2.1°C, -2.8°C and -3.5°C). We found that touch enhanced the perceived intensity of focal non-tactile cooling within this range. However, power law fitting revealed that touch decreases the rate at which the intensity of a cold sensation increases as a function of the cooling amplitude. In other words, touch slows down how intense a cold sensation becomes as the amplitude of the stimulus increases. Altogether, our results from psychophysical studies in humans suggest two modes of interaction between cooling and tactile inputs during thermotactile perception. First, tactile inputs reduce detection of near-threshold cooling signals. Second, tactile inputs increase the perceived intensity of cooling signals. Further research can now study the neural mechanisms mediating these modes of interaction between cooling and tactile inputs.
5.2 Introduction

A fundamental question in perception is whether conscious detection of a sensation always means conscious perception of all its features. For instance, if a participant feels a temperature change, are they also conscious of its intensity? The traditional view is that detection can be dissociated from other perceptual processes such as discrimination and intensity judgements (Pöppel et al., 1973; Zihl, 1980). The strongest evidence for this view is ‘subliminal perception’ and ‘blindsight’. In both phenomena, information that is not consciously perceived influences the behaviour of people. However, a recent study found that the thresholds for detection and discrimination are effectively the same in a visual task, challenging the classical notion that perceptual processes can be dissociated during conscious experience (Peters & Lau, 2015). We still cannot clearly answer whether we are simultaneously conscious of all the features of a sensation.

Researchers have studied this question by following two different strategies primarily in the visual system. First, studies can compare perceptual and metacognitive performances in the same task. Metacognition is the ability of people to monitor their performance in a perceptual task (Fleming, 2017). If metacognition differs between two perceptual tasks with matched performance, it suggests different perceptual processes are in play. In this line, a recent imaging study has suggested that detection and discrimination of a visual stimulus recruits different neural processes (Mazor et al., 2020). Second, we can apply a specific manipulation to one sensory channel and compare the perceptual performance across different tasks. For instance, a series of studies found a dissociation between detection and localisation of touch mainly on neurological patients (Paillard et al., 1983; Rossetti et al., 1995), but was then challenged by studies on healthy participants (Harris et al., 2004, 2006). Traditionally, these strategies have been applied within one sensory system, mainly the visual one. Therefore, it is unclear whether the characteristics of a multimodal sensation can be dissociated or not.

The thermotactile system is an ideal model to study multisensory sensations. Tactile and thermal pathways have dedicated peripheral sensory afferent neurons expressing different ion channel receptors, but everyday perceptual experience tells us that thermal and tactile signals are integrated during haptic exploration of objects. In the noxious warm range, touch reduces both the detection of as well as the perceived intensity of nociceptive inputs (Mancini et al., 2014, 2015). In the innocuous cooling range, we found that touch reduces detection performance of focal cooling
(Chapter 3), but we do not know the influence of touch on the perceived intensity of non-tactile cooling. We can now compare cooling sensations across tasks with and without touch to investigate whether processes during conscious thermotactile perception can be dissociated and possibly mediated by different networks.

We have therefore studied the perceived intensity of cooling with our novel non-tactile cooling stimulator. Specifically, we used the scaling method to investigate magnitude estimation to different levels of cooling in the presence or absence of touch. We found that touch enhanced the perceived intensity of focal non-tactile cooling. However, power law fitting revealed that touch decreases the rate at which the intensity of a cold sensation increases as a function of the cooling amplitude.

### 5.3 Methods

#### 5.3.1 Participants and Ethics

A total of 12 human participants took part in Experiment 8 (mean age: 21.75 ± 2.78 SD; 9 females). There were no a priori exclusion criteria, hence none of the participants were excluded from the analysis of Experiment 8. The individuals included in the study did not have any underlying skin conditions or conditions that would affect skin sensations. Furthermore, there was no current evidence or history of neurological or psychiatric disease present in any of the participants, nor were they taking psychoactive medication. They had not previously participated in experiments using this method, and prior to participation, written informed consent was obtained from all participants.

We implemented measures to treat potential risks around handling dry ice, having mobile parts in the setup and warming up the hand with an infrared lamp. UCL Research Ethics Committee approved this research (ID number: ICN-PH-PWB-0847/010).

#### 5.3.2 Experimental Set-up

The experimental set-up was similar to the one used in Experiments 2, 3 and 4 (Chapter 3).

In the current experiment, the tactile stimulation was again two von Frey filaments. Both cooling and tactile stimuli were delivered to the back of the left hand. The stimulation locations in the skin were arranged in a square grid with a spacing of 1 cm as described in previous experiments (Figure 3.1B). Similarly, the distance between
the nozzle and the skin was established for each relative temperature decrease based on the linear regression model obtained in Chapter 2 (Figure 2.2).

5.3.3 Experimental Design and Task

Experiment 8 was composed of two psychophysical procedures: a staircase and a scaling paradigm. The staircase procedure was used to obtain an estimation of the relative temperature decrease without touch which participants could detect at a fixed probability. In this experiment, the staircase procedure was identical to the one described in Experiments 5, 6 and 7. The boundaries of the staircase were \(-0.2^\circ C\) and \(-3^\circ C\).

The scaling paradigm was used to study the perceived intensity to focal non-tactile cooling with and without touch. Participants provided numerical ratings to indicate the perceived intensity of the cold sensation (Gescheider, 1988; Marks and Stevens, 1972). Participants were exposed to 12 different experimental conditions. These conditions were a combination of 6 relative decreases of temperature from \(0^\circ C\) to \(3.5^\circ C\) in 0.7 \(^\circ C\) steps (\(0^\circ C\), \(-0.7^\circ C\), \(-1.4^\circ C\), \(-2.1^\circ C\), \(-2.8^\circ C\) and \(-3.5^\circ C\)) in either the presence or absence of touch.

After each break, there was a training section composed of two parts: presentation and anchoring. During presentation, participants were exposed to a trial with no temperature change (\(0^\circ C\)) and a trial with a temperature decrease of \(-3.5^\circ C\). After each trial, they were instructed to associate the perceived intensity to a magnitude of 0 (\(0^\circ C\)) and of 100 (\(-3.5^\circ C\)), respectively. Then, during anchoring, participants were randomly exposed twice to both a change of \(0^\circ C\) and of \(-3.5^\circ C\). After each trial, they were asked to rate the perceived intensity with either 0 or 100. All cooling stimuli in these sections were delivered in the absence of the tactile stimulation. Once the training section was completed, the testing section took place. In this section, participants were instructed to provide numerical ratings of the perceived intensities during each trial based on the sensations and ratings of the training section.

In this experiment, there were 14 trials for each of the 12 experimental conditions, which were presented randomly to the participant. The structure of a trial in the scaling paradigm was similar to the structure of the trials in the signal detection paradigms of Experiments 2, 3, 4, 5, 6 and 7 described in Chapters 3 and 4. Participants were asked to type their numerical ratings on a keypad (Pauk10, Targus International LLC) in response to the following prompt: ‘Please rate the intensity of the temperature change from 0 to 100’.
5.3.4 Data Analysis and Statistics

The staircase percent-correct point was calculated identically to the way it was computed in Experiments 5, 6 and 7 in Chapter 4.

To assess whether touch modulates the perceived intensity of focal cooling, we modified Stevens’ power law (Stevens, 1957) to include a category term (no touch or touch) and an interaction term:

\[
(\Psi) = kI^a + a_1 \times \gamma + a_2 \times I^a \times \gamma + a_3,
\]

where \(\Psi(I)\) is the perceived intensity, \(I\) is the stimulus magnitude, \(k\) is a proportionality constant, \(a\) is an exponent, \(a_1\) is the coefficient of the category term, \(\gamma\) is the category term (0 or 1), \(a_2\) is the coefficient of the interaction term and \(a_3\) is an intercept term.

We fit this equation to the mean perceived intensities of each participant for both conditions. Then, both the exponents (\(a\)) and the constants (\(k\)) were submitted to a one-tailed paired t-test as we hypothesized that, if there was a difference in either value, the value would be greater for the ‘Cold’ condition than for the ‘Cold & touch’ condition. The Python package scipy was used for curve fitting. Specifically, curve_fit from scipy.optimize to optimise the modified power law function.

5.4 Results

5.4.1 Experiment 8: Intensity Magnitude Estimation

![Figure 5.1. Experiment 8: staircase procedure. A) An example percent-correct point estimation with a staircase procedure from one participant. The black dots indicate the trials in which the participant said ‘Yes’ to the prompt: ‘Was there a temperature change during the tone?’. The light red dots indicate the first three trials in which the participant said ‘No’. The red dots indicate the trials in which the participant said ‘No’. The value of these trials was used to estimate the relative temperature decrease. B) The percent-correct point estimation for each participant. The x-axis represents the percent-correct point values, and the y-axis represents the number of participants.](image)
calculate the final percent-correct point for one participant. The horizontal dashed line is the final percent-correct point for one participant. The horizontal solid black lines represent the clamp boundaries (-0.2, -3) of the staircase algorithm. B) Each datapoint represents the percent-correct point of one participant (blue dots) based on a staircase procedure (figure A). The horizontal blue line is the mean across participants (n = 12).

Percent-correct point estimation

The relative temperature decrease that participants could detect 80% of the time was -1.26°C ± 0.80 (Figures 5.1A & B).

Figure 5.2. Experiment 8: intensity magnitude estimation. A) Schematic displaying the temporal sequence of events in a scaling trial. People responded with a numerical rating to the prompt: ‘Please rate the intensity of the temperature change from 0 to 100’. They used a keypad to input their response. B) The perceived intensity ratings as a function of the change in temperature. Each coloured cross is the mean ratings across participants for a given temperature decrease and condition. Each light-coloured dot represents the mean average of the ratings for a given participant for a given temperature decrease and condition. Each coloured cross is the mean average across participants for a given temperature decrease. The solid lines are the power law fit to the datapoints for each condition.

Magnitude estimation

Participants reported numerical ratings for the intensity of the cold sensations upon cooling with (‘Cold & touch’) and without touch (‘Cold’). We found that people reported larger magnitudes for larger stimulus intensities (Figure 5.2B). Furthermore, perceived intensity was higher with touch than without touch at all stimulus intensities (Figure 5.2B). We fit Steven’s power law to our data to compare the relationship between perceived intensity and magnitude intensity. We found that the exponent for both conditions was below 1, meaning the perceived intensity increases at a slower rate than the stimulus magnitude (Cold exponent = 0.73 ± 0.3; Cold & touch exponent = 0.44 ± 0.28). Crucially, the exponent was significantly higher without touch than with touch (one-tailed paired sample t-test; \( t_{11} = 3.26.16; p = 0.004; d = 1.08 \)). This means that touch decreases the rate at which the intensity of cold sensations increases as a function of stimulus amplitude. In other words, touch slows down the intensity of cold
sensations as the temperature decreases compared to when there is no touch. In contrast, the constant value $k$ was not significantly different between the two conditions (Cold $k = 5.22 \pm 4.17$; Cold & touch $k = 49.18$; one-tailed paired sample t-test; $t_{11} = -0.13; p = 0.55; d = -0.06$).

