THIN-FIBRE SIGNALLING IN HUMANS
CORTICAL PROCESSING OF SENSORY AFFERENCE AND AUTONOMIC EFFERENCE

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Cover illustration: Brain activity covarying with right anterior insula activity at thin-fibre stimulation. Adapted from Paper IV.

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Cortical processing of sensory afference and autonomic efference

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Abstract
Thin nerve fibres innervate the entire human body and mediate sensations such as pain, temperature and visceral sensory input. Moreover, a special class of unmyelinated afferents responsive to light touch has recently been found in humans: C-tactile (CT) fibres. In the efferent side, C-fibres are the path for signalling in the autonomic nervous system, controlling the internal milieu of the body. There is growing evidence that C-fibres form the basis for monitoring and regulating the physical status of the body. This thesis focuses on central projections of mild thin-fibre input and their integration with autonomic reactions. Brain activity was studied with functional magnetic resonance imaging (fMRI).
The first paper examined cortical activation of selective CT-stimulation by soft tactile stimulation in two rare patients lacking Aβ fibres. The results confirmed previous findings based on one of these patients, showing that CT stimulation activates the insular cortex. In addition, CT stimulation deactivated somatosensory cortices.
The second paper further investigated cortical effects of CT stimulation in healthy controls by comparing rapid vibration (predominantly activating Aβ fibres) and soft brush stroking (combined Aβ and CT activation) on the skin. The ventromedial prefrontal cortex was significantly more activated by brushing than by vibration, an area previously implied in coding for the expected emotional value of an event.
The third paper focused on the role of CT fibres and autonomic function. We again studied the two Aβ deafferented patients to examine whether CT stimulation could evoke an autonomic response. We also examined their ability to localise the CT stimulations to the correct limb. Capacity for localisation of the stimulus was poor but above chance. Despite producing only a vague percept in the patients, the CT stimulus gave rise to a skin sympathetic reaction which was indexed by a galvanic skin response.
The fourth paper studied the cortical mechanisms behind a restricted autonomic response elicited by a perceptually weak C-fibre input in healthy subjects. We used low-intensity rectal distension while recording autonomic variables and cortical responses. Rectal distension activated insular cortex. Central activation specifically related to the skin sympathetic response was, in addition to the brainstem, limited to the right inferior frontal gyrus (IFG).

The CT evoked insular activation and the Aβ-denervated patients' poor ability to localise a CT stimulation support the concept that these fibres underpin affective rather than discriminative aspects of touch. The rectal distension study indicated that insular activation via low-threshold mechanovisceral thin fibres predominantly reflects afferent processing whereas IFG and the brainstem may be important in the generation of autonomic responses. Further, the studies suggest that stimulus perception is a prerequisite for cutaneous autonomic responses to both CT and visceral thin fibre stimuli. These findings set the stage for future studies of thin nerve fibre function, including neural mechanisms of hedonic processing as well as pathophysiological studies of conditions such as irritable bowel syndrome, which may tease out putative contributions from afferent input, cognitive processing and autonomic consequences.
Keywords: *CT afferent, thin fibre, autonomic, insula, fMRI*
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POPULÄRVETENSKAPLIG SAMMANFATTNING

Människans nervsystem kommunicerar genom olika typer av nerver med specialiserade funktioner. Tunna nerv-typer signalerar t.ex. smärta och temperatur, och känselintryck från inre organ till hjärnan. Det tycks vara så att information från tunna nervfibrer aktiverar delar av hjärnan som är viktiga för känslolivet. Nyligen har man upptäckt en ny klass tunna nervfibrer som är specialiserade på att reagera på långsam beröring av människans hud; s.k. taktila C-, eller CT-fibrer. Tunna nervfibrer utgör också infrastrukturen för signalering i det autonoma nervsystemet, som kontrollerar kroppens inre miljö.

Syftet med denna avhandling var att undersöka vilka områden i hjärnan som bearbetar information som signaleras via tunna nervfibrer i kroppen. Speciellt undersöktes vilka hjärnområden som aktiveras av (inkommande) signaler från CT nerver, men också hur hjärnan förmedlar (utgående) reaktioner i autonoma nervsystemet vid lätt mekanisk stimulering av huden eller inre organ (lätt vidgning av tarm). De grundläggande områdena för autonom kontroll finns i hjärnstammen och kan, som namnet påvisar, arbeta självstyrande, men medvetandet (och därmed högre hjärnfunktioner) kan påverka denna kontroll.

Vi studerade tunnfiberfunktion hos friska försökspersoner, samt hos två patienter som genom sjukdom har förlorat sina vanliga (tjocka) känselnerver och därför inte har kvar någon känsel i vanlig bemärkelse. Däremot har de intakta CT nerver, vilket ger ett unikt tillfälle att studera CT nerver enskilt eftersom dessa hos friska aktiveras parallellt med vanliga känselnerver. Vi mätte hjärnaktivitet med funktionell magnetresonansavbildning, fMRI, under tiden som vi aktiverade tunna känselnerver (CT-nerver genom att borsta med en mjuk pensel på huden i tre studier och tunna känsel-nerver i tarmen i en studie). Vi mätte också samtidigt autonoma reaktioner på stimuleringarna (bl.a. kärl- och svettreflexer i huden).

