SMALL DIAMETER CUTANEOUS AFFERENT STIMULATION AND ITS EFFECT ON BEHAVIOR IN HUMANS

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Abstract
Sensation and behavior are linked dimensions in human lives. Experiencing a gentle caress from a loved one is very different than getting our hand burnt on a hot stove. However both those stimuli are signaled in small diameter cutaneous afferents and have an inherent affective valence that modulates our actions. Pain is transmitted by thinly myelinated A∂ fibers and unmyelinated C fibers, and affective touch is mediated by unmyelinated C-Tactile mechanoreceptors (CT). Both critical for survival, pain and pleasure sit on opposite ends, with pain serving avoidance and pleasure eliciting approach motivation. This thesis investigates the impact of painful and pleasant stimuli on our behavior and the brain mechanisms involved in these processes. Our research population includes healthy subjects and a group of carriers with a rare hereditary sensory and autonomic neuropathy type V (HSAN-V), causing a selective loss of small diameter afferents. In **Paper I** we addressed whether in healthy subjects part of the activation during pain can be accounted for motor processing, supporting the idea of a central multidimensionality of pain. Areas including the cingulate, motor cortex, thalamus and cerebellum serve a motoric role during pain. In **Paper II** we focused our attention on the perception and reaction to thermal pain in a group of HSAN-V patients. Using the same design as in Paper I, we addressed the effects of lower density of small diameter cutaneous fibers on the experience of pain. The patients showed difficulties in recognizing and reacting to pain suggesting that their peripheral fiber loss resulted in unreliable and less adaptive responses to acute pain. In **Paper III** we addressed the patients' ability to appreciate affective touch, conveyed by CT fibers. The critical characteristic of CT fibers is their velocity dependent response pattern for stroking stimuli, with higher firing for intermediate speeds (~3 cm s^{-1}) compared to very fast or very slow ones. This firing pattern matches linearly with the touch pleasantness ratings in healthy subjects. The patients did not show the same pleasantness ratings pattern across velocities suggesting an alternative route for affective touch processing. In **Paper IV** we investigated the relationship of CT fibers to the reward system in the brain by creating a feedback-based task in which healthy subjects could decide to receive the stimulation they preferred the most. CT optimal speeds were the most preferred and elicited activation in reward related areas like the caudate, insula and prefrontal cortex. In conclusion, this thesis provides an understanding of the cerebral and behavioral mechanisms underlying the experience of painful and pleasant somatosensory stimuli in healthy individuals and following thin fiber neuropathy.

Keywords: *pain, cingulate, action, fMRI, touch, hairy skin, reward, NGFB mutation.*
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LIST OF PUBLICATIONS

This thesis is based on the following studies, which are referred to in the text by their Roman numerals.

I. Perini I, Bergstrand S, Morrison I. Where pain meets action in the human brain.
In press, Journal of Neuroscience

II. Perini I, Ceko M, Olausson H, Minde J, Morrison I. Effects of a human nerve growth factor beta (NGFB) mutation on cerebral structure and function in pain.
Manuscript

III. Morrison I, Löken L, Minde J, Wessberg J, Perini I, Nennesmo I, Olausson H. Reduced C-afferent fiber density affects perceived pleasantness and empathy for touch.
Brain 2011: 134; 1116–1126

IV. Perini I, Olausson H, Morrison I. The relationship of pleasant touch pathways to reward processing: an fMRI study.
Manuscript

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Abbreviations

ACC Anterior Cingulate Cortex
ADHD Attention-Deficit Hyperactivity Disorder
ATP Adenosine Triphosphate
ATS Advanced Thermal Stimulator
BOLD Blood Oxygenated Level Dependent
CMA Cingulate Motor Area
CT C-Tactile fibers
dlPFC Dorsolateral Prefrontal Cortex
EIN Excitatory and Inhibitory Networks
fMRI Functional Magnetic Resonance
LFP Local Field Potential
HDR Hemodynamic Response
HSAN-V Hereditary Sensory and Autonomic Neuropathy – type five
NGFB Nerve Growth Factor Beta
NWR Nociceptive withdrawal reflexes
pSTS Posterior Superior Temporal Sulcus
QST Quantitative Sensory Testing
SDT Signal Detection Theory
STT Spinothalamic Tract
VAS Visual Analogue Scale

INTRODUCTION

Behavior: a window on sensation

"Pronto a far tutto, la notte e il giorno
sempre d'intorno, in giro sta."

"Ready for everything by night or by day,
always in bustle, in constant motion."

-Il Barbiere di Siviglia-

We often think of behavior in terms of the consequences that it can produce on us. We look at how a behavior is caused, where it leads to and what is its function. Behavior is a fundamental "tool" used by the body and the brain to benefit the entire system. Constant information and feedbacks are transmitted between the periphery and the brain to inform whether the outcome of an action has fulfilled the body's needs. Such continuous communication and reciprocal modulation allow for an adequate protection and a good functioning of the system. Behavior is crucially linked to sensation and in this thesis we regard it not only as a consequence but also as an important perspective for understanding sensation. Most importantly we aimed to understand what brain mechanisms are crucial for the implementation of a certain action relevant for the well being of our system.

This thesis investigates the link between salient stimuli and behavior with a particular focus on the brain structures behind the scenes of such mechanisms. In particular we looked at two very different categories of stimuli with high affective valence but opposite effects on our behavior: pain and pleasure.

In Paper I we investigated behavioral and cerebral mechanisms involved in the experience of acute thermal pain. Most of the cortical activation during pain is not pain specific but is also involved in other sensory modalities (Mouraux *et al.*, 2011). We propose that the urgency to react to pain is such a major aspect during the experience of pain that parts of the activation during painful stimulation are responsible for motor reactions to it. We addressed such issue in Paper I.

In Paper IV we investigated the link between pleasurable tactile stimulation and the reward system in the brain. We did this by letting the subject control the stimuli he/she received by selecting the most preferred stimulations, consisting of soft brush strokes at optimal and non-optimal C-tactile fibers velocities. It has been shown that the activation pattern of C-tactile fibers is velocity dependent and correlates with subjective pleasantness ratings (Löken *et al.*, 2009; Morrison *et al.*, 2011). Intermediate velocities are reported as more pleasant than very slow or very fast ones, and such pattern matches the CT fibers firing. Importantly we addressed pleasantness by investigating

behavioral changes (preferences) and therefore by looking at whether the response was triggered by the reward system, without the need of any subjective rating.

The motivation to act and react to salient stimuli is highly dependent on the ability to perceive them adequately. If a message is transmitted poorly, then the content will not be fully understood. Likewise, inefficient peripheral inputs obscure the message that reaches the cortex. In Paper II and III we investigated the importance of the efficiency of a signal during pain and affective touch in a population of subjects with a rare type of hereditary sensory and autonomic neuropathy (HSAN, type V) causing a selective loss of small diameter afferents, known to convey pain and affective touch. The disruption of the peripheral system results in a less efficient signaling, in altered sensation and less adaptive behavioral response.

The somatosensory system: from pain to affective touch receptors

A ladybug flying onto your leg, the gentle caress of a welcome friend on your shoulder, a vigorous grasp of your arm, or a painful pinprick on your back; all these events give distinct sensations that originate in the peripheral receptors in the skin. The various specialized receptors and peripheral nerve fibers that support this information are categorized according to their diameter and conduction velocity - parameters that are linearly correlated (Gardner, & Johnson, 2012). Large diameter fibers are the fastest because their axons are surrounded by myelin sheaths that provide a better insulation and facilitate the propagation of the signal. This thesis mainly focuses on small diameter fibers that include both thinly myelinated A∂ and unmyelinated C fibers and have comparatively slow conduction velocities (0.5-30 m/s) (Basbaum, & Jessell, 2012). These fibers respond to noxious, thermal and mechanical stimuli.

Noci-ceptor (Latin, *nocere*, to harm) is a term coined in the 1906 by the famous physiologist Sir Charles Sherrington to describe afferent neurons signaling information on tissue-threatening stimuli: *"Remembering that the feature common to all this group of stimuli is that they threaten or actually commit damage to the tissue to which they are applied, a convenient term for application to them is 'nocuous'. In that case what from the point of view of sense are cutaneous pain-nerves are from the point of view of reflex reaction conveniently termed noci-ceptive nerves."* (Sherrington, 1906). The ability to detect injury has a clearly adaptive implication not only because it allows the localization of harmful stimuli, but also because it informs the body of what could potentially result in tissue damage. In addition, it also sends continuous signals from an already damaged tissue. Such properties highlight the essential

protective role of nociceptors. A∂ afferents in particular are responsible for a sharp and pricking first pain sensation (Greenspan, & McGillis, 1991; Torebjork *et al.*, 1984) such as when stepping on a sharp object or inadvertently putting a hand on a hot stove. This information, supported by discriminative details mediated by large diameter Aß fibers, is essential for a fine localization of the harmful stimulus and a proper reaction to it. As an additional, C fibers mediate a diffuse, burning second pain sensation (Basbaum, & Jessell, 2012).