5.5 Discussion

We investigated the influence of touch on the perceived intensity of non-tactile cooling. Our results show that the presence of touch increased the perceived intensity of cooling. However, touch decreases the rate at which the intensity of a cold sensation increases as a function of stimulus amplitude. Based on the results of our previous experiments, we expected touch to reduce both the perceived intensity and the rate of change. Here, we first discuss the findings of this experiment in the context of the literature and then in relation to our previous results.

Previous experiments have used the scaling method to study the perceived intensity of thermal stimuli (Berglund and Harju, 2003; Marks and Stevens, 1972). Our observations are in line with previous results in that larger temperature decreases yield higher magnitude estimations. However, the parameters of our power law fits differ from those obtained by Marks and Stevens (1972) for non-contact cold sensations. In their experiment, participants were lying down in a cold room at 3-4°C. A lamp was used to induce thermal neutrality over a skin region and cold sensations were elicited by turning down the power of the lamp. Crucially, ambient temperature influences object temperature perception (Halvey et al., 2012). Therefore, our stimuli and experimental set-ups are fundamentally different, limiting the comparison of our results. Altogether, our data further supports the use of the scaling method to study thermal perception and validates the use of our novel stimulator to study touch and cold interactions.

In previous chapters, we found that touch decreases detection of non-tactile cooling, which is perhaps analogous to the well-established ‘gating’ of pain by touch. Therefore, we reasoned touch would also attenuate the perceived intensity of cooling (Kakigi & Watanabe, 1996; Mancini et al., 2015). We found the rate of intensity judgement was lower with touch than without it, but, at the studied range of temperature decreases, we found touch increases the perceived intensity of non-tactile, focal cooling. Together, these results suggest the intriguing possibility that, for cold sensation, detection and intensity judgement are mediated by different mechanisms. A similar dissociation has been suggested for other modalities. For instance, detection and localisation of stimuli has been proposed to be supported by
different mechanisms for vision and touch (Paillard et al., 1983; Pöppel et al., 1973; Rossetti et al., 1995; Zihl, 1980). More recently, a psychophysical and neuroimaging study revealed a neural dissociation between discrimination and detection of visual stimuli (Dijkstra et al., 2023). What might be the mechanisms supporting detection and intensity judgment of cooling?

Crucially, both the mechanisms supporting detection and intensity judgement of cooling are influenced differently by touch. This suggests that the mode of interaction between cooling and tactile signals differs between the mechanisms. First, touch seems to gate the cooling signals. We have proposed this gating mechanism occurs at the spinal cord given the role of inhibitory interneurons in processing somatosensory information in this region (Abraira & Ginty, 2013; Goulding et al., 2014; Melzack & Wall, 1965). Second, touch seems to enhance the perceived intensity of detected cooling signals. We propose this facilitatory mechanism occurs at the cortical level for three reasons. First, a previous psychophysical study found an overestimation bias in intensity judgements during two-point stimulation for pain, contact-warm and contact-cold (Walsh et al., 2016). Second, responses to cooling and touch are robust and co-localised in the rodent primary somatosensory cortex (Milenkovic et al. 2014; Vestergaard et al., 2023). Third, a cortical mechanism for magnitude encoding has been described in humans and rodents (Pardo-Vazquez et al., 2019; Sheahan et al., 2021). In our experiment, cold and tactile sensations may have been integrated possibly in the primary somatosensory area. Then, this integrated signal may have been used for magnitude estimation of cold sensations. In other words, the intensity of both sensations may have biased the magnitude estimation of cold sensations. These conclusions are limited by the lack of control conditions in which we measured perceived intensity of cooling during stimulation of another sensory channel. If there is a cross-modal magnitude estimation mechanism (Pardo-Vazquez et al., 2019; Sheahan et al., 2021; Walsh et al., 2016), we would expect increased intensity judgements for any pair of multimodal stimuli.

In conclusion, our psychophysical results in humans suggest that cooling and tactile signals interact in two ways. First, touch reduces the sensitivity to non-tactile, focal cooling. Second, touch enhances the perceived intensity of non-tactile, focal cooling. The inhibitory interaction might occur at the spinal cord, whereas the facilitatory interaction may take place in the cortex. In the following two chapters, we will study thermotactile interaction in the cortex. Specifically, we will investigate cortical encoding of thermotactile stimulation in the primary somatosensory cortex of anaesthetised mice with widefield imaging and electrophysiological recordings.
6 The Mouse Cortex Non-Linearly Integrates Non-Contact Cooling and Vibrotactile Stimulation
6.1 Summary

In the previous chapters, we studied how touch modulates non-tactile cooling in a series of psychophysics experiments with humans. We found that touch reduces detection of near-threshold cooling, whereas it enhances the perceived intensity of cooling. In this chapter, we study with widefield imaging the activity of the mouse forepaw primary somatosensory cortex (fSI) during non-tactile cooling and vibrotactile stimulation. Neurons in this brain region respond to contact cooling and tactile stimuli, so we can study how primary cortical neurons integrate multisensory information. We showed, for the first time, that non-tactile cooling elicits cortical responses. Crucially, we found that the arithmetic sum of the unimodal responses was significantly higher than the bimodal (thermotactile) response. We replicated this finding with two different analysis strategies. In conclusion, the mouse cortex non-linearly integrates non-tactile cooling and vibrotactile stimulation.
6.2 Introduction

A principal role of the brain is to integrate sensory inputs from different modalities. The cortex is necessary for sensory perception and is involved in multisensory integration (Avillac et al., 2007; Stein & Stanford, 2008). However, most studies on multisensory integration have focussed on audiovisual and visuotactile tasks (Avillac et al., 2007; Meredith & Stein, 1986; Meredith & Stein, 1983; Wallace et al., 1998). To our knowledge, there are no studies on the integration of thermal and mechanical signals in the cortex.

Neuroscientists have traditionally explained multisensory integration in the cortex as a hierarchical process. Namely, primary regions are unimodal and provide input to secondary regions, which are multimodal and integrate information from various sensory channels (Felleman & Van Essen, 1991). Recently, anatomical and neurophysiological studies have suggested that even the earliest stages of cortical sensory processing play a significant role in multisensory integration, challenging traditional views (Bizley et al., 2007; Falchier et al., 2002; Fishman & Michael, 1973; Godenzini et al. 2021; Ghazanfar & Schroeder, 2006; Morrell, 1972; Iurilli et al., 2012). However, the responses of primary cortical sensory areas to a secondary modality are typically sparse and weak. Therefore, researchers have considered secondary modalities to merely play a modulatory role in these regions (Iurilli et al., 2012; Lakatos et al., 2009; Ghazanfar et al., 2008, Kayser et al., 2008; Bizley et al., 2007). On the other hand, a recent study suggested that auditory responses in the visual cortex reflect small body movements rather than sound encoding (Bimbard et al., 2023). The significance of the responses to secondary modalities play in primary cortical areas therefore remains unclear.

The thermotactile system is an ideal model for studying the cortical encoding of multisensory stimulation. Tactile and thermal pathways have dedicated peripheral sensory afferent neurons expressing different ion channel receptors, but everyday perceptual experience tells us that thermal and tactile information are integrated during haptic exploration of object surfaces. Intriguingly, somatosensory illusions also hint at a profound interaction between thermal and tactile pathways even at early stages of the sensory pathway. For example, the lack of dedicated wetness hygroreceptors in primary somatosensory afferent neurons indicates that our sense of wetness is created centrally by the integration of thermal with tactile information (Filingeri, 2016). Further, Weber’s ‘thaler illusion’ shows that cooled objects appear heavier than those of a neutral temperature, and ‘thermal referral’ shows that a
warmed coin placed on the skin next to a cooled coin starts to feel cold (Cataldo et al., 2016; Green, 1977; Ho et al., 2011; Stevens & Green, 1978). Crucially, the mouse primary forepaw somatosensory cortex (fSI) has robust, co-localised neural responses during cooling and tactile stimulation of the glabrous skin of the forepaw (Milenkovic et al. 2014; Vestergaard et al., 2023). This suggests that fSI could play a role in thermotactile integration. However, how the cortex integrates cooling and tactile inputs has not been examined, in part because of the difficulty of unimodal stimulation.

The rodent fSI is therefore a suitable candidate for studying cortical integration of cooling and mechanical signals. We have therefore studied the activity of fSI during non-tactile cooling and vibrotactile stimulation. In this experiment, we performed widefield imaging in anaesthetised mice and compared the output activity of this region between unimodal and bimodal (thermotactile) stimulation. We adapted our non-tactile cooling stimulator for targeting the paw of mice. Widefield imaging allowed us to measure the output activity of cortical neurons encoding thermotactile information. Mice were anaesthetised to rule out attentional and motion confounds, and to facilitate stimulation with our non-tactile cooling device. Our results show that thermotactile stimulation excited fSI more efficiently than unimodal stimulation, but the integration of the information is sub-linear.

6.3 Methods

6.3.1 Mice and Ethics

A total of 5 mice were part of Experiment 9 (age: > 2 months; 5 females). Two additional mice were used for training purposes and culled at the end of the surgery. The surgery was performed on 4 additional mice for testing purposes, but they could not be used for Experiment 9 due to scarring and bleeding on the cortical surface. The 5 mice used for Experiment 9 were not culled after the end of the experiment as they were used for other experiments in the lab.

Mice were maintained on a 12:12 hr light-dark cycle. All recording sessions were performed during the light phase of the cycle. Mice were housed in groups at 22 ± 2 °C temperature and 55 ± 10% humidity with ad libitum access to food and water. Thy1-GCaMP6s mice (C57BL/6J-Tg(Thy1-GCaMP6s)GP4.3Dkim/J) were used for calcium imaging (The Jackson Laboratory, stock no. 024275, USA). GCaMP6s is expressed in a subset of excitatory neurons (Chen et al., 2013; Dana et
al., 2014). All experimental procedures were carried out in accordance with the State of Berlin Animal Welfare requirements and European animal welfare law.