Low-threshold mechanoreceptors signal touch and are mainly innervated by fast conducting Aß fibers. There are different receptors that code different aspects of touch and are classified according to their adaptation properties to a long-lasting stimulus and differ in their location in the skin. The hairy, but not glabrous, skin has a type of mechanoreceptor that is innervated by an unmyelinated (C) fiber, and responds to innocuous mechanical stimulation, in particular to slowly moving touch, usually reported as pleasant (Löken*, et al.*, 2009). This characteristic suggests that these fibers are likely to mediate hedonic properties of gentle touch.

Different stimuli encoded by the somatic sensory system give rise to distinct sensations that enable us to discriminate whether we are feeling a caress or we've been bitten by a mosquito. The way the nervous system orchestrates sensation is not fully understood and there are mainly two hypotheses regarding such issues (Perl, 2007). The first one suggests that the nervous system is specialized according to the different sensory modalities, with a modality specific direct communication between periphery and central areas. This "labeled line" view originated during the late 19th century, following the observation that specific spots in the skin evoked different sensations (Norrsell *et al.*, 1999). The other hypothesis suggests a more dynamic pattern of converging inputs of somatosensory afferents within a central network (Craig, 2003b). Looking specifically at pain, it is proven that there are specific cells carrying nociceptive information. However, there is also evidence of the involvement of unspecific cells (*i.e.* cells responsive to various aspects of tactile sensitivity) providing homeostatic information during the experience of pain (Craig, 2003b). This suggests that the neural representation of pain probably involves both specific and converging mechanisms, from pure nociceptive inputs to homeostatic and motivational regulations.

Pain pathways

Information about potential or actual tissue damage but also thermal changes is conveyed by thinly myelinated A∂ and unmyelinated C afferents via the

spinothalamic (STT) tract. The first-order axons proceed into the superficial layers of the dorsal horn and synapse with the second order (Basbaum *et al.*, 2009; Basbaum, & Jessell, 2012). Here, at the spinal cord level, they decussate and ascend to the thalamus in the STT tract. Then the axons reach thalamic regions including the ventro posterior and the ventro medial nuclei (VMpo (Craig *et al.*, 1994)) where they synapse with third order neurons. The STT tract arrives at 3rd-order synapses in the contralateral somatosensory cortex SI. Other projection neurons via connections in the brain stem, project to cingulate, insula and the amygdala, contributing to the affective component of the pain experience. (Basbaum*, et al.*, 2009).

Touch pathways

Discriminative information of touch, conveyed by large myelinated Aß fibers, reaches the cortex via the dorsal column medial lemniscal system (Gardner, & Johnson, 2012). The cell bodies of the first order neurons are located in the dorsal root ganglia. The axons of the first order neurons proceed in the dorsal column that constitutes of the gracile and cuneate fasciculi, and reaches the homonymous nuclei at the level of the medulla where they synapse with the second order neurons. Here the axons of the second order neurons decussate and synapse with the third order neurons in the ventral posterior nucleus of the thalamus and finally reach the cortex. More specifically, the dorsal column medial lemniscal system terminates in the contralateral somatosensory cortices SI and SII (Maeda 1999) in a somatotopic fashion (Penfield, & Boldrey, 1937; Ruben *et al.*, 2001) and in the insular cortex (Schneider *et al.*, 1993).

CT fibers and affective touch pathways

The skin is an effective organ in determining whether the page we're touching is smooth or if the table is sticky, but is also efficient in sensing the affective value that touch may have in a social interaction. It can sense whether the touch we are receiving has the characteristics for being potentially emotionally relevant. The hedonic valence of a caress is not only a product of central mechanisms but starts already in the skin that, far from being purely discriminative, can be considered a "social organ" (Morrison *et al.*, 2010). A subtype of slowly conducting, unmyelinated, low-threshold C-fibers afferents in humans has been shown to signal dynamic gentle stroking on hairy skin. Like Aß afferents, they are very sensitive to deformation and respond to forces as low as 0.3 mN (Vallbo *et al.*, 1999). These afferents have been classified as C-tactile (CT afferents) and have been found in hairy skin only, specifically on

arm, leg and face (Nordin, 1990) but never on glabrous skin such as the palm of the hand and the soles of the feet (Vallbo*, et al.*, 1999; Wessberg *et al.*, 2003). They respond at a high frequency of 50-100 impulses/s to innocuous stimuli such as slow, soft, light stroking (Vallbo *et al.*, 1993).

CT impulses are conducted at a slow speed of about 1m/s (range 0.6m/s – 1.3 m/s (Vallbo*, et al.*, 1999). CT activity is highly dependent on previous stimulation, showing a decrease of response as several identical stimuli are presented. CT fibers show poor response to high-frequency (> 50 Hz) vibration (Wiklund Fernström, 2004) and to rapidly changing stimuli.

Perhaps the most intriguing property of these fibers is their dependence on stimulus' velocity. Unlike Aß fibers that show higher firing frequency the faster the stimulation's speed, CT fibers show peak impulse frequency in response to stoking stimuli at mid slow velocities (Vallbo*, et al.*, 1999). When the skin is gently stroked at different velocities ranging from 0.1 cm s^{-1} to 30 cm s^{-1}, the CTs respond most vigorously to intermediate speeds ranging from 1-3 cm s^{-1}. Crucially when subjects are stimulated at the same velocities and asked to report the pleasantness of the stroking, they perceive these intermediate velocities as the most pleasant.

What cerebral areas are involved in the stimulation of CT fibers? Evidence suggests that brush stroking on hairy skin at intermediate velocities activates posterior insula and posterior superior temporal sulcus (pSTS), prefrontal cortex and caudate (Bennett *et al.*, 2013; Gordon *et al.*, 2013; May *et al.*, 2013; Morrison*, et al.*, 2011). As mentioned before unmyelinated fibers synapse in lamina I and II (Craig, & Blomqvist, 2002; Kumazawa, & Perl, 1977; Sugiura *et al.*, 1986), reach the posterior portion of the ventromedial nucleus of the thalamus (Craig, 2008; Craig, & Blomqvist, 2002; Craig*, et al.*, 1994) and finally the insula via the STT tract (Coghill *et al.*, 1999). This tract is well-suited for interoceptive information and therefore suggests that CT fibers might be providing affective more than discriminative information of touch (Morrison, 2012).

Studies on two patients lacking Aß fibers as a result of a rare neuronopathy, offered a unique chance to inspect activation following selective stimulation of CT fibers by gentle brush stroking on the arm (Olausson *et al.*, 2002; Olausson *et al.*, 2008). Such pure CT stimulation in healthy subjects is impossible to achieve since any tactile stimulation will always activate myelinated fibers as well. The lack of Aß fibers in the patients compromised their discriminative tactile ability, but not the ability to detect gentle stroking on their arms which was experienced as vague, weak and pleasant in a forced choice rating (Olausson*, et al.*, 2002). Such ability was not seen when the patients were stimulated on the palm, where CT fibers have never been found. Furthermore

activation in posterior insula following gentle brush stroking was seen in these patients, suggesting that the insula is a cortical target area of CT fibers. Additional studies show a somatotopical organization for CT processing in the insula (Bjornsdotter *et al.*, 2009) similar to what has been found for painful stimuli (Brooks *et al.*, 2005; Hua le *et al.*, 2005).

Patients

"In tanto buio lo sguardo è nullo"

"It was so dark I could not see."

-Rigoletto-

The patients investigated in Paper II (n=7) and Paper III (n=10) are diagnosed with hereditary sensory and autonomic neuropathy type five (HSAN-V). There are five types of HSAN, classified according to mode of inheritance, neuropathology and clinical symptoms (Dyck *et al.*, 1983). Generally the HSAN condition implies autonomic symptoms, mild to severe retardation and insensitivity to pain, often manifested by painless fractures, burn injuries, scars and distal mutilation (Minde, 2006). Such symptoms appear early in life, often during childhood. More specifically, HSAN-V, which is the most rare of all types, is an autosomal-recessive condition with the mutation located on chromosome 1 and affecting the nerve growth factor beta (NGFB) gene (Einarsdottir *et al.*, 2004).

This mutation selectively alters the development of thin-diameter sensory afferents, without interfering with other aspects of the central nervous system. Differently from other types of HSANs, the patients do not face cognitive abnormalities, and no autonomic-related deficits were detected in R-R variations during normal and deep breathing and sympathetic skin response to electrical stimuli was normal in the youngest homozygous but absent in the other two patients (Minde *et al.*, 2004; Minde, 2006). HSAN-V patients have difficulties in perceiving pain yet have intact discriminative abilities like touch direction discrimination, pressure and vibration. A moderate loss of thinly myelinated A∂ and a severe loss of unmyelinated C fibers are the major consequences of the mutation, leading to bone necrosis, painless fractures, osteochondritis and neuropathic joint destruction. The carriers live in Norrbotten, the most northern region of Sweden. Dedicated investigations of the genealogy of this condition allowed to identify the common ancestor, a man who in the 1600s founded Vittangi, a small town in the Tornio Valley ("Tornedalen") (Minde, 2006). Consanguinity allowed the mutation to persist

into the present population. At present there are three homozygous patients that are severely affected with limited mobility due to join destruction, and sixty-two heterozygous patients that are either less affected or entirely asymptomatic. The three homozygous patients were investigated in both papers and a brief summary of their clinical conditions will follow.