### 6.3.2 Surgery

Deep anaesthesia was induced with 3-4% isoflurane in 100% O₂ and maintained at 1.5-2% isoflurane. Mice were injected subcutaneously with metamizol (200mg/kg) for pain management post-operation. Anaesthetised mice were fixed with a nose-clamp. Eye gel (Vidisic, Bausch+Lomb, Laval, Canada) was applied to both eyes to maintain their hydration. Their body temperature was maintained at 37-38°C using a heating pad and rectal probe (50300, Stoelting, Wood Dale IL, USA). After surgery, mice were placed in a cage situated over a warm plate at 38°C, and monitored until they recovered from anesthesia. Metamizol was mixed in the drinking water to avoid post-operative pain for at least 48h after surgery. The first recording was performed at least a week after the surgery.

**Implantation of cranial windows**

A cranial window was implanted over left SI and centred at the representation of the forepaw. To locate the representation of the forepaw, its anatomical position with respect to bregma (1.5-2.5 mm lateral and 0.5-1 mm anterior) was located and marked with a caliper (Castroviejo Surgical Caliper, Fine Science Tools, USA). Then, a circumference was marked on the skull with a 3-mm diameter biopsy punch (3-mm biopsy-punch, Stiefel Laboratories, USA), which was centred over the identified location of forepaw SI. A craniotomy was performed on the marked location. Then, Ringer’s solution was used to rinse the surfaces and 2 glass coverslips were placed on the surface of the cortex. The lower glass (3-mm diameter) and the upper glass (4-mm diameter) were glued together with optical adhesive (NOA 61, Norland Products, USA). The lower glass was introduced in the craniotomy, while the upper coverslip was resting on the skull and attached to it with cyanoacrylate glue and dental cement. A metal head post was also attached to the skull with glue and cement.

### 6.3.3 Sensory Stimulation

**Thermal**

Non-tactile cooling was delivered with sublimated CO₂ gas using dry ice powder (Figure 6.1). The dry ice was held in a 10 ml syringe with a 4-cm needle (BD Emerald Hypodermic Syringe - Luer Slip Concentric, BD, USA) and it was renewed every 5 trials. The needle was attached to the syringe through a 2-cm long flexible silicone tube, which allowed manipulation of its position and orientation. The needle was
attached to a lever, which was rotated by a servo motor (FS90 Mini Servo 120° 9g, Feetech, China) and controlled by an Arduino Leonardo. The syringe was secured on a 3D printed holder (Material: Tough Black PLA, Ultimaker BV, Netherlands; Printer: Ultimaker S3, Ultimaker BV, Netherlands). The 3D printed holder was positioned on a miniature metallic linear rail (Figure 6.1A). To adjust the position of the non-tactile cooling stimulator, the metallic rail was attached to a micromanipulator (MM-33, Harvard Bioscience Inc, USA) at an angle of approximately 60° with respect to the horizontal plane. The syringe was positioned at this angle to avoid the stimulator from colliding with the imaging set-up and have access to the paw. A thermal cover made of cardboard was positioned just above the wrist with an articulated arm to avoid cooling of the rest of the forearm and body.

During the intertrial intervals, the needle was pointing away from the mouse to avoid cooling any skin or fur region. To initiate non-tactile cooling stimulation, the lever rotated and oriented the needle pointing towards the paw. Thermal stimulation lasted 2 s and it was terminated when the lever rotated back to the initial position. The temperature of the paw below the syringe tip was measured with a thermal camera (module temporal resolution: 8.7 Hz, Field of View: 57° & camera resolution: 60 x 120) (Lepton 3.5, Teledyne FLIR), interfaced with a computer through a I/O module (PureThermal 2 - FLIR Lepton I/O Smart module, Teledyn FLIR). The thermal camera was enclosed in a protective 3D printed case. The camera was mounted on an articulated arm to control its position (Fisso strato line XS-13, Baitella AG, Switzerland). A region of interest (ROI, Figure 6.1B) under the needle tip was selected for online image analysis. The pixel values in degrees Kelvin (K) were transformed into degrees Celsius (°C). The temperature of the ROI was obtained by performing the mean across all the pixels within the ROI. During the 2-s stimulation, the temperature of the paw decreased on average to 24.08°C ± 2.23). This was an average relative temperature decrease of -5.64°C ± 2.07).

**Vibrotactile**

Vibrotactile stimuli (80 Hz) were generated by a piezoelectric actuator (PL127, Physik Instrumente GmbH & Co. Karlsruhe, Germany). A bent 2-mm diameter glass rod was attached with cyanoacrylate glue to the tip of the actuator (Figure 6.1). To match the surface area of thermal stimuli, a 3-mm diameter glass coverslip was glued to the tip of the glass rod. To control the position of the vibrotactile stimulator, the actuator was mounted on a glass rod which was attached to an articulated arm. Vibrotactile tactile stimuli had a duration of 2 s.
6.3.4 Widefield Imaging

Setup

Imaging was performed using a custom tandem lens epifluorescence macroscope (Augustinaite & Kuhn, 2020; Couto et al., 2021; Holtmaat et al., 2009; Ratzlaff & Grinvald, 1991). Blue excitation light was emitted from an LED (Thorlabs, M470) and bandpass filtered (469 ± 17.5 nm). Emission light was band pass filtered (525 ± 19.5 nm) before recording with a sCMOS camera (ORCA-Flash4.0 LT, Hamamatsu). Images were acquired at a rate of 50 Hz and 20 ms exposure time. Frame size was 512x512 pixels (4x4 binning) and the Field of View was ~3.9x3.9 mm. Data collection was controlled by custom written code in Python, Arduino and Matlab.

Widefield image analysis

Video recordings were motion corrected. A mask was manually drawn to leave out of the analysis the pixels of the recordings outside the brain. The same mask was used across all trials within the same mouse. The relative change in fluorescence ($\Delta F/F$) defined as: $\Delta F/F = (F(t) - F_0)/F_0$, was calculated across the masked field-of-view. $F_0$ is the median activity before stimulus onset. An 8x8-pixel mean filter was applied on $\Delta F/F$. Then, we calculated an average response for each condition. Next, a rectangular region of interest (ROI) was defined for each condition and mouse. Following this point, all steps were applied on two different types of analysis: noise- and median-based. In the noise-based analysis, the noise floor for each pixel was calculated by finding the 95th quartile of $\Delta F/F$ before stimulus onset. Then, the pixels were binarized at each frame based on their corresponding noise floor. In other words, if a pixel value was above its own noise floor, then the pixel was set to 1, and, if the value was below its noise floor, then the pixel was set to 0. This binarized data was then used for subsequence analyses. In the median-based analysis, the pixels at each frame that were below the median of the given frame were set to 0. If the value was above the median, they retained their original value. This thresholded video was then used for subsequent analyses.

To define the ROI, the centre of geometry, width and height of the response was computed at each frame from stimulus onset to the peak of the response. In the noise-based analysis, the peak response was at the frame with the most pixels above noise floor. In the median-based analysis, the peak response was at the frame with the maximum $\Delta F/F$. In both analyses, the median of the centre of geometries, widths and heights was taken to define the ROI. To compare the intensity of the cortical
response (ΔF/F), a mean ROI was calculated for each mouse and fixed across conditions (Figure 6.2B and Figure 6.3).

Response onset estimation

The noise-based analysis was used to define the onset of the response. Across mice and condition, the same value was used to estimate onset latency. First, for each mouse and condition, we calculated the 99th quantile of the number of pixels above their own noise floor before stimulation onset. Then, the 99th quantile was again calculated for the values obtained in the previous step. This was the threshold used across mice and conditions. Therefore, the onset of the cortical response was defined as the point at which the number of pixels above their own noise floor crossed this threshold.

6.3.5 Experimental Design

During all trial types, the right paw was tethered onto a 20x20-mm Peltier element (CP30238H, CUI devices) and its temperature was regulated by a feedback-controlled stimulator (custom-made device, ESYS GmbH Berlin). The dorsal hairy side of the paw was in contact with this Peltier, whereas the ventral hairless side of the paw was facing upwards. On the paw, thermal and vibrotactile stimuli were targeted to the most prominent walking pads proximal to the wrist (Figure 6.1B). On the arm, thermal and vibrotactile stimuli were targeted at the boundary between the regio brachii and the regio antebrachia (Komárek, 2004).

To recover the temperature of the paw between trials and maintain a consistent baseline temperature throughout the whole experimental session, the 20x20-mm Peltier was turned on manually during all intertrial intervals. The set temperature of the 20x20-mm Peltier was established at the beginning of each experimental session to maintain a baseline temperature between 29-32°C as recorded by the thermal camera.

In this experiment, there were 4 conditions (Figure 6.1):

1. Touch paw: vibrotactile stimulation on the paw.
2. Touch arm: vibrotactile stimulation on the forearm.
3. Cold: Non-tactile cooling on the paw.
4. Thermotactile: Non-tactile cooling on the paw and vibrotactile stimulation on the forearm.

All conditions were tested on the same experimental session per mouse. Trials were presented in blocks. Each block contained 10 trials from a single condition. All
conditions were tested once before repeating any condition. The experimenter was not blind to the order of the blocks, but this order was randomised at the start of the experiment.

The duration of all stimuli was 2 s. Imaging started 3 s before stimulus onset and finished 3 s after stimulus offset. The intertrial intervals defined as the period between start and end of imaging was 25 s.

6.3.6 Data Analysis and Statistics

The sample size was not predetermine using statistical methods, but our sample size is similar to that use in previous publications. The experimenter was not blind to trial order, but trial order was randomized during the experiment. Data analysis was performed with custom-written Matlab and Python code.

![Figure 6.1. Experiment 9: experimental set-up. A) Schematic of the set-up. Elements are not represented to their true scale. The mouse’s paw rested on a 20x20mm Peltier device. Vibrotactile stimuli were delivered by a piezoelectric actuator, which had attached a glass rod with a glass coverslip at the tip. Non-contact cooling stimuli were delivered with sublimated CO₂ gas from dry ice powder, which was contained in a syringe. The needle was attached to the syringe with a flexible, silicone tube. The needle was oriented towards or away of the paw by a servo motor. Temperature of the paw during non-contact cooling was measured with a thermal camera. B) The top panel shows a lateral, schematic view of a mouse’s upper body. The light blue lines delineate body regions as described by Komárek (2004). The vibrotactile stimulus was delivered on the paw and forearm (dark green circle). Non-tactile cooling was delivered on the paw (dark blue semicircle). The black circles on the ventral side of the paw](image-url)
(palm) represent the walking pads. In the paw, the vibrotactile and cooling stimuli were targeted onto the two most proximal walking pads. Adapted from Komárek (2004). The bottom panel shows a thermal image during stimulation. The distal paw and fingers are not clearly visible because they have the same temperature as the Peltier. C) Schematic of the 4 experimental conditions. An additional control condition in which there was no stimulation was performed and included in the analysis. D) Schematic representation of the trial structure. Recording includes thermal camera and widefield imaging.

6.4 Results

6.4.1 Experiment 9: Widefield Imaging of fSI

To examine the integration of non-tactile cooling and mechanical inputs in fSI, we performed widefield calcium imaging through a glass window in paw-tethered, anaesthetised mice expressing the calcium indicator GCaMP6s in cortical excitatory neurons (Figure 6.1 & Figure 6.2A). We stimulated the ventral side of the paw and the forearm with non-tactile cooling and a vibrotactile stimulus (Figures 6.1B & C). Vibrotactile stimulation of the paw elicited the strongest (response intensity: 0.045 ΔF/F ± 0.01), widest (area: 31053.54 pixels² ± 8837.94) and fastest (onset: 112 ms ± 7) response (Figures 6.2B, C, D & Figure 6.3B) among the unimodal conditions (Vibration paw, Vibration arm and Cooling arm).
Figure 6.2. Experiment 9: responses in SI to cooling and tactile stimuli. A) Snapshots of the cortical surface during in vivo widefield imaging and thermotactile stimulation from an example mouse. Each image is the average across 20 trials. B) Widefield responses to thermotactile stimulation for different conditions within a fixed ROI obtained with the median-based analysis. The coloured lines are the population mean (n = 5) and the light-coloured shade around the mean indicates the standard deviation. The light-coloured lines show the responses from individual mice. The black shaded region indicates the duration of the stimulation. C) The area of the ROI obtained from noise-based analysis at each condition. The horizontal-coloured lines are the population mean. The coloured dots indicate the areas for individual mice and the grey lines connect datapoints from the same mouse. D) The onset of the cortical response since the start of the stimulation based on a noise-based analysis. The horizontal-coloured lines are the population mean. The coloured dots indicate the onsets for individual mice and the grey lines connect datapoints from the same mouse.

Furthermore, we found cortical responses to non-contact cooling in the SI of mice upon stimulation of the paw for the first time. This is consistent with previous work that has found cortical responses to contact cooling in this region (Milenkovic et al., 2014; Vestergaard et al., 2023). The cortical responses were weaker (response intensity: 0.028 ΔF/F ± 0.01), smaller (area: 20401.80 pixels² ± 15037.87) and slower (onset: 260 ms ± 6) compared to the responses seen upon vibrotactile stimulation of the paw (Figures 6.2B, C, D & Figure 6.3B). This is again in line with previous cortical studies and the conduction velocity of cooling-sensitive afferents (Campero et al.,
2001; LaMotte & Campbell, 1978; Darian-Smith et al., 1973; Iggo, 1969; Milenkovic et al., 2014; Vestergaard et al., 2023). Then, we found thermotactile stimulation (Cooling paw & Vibration arm) to elicit a response stronger (response intensity: $0.042 \Delta F/F \pm 0.01$) and wider (area: $44690.14 \text{ pixels}^2 \pm 16549.16$) than the corresponding unimodal stimulations (‘Cooling paw’ and ‘Vibration arm’) (Figures 6.2B, C & Figure 6.3B). However, the onset of the cortical response to the thermotactile stimulation (‘Cold & touch’) was faster (onset: $148 \text{ ms} \pm 6$) than to non-tactile cooling (onset: $260 \text{ ms} \pm 6$), but slower than to vibrotactile stimulation of the paw (onset: $112 \text{ ms} \pm 7$) (Figure 6.2D). Altogether, these results show that thermotactile stimulation drives the circuit in fS1 more effectively than unisensory stimuli. Crucially, we have observed, for the first time, cortical responses in the mouse’s SI to non-tactile cooling.

Figure 6.3. Experiment 9: thermotactile integration is sub-linear. A) Difference between the ROI coordinates for Vibration paw and the other conditions. The centre of the plot is the mean ROI coordinates for Vibration paw. The coloured dots are the mean differences per condition. The light-coloured dots are the differences per condition and mouse (0-3). The coloured rectangles show the width and height of the ROIs for the corresponding conditions. B) Widefield responses to thermotactile stimulation for different conditions, including the arithmetic sum (‘Sum unimodal’). These responses were obtained from the median-based
Next, we aimed to evaluate the integration of non-tactile cooling and mechanical inputs. Even though our stimulations were targeted at different points on the forearm during the thermotactile condition, we found the ROIs defined for the unimodal conditions, ‘Touch arm’ and ‘Cold’, largely overlapped, meaning the cortical responses were co-localised (Figure 6.3A). Therefore, we could compare the widefield responses to multisensory stimulation (‘Thermotactile’) with the arithmetic sum of the widefield responses of the corresponding unimodal stimuli (Figures 6.3B, C & D). We found the arithmetic sum of the unimodal response (black in Figures 6.3B, C & D) to differ from the thermotactile responses. Specifically, the intensity at the peak of the widefield response was significantly greater for the arithmetic sum of the unimodal conditions than for the thermotactile condition (sum unimodal $\Delta F/F = 0.061 \pm 0.01$; Thermotactile $\Delta F/F = 0.042 \pm 0.01$; two-tailed paired sample t-test; $t_4 = 2.969$; $p = 0.041$; $d = 1.476$) (Figure 6.3C). Additionally, we replicated this observation for the noise-based analysis (Sum unimodal $\Delta F/F = 0.046 \pm 0.01$; Thermotactile $\Delta F/F = 0.033 \pm 0.01$; two-tailed paired sample t-test; $t_4 = 3.069$; $p = 0.038$; $d = 1.375$). In conclusion, our results suggest non-linearity in cortical responses at the population level during thermotactile integration.

6.5 Discussion

Prior work had shown that mouse fSI neurons respond to both cooling and tactile stimulation of the forepaw (Milenkovic et al., 2014, Vestergaard et al., 2023), but had not investigated the activity of fSI during multimodal, thermotactile stimulation. Here, we confirm that fSI has robust responses to cooling and tactile stimulation (Figure 6.2). For the first time, we show cortical responses to non-tactile cooling. The overall cortical response strength was higher for thermotactile than unisensory stimulation. Moreover, cortical responses to tactile stimuli were shorter latency than to cooling. The mean onset latency to thermotactile stimuli was in between the cooling and vibrotactile response latencies, possibly reflecting the fact that the responses of thermotactile neurons can be dominated by either modality.

The main finding in this study is the sub-linear integration of non-tactile cooling and vibrotactile stimulation in the mouse fSI. If thermotactile integration was linear, we
would expect the arithmetic sum of the unimodal responses to be equal to the thermotactile response. However, our results show that the sum of the unimodal responses is less than the thermotactile response. This suggests there is a suppressive non-linear interaction along the thermotactile system before or when the signals reach the cortex. Previous studies in both cortical and subcortical areas have also found that multisensory integration is non-linear (Avillac et al., 2017; Meijer et al., 2018; van de Rijt et al., 2019; Holmes, 2007; Holmes, 2009). This non-linearity is captured in a well-established feature of neural responses in the superior colliculus during audiovisual stimulation—the principle of inverse effectiveness. Briefly, this principle states that the smaller the unimodal response the greater the multimodal gain (Meijer et al., 2018; van de Rijt et al., 2019; Holmes, 2007; Holmes, 2009). In other words, multimodal integration is the strongest when unimodal stimuli are close to their thresholds, and the multimodal gain decreases as the intensity of the unimodal stimuli increases. Our results cannot confirm or rule out whether this principle applies in fSI because we only used one stimulus intensity for both cooling and touching. Additionally, this principle is traditionally studied at the cellular level. Future studies should aim to measure the electrophysiological responses of neurons in fSI over a broad range of thermal and mechanical stimulus amplitudes to investigate whether fSI follows the principle of inverse effectiveness during thermotactile integration.

A second empirical feature of neural responses to multisensory stimulation is the spatiotemporal principle of multisensory enhancement. This principle states that the effectiveness of multisensory integration is influenced by the spatial and temporal proximity of the stimuli (Stein & Meredith, 1993). Specifically, multisensory enhancement is generally more pronounced when the stimuli are presented closer in space and time (Wallace et al., 1996). In our stimulation paradigm, the stimuli in the thermotactile condition started at the same time. However, non-tactile cooling was delivered to ventral side of the paw, whereas vibrotactile stimulation was targeted to the arm. Despite this spatial separation, we observed overlapping responses in fSI during unimodal stimulation (Figure 6.3C), suggesting that the integration of thermotactile stimulation occurred effectively in the cortical region.

A novel finding in this study is the cortical response to non-tactile cooling. Our stimuli are not ecological, in the sense that mice do not encounter focal, rapid, and non-tactile cooling in nature. However, our stimulation paradigm dissociates thermal from mechanical information. Previous studies had found cortical responses in mouse fSI during contact cooling (Milenkovic et al., 2014, Vestergaard et al., 2023). Together
with previous results, our finding highlights the role of SI in the encoding of cooling, but the functional implications of this are still unknown. Filingeri and other colleagues (2014, 2017) have proposed that the sensations of wetness result from the central integration of cooling and mechanical inputs in brain regions such as SI. Future studies should compare cortical responses to non-tactile cooling and contact cooling with matching spatiotemporal properties and develop a behavioural test to evaluate wetness perception.

Here, we have presented widefield imaging while mice were anaesthetised with isoflurane. Therefore, we cannot draw strong conclusions about the role of fSI in thermotactile integration during awake perception. Isoflurane alters network activity in the mouse cortex. Specifically, it decreases functional connectivity between bilateral cortical regions, disrupts horizontal activity and inhibits the facilitation of thalamo-cortical responses (Bukhari et al., 2018; Hentschke et al., 2017). These disruptions are largely due to increased activation thresholds in excitability and are counteracted by increases in stimulus intensity (Hentschke et al., 2017). Thus, it is likely that our results mirror the patterns of activity observed during awake perception, but have weaker amplitudes. For instance, a previous study in anaesthetised monkeys found evidence of brain activity following the principles of inverse effectiveness and spatiotemporal congruency with fMRI (Kayser et al., 2009). Crucially, our recordings rule out motion and arousal effects, which have confounded our understanding of multisensory encoding (Bimbard et al., 2023). Our results should be compared with recordings performed during awake perception to disentangle what response features are due to thermotactile perception from those resulting from motion, arousal, or anaesthesia.