The youngest of the homozygous patients (Figure 1A), born in 1992, has a hist ory of painless fractures starting since the age of four. He was admitted to the hospital for a swollen painless foot that was revealed being caused by multiple painless fractures. Few years later he was faced again with painless fractures and gradually developed neuropathic deformities in both ankles. The following years he started suffering from arthritis, osteochondritis and knee joint neuropathies. Already at age of twelve his mobility was severely affected, forcing him on a wheelchair for most of the time.

Even if deep pain sensation is mostly affected, superficial pain is also altered causing painless burns and difficulties in detecting painful stimulation such as detecting hot water when showering.

A young woman (Figure 1B), born in 1983,, first presented with painless fractures in her right leg when she was seven years old. In the following years she developed neuropathic joints, accompanied by fractures in her left leg and right hip destruction leading to leg length disparity of 12 cm. As with the previous patient, she presents with alterations in superficial pain with a reduced ability to feel burning sensation and with lack of protective reflexes (Minde*, et al.*, 2004).

The third homozyguous patient investigated is a man born in 1965 (Figure 1C). When only seven years old he suffered from a destruction of the right knee following a fracture of the tibia and during the next year he fractured both his ankles. He presented with neuropathic arthropathies in his knees and ankles by the age of 11, and later on, when 32, in his lower back. A few years later he developed spondylolisthesis in his lower back, and myelopathy. However, this patient does not suffer from painless burns suggesting he has an adequate perception of superficial pain (Minde*, et al.*, 2004).

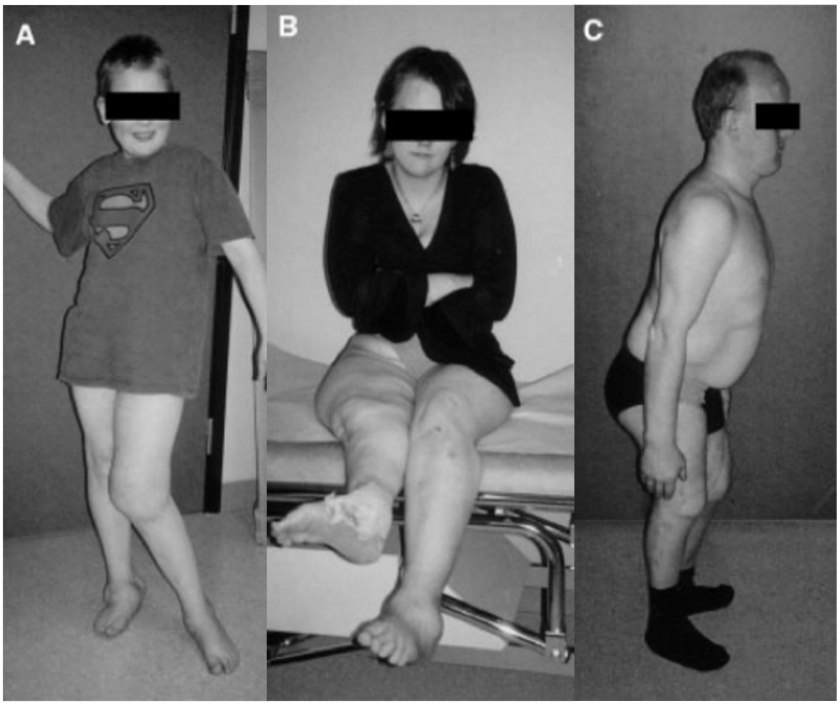

Figure 1. The three homozygous patients investigated in Paper II and Paper III, with severe neuropathic arthropathies at knee and ankle joints *(with permission of Jan Minde)*.

AIMS OF THE THESIS

Paper I. The urgency to react is tightly linked to pain sensation. What brain areas reflect motor reactivity during pain?

Paper II. What are the effects of a hereditary small fiber neuropathy on motor reactivity during pain?

Paper III. What are the effects of a hereditary small fiber neuropathy on affective touch perception and empathy for touch in others?

Paper IV. From subjective ratings to behavior. Does affective touch trigger the reward system?

METHODOLOGICAL CONSIDERATIONS

PAPER I. STIMULI AND DESIGN

Thermal painful and nonpainful stimulation were applied manually to the left hand of neurologically intact participants using a thermode that reached the target temperature before it was placed on the skin. Visual cues displayed on a monitor inside the scanner guided the experimenter in delivering the stimulation at the correct time. The order of the stimulation was pseudo-randomized, and there were four runs in total. Subjects had to respond to either painful or nonpainful stimulation by making a button press with their right hand, at a visual cue onset. The instructions for each run were counterbalanced, with half of the runs (4 runs in total) requiring responses during painful stimulation, and half during nonpainful.

PAPER I. DESIGN CONSIDERATIONS

The aim of this study was to address whether an ongoing noxious stimulation would modulate motor action differently compared to an innocuous one, and at the same time to address the contribution of motor related areas to the general cerebral activation during pain. Four different stimulation temperatures (painful and nonpainful heat and cold) were used in the design. Painful heat has almost immediate damaging consequences on the tissue whereas painful cold takes longer even to be perceived as discomforting, especially on a small skin surface (the stimulation area in this study was 9cm^2). For this reason painful heat was the most adequate for the investigation of motor reactions to acute pain and we could have addressed our scientific questions by using heat temperatures only. However the decision to use both heat and cold temperatures was mainly to have a complete set of thermal stimulations and to investigate possible differences in painful cold and heat processing between healthy subjects and the HSAN-V patients (Paper II).

Thresholding

Prior to the experiment in the scanner, quantitative sensory testing (QST) was used to examine the subject's thermal detection and thermal pain thresholds. The measurements were obtained with a PATHWAY Advanced Thermal Stimulator (ATS) machine (PATHWAY Model ATS, Medoc Ltd., Ramat Yishai, Israel). A 3 x 3 cm ATS thermode was firmly placed on the dorsal part of the left hand, while the right hand was holding a mouse pad. The thermode started at a baseline temperature of 32°C and decreased for cold threshold or increased for warm thresholds at a rate of 1°C/s. During detection thresholds testing, subjects were instructed to press a mouse button as soon as they felt a change in the temperature. During painful thresholds testing, subjects had to press the button as soon as the stimulation was

painful. Such method is widely used in clinical testing and it was sufficient in this study for detecting painful versus nonpainful stimulation temperatures. However, the study would have perhaps benefitted of a more thorough investigation of the experience of pain. The method of limits does not directly address qualitative aspects of pain because no evaluation of subjective feelings are recorded when the subject reaches the pain threshold. Such details are highly relevant for a full characterization of the subjective feeling of pain. An investigation of the perceived intensity of the stimulation by using a verbal numeric rating scale and descriptors provided by questionnaires (*i.e.* McGill pain questionnaire) would have offered a more integral picture of the individual's experience.

Task and Laterality

Motor facilitation during pain is a common phenomenon experienced on a daily basis. Holding a hot cup speeds up our behavior in order to maximize the outcome of our actions. Faster movements allow us to reduce tissue damage and at the same time avoid dropping our favorite drink. We tried to recreate a similar context by investigating the actual changes in voluntary behavior when an individual is exposed to painful sensation. A more ecological way to address such behavioral changes would have been to have stimulation and button press response on the same side. However, we decided to have stimulation and button press on separate hands, which would help in separating stimulus processing and motor output at the cortical level.

PAPER II. STIMULI AND DESIGN

Thermal painful and nonpainful stimulations were applied to HSAN-V patients and delivered in the same fashion as in Paper I. A practice trial inside the scanner was performed to make sure that the patients had understood the instructions.

PAPER II. DESIGN CONSIDERATIONS

Cognitive abilities

The patients have been reported to have normal cognitive abilities (Minde, 2006) and normal social and work lives. Except the youngest homozygous, diagnosed with attention-deficit hyperactivity disorder (ADHD), who took longer time during the practice trial, no obvious abnormalities during the trial and experimental sessions were noticed. However no specific cognitive testing was carried out. Future investigations will consider adding cognitive

tests in order to confidently exclude the possibility of a subtle cognitive confound in task understanding.

Thresholding

The patients showed similar pain detection thresholds as controls whereas their performance in the pain-motor task, highly based on the ability to discriminate painful and nonpainful sensations, was poor. As previously discussed, the method of limits, widely used in the clinic, does not provide a thorough understanding of the subjective experience of pain, but offers basic information that the experimenter can start building from. This is particularly true in the case of this population of carriers whose discrepancy in their thresholds and task performance reveals how the method of limits might characterize pain sensation in the specific context of clinical testing but can not necessarily be generalized to other contexts, from experimental settings to everyday life situations. Such differences between thresholding and task result, might be explained by the different methodologies used during the thresholding session and during the task. During the thresholding, the subject felt the temperature raising or decreasing and decided when the temperature felt painful. In contrast, during the experimental setup the patient received the stimulation at a set temperature. In the first method, the patient indicated a perceived change in a ramping temperature whereas in the second method during the experimental task, the patient reacted to the stimulation temperature per se. The thresholding method provided the subject with more information than the other method, especially in the case of painful temperatures. In fact during thresholding the subject knew that the thermal changes were heading towards heat or cold extremes, whereas during the experimental task they had no other cues other than their sensation to a set stimulation temperature. This might explain the discrepancy in the findings and it raises the issue of the plausible necessity of orthogonal methods in the assessment of pain.