In conclusion, our results suggest that cooling and tactile inputs have a suppressive non-linear interaction (Figure 6.3). Specifically, the responses to thermotactile stimulation are generally smaller than the sum of the responses to unimodal stimulation. Therefore, SI should be integrated in theories and studies of multimodal somatosensory perception. In the next chapter, we perform single-unit recordings in the SI of anaesthetised mice during thermotactile stimulation to further understand the mechanisms behind thermotactile integration.
7 Thermotactile Stimulation Changes the Response Dynamics of Cortical Neurons
7.1 Summary

In the previous chapter, we studied with widefield imaging in mice the cortical responses to unimodal and bimodal, thermotactile stimulation. We found fSI integrates non-linearly non-tactile and vibrotactile stimulation. In this chapter, we study with dense multi-electrode arrays the activity of individual cortical neurons in the same brain region during thermodactile stimulation. In this experiment, we used a contact thermal stimulator and a vibrotactile stimulus. We found that thermodactile stimulation recruits silent fSI neurons that are inactive during unimodal stimulation. Additionally, we show multisensory stimulation to boost responses by increases of the firing rate of neurons between unimodal and thermodactile stimulation. Consistent with previous studies in the cortex, we found units with supra-additive, additive or sub-additive responses to thermodactile stimulation. Interestingly, only sub-additive neurons show intensity-dependent modulation during bimodal, thermodactile stimulation. Finally, thermodactile stimulation prolonged the response window of supra-additive cortical neurons. Altogether, our recordings show, for the first time, the features of cortical integration in SI during thermodactile integration.
7.2 Introduction

Sensory pathways converge on multisensory neurons in the central nervous system (Godenzini et al., 2021; Meredith & Stein, 1986). These neurons integrate information from different modalities. Presumably, populations of these neurons support multisensory perception (Fardo et al., 2020; Treue et al., 2000). However, most studies on multisensory integration have recorded neurons in the superior colliculus during audiovisual tasks (Meredith & Stein, 1986; Meredith & Stein, 1983; Wallace et al., 1998). Therefore, research on multisensory perception would benefit from single-unit recordings in the cortex during multimodal stimulation.

Single-unit recordings in the superior colliculus have shown that neurons in this region follow the principles of inverse effectiveness and spatiotemporal congruency upon audiovisual stimulation (Meredith & Stein, 1986; Meredith & Stein, 1983; Wallace et al., 1998). Both imaging and neurophysiological studies in different animal models have also revealed cortical areas to follow these principles (Bizley et al., 2007; Kayser et al., 2009). Additionally, an EEG study found evidence for the principle of inverse effectiveness in humans during a visuotactile integration (Kanayama et al., 2015). It is unclear whether these principles are specific to multimodal integration with vision or common to different forms of multisensory integration.

Multisensory integration at the single neuron level is not predicted by the population signals and the behavioural performance in multimodal tasks. Specifically, recordings in the superior colliculus and audiovisual regions have identified three types of responses: supra-additive, additive, or sub-additive (Bizley et al., 2007; Stein & Stanford, 2008; Stanford et al., 2005). Therefore, multisensory responses at the cellular level are more heterogeneous than such responses at higher levels of observation. Thus, equal numbers of sub-additive and supra-additive multisensory neurons might lead the population as a whole to appear to have a purely linear response, leading to the incorrect inference that there were no multisensory neurons present. We do not understand how the diverse response types at the neuronal level mediate the principles observed at higher levels and whether this heterogeneity is specific to audiovisual regions.

We have therefore performed single-unit recordings with dense multi-electrode arrays during thermotactile stimulation. Our recordings were performed in the fSI of anesthetised mice and contribute to our understanding of neural encoding in cortical neurons during multisensory integration. Our results show that thermotactile
stimulation activates unimodally-silent cortical neurons and changes neuronal response dynamics.

7.3 Methods

7.3.1 Mice and Ethics

A total of 6 mice were part of Experiment 10 (age between P52-P198; 6 males). Two additional mice were used for training purposes and culled at the end of the surgery. An additional 5 mice were used for testing but no data was obtained from them due to bleeding or technical problems with the set-up. Mice were maintained on a 12:12h light-dark cycle. All recording sessions were performed during the light phase of the cycle. Mice were housed in groups at 22 ± 2 °C temperature and 55 ± 10% humidity with ad libitum access to food and water before the recording day. C57BL/6J mice were used for the recordings. Mice were decapitated at the end of the experiment under deep anaesthesia and their brain was immersed in paraformaldehyde (PFA) for histology (see Histology). All experimental procedures were carried out in accordance with the State of Berlin Animal Welfare requirements and European animal welfare law.

7.3.2 Sensory Stimulation

Stimulus presentation was controlled via custom-written MATLAB-scripts and a DAQ-board (NI-6232, National Instruments, Austin TX, USA). Stimuli were synchronised with the recording setup (see below) via TTL pulses.

**Thermal**

Temperature stimuli were delivered via 5 high-performance 3.2x2.4mm Peltier elements (TCSII, QST Labs, Strasbourg, France) to the ventral side of paw. The stimulus had the shape of a pulse and it lasted 3 s. The baseline temperature was 32°C and there were 5 different amplitudes: 0, -1°C, -2°C -4°C and -8°C. The ramp speed was 300°C/s and the resolution of the stimulus was 0.1°C.

**Vibrotactile**

Vibrotactile stimuli were delivered to the top of the paw with a force-feedback lever system (300C, Aurora Scientific, Aurora ON, Canada). The vibration lasted 1 s and followed a sinusoidal shape at 60 Hz. The amplitudes were calibrated to result in vibrations with a force of 0, 5, 10 and 20 mN. The force at the tip of the lever was
recorded to implement an online feedback correction to keep the stimulus intensity consistent across trials in case the animal moved during the recording.

7.3.3 Electrophysiological Recordings

Set-up

All recordings were performed with Neuropixels 1.0 probes (imec, Leuven, Belgium) and the spikeGLX acquisition package (Bill Karsh, Janelia Research Campus, Ashburn VA, USA) via a PXI interface (NI-PXIe-1071, National Instruments, Austin TX, USA). Probes were coated with Dil (Invitrogen, Waltham MA, USA) and lowered into the tissue automatically via micromanipulators (LN25, Luigs&Neumann, Ratingen, Germany) to a recording depth of 1500-1800 µm at a speed of ~2 µm/s. Raw data was acquired at ~30000 Hz (actual rate calibrated for each probe separately) from up to 384 electrodes.

Intrinsic imaging

We used intrinsic optical imaging to locate the forepaw region in the primary somatosensory cortex of the right hemisphere. On the day of recording, mice were deeply anesthetised and then maintained at light anaesthesia levels (1-1.5% isoflurane) until the end of the experiment. To locate the forepaw region, the forepaw was stimulated with a 100-Hz sinusoidal vibration for 5 s using a Piezo element (PL127.11, Physik Instrumente, Karlsruhe, Germany). During the stimulation, a video of the cortical surface (centred at 1.5-2.5 mm lateral and 0.5-1 mm anterior with respect to bregma) was recorded with a CMOS camera (QICAM Fast 1394, Teledyne, Surrey, Canada). The video was analysed online using custom-written software in MATLAB (Mathworks, Natick CA, USA). After every stimulation, the analysed video was visually examined to locate a cortical response. Once an area with a robust response was detected, a craniotomy was performed over the corresponding region. Then, the dura was incised to facilitate probe insertion later. Mice were then immediately moved to the electrophysiology set-up where the level of anaesthesia was maintained during extracellular recordings.

Extracellular spike sorting

Spike sorting was performed offline using Kilosort 2.0 (Pachitariu et al., 2016). Manual curation of the sorted units was performed using the Phy2 package (https://github.com/cortex-lab/phy). Splitting and merging of clusters was kept to a minimum and manual curation was mostly used to tag putative single units and exclude drifting units and artifacts for subsequent analysis. After manual curation, all
remaining units were evaluated by 3 main quality metrics. First, units with less than 1000 spikes were removed. Second, units with more than 0.5% refractory period (1.5 ms) violations were removed. Third, units with more than 10% of missing spikes were removed. Missing spikes were estimated with the overlap of the gaussian distributions obtained from the waveform and the noise.

Spike sorting was performed by Dr. Philipp Schnepel.

Electrophysiological recordings analysis

Offline analysis of stimulus responses was performed for each putative single unit using custom MATLAB scripts and existing code (https://github.com/cortex-lab/spikes). Responses were defined as the average, baseline-corrected firing rate (or z-score) in response windows of varying size (default: 1 s) after stimulus onset. Units were considered ‘responsive’ to a particular stimulus if the number of action potentials measured in the response window deviated significantly from the spiking probability of a Poisson process with that unit’s mean baseline firing rate by using a ‘binless’ method (i.e. using ‘bins’ of 1 ms, α < 0.01). The first ‘bin’ to cross the significance threshold of α < 0.01 was used as the latency of this unit’s response. Units were tagged as ‘overall responsive’ if at least 2+x stimuli elicited significant responses with x being the rounded false positive detection rate of the binless method determined from blank trials multiplied by 20 (average over 6 anesthetised recordings: x = 10.22 ± 2.91%).

PSTHs were constructed using an optimal bin size algorithm. Instantaneous firing rate was calculated using a gaussian KDE (kernel density estimate). Hierarchical clustering was performed on the z-scored KDE over the whole trial of a particular stimulus combination (maximum unimodal stimulus intensities (20 mN/-8°C) for 2x2 clustering; ‘best’ stimulus combination from additivity analysis for 3x3 clustering, see below) for each unit using the ‘linkage’ and ‘cluster’ functions in MATLAB.

The additivity of each unit was determined as follows. First, the distribution of the arithmetic sum of the unimodal response values was bootstrapped for each possible stimulus combination (10,000 iterations, 12 stimulus combinations). Then, the distribution was z-scored. If the z-score of the actual thermotactile response surpassed ±1.96, this response was considered supra- or sub-additive, respectively. It was considered additive otherwise. The stimulus combination which had the maximum absolute z-score was tagged as the ‘best’ combination. The Multisensory Index (MSI) was calculated as the difference between the multimodal and the
summed unimodal responses normalized by the sum of both (MSI = M - (T + C) / M + (T + C)).

This analysis was entirely implemented by Dr. Philipp Schnepel. The figures were also produced by Dr. Philipp Schnepel.