PAPER III. STIMULI AND DESIGN

To address if the peripheral abnormalities associated with the nerve growth factor beta gene (NGFB) mutation have an effect on social touch, we explored the subjects' first-person experience of affective touch and their ability to perceive hedonic touch in others. In addition, an introspective questionnaire investigating touch communication (TACTYPE questionnaire) was added to

the testing, and discriminative abilities were tested using a tactile direction discrimination psychophysical test (Deethardt, & Hines, 1983).

PAPER III. DESIGN CONSIDERATIONS

TACTYPE

Understanding tactile communication via an introspective evaluation of one's social experience of touch is the main goal of the 15-items TACTYPE questionnaire. Such questionnaire gives a basic understanding of the conscious communicative experience of interpersonal touch with a focus on the active communicative effects (Deethardt, & Hines, 1983).

Discriminative touch

Tactile perception abilities were investigated for both discriminative and affective touch in order to rule out the possibility of a general alteration in touch sensitivity. Another aim was to demonstrate that loss of small diameter fibers has no impact on discrimination, typically conveyed by large diameter fibers. Tactile direction discrimination was measured by manually moving a probe at 1 cm s^{-1} in a proximal or distal direction over the skin of the left arm (Norrsell *et al.*, 2001; Olausson *et al.*, 1997). The subjects had to report the direction of the movement, keeping eyes closed. The task performance results in a response profile area, representing the level of tactile direction sensibility.

Felt and seen affective touch

Touch communication relies not only on the ability to decipher other's intention towards us but also by understanding the way others interact with each other. To investigate both aspects the subjects were presented with either tactile stimulation or with videos of other people being touched. Given that speed is the crucial aspect in linking CT fiber activity to pleasant sensations, different velocities of stroking were used for both felt and seen touch. Touch stimulation was delivered manually by single brush strokes over 10 cm of left forearm skin at five different velocities: 0.3, 1,3, 10 and 30 cm s^{-1}. Videos showed arms being caressed using the same five different velocities. After each stimulation subjects were instructed to rate how pleasant it felt on a visual analogue scale (VAS) displayed on a computer screen, with endpoints unpleasant to pleasant, midpoint being neutral. After each video clip, subjects had to rate on the same VAS how they thought the person in the video felt the stimulation.

PAPER IV. STIMULI AND DESIGN

A series of single low force, gentle brush strokes at 5 different velocities (0.3, 1, 3, 10, 30 cm s^{-1}) were manually delivered on the dorsal part of the left arm while the subject was lying in the scanner. A 7 cm wide soft artist brush was used to deliver the stimulation. After the stimulation, the subjects were instructed to indicate whether they wanted to receive the same stimulation or change to another one. A speed-meter on the monitor inside the scanner (invisible to the subjects) guided the experimenter in the delivery of the correct stimulation speed. A cue (visible to the subjects) signaled when the subject had to make the choice by pressing a button. The subject could choose to repeat the stimulation up to two times, with a resulting maximum of three stimulations in a row (to avoid "stay" biases). Each stimulation velocity was repeated for at least 6 times.

PAPER IV. DESIGN CONSIDERATIONS

Task

Investigating the reward system calls not only for an evaluation of pleasantness but also for behavioral preference. The aim of the task used in this study was to address the behavioral changes following the activation of the reward system and hence characterizing pleasantness by means of choice rather than rating. Such design is innovative because it offers an alternative method of pleasantness investigation, in which the subject is asked to "translate" the sensation into a rating scale system. Such discrete choice (repeat or change) is a straightforward way to address how the reward system works and provides an orthogonal measure for exploring preferred stroking velocities.

Velocity vs. duration of stimulation

By definition, dynamic touch is related to the speed of stroking and traveled distance. Since velocity of stroking is an essential aspect in touch hedonics we could not avoid the issue of having different stimulation times for different strokes, since we considered it important to keep the stimulation distance constant. We could have had same duration and different number of strokes but we preferred to have the subject focusing on the characteristics of one single stroke avoiding possible confounds related to the number of stimulations or the direction of the stroking. In addition previous studies that took those issues into account, by either keeping same distance or same timing, showed no difference in the results, meaning that stimulation speed

is the most important parameter when it comes both to perceived pleasantness and cerebral activation (Morrison*, et al.*, 2011).

FUNCTIONAL MAGNETIC RESONANCE IMAGING

Functional magnetic resonance (fMRI) is a widespread technique to investigate cerebral functioning. Its non-invasive character has made it one of the most used methods for shedding light on brain mechanisms. The major assumption behind fMRI is that active areas (*i.e.* neuronal activity) demand higher amounts of glucose and oxygen compared to areas in a baseline state. Adenosine triphosphate (ATP) is the main energy supply to the cells and is produced by aerobic glycosis in the mitochondria. Glucose and oxygen are therefore critical substances for the production of ATP. Given that blood supplies the tissue with these substances, fMRI offers an indirect measurement of neural activity by detecting hemodynamic metabolic changes in the brain. More important than the increase of oxygen per se is the proportion of oxygenated and deoxygenated blood, the crucial property for fMRI. When a brain area is at a baseline state, there is a balance in the amount of oxygenated and deoxygenated hemoglobin in the vessels and capillaries. When an area is activated, the delivery of oxygen results in a lower amount of deoxygenated compared to oxygenated hemoglobin in the local increase of blood flow and blood volume. Such disproportion is due to the fact that the amount of oxygen delivered exceeds the actual need resulting in a higher local concentration of oxygenated blood that can be measured thanks to the magnetic properties of hemoglobin. Oxygenated hemoglobin is diamagnetic whereas deoxygenated hemoglobin is paramagnetic. In a basal state the presence of deoxygenated hemoglobin results in the creation of microscopic field gradients around the vessels and capillaries causing a decrease in the signal of a gradient-echo T2* sequence (Jezzard, & Ahmed, 2005). When an area is active, the increase of oxygenated hemoglobin decreases the relative amount of deoxygenated blood, attenuating the field gradients and restoring the gradient-eco signal. fMRI benefits of the attenuation of what in principle is an artifact in the signal induced by the magnetic properties of the blood. This effect is the foundation of fMRI and is called the blood oxygenated level dependent (BOLD) contrast (Ogawa *et al.*, 1990). The BOLD signal increase after the onset of neuronal activation is delayed and follows a typical pattern characterized by an initial dip, an overshoot peak and a final undershoot. The hemodynamic response (HDR) typically lags the neuronal activity by around 2 seconds (*i.e.* the time needed for the vascular system to provide the needed energy supply) and it lasts for about 12 seconds, reaching the peak only after 6 seconds. Given that

arteries are fully oxygenated in a normal state, the major signal change occurs at the level of capillaries, venules and draining veins (Menon, & Kim, 1999). The anatomical characteristics of the vascular system in the brain can differ quite substantially. Differences in the diameter of the vascular system can vary from μm in the capillaries to mm in the draining veins affecting the localization of the neuronal activity, which is more accurately localized in small capillaries than in bigger veins. However the spatial resolution scale is fairly good, with the hemodynamic response reflecting neuronal activity in a range of a few millimeters (Kim *et al.*, 2004). As mentioned before, fMRI offers an indirect measurement of neuronal activity and issues related to neurovascular coupling are still a matter of discussion.

An elegant study by Logothetis et al. (2001) investigated the relationship between the BOLD contrast and neuronal signals by simultaneously recording neuronal activity intra-cortically while running fMRI in *Macaca mulatta* monkeys. The results provide a useful insight on neurovascular coupling, and show that fMRI does not measure soma spiking activity (*i.e.* the output of a neural population) but more the local field potential (LFP) that reflects the presynaptic local activity (Logothetis, 2008; Logothetis *et al.*, 2001). In addition the hemodynamic responses reflect a mass action of neurons not necessarily linked to stages of sequential neuronal excitation but more a modulatory balance between excitatory and inhibitory networks (EIN) necessary for an adequate cerebral function (Logothetis, 2008).

The useful ferromagnetic properties of blood are the crucial tool used in fMRI. However the magnetic signal change that follows a metabolic increase in oxygen in a certain area, is small and noisy. This aspect adds complexity to the experimental design and stresses on the need of following statistical analysis for the understanding of the data provided by the fMRI.