**Histology**

After the recording, mice were decapitated under deep anaesthesia and the brain was immersed in paraformaldehyde (PFA) for at least 24 h. Next, thick slices of 100µm were cut with a vibratome (VT12000S, Leica, Wetzlar, Germany). Then, the slices were imaged on a fluorescence microscope (AxioImager.M2, Karl Zeiss Microscopy GmbH, Göttingen, Germany) to visualise the DiI which coated the probe. Slice images were subsequently inspected to confirm the location of the recording in fSI.

### 7.3.4 Experimental Design

Throughout the recording session, the paw was tethered to the thermal stimulator and the vibrotactile stimulator rested on the back of the paw. Therefore, thermal stimuli were delivered to the ventral side of the paw, where vibrotactile stimuli were delivered to the dorsal side. Trials lasted a total of 5 s. Stimulus onset was 1 s after the beginning of the trial. The intertrial interval was 8 s. There were 5 thermal stimuli (0°C, -1°C, -2°C, -4°C and -8°C) and 4 vibrotactile stimuli (0, 5, 10 and 20 mN). Thermal and tactile stimuli were combined for a total of 20 combinations. There were 25 trials per combination for a total of 500 trials.

### 7.3.5 Data Analysis and Statistics

Statistical tests were performed using MATLAB built-in functions. A Wilcoxon signed-rank test or a paired t-test was used to test the difference between two conditions. An ANOVA (Kruskal-Wallis for multiple comparisons with Dunn-Sidak correction) was used to test differences between several conditions. If not otherwise noted, values are reported as mean ± SEM (standard error of the mean).
Figure 7.1. Experiment 10: extracellular recordings in fSI during contact cooling and vibrotactile stimulation. A) Schematic of the recording set-up. Anaesthetised mice were head-fixed and received thermotactile stimulation to their right paw. B) Schematic of the stimulation paradigm. The cooling stimulation had a ramp-hold-return shape and lasted 3 s. The vibrotactile stimulation had a sinusoidal shape with a frequency of 60Hz and a duration of 1s. C) Example penetration with a Neuropixel probe. The left panel shows the pattern of electrodes used during recording. Then, there is a reconstruction of the location of probe sites across cortical layers (abbreviation of brain areas: SSp-ul: primary somatosensory area upper limb; scwm: supra-callosal cerebral white matter; ccb: corpus callosum body; CP: caudoputamen). The right plot shows the z-scored firing rate responses across the probe shank for cooling and vibrotactile stimulation. D) The first panel shows a craniotomy under brightfield illumination. The second panel is a corresponding snapshot during intrinsic signal optical imaging. Darker area denotes response to tactile stimulation of the forepaw. Green dot denotes the same spot in both panels. Scale bar is 1mm. The third panel shows DiI-staining in a horizontal brain slice (100μm thickness) of two Neuropixel probe tracts. E) Raster plots and corresponding peri-stimulus time histogram of four example units during cooling and vibrotactile stimulation.
7.4 Results

7.4.1 Experiment 10: Electrophysiological Recordings in fSI

*Cooling and vibrotactile stimulation excite and inhibit fSI neurons*

We performed extracellular recordings from fSI neurons in anaesthetised mice (Figure 7.1). We targeted recordings to the left fSI using intrinsic optical imaging and confirmed the recording site post-hoc using Dil staining of the probe tract (Figure 7.1). We stimulated the right forepaw of mice with contact cooling, vibrotactile stimulation, or simultaneous cooling and vibrotactile stimuli. We first examined single unit responses to unimodal stimuli. We recorded a total of 259 neurons. Of the recorded neurons, we found that 39% responded to both cooling and touch, 24% only to touch and 7% only to cooling. The remaining neurons (30%) were unresponsive to the stimuli (Figure 7.2B). These results show that fSI neurons exhibit varying levels of modality-specific activity.
Figure 7.2. Experiment 10: thermotactile stimulation boosts responses and recruits silent neurons. A) Raster plots and corresponding peri-stimulus time histogram of one example unit during cooling, vibrotactile and thermotactile stimulation. This unit exemplifies the response boost by thermotactile stimulation (scale bar: 500 ms). The Kernel Density Estimate (KDE) of the example unit is shown to the right. B) Fractions of responsive (cooling, tactile and thermotactile stimulation) and unresponsive (NR) single units at maximum intensity stimulation. C) The percentage of recruited units as a function of stimulus intensity. D) The percentage of cumulative additional units recruited as a function of stimulus intensity. E) Raster plots and corresponding peri-stimulus time histogram of one example unit during cooling, vibrotactile and thermotactile stimulation. This unit exemplifies recruitment by thermotactile stimulation (scale bar: 500 ms). F) Average firing rate of all responsive units (n = 259) to increasing intensity of cooling, vibrotactile and thermotactile stimulation. G) Median response strength for all responsive units per condition. H) Median onset latency for all responsive units per condition.
Multisensory stimulation boosts responsiveness and recruits previously silent neurons

We next studied excitatory responses in fSI during multisensory stimulation compared during unimodal stimulation. Here, we defined fSI neurons as significantly responsive if they were excited by either cooling, touch or both. Then, we compared their responses to unisensory (cooling or touch) with multisensory (combined cooling and touch) stimuli at different amplitudes (Figures 7.2E, F, G & H). First, the median excitatory response strength to the maximum intensity of touch was significantly higher than for cooling (touch: 1.26 Hz; cooling: 0.71 Hz, respectively, p = 0.0015). Second, the strength of the thermotactile response was significantly increased compared to both cooling and touch (2.15 Hz, p = 6 x 10^{-11} and p = 0.0099, respectively, Figures 7.2F & G). Third, the median fSI neuron response latency to thermotactile stimulation was faster (24 ms) than to contact cooling (53 ms; p = 8.2 x 10^{-6}), but slower than to vibrotactile stimulation (21 ms; p = 0.72; Figure 7.2H). Together, these results show that thermotactile stimulation drives the circuit in fS1 more effectively than unisensory stimuli.

In prior work, little attention has been paid to the role of neurons with silent or suppressed responses to unimodal stimuli. In our recordings, we found a higher number of neurons responsive to the multisensory stimulus than to cooling or touch alone (Figure 7.2B). Therefore, we reasoned that unimodally silent neurons were unmasked by the combined multimodal drive and thus recruited to increase the numbers of cells representing the multisensory stimulus in fSI (e.g. unit in Figure 7.2A). To quantify this, we examined the dependence of neuron recruitment on stimulus amplitude and calculated the fraction of responsive units at increasing stimulus intensities during unisensory and multisensory stimulation (Figure 7.2C). As expected, the number of recruited neurons increases with increasing unisensory cooling intensity (grey line). When including a simultaneous tactile stimulus (green lines), the number of recruited neurons increases, which is seen in the vertical shift of the green lines at a given cooling amplitude (Figure 7.2C). This increase in recruitment plateaus towards the maximum intensity of cooling and vibrotactile stimulation, which might be due to the network operating at the upper end of its dynamic range (i.e. a ceiling effect). Thus, at low stimulus intensities, adding a second modality results in relatively more units being recruited than at higher stimulus intensities. This can be seen by the steeper increase in the cumulative additional units for weak cooling during tactile stimulation than for strong cooling during tactile stimulation (light blue compared to dark blue lines in Figure 7.2D). This suggests the
recruitment of unimodally silent neurons is a critical feature of multisensory stimulus representation in fSI.

**Non-linear processing in fSI during thermotactile integration**

A key question in multisensory integration is whether the responses of neurons to multisensory stimuli are predictable from their responses to unisensory stimuli. A widely used metric to address this is response additivity. Classically, the response of a unit to multisensory stimulation is compared to the arithmetic sum of the corresponding unimodal responses and units are categorized as supra-additive, additive or sub-additive (Figure 7.3). In our recordings, we saw examples of supra-additive (Figure 7.3B, top, MSI > 0), additive (Figure 7.3B, middle, MSI =~ 0) or sub-additive units (Figure 7.3B, bottom, MSI < 0). The majority of fSI units were classified as supra- or sub-additive (0.37 and 0.43, respectively) and only a small fraction as additive (0.17; Figure 7.3E). This suggests most multimodal responses in fSI are non-linear and not predictable from their unimodal response.

Furthermore, a ‘Multisensory Index (MSI)’ can be calculated as a normalised quantification of multimodal enhancement/suppression. Based on this index, classic studies in the superior colliculus have proposed a principle in multisensory integration: inverse effectiveness. Specifically, it states that a neuron’s MSI is inversely proportional to the unisensory response strength (Figures 7.3C & D). The focus of multimodal integration is frequently on those neurons showing enhancement of responses as compared to unimodal responses. However, suppression of neural activity might be a critical feature of cortical encoding.

In our recordings, we observed a significant proportion of neurons with sub-additive multisensory effects. Therefore, we compared MSI across our three additivity populations to study inverse effectiveness across the different groups. Specifically, we plotted our data in a semi-log space and fitted separate regressions for each additivity group. We observed trends resembling inverse effectiveness (Figure 7.3F). While supra-additive units showed weak, positive inverse effectiveness ($r = -0.14$), sub-additive units exhibited stronger, but ‘negative’ inverse effectiveness ($r = 0.45$). This means the MSI of a sub-additive unit is directly proportional to its unisensory response strength, which is opposite to the classical view in inverse effectiveness (Figures 7.3C & D). Moreover, additive units also trended towards ‘negative’ inverse effectiveness ($r = 0.22$). Interestingly, the correlation was only significant in the sub-additive population ($p = 0.000076$), but not in the supra-additive ($p = 0.37$) and the additive ($p = 0.52$) populations. Altogether, these results highlight a large diversity of
non-linearity in cortical responses during multisensory integration in fSI with both multisensory suppression and enhancement during thermotactile integration.

**Figure 7.3. Experiment 10: thermotactile integration at the cellular and population level.**

A) Comparing the arithmetic combination of touch and cold responses (\(\sum\)) to the response in the multimodal condition (\(M\)) allows the definition of additive (gray) and sub-/supra-additive (yellow, red) sub-populations of neurons (see Methods). B) Example units illustrating additivity: supra-additive (top), additive (middle) and sub-additive (bottom). Each row shows (from left to right): Raster/PSTH for touch, cold and multimodal stimulus, kernel density estimate (KDE) of the firing rate for \(\sum\) vs. \(M\) (scale-bar: 500msec), normalized response strength for \(\sum\) and \(M\) (left axis) and corresponding MSI (gray bars, right axis) for one temperature (the example’s stimulus combination is marked next to the KDE plot, e.g. -4°C/20mN). C) The principle of inverse effectiveness postulates that the strength of the multisensory modulation (MSI, Multisensory Index, see Methods) is inversely correlated to the unimodal stimulus/response strength. D) Example unit illustrating inverse effectiveness. The modulation strength (difference between KDE for \(\sum\) vs. \(M\), gray bar) is decreasing with increased intensity of one modality (tactile strength increase from left to right, green bar; cool is fixed at 8°C). This is summarized in the modulation plot as in B). E) Fraction of units in respective sub-populations after determining additivity. F) Multisensory Index plotted against the arithmetic sum of the corresponding unimodal stimulus response. Inverse effectiveness is estimated by linear fits to the different sub-populations of neurons. G) Normalized, average response of all sub-populations.