Image acquisition and analysis

The information obtained from an fMRI run is essentially a time course of the hemodynamic changes across the brain following a predetermined experimental design. During an fMRI session, a low-resolution functional volume of the brain is acquired several times every few seconds. Each volume is a 3D reconstruction of the brain's metabolic changes and is made up of small voxels (volumetric pixels), cuboid elements representing the smallest unit in which the acquisition image is divided (Smith, 2001). Several brain volumes (100 or more) are acquired during an fMRI scan because of the aforementioned poor signal evoked by hemodynamic changes following a unique event. The goal of fMRI is to isolate a cognitive function by maximizing between

condition variability and minimizing within condition variability. Different conditions are included in an fMRI investigation and compared by subtracting one condition to the other. For example, if one is interested in activation during thermal pain, the design will have intervals of painful stimulation and intervals where no stimulation is presented. The difference between the two conditions will reveal activation related to tactile painful stimulation.

BOLD signal changes within each voxel are collected during the brain scan and used for the subsequent preprocessing and statistical analysis. We implemented a linear modeling approach for the statistical analysis of our data, a method widely used in fMRI data analysis (Friston *et al.*, 1994). During lineal modeling a time course of the BOLD response during the experiment at each voxel is created and fitted to the experimental design pattern (model), giving an estimation of which voxels are involved in the aspects investigated in the design. Such method, aims at explaining the variance in the time course as a linear combination of explanatory variables (design variables) and noise and it is described by the following equation:

$$y(t) = x(t)ß + c + e(t),$$

where *y(t)* is the data, *x(t)* is the set of model's regressors, ß is the parameter estimate for *x(t), c* is a constant and *e* is noise. The parameter estimate value ß is the key element in the analysis, reflecting the amplitude of the regressors. A large value of ß will reflect a strong fit to the model. Following our previous example, if the voxels lie in an area involved in pain processing, they will show higher BOLD signal (and therefore a higher ß value) during pain compared than rest and their activity over time will match the pattern used in the experimental design.

To make a statistical use of the parameter estimates, their value is compared to their uncertainty (*i.e.* ß/standard error (ß)), resulting in a statistical map of T value for each voxel in the brain. Such value is in turn transformed into either P (probability) or Z statistics and it represents how significantly the data are related to the model. In order to define what areas of the brain are significantly activated, the statistical map is thresholded resulting in a color-coded activation map of the brain.

PATHWAY

The PATHWAY Pain & Sensory Evaluation System (Medoc Ltd; Ramat Yishai, Israel). was used in Paper I and II for thermal thresholds testing and during the experiment in the scanner. The PATHWAY model ATS (Advance

Thermal Stimulator) can deliver temperatures ranging from -10˚C to 50˚C with heating and cooling rate up to 8°C/s. It is a machine widely used in clinical settings for Quantitative Sensory Testing (QST) and for research purposes. It constitutes of an electronic base unit, a heavy-duty integrated cooling unit and a thermode, attached to a cable that ultimately is connected to the electronic base unit. To allow the use of the PATHWAY system in the scanner and avoid magnetic field alterations that would affect the quality of the imaging and the functioning of the machine, an fMRI filter was installed on the wall between the control room and the scanner room. After connecting the thermode to the fMRI filter, the thermode could be used in the scanner room with no additional need of shielding. All the other components were kept in the control room (Figure 2). The ATS thermode is a 3x3 mm probe based on Peltier elements that consist of semiconductor junctions, which produce a temperature gradient, between the upper and lower stimulator surfaces, produced by the passage of an electric current.

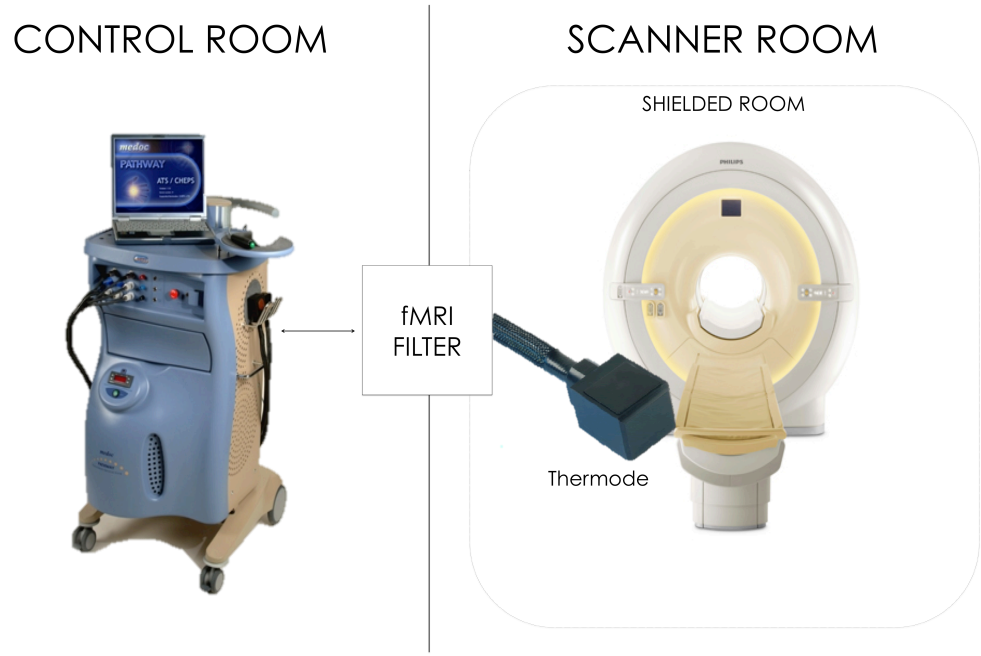

Figure 2. The experimental setup. The PATHWAY machine is kept in the control room. The thermode reaches the scanner room via a connection to the fMRI filter fixated on the wall between the two rooms.

RESULTS

PAPER I. INVESTIGATING THE MOTOR COMPONENT OF PAIN WITH MRI

This study addressed the involvement of motor activation during the experience of pain. We implemented a speeded-response button-press task during painful and nonpainful stimulation to discover any common activation between pain and motor responses. Discovering such common substrates for motor task during both painful and nonpainful stimulation allowed us to localize areas that might serve a generally motoric role during pain. In addition, the task allowed us to investigate whether pain entailed our ability to adjust actions in an appropriate and adaptive way, according to the quality of the stimulation. Subjects were asked to discriminate painful versus nonpainful stimulation by pressing a button. This simple task allowed us to investigate both the ability to recognize noxious versus innocuous stimulation, and the effect of the different stimulations on the motor reactivity.

Pain speeded up reaction times

Performing a motor task while experiencing pain gave rise to a general decrease in reaction times in healthy subjects. Subjects were faster in making a button press response even though the stimulation was on the contralateral side of the response button. This suggests that pain prompted motor reactivity not only specifically to the site of stimulation but at a more general level, affecting the reactivity of the entire system.

Brain structures underlying motor aspects of pain response

The motor task during painful or nonpainful stimulation elicited fMRI activation in the primary motor cortex, cingulate (cingulate motor areas CMA), thalamus, and cerebellum. Such activations were independent of stimulation characteristics *(i.e.* painful or nonpainful) and reflected voluntary button press responses, suggesting that their contribution were mainly motoric.

Brain structures underlying non-motor aspects during pain

Activation in bilateral anterior and right posterior insulae during painful stimulation were found to be related to the stimulation characteristics (*i.e.* painful stimulation) and not to the task, therefore having a role in coding the nociceptive characteristics of the stimulation. Given that insula is one of the classical structures coding pain and the only structure in the cortex that evokes pain when stimulated (Ostrowsky *et al.*, 2002), this results confirm its crucial role in pain perception. However it is well known that the insula is involved not only in many other types of sensations arising from our body

such as gustation, olfaction, sexual arousal and disgust (Craig, 2002) but also in different cognitive processes (Kurth *et al.*, 2010).

PAPER II. INVESTIGATING PAIN PERCEPTION IN PATIENTS WITH A REDUCTION OF SMALL DIAMETER FIBERS

The perception of any stimulation starts from an efficient peripheral coding, further processed centrally in the brain. Peripheral and central systems orchestrate the information available from either an internal (*i.e.* thirst) or external trigger (*i.e.* holding a hot cup) for a global understanding of the condition of the body and for an appropriate behavioral response. This study investigated how alterations in the periphery alter sensation, perception and reaction to acute thermal pain. The decrease of small diameter fibers in a population of HSAN-V patients offered a unique chance to address such issues. Using the same experimental protocol as for the study in healthy subjects (Paper I), we investigated the ability of the patients to discriminate painful versus nonpainful stimuli and if there were abnormalities in the reactivity to such stimuli.

Pain recognition and reaction times

Standard testing of discriminative and painful thermal thresholds did not show any evidence of peripheral deficits since results were surprisingly similar to healthy subjects, although with a higher variance at group level. In contrast, the results from analyses using signal detection theory (SDT) (Wickens, 2001), a model that looks at the ability of a subject to detect the difference between signal and noise and specifically in this case the ability to discriminate painful from nonpainful stimulations, showed that the patients had major difficulties in separating painful and nonpainful stimuli. The patients' poor performance did not allow for balanced comparisons of reaction times for painful and nonpainful stimulations but we concluded that pain did not facilitate motor response in the patients since no difference was found in their reaction times across conditions.

Brain activation and structural aspects

Addressing motor related activation during pain perception was not possible given the patients' high number of erroneous button presses. However, we investigated the fMRI activation during pain stimulation. In healthy subjects we observed that insula was the major site of nociceptive evaluation, whereas in the patients, insula activation was not found at the group level. The striking lack of insular activation at the group level was consistent with

their difficulties in performing the task and could reflect either a lower peripheral signal to the posterior insula via the STT, giving rise to a less obvious percept or an altered assignment of saliency at the central level. Furthermore additional structural findings suggest the possibility of a cortical reorganization following small diameter fibers loss. Supporting this, the patients had a thinner right anterior insula compared to age matched controls. Rostral anterior cingulate cortex was activated in the patients, consistent with pain literature and possibly reflecting a high cognitive load in stimulus perception.

PAPER III. INVESTIGATION OF AFFECTIVE TOUCH PERCEPTION IN PATIENTS WITH REDUCED SMALL DIAMETER FIBERS

Small diameter somatosensory fibers include not only nociceptors, thermoreceptors, and prurireceptors but also affective touch mechanoreceptors. This study addressed the perception of discriminative and affective touch in the HSAN-V patients. The patients were asked to perform a standard discrimination test used clinically to establish touch discriminative abilities. In addition, pleasantness ratings for perceived touch and seen touch in others were collected, and the patients filled in a questionnaire regarding social touch interactions. Cerebral mechanisms following CT optimal stimulation were also investigated. In a previous study, contrasting CT optimal versus non-optimal stimulation on the arm of healthy subjects, activation was shown in the posterior insular cortex (Morrison*, et al.*, 2011). In the same fashion, the patients were stimulated at CT optimal and non-optimal velocities to investigate how the loss of CT fibers might affect central processing and subjective feeling following affective tactile stimulation.

Discriminative and pleasant touch

The ability to appreciate the discriminative aspect of touch was intact in the patients as no difference was seen compared to the controls. However, we demonstrated differences in the evaluation of affective touch. In healthy subjects pleasantness ratings across velocities followed an inverted U shaped curve, with higher pleasantness ratings for mid slow velocities compared to slower or faster velocities. Such relationship, best fitted by a negative quadratic regressor, was not seen in the patients, who instead showed a better fit to a linear regressor.

The coupling between felt and seen touch

When rating how pleasant gentle stroking feels in others, healthy subjects are influenced by their own experience, as seen by the striking similarity of the ratings for felt and seen touch (Morrison*, et al.*, 2011). Such a finding suggests that the subjective feeling of affective touch is a major tool for understanding other people's interactions and feelings. This evidence is corroborated by the findings in the patients. As previously described, the patients' pleasantness ratings were significantly different from healthy subjects. Compared to healthy subjects, patients showed not only general lower rating values but also an increased pleasantness for faster velocities. However, as in the healthy subjects, the patients showed similarities in the ratings for felt and seen touch, hence, the patients' understanding of hedonic touch in other people is matched with their own experience of affective touch and does not resemble the ratings of the healthy subjects. Such finding allows us to shed light not only on the differences between healthy subjects and patients but also on the basic mechanisms of empathic behavior, highly grounded on first person's experience.

Differences between healthy subjects and patients in pleasant touch processing in the brain

Previous investigations of optimal versus non optimal CT stimulation in healthy subjects showed somatotopical activation in contralateral posterior insula, suggesting a first stage processing of affective touch there, and supporting the idea of the posterior insula as a first cortical relay site following CT stimulation (Bjornsdotter*, et al.*, 2009). An investigation of the posterior insula activity in the patients failed to show similarities with the healthy subjects. In the patients no velocity-related modulation was seen in the insula, and an additional analysis not presented in this Paper showed contralateral parietal opercular activation for CT optimal versus non-optimal velocities. These findings suggested that the patients might be relying more on sensory and discriminative inputs given that their system receives predominantly large myelinated fiber inputs, rather than thinly- and unmyelinated ones.

PAPER IV. INVESTIGATION OF THE REWARDING VALUE OF AFFECTIVE TOUCH

The sense of touch has important implications in individuals' lives (Ardiel, & Rankin, 2010). Affective touch is an intimate form of communication highly linked to physical and cognitive development in early age and to wellbeing

throughout people's lifespan. From childhood to adulthood, we seek contact with our loved ones because of its pleasurable effects on our bodies and minds (Hertenstein *et al.*, 2006; Muir, 2002). In this study, we investigated whether pleasant touch affected our behavior via reward-related processing. Subjects received different soft brush strokes, of which the only difference was in the speed of stroking. The speed of stroking is an important factor for peripheral unmyelinated CT fiber response, and their degree of firing is related to touch pleasantness (Löken*, et al.*, 2009). We used five velocities of stroking (ranging from 0.3 cm s^{-1} to 30 cm $s^{-1,}$ with CT optimal speeds being 1-10 cm s^{-1}) as a tool for investigating touch hedonics. We asked the subjects to choose their preferred stroking speeds by deciding to receive the same stimulation again or to switch to another random one, following each trial. To illustrate the areas involved in such process, we focused our analysis to three contrasts. First we looked at activation for tactile stimulation (Figure 3 "STIMULATION").Then we focused on the interval during the choice-making process (Figure 3 "EVALUATION"). Finally, we looked at the representation of the different hedonic value of the different speeds (Figure 3 "RELATIVE PREFERENCE").

Hedonic touch affected choice

CT optimal velocities were chosen the most, suggesting that CT signaling affects touch hedonics and triggers a seeking behavior. The subjects' behavioral pattern across the five velocities resembled previous subjective pleasantness VAS ratings (Löken *et al.*, 2011; Löken*, et al.*, 2009), with slowest and fastest speed less preferred than intermediate ones. There was a match between behavioral preference and previously published subjective pleasantness ratings across velocities (Löken*, et al.*, 2009) suggesting a link between pleasurable experience and behavioral preferences.

Brain correlates of tactile stimulation on arm and palm

All tactile stimulation (*i.e.* both preferred and non preferred stimulation) activated posterior insula and somatosensory cortices. When looking at arms and palms separately we found greater activation in somatosensory cortices for palm. Posterior insula was activated for both arm and palm stimulation. However, arm activation was limited to posterior insula, whereas palm activation spread to secondary and primary somatosensory cortices. Such greater somatosensory input for palm stimulation is consistent with peripheral and receptive field characteristics of this skin area, with a higher density of Aß fibers.

Brain correlates of evaluation of hedonic stimulation

The evaluation period (after the stimulation period) showed activation in similar areas for both preferred and non-preferred speeds. The activation included bilateral anterior insula consistent with interoceptive evaluation of the stimulation. However, direct comparison of preferred versus non-preferred stimulation showed activation in the head of the caudate, a basic structure involved in goal directed behavior. Such activation is particularly interesting given that the evaluation interval reflected computations before behavioral choice.

Brain correlates of the representation of different hedonic values

To get an appreciation of the representation of the value of different stroking speeds in the brain, we looked at activation following the behavioral choice, in which mid-slow velocities were repeated above chance compared to the slowest and fastest. We addressed brain activations of this ratio of repeat to change choices and found activation in posterior insula and dorsolateral prefrontal cortex (dlPFC). Activation in posterior insular cortex have been shown to be related to CT fiber input (Morrison*, et al.*, 2011; Olausson*, et al.*, 2002), and in this case it could provide a first stage velocity dependent estimation of pleasantness. During the task, dlPFC might have played a role in aiding relative rewarding value for an efficient behavioral response (Wallis, 2007; Wallis, & Miller, 2003).

DISCUSSION

Peripheral signaling and behavior

In most cases, our behavior can be modulated by the effects of external and internal stimuli. Behavior can be seen as a tool for regulation of dispositions, influencing both approaching and avoiding actions toward adaptive solutions. The studies in this thesis have addressed brain areas involved not only in the experience of affectively relevant stimuli but also in the initiation of purposeful movements. It has been proposed that stimuli have discriminative and affective dimensions (Löken*, et al.*, 2009; Rainville *et al.*, 1997) referring to the peripheral and brain areas carrying such distinct information. However, this might be an over simplification of the roles of peripheral and central processes. For both these dimensions, the saliency of the stimuli we encounter—for example their intensity or affective relevance—often results in an adaptive modification of our actions. In this view, behavior becomes an additional relevant dimension in the somatosensory experience.

A central role of small diameter somatosensory fibers as a class is tomediate nociception, temperature and itch but also low-threshold mechanoreception. These afferents include thinly myelinated A∂ and unmyelinated C fibers that via the STT tract project to somatosensory cortices and the insula (Gardner, & Johnson, 2012). In addition, a large body of evidence shows that small diameter fibers project to areas involved in motor functions too. In particular medial wall premotor areas, located on the cingulate gyrus of the frontal lobe not only receive projections from the STT tract but also contain corticospinal neurons and project to primary motor cortex (Dum *et al.*, 2009; Dum, & Strick, 1991; Vogt, & Morecraft, 2009; Vogt, & Sickes, 2009). Such evidence reinforces the idea that sensation and action are often in mutual interaction. This thesis aimed at showing some relationships between thermal pain, affective touch sensation and behavior.