**Multisensory responses in fSI are prolonged**

In MSI analysis, non-linear boosting of responses is reflected by a quantitative change in the absolute number of action potentials during the entire stimulus period. However, a neuron’s response can also be changed qualitatively by altering its
temporal response dynamics. To examine in our recordings, we averaged and normalized the multimodal responses across the sub-, supra- and additive populations (Figure 7.3G). Visual inspection shows a more prolonged multisensory response for the supra-additive neurons as compared to the sub-additive and additive populations. To quantify this effect, we calculated a ‘duration index’ for each unit that compares the strength of the first and last 200 ms of the response. We found that the distribution for supra-additive units was shifted to the left compared to sub-additive units, indicating longer responses (supra-additive median: 0.37 duration index; sub-additive median: 0.55 duration index; \( p = 0.0008 \)).

7.5 Discussion

Prior work had shown that mouse fSI neurons respond to both cooling and tactile stimulation of the forepaw (Milenkovic et al., 2014, Vestergaard et al., 2023), but had not investigated the neuronal response properties during thermotactile stimulation. Here, we replicate the widefield imaging results and extend them at the cellular level. Specifically, fSI neurons show robust responses to cooling and tactile stimulation (Figure 7.2) and that most responsive neurons encode both modalities (Figure 7.2B). The overall neural response strength was higher for thermotactile than unisensory stimulation. Moreover, cortical responses to tactile stimuli were shorter latency than to cooling. The mean onset latency to thermotactile stimuli was in between the cooling and vibrotactile response latencies, possibly reflecting the fact that the responses of thermotactile units can be dominated by either modality.
An intriguing finding was that many neurons with no change or a reduction in firing rates during unimodal stimulation showed significant responses during multisensory, thermotactile stimulation. In this sense, we have found unimodally-silent multisensory neurons. This uncovering of responses could be key to an improvement of the stimulus representation in fSI. One possibility is that the recruitment of previously silent cells could result from alterations in the level of local synaptic inhibition during multisensory stimulation (Iurilli et al., 2020). Future experiments could address this hypothesis with whole-cell, membrane potential recordings from cortical neurons combined with activity manipulations of cortical GABA-ergic inhibitory interneurons.

A central question regarding cortical multisensory integration is whether stimuli are combined in a linear or non-linear fashion. We tested this by comparing the linear addition of unimodal responses with the combined, multisensory response. Across the entire population of neurons responding to at least one modality, we found evidence for different forms of integration including non-linear supra- and sub-additive responses as well as linear additive responses (Figure 7.3) (Stanford et al., 2005). Interestingly, we found opposite patterns of intensity-dependent multisensory integration for supra- and sub-additive subgroups. Specifically, as the sum of the
unimodal responses increased, the MSI of supra-additive neurons decreased (a negative correlation), whereas the MSI of the sub-additive increased (a positive correlation). These patterns are analogous to the principle of inverse effectiveness (Alvarado et al., 2007; Lakatos et al., 2009; Meijer et al., 2018; Meredith & Stein 1986; van de Rijt et al., 2019).

Inverse effectiveness is the most accepted form of non-linear integration and has been proposed as a key mechanism of multisensory integration in several systems. Inverse effectiveness is traditionally associated with enhancement of behavioural and neural responses. Namely, the greatest multisensory enhancement occurs for weak stimuli. One might think of inverse effectiveness as a specific example of a much more general effect in multisensory integration: the interaction is itself intensity-dependent. In our recordings, we found the strongest intensity-dependent integration was sub-additive. Additionally, there are many caveats such as the ‘ceiling/floor effect’ or ‘regression towards the mean’ that limit our understanding of inverse effectiveness (Holmes, 2007; Holmes, 2009). In our case, we partly mitigate the problems associated with ‘regression towards the mean’ (Holmes, 2009) by using an ‘a priori’ approach. Specifically, our stimulation paradigm samples responses over a range of defined stimulation intensities instead of sorting neuronal responses by strength. However, this approach still has issues with noisy estimations of small responses (floor effect) and finite maximum firing rates of neurons (ceiling effect). This makes it difficult to unambiguously identify inverse effectiveness as the key mechanism of multisensory enhancement in our recordings. Altogether, we have identified for the first time the presence of sub-populations with intensity-dependent modulation during thermotactile stimulation in the mouse SI.

Moreover, we observed that the supra-additive population has prolonged responses during thermotactile stimulation (Figure 7.3G), which was not the case in the sub-additive or additive populations. A prolongation of responses especially at low saliency levels could favour a more robust representation over time for hard-to-detect stimuli by increasing the effective integration time window. The prolongation of the cortical response suggests that the accumulation of evidence is enhanced over time.

Prior work in the audiovisual system suggests that multisensory modulation is enhanced if two stimuli are presented at the same environmental location (spatial congruency), the same time (temporal congruency) and at threshold stimulus levels (inverse effectiveness). To achieve spatial congruency, we delivered both cooling and vibrotactile stimuli to the right forepaw. However, a limitation of our study is that the
vibrotactile stimulation was delivered to the hairy skin on the back of the forepaw, whereas cooling was delivered to the glabrous skin on the ventral side of the forepaw. Although our vibrotactile stimulation was delivered to the back of the paw, it vibrated the whole forepaw and the forepaw was taped onto the thermal stimulator. Additionally, during intrinsic optical imaging, we used vibrotactile stimulation on the ventral side of the forepaw and placed the electrodes in the identified area. Both cooling and vibrotactile stimuli evoked strong and reliable neural responses in fSI. Therefore, we assume that the spatial congruency of our stimuli was sufficient to study thermotactile integration in our set-up.

We cannot draw strong conclusions about the role of fSI in thermotactile perception because our electrophysiological recordings were performed in anaesthetised mice. Given the effect of isoflurane on cortical activity, our results contribute to our understanding of thermotactile integration in fSI. Crucially, our recordings can now be compared to identical studies in awake mice to disentangle what neural features are due to thermotactile integration from those resulting from motion, arousal, or anaesthesia.

In conclusion, our results shows that the integration of thermal and tactile inputs in cortical neurons is non-linear. The data suggest that the recruitment of additional cortical neurons with longer integration times are key encoding changes in fSI (Figure 7.4). Rather than an area containing anatomically segregated cortical neurons processing different modalities of somatosensory input, fSI should be considered as an interconnected, multisensory region with strong multisensory interactions. Crucially, it is an accessible region for identification of neuronal principles of cortical multisensory integration. Future studies should consider the temperature of surfaces as a fundamental component of tactile sensation and attempt to dissociate thermal from tactile information (Ezquerra-Romano 2023). While these findings place multisensory integration at the heart of primary cortical processing, recordings and manipulations during behavioural tasks will be required to further link perception to cortical neuronal activity.
8 Conclusion
8.1 Summary

In this thesis, we have studied how mechanical touch modulates perception and neural encoding of focal, non-tactile cooling. In a series of psychophysical studies in humans, we found two modes of interaction between cooling and tactile information. Namely, touch decreases detection of near-threshold cooling, whereas it enhances the perceived intensity of cooling. In a couple of neurophysiological studies in rodents, we found the principles of neural encoding during thermotactile stimulation at both the population and cellular level. Specifically, we found that the primary cortex integrates non-linearly cooling and tactile inputs. Additionally, thermotactile stimulation recruits silent neurons as well as boosts the firing rate and prolongs the response of cortical neurons. In this chapter, we discuss the findings and propose a new model for thermotactile interactions. We also outline the limitations of our research and suggest future search directions based on the advances of this thesis.
8.2 A New Model for Thermotactile Interactions

Thermal and mechanical signals interact in various ways from the skin to the perception of objects. Therefore, the logic way to study their interactions is to selectively stimulate one channel and then compare the responses with and without stimulation of the other channel. Most studies on thermal sensation fail to selectively stimulate the thermal pathway because they use stimulators which involve a degree of mechanical stimulation. Therefore, these studies are confounded by the presence of touch. Here, we use a novel non-tactile stimulator to study how touch modulates perceptual and sensory responses to non-tactile focal cooling (Table 3).

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<td>Method of Limits</td>
<td>Non-tactile cooling can give psychophysical measures for cold perception</td>
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<td>2</td>
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<td>3</td>
<td>Human</td>
<td>Non-tactile (back of hand)</td>
<td>Signal detection paradigm</td>
<td>Touch decreases detection of focal cooling</td>
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<tr>
<td>4</td>
<td>Human</td>
<td>Non-tactile (back of hand)</td>
<td>Signal detection paradigm</td>
<td>Touch decreases detection of focal cooling</td>
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Table 3. Summary of experiments: methods and results.
In a series of signal detection studies, we found that mechanical input reduced the ability of people to detect focal, non-tactile cooling (Figure 3.3; Figure 3.5; Figure 3.7; Table 3). On the other hand, we found that mechanical input increased the perceived intensity of focal, non-tactile cooling in a scaling experiment (Figure 5.2; Table 3). We fitted a power law function to this data. Although touch increased the perceived intensity of cold sensations, it slowed down the rate at which cold sensations intensify as a function of stimulus amplitude. Together, these results suggest touch modulates detection and magnitude estimation of cooling inputs in different ways.

Our detection results are analogous to other inhibitory interactions between somatosensory modalities. Namely, touch inhibits pain, pain inhibits itch, cold inhibits pain and static touch inhibits vibration touch (Cataldo et al., 2021; Mancini et al., 2014, 2015; Proudfoot et al., 2006; Yosipovitch et al., 2007). Similarly, we propose that touch inhibits cold. Where does this inhibitory interaction take place? Following the tradition in the pain-touch literature (Braz et al., 2014; Mancini et al., 2015), we propose a model in which this interaction occurs in the spinal cord. Specifically, mechanosensitive fibres inhibit incoming cooling-associated activity at the level of the spinal cord through inhibitory interneurons (Figure 8.1). Then, cooling-associated activity is transmitted to the brain and integrated in brain circuits involved in stimulus detection. Importantly, cooling signals are not integrated with mechanical signals during the detection process (Figure 8.1). Therefore, if cooling signals are dampened by mechanical stimulation at the level of the spinal cord, then stronger cooling-associated activity will be required to reach the detection threshold in the brain circuits involved in stimulus detection.