Central multidimensionality of pain

"Alla fin trabocca e scoppia, si propaga, si raddoppia
e produce un'esplosione"

"Finally with crack and crash, it spreads afield, its force redoubled,
and produces an explosion"

- Il Barbiere di Siviglia -

The significance of a response to pain is obvious. It provides a fundamental protection for avoiding body injuries. Pain can manifest in many ways and

painful stimulation can range from triggering a withdrawal reflex to a more "conscious" behavioral adjustment. In Paper I we challenge the classical view of the "pain matrix" as being a signature for nociception (Wager *et al.*, 2013). The concept of the "pain matrix" comes from the consistent activation of certain areas during pain (Brooks, & Tracey, 2005; Talbot *et al.*, 1991). Primary and secondary somatosensory cortices, together with anterior cingulate cortex (ACC), insula, prefrontal cortices, and thalamus are typically considered part of the pain matrix (Apkarian *et al.*, 2005).

In Paper I we show that several areas usually activated during pain are not pain-specific but that their role can be accounted for by motor behavior. This suggests that the global activation following pain involves motor related processing that nevertheless is part of the experience of pain. We proposed that motor adjustments are embedded in the experience of pain because of their adaptive value. The ability to escape or avoid a threatening stimulus has massive beneficial effects for survival. It is then not surprising that motoric aspects might be part of the activation to pain. In line with our findings a recent study has reported "pain matrix" activation in response to all kinds of sensory stimulation: painful touch, nonpainful touch, visual and auditory stimuli (Mouraux*, et al.*, 2011). The high level of overlap between the areas involved in all these stimuli challenges the idea of these areas being pain specific. The authors suggest that such multidimensional network is not specific to pain but more to the saliency of any sensory input (Iannetti, & Mouraux, 2010). We assume that such view best represent and supports our current findings and suggest that although specific peripheral receptors are specialized in coding potential or actual tissue damage, there is need of additional research on whether centrally we can find areas exclusively coding for pain.

Dolor dictat

"Nessun dorma! Nessun dorma!"

"Nobody shall sleep!"

- Turandot -

Pain is a sensation. However the evident link between acute pain and behavioral response suggests that boundaries between action and sensation are not distinct. Sensation and action are likely to be fused during the experience of pain. Acute painful information comes with a necessary question about how easily and efficiently we can terminate an uncomfortable sensation. A feeling of pain is not naturally endured but it automatically gives rise to proactive

actions aimed at avoiding the possibility of tissue damage. In fact nociceptive activation does not necessarily signal tissue damage but more a realistic prediction of it. For example, tissue damage occurs at higher temperatures than nociceptors firing threshold (Raja *et al.*, 1999). This suggests that pain processing constitutes a smart protective system, implemented at different stages of pain experience, and its behavioral outcomes range from withdrawal reflexes to behavioral adjustments to a warning stimulus. Nociceptive withdrawal reflexes, occurring at the spinal cord level, provide an efficient defense to harmful stimuli. Their immediate and accurate response to pain is an important characteristic and is their most obvious quality.

However, the highly stereotyped nature of spinal reflexes and simple withdrawal actions would not be sufficient in more complex situations where the implementation of actions involves trading between options and outcomes. The cortex "lifts" reflex-mediated responsiveness to a higher level of integration with spatial, temporal and sensory information available. The ability not only to react but also to control our movements during painful stimulation to guarantee an adaptive behavioral outcome are tuned and adjusted during the experience. Imagine the situation in which you're grabbing a cup of warm tea, knowing that holding it for too long would cause you serious pain. After grabbing the mug one might quickly move one's hand away to get rid of the uncomfortable sensation. Such a bad sensation reflects more of a forecast than an actual damage. The tissue is not damaged yet but is likely to be if the contact with the cup is prolonged.

This ability of our brain to make and utilize predictions about potentially dangerous stimuli is tightly linked to the way we perform our actions. A faster reaction will lead to a less painful sensation and a lower tissue damage risk than a slower one. Behavior is therefore not only a consequence of pain processing but is an essential component of it. What we experience as a behavioral consequence to a harmful stimulation is already processed in the brain during sensation. Several areas in the brain reflect this processing of adapting our actions for an optimal outcome, probably reflecting the typical urge to react experienced during pain. Cortical targets of nociceptive inputs lie in the posterior insula and the cingulate cortex (Craig, & Zhang, 2006; Dum*, et al.*, 2009). Posterior insula is a site of interoceptive integration of somatosensory signals and is the only area in the brain able to evoke pain if stimulated electrically (Ostrowsky*, et al.*, 2002). The cingulate and in particular the dorsal part of the anterior cingulate cortex has regions involved in motor processing called cingulate motor areas. Interestingly, when electrically stimulated the ACC does not induce pain but more feelings of urgency (Bancaud *et al.*, 1976; Matsumoto *et al.*, 2003).

In Paper I we found that areas in the cingulate, motor cortex, thalamus and cerebellum are involved in motor reactions during pain. Such finding supports the hypothesis that activations during pain are multisensory contributions from areas not necessarily pain-specific. We show that a large portion of activation during pain is essentially related to motor action. In particular we were able to support our hypothesis on the cingulate motor areas, showing not only that their behavior in humans resembles their function in monkeys but also to put a label on the specific contribution of the cingulate during pain, for long only considered to reflect its affective value. In addition we were able to show that when it comes to pain the boundaries between action and sensation are basically non-existent. Action is an essential characteristic of pain because it contains the intrinsic urgency to adaptively react to it. Action reflects the need of our system to reject stimuli that can harm it. Pain without action would fail to be pain itself and therefore a symptom of self-damaging system.

Altered experience of pain

In Paper II we explored the consequences to pain reactivity in patients with a hereditary neuropathy causing a severe loss of unmyelinated C afferents and a moderate loss of thinly myelinated A∂ fibers. This condition, caused by a mutation on the nerve growth factor beta gene (NGFB) leads to an impaired ability to accurately perceive pain with complications at the joints level including arthropathies and Charcot joints (Minde*, et al.*, 2004). The symptoms are most severe in the three homozygous carriers, who present with serious impairment in mobility due to joint destruction, and two of them have relied on wheelchairs already during adolescence or young adulthood. The heterozygous are not as impaired, and range from manifestations of Charcot joints to no evident phenotypical signs of the mutations. We tested the effects of the mutation on the carriers' ability to recognize and react to pain. Using the same paradigm as in Paper I we delivered series of painful and nonpainful stimulations on the left hand of the carriers. The task in the scanner was relatively simple: in one run they had to respond only when the stimulation was painful and in the other run only when it was nonpainful. As in Paper I we were interested in the participants ability to discriminate the two kinds of stimulation and whether they had different reactivity to them. In contrast to the controls, who showed faster responses to painful stimuli, patients showed no difference in reaction times when responding to painful or nonpainful stimulation. In addition, the patients' sensitivity to the task was poor.

We investigated their ability to perform the task adequately by looking at whether carriers could distinguish signal from noise (each of which were

sometimes painful and sometimes nonpainful stimulation) during the task. The results showed that this ability was low, and their sensitivity to the task was compromised by their difficulty in telling apart the two kinds of stimulations. In addition, above-threshold painful stimulation mainly activated the rostral part of the anterior cingulate cortex, failing to match the healthy subjects' activation map, which was characterized by a vast bilateral activation in the insula. The carriers' deficit in appreciating pain is especially compromised when they are required to apply their perceived painful and nonpainful thermal sensations to a behavioral task. Yet this was not reflected in the pain and discriminative thresholding, suggesting that the method of limits per se might not be a sufficient procedure for revealing decrease in nociceptive fibers, or that the carriers' low thin-afferent density might be sufficient to detect thresholds. A thorough investigation with additional testing from psychophysics to subjective reports and structured interviews would offer a more complete overview on their conditions. In addition, a quantification of their fiber loss would be a crucial piece of information in linking peripheral loss to behavioral performance and cerebral activations. Such quantification has been previously done in some of the patients using invasive nerve biopsies. We aim at using a non-invasive, fast technique called confocal corneal microscopy that will guarantee same efficiency as the nerve biopsies but no unnecessary burden or discomfort in the patients. Such technique will allow us to specifically address whether the extent of fiber loss is linked to perception, reactivity and central alterations to pain and it would provide the patients with a clearer picture of their condition.

Altered experience of touch

In Paper III we address whether affective touch processing in hairy skin is affected in the patients, given that CT fibers are highly liked to be diminished. In healthy subjects, touch is mediated by myelinated Aß and unmyelinated CT fibers; the Aß mainly code for discriminative aspects of touch and CT fibers for its pleasurable effects (Löken*, et al.*, 2009; McGlone, & Reilly, 2010; McGlone *et al.*, 2007). Tactile sensation on hairy skin recruits both the dorsal column and the spinothalamic tracts, projecting to somatosensory and interoceptive areas. Aß fibers show a velocity dependent pattern that linearly correlates with speed of stroking: the faster the stimulation the higher the firing. CT fibers show a velocity dependency pattern of activation that does not correlate linearly with speed of stroking but with subjective pleasantness ratings. When healthy subjects are stroked at different velocities on the arm, their preferences follow an inverse U-shaped pattern, with intermediate velocities most preferred

compared to very slow or very fast ones (Löken*, et al.*, 2011; Löken*, et al.*, 2009; Morrison*, et al.*, 2011). The same pattern is seen for CT firing rate across velocities (Vallbo*, et al.*, 1999).

CT fibers are proposed to project via the posterior part of the ventromedial nucleus of the thalamus to the mid-posterior insula, a region of sensory and interoceptive integration (Craig, 2002, 2003a, 2009; Paulus, 2007). Here we addressed whether a reduction of CT fibers might result in an altered pleasantness rating. We investigated subjective ratings of pleasantness of stroking using five different stroking velocities, previously used to assess CT fibers characteristics (Löken*, et al.*, 2009). The patients' pleasantness ratings differed from the controls, showing a flattened rating shape that recalls more an Aß than a CT firing rate pattern. Interestingly, we observed a match between felt and seen pleasantness ratings in both groups, suggesting that our own percept is a measuring tool for the comprehension of other people's states.

This intriguing result suggests that at this level of investigation there are differences in the way the carriers perceive CT mediated touch and the way they evaluate it in other people. However, no between-group differences were found in the TACTYPE questionnaire that investigates general attitudes towards social tactile interactions. This suggests that alteration of CT firing is not sufficient to generally disrupt the communicative aspects of social touch per se and that mechanisms relying on myelinated afferents might be quite effective in compensating for the loss of CTs. However an additional and more thorough scrutiny of social aspects of touch might reveal differences that were not noticeable at the level of investigation of the Tactype. This 15 items questionnaire investigates very common tactile interactions concerning mainly romantic relationships: *"I touch my girlfriend/boyfriend when about to leave on a trip."* In addition the items never specify the kind of tactile interaction but only uses the general word "touch" that can range from a cold static pat on a shoulder, to a warm caress or a hug. Taking all the results together the differences between carriers and healthy subjects do not allow us to draw any conclusions about general abnormalities in their affective tactile interactions in daily life. It is important to stress the fact that in healthy subjects myelinated Aß afferents are always stimulated whenever we are touched. Their role is extremely crucial for the discriminative aspects of touch but they can also trigger pleasant sensations, since gentle touch on the palm is also perceived as pleasant.

The rewarding value of affective touch

"Che gelida manina, se la lasci riscaldar.."

"This little hand is frozen, let me warm it here in mine."

- La Bohème -

In Paper IV we more closely investigated the rewarding aspects of pleasant touch. We explored whether CT optimal stimulation was more preferred than CT non-optimal ones by looking at the overt behavioral choices of our subjects. The ability to influence the type of stimulation one is experiencing is rarely seen in fMRI studies. Usually a subject receives a stimulation that is decided by the experimenter with not much freedom but more a controlled acceptance of the experimental setting. Here, the subject was presented with various gentle stimulations and could decide to receive more of the stimulation he/she preferred the most. Such paradigm even if in the artificial setting of the MR scanner, likely triggered a more ecological process aimed at recruiting structures that in everyday conditions are responsible in driving our behavior to beneficial outcomes.

A vast number of studies highlights the crucial role of touch in normal development in humans and across species (Ardiel, & Rankin, 2010). Psychological studies investigating dyads' tactile interactions or effects of mechanosensory stimulation in infants show that the beneficial effects of touch are critically related to body growth and cognitive development. The role of the reward system in coding affective touch might be in signaling such positive effects and reinforce the engagement in touch related activities. It should be emphasized that tactile communication is bi-directional and dynamic (Muir, 2002). Therefore the ability to recognize the pleasurable and beneficial aspects of touch might relate to the ability to give it to others and to invest in such interaction for the wellbeing of our system.

In Paper IV we directly addressed the link between affective dynamic touch and reward processing via a feedback-based behavioral task in which subjects had a degree of freedom in choosing the stimulation they preferred the most. The behavioral results suggested that CT optimal velocities were the most preferred across a range of speeds and were able to trigger subcortical basic reward mechanisms, highlighting the importance that CT mediated touch has for the organism.

Somatosensory processing was involved during tactile stimulation, including activation in primary and secondary somatosensory areas and posterior insula. Activation in dlPFC, insula and caudate for most preferred velocities reflected value-based choice and goal directed behavior. In line with our findings a

recent study shows that gentle touch stroking can trigger activation in the insula and caudate both on hairy and glabrous skin (May, *et al.*, 2013). A summary of the central processing during stimulation interval and evaluation stage, are shown in Figure 3.

The absence of CT fibers in glabrous skin has lead to the idea that CT optimal velocities are not similarly experienced in the palm. However we show here that there is more in common between social touch processing for glabrous and hairy skin than hypothesized, suggesting that these skin domains do not process information on social touch independently but might interact to produce a coherent pleasantness feeling in the entire body. Despite this, preferred stroking speeds in our experiment activated distinct yet partly overlapping areas for the two skin types. We speculate that fine differences between arm and palm might related to the different roles that they have in social tactile interactions, with arm being more the "receiver" and palm being more the "explorer", playing different but complementary characters in the drama of affective touch play takes place on our skin.

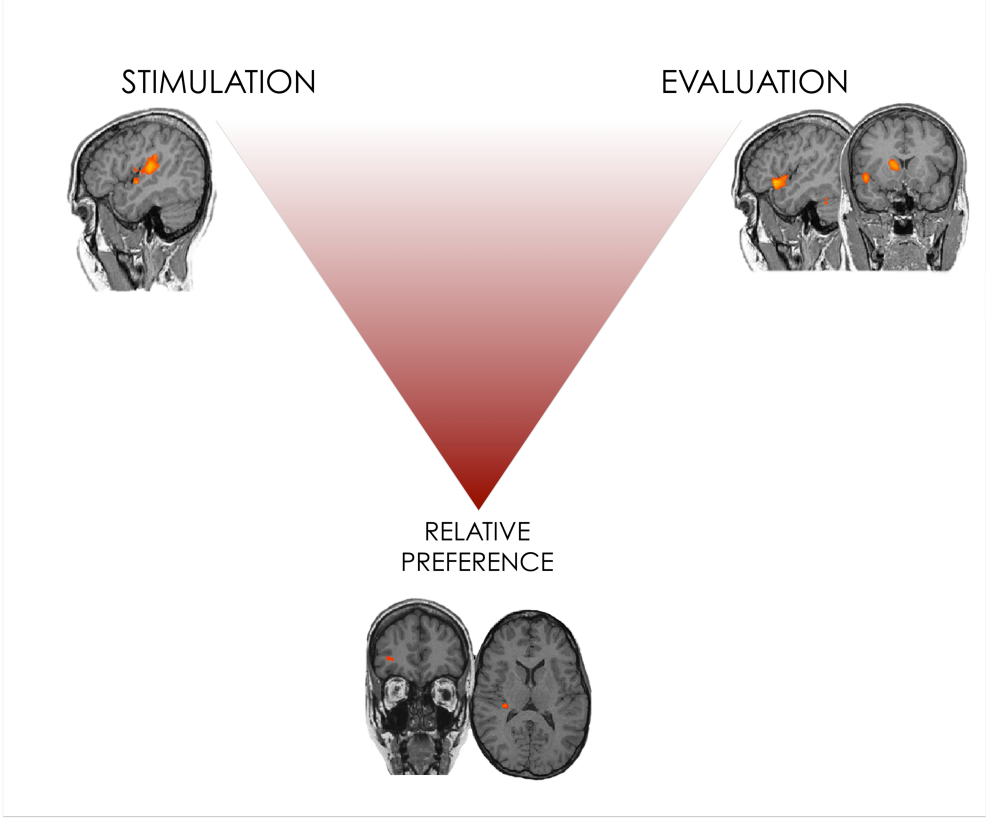

Figure 3. Areas involved in the experimental trial. Somatosensory processing was predominant during the tactile stimulation interval ("STIMULATION"), whereas areas involved in interoception and reward were activated during the choice-making process ("EVALUATION"), and in the representation of the hedonic valence of the different speeds ("RELATIVE PREFERENCE").

CONCLUSIONS

Paper I. In healthy subjects, cingulate motor areas, primary motor cortex, and the cerebellum were important for motor reactions to pain, whereas the insular cortex was important for coding the sensory characteristics of the painful stimulation.

Paper II. Hereditary loss of small diameter afferents impaired the ability to perceive and react adaptively to acute thermal pain. However, clinical QST thresholds were close to normal range.

Paper III. Hereditary loss of small diameter afferents resulted in abnormal perceptions of gentle touch.

Paper IV. In healthy subjects, CT-optimal stimulations were preferred compared to non-optimal ones. The preferred stimuli activated regions involved in reward processing including the caudate nucleus.

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