Our magnitude estimation results are however inconsistent with other somatosensory interactions. Namely, touch attenuates pain intensity and static touch reduces the intensity of vibration touch (Cataldo et al., 2021; Mancini et al., 2014, 2015). This suggest pain intensity is computed with the same signal used to make detection judgements. In contrast, we propose that magnitude estimation of cooling is computed with a different signal to the one used for cooling detection. If the same signal was used for detection and magnitude estimation of cold sensations, then we would presumably have found that touch decreases the perceived intensity of non-tactile focal cooling. However, we observed the opposite.

Detection and magnitude estimation differ in one important cognitive respect. A recent study found that detection and discrimination of a visual stimulus recruit different neural processes (Mazor et al., 2020). Detection performance is thought to require
perceptual judgements about absence (i.e., correct rejections). In particular, a clear counterfactual model about what the percept would be if it had been present is needed to decide that the stimulus is absent in a detection paradigm. In contrast, tasks like discrimination and magnitude estimation can be performed by simple readout of an idealised projection neuron. Therefore, detection tasks are thought to be intrinsically metacognitive in a way that discrimination and estimation tasks need not be (Mazor, 2022). Our results show detection and magnitude estimation judgments of a cooling input are modulated differently by touch, which suggests they might be processed by different neural processes.

The neural process involved in intensity judgement seems to integrate cooling and mechanical signals. Specifically, cortical neurons integrate both mechanical- and cooling-associated activity from ascending projections. We suggest that two quite distinct forms of thermotactile interaction may occur along this pathway. First, cooling signals are inhibited by mechano-sensitive inputs, possibly at the level of the spinal cord (Figure 8.1). Next, the two signals may interact in the cortex, through a form of integration involving convergent projections onto higher-order neurons. These higher-order neurons may form the basis for sensory magnitude estimation. Our mouse recordings suggest SI could be the site where this integration occurs. However, we should be cautious when drawing conclusions across species.

Importantly, our observations in rodents do not rule out our model for cooling and tactile interactions. We found that bimodal stimulation elicits greater cortical activity than the activity elicited with cooling alone. This is consistent with the increase in perceived intensity observed in humans. Moreover, we found that the arithmetic sum of the cortical responses to unimodal cooling and mechanical stimulation was greater than the response to bimodal, thermotactile stimulation. Although there is integration, it is sublinear at the population level in the cortex. This suggests there are inhibitory interactions in the thermotactile pathway before cortical integration. If there were no interactions, we would expect the arithmetic sum of the unimodal responses to be equal to the bimodal response. In other words, the response to the whole (bimodal) is less than sum of the parts (unimodal). Strikingly, we found in humans that the rate at which the intensity of cold sensations increases is slower with touch than without touch, highlighting that touch introduces non-linearities in the perception of cold.

Although the observation in rodents do not rule out our model for cooling and tactile interactions, they cannot confirm it either. Importantly, our imaging results are only from a subset of excitatory neurons which express GCaMP6s, whereas our
electrophysiological recordings were performed across cortical layers and were not specific to a neuron population. Moreover, we only performed recordings at a single region, SI, which might not be the main brain region supporting cooling sensation. A recent study proposed the insular cortex (IC) to contain the primary cortical representation of skin temperature (Vestergaard et al., 2023). They showed that optogenetic inhibition of IC impaired cooling detection more effectively than inhibition of SI. Future studies should compare the responses of neurons in IC to temperature changes with and without touch. What is then the role of SI in thermotactile integration? The sensation of wetness is an experience qualitatively different to cold and touch alone. It has been suggested that the sensation of wetness is an integration of cooling and tactile signals. Therefore, the role of SI could be to mediate the sensation of wetness.

The thermal sensation experienced when touching a cooled object are different to the ones felt when a thermally neutral object in contact with our skin decreases of temperature (Green & Schoen, 2005; Green, 2009). Researchers have proposed that thermosensation is an interoceptive sense- it signals the physiological condition of our body. Although we often perceive thermal sensations as external inputs (i.e. the temperature of the object), these sensations are a result of changes in the temperature of our own skin. In this sense, touch externalises thermal sensations. Thus, the value of detecting temperature changes is fundamentally different with and without external inputs. The consequences of small changes in body temperature without external events can be damaging or lethal. However, small changes in body temperature due to external events are natural and experienced in a daily basis. On the other hand, big changes of temperature due to external events are possible and suppose a threat to our body integrity. In this sense, we expect small changes in temperature to be facilitated when they are not accompanied with external events. On the other hand, the thermotactile system combines thermal and mechanical signals during haptic exploration to generate the mental representations of objects (Ho & Jones, 2004; Ho & Jones, 2007; Ho & Jones, 2006; Ho, 2017). Therefore, we expect thermal and tactile information to integrate and facilitate each other during conscious experience of objects.
Figure 8.1. Model of cold-touch interactions. Schematic representation of the interactions between cooling and tactile signals in the nervous system. Populations of cooling- (blue), mechano- (green) and thermotactile-sensitive (yellow) neurons are represented with a schematic of a single unit. Tactile signals dampen incoming cooling signals by exciting an inhibitory interneuron (black) in the spinal cord, which inhibit cooling-sensitive secondary afferents. Cooling-sensitive neurons in the spinal cord project to the thalamus, where they synapse with thalamocortical projections. Mechanosensitive neurons in the spinal cord project to the medulla (dashed line), where they synapse with neurons that have ascending projections to the thalamus. In the thalamus, there are inhibitory interactions between cooling- and mechano-sensitive neurons, mediated by inhibitory interneurons. Here, we propose that two different thalamocortical pathways emerge. One pathway carries cooling information to unimodal cortical neurons involved in the detection of cooling inputs. The other pathway carries cooling and tactile information to thermotactile cortical neurons involved in multisensory object perception which includes intensity estimation. These pathways could be co-localised in the same cortical region or target different brain regions.

8.3 Limitations and future directions

Our non-tactile cooling stimulator has limitations that should be addressed in future research. First, our stimulator does not control for airflow intensity online. We measured offline the airflow generated by dry ice sublimation and concluded that it is unlikely to stimulate mechanosensitive fibres. However, we cannot be certain that the airflow was always below threshold for activating mechanosensitive fibres. Second, our stimulator works optimally within a limited temperature range. We can only deliver innocuous cold sensations. The stimulator is capable of eliciting noxious cold sensations, but at a slower rate compared to available contact cooling stimulators.
(Leone et al., 2019). Our stimulator cannot deliver non-tactile warming, so we have limited our studies to cold sensations. We therefore cannot draw conclusions about the interactions between warming and mechanical signals. Strikingly, laser studies, which can heat the skin without any mechanical input, have only focussed on noxious warm sensations.

Addressing these limitations would strengthen the conclusions that can be drawn from the results and further understand the interactions between thermal and mechanical signals. Additionally, we showed in Chapter 2 our non-tactile cooling stimulator can deliver different stimulus profiles. In this thesis, we have used one type of stimulus profile for all experiments. Future studies can leverage other stimulus profiles to study focal, cold sensations during longer periods of time. For instance, a study found that the Thermal Grill Illusion is not affected by tactile input, challenging the notion that this illusion is a result of spinal interactions. However, their non-tactile cooling lacked spatiotemporal control (Ferrè et al., 2018). The site of the mechanism underlying this illusion is still unclear. We could combine our stimulator with a warming one of similar characteristics to study the Thermal Grill Illusion and further understand somatosensory interactions.

In both our rodent experiments, mice were anaesthetised with isoflurane. This does not retract the validity of our data given the effects of isoflurane on neural activity. In fact, our recordings likely reflect features of thermotactile integration without attentional and motion confounds (Bimbard et al., 2023). However, it limits the conclusions we can draw from this data about conscious, awake thermotactile perception. Identical recordings were performed in awake mice (Schnepel et al., 2023). The main findings of our anaesthetised recordings were also observed in the awake recordings. Additionally, results from behavioural experiments were in line with cortical encoding of bimodal, thermotactile stimulation. Namely, the perception of threshold level cooling or mechanical stimuli was enhanced when they were presented simultaneously compared to single presentation. Strikingly, this observation is inconsistent to what we found in humans for detection of near-threshold cooling.

There are several differences between our human and mice experiments. This limits the strength of the connections we can draw between the findings presented in this thesis. First, the stimuli had different spatiotemporal arrangements due to practical and methodological constraints. For instance, cooling and touch were delivered to the back of the hand in the human experiments in Chapter 3. Additionally, touch
preceded cooling by 2 s and the filaments were bracketing the cooling point (Figure 3.1). On the other hand, in the rodent experiment in Chapter 6, cooling was delivered to the palm and started at the same time as touch, which was targeted to the arm. Second, the tactile stimulators in the human experiments were von Frey filaments, whereas we used a vibrotactile stimulus in the rodent experiments. Each stimulator preferentially activates one mechanosensitive fibre type (Saal et al., 2017) and each may interact differently with cooling-sensitive fibres. Third, our electrophysiological recordings were performed with a contact cooling stimulator due to logistic and methodological constraints, whereas widefield imaging was performed with our non-tactile, focal cooling stimulator.

Moreover, we did not perform any human recordings to compare the encoding features across species. This does not retract the validity of our rodent recordings, which, for the first time, show cortical responses to thermotactile stimulation in the primary somatosensory cortex. However, we cannot directly extrapolate the findings of our neural recordings to explain how the human brain encodes thermotactile information. Additionally, our psychophysical experiments were designed to provide insights about neural interactions at different steps in the thermotactile pathway. On the other hand, our recordings in mice were all targeted to one region, the primary somatosensory cortex. Therefore, we lack neural recordings along the thermotactile pathway that would allow us to confirm or reject our proposed model for thermotactile interactions. To this end, we need presynaptic and postsynaptic recordings at the spinal cord, thalamus, SI and IC during unimodal and bimodal thermotactile stimulation. Finally, bridging human and rodent work would be facilitated by computational work. For instance, the model of divisive normalisation seems to explain the behavioural results and neural recordings observed in the superior colliculus during multisensory integration (Coen-Cagli & Solomon, 2019; Ohshiro et al., 2011). However, similar computational models are lacking for the thermotactile system.
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