Resultaten visar att stimulering av CT nerver genom att borsta på huden med en mjuk pensel aktiverar insulära kortex, ett område i hjärnan som aktiveras även av andra tunnfiber-modaliteter (t.ex. smärta, temperatur) och som är viktigt för uppkomsten av emotioner och kroppsuppfattning. Patienterna, som saknade tjocka känselnerver, hade svårt att lokalisera på vilken del av kroppen de fick CT-stimuleringen men stimuleringen gav ändå upphov till en vag känsla av beröring och en begränsad autonom reaktion. Tillsammans stöder detta hypotesen att systemet som består av CT nerver är viktigt för att förmedla emotionella snarare än diskriminativa aspekter av beröring. Resultaten talar vidare för att högre hjärnfunktioner, via varseblivning av dessa diskreta stimuleringar, tillåter det autonoma nervsystemet att reagera men den tydligaste hjärnaktiveringen relaterad till den autonoma reaktionen sker i hjärnstammen.

Denna och ytterligare studier av hur hjärnan hanterar inkommande och utgående tunnfiber-funktion är viktiga för att bättre förstå betydelsen av beröring för människans välbefinnande, men också för att förstå mekanismer bakom t.ex. psykosomatiska sjukdomar med störningar i autonom funktion.

LIST OF PUBLICATIONS

This thesis is based on the following papers, which are referred to in the text by their roman numerals:

I **Unmyelinated tactile afferents have opposite effects on insular and somatosensory cortical processing.**
Olausson HW, Cole J, Vallbo A, McGlone F, Elam M, Krämer HH, Rylander K, Wessberg J, Bushnell MC
Neuroscience Letters 2008 May 9;436(2):128-32

II **Cortical processing of tactile C-fibre stimulation.**
Rylander K, Elam M, Olausson H
Manuscript

III **Functional role of unmyelinated tactile afferents in human hairy skin: sympathetic response and perceptual localization.**
Olausson H, Cole J, Rylander K, McGlone F, Lamarre Y, Wallin BG, Krämer H, Wessberg J, Elam M, Bushnell MC, Vallbo A
Experimental Brain Research 2008 Jan;184(1):135-40

IV **Central nervous control of cutaneous sympathetic responses: disentangling afferent and efferent processing of mild rectal distension.**
Rylander K, Posserud I, Simrén M, Olausson H, Elam M
Submitted manuscript

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Abbreviations

ACC	anterior cingulate cortex
AIC	anterior insular cortex
ANS	autonomic nervous system
BOLD	blood oxygenation level dependent
CNS	central nervous system
CT	C-tactile (afferents)
fMRI	functional magnetic resonance imaging
GI	gastrointestinal
GSR	galvanic skin response
IC	insular cortex
IFG	inferior frontal gyrus
MRI	magnetic resonance imaging
ROI	region of interest
SPM	statistical parametric map
VAS	visual analogue scale

1 Introduction

The human brain is continuously bombarded by sensory input from the body. The input may arise from our body (such as feeling our stomach moving, the sensation of blushing or basic homeostatic information about blood pressure level) or from interactions with the surroundings (like being touched on the skin or the pain from stepping on a needle). Some input reaches perceptual levels whereas some remains subliminal. Physiological systems in the body respond to inner and outer challenges or input and aim to maintain a stable inner environment. All sensory input is integrated in the brain, to give a total picture of the state of our body, mentally and physiologically.

In the past decade, the advent of brain imaging techniques such as functional magnetic imaging (fMRI) has revolutionized brain sciences, providing a non-invasive method to explore brain function. Brain imaging studies of human sensory perception concerning thin fibre function have shown that sensory qualities mediated by thin nerve fibres often activate brain areas thought to be involved in emotions, so called limbic or para-limbic structures. Evolving from these and additional anatomical evidences, a hypothesis about the overall function of thin nerve fibres has been formulated (Craig, 2002).

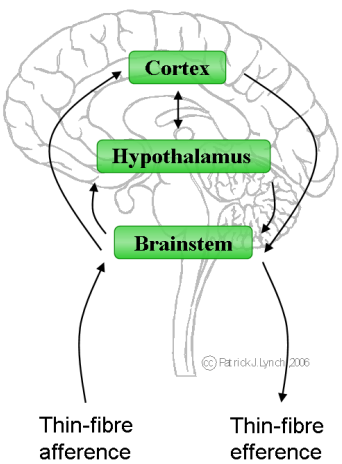

Figure 1 *Simplified illustration of the central circuits of the proposed thin-fibre network (for a detailed picture, see Craig, 2002)*

The theory proposes that thin nerve fibres (in the periphery and their central projections/origins) form a network where the afferent limb is sensory thin-fibre input from the body and the efferent limb is the autonomic nervous system (in which signals are also conveyed in the periphery by thin fibres), see Figure 1. The afferent and efferent limbs are integrated at different levels in the central nervous system hierarchy.

Central cortical structures in this thin fibre network include the cingulate and insular cortices. Specifically, right anterior insula cortex (rAIC) is thought to substantiate an interoceptive cortex, where an integration of all thin-fibre input forms a representation of the physiological condition of the body. Anterior cingulate cortex (ACC) is often conjointly activated with insula by thin-fibre input, and the insula and cingulate are considered to be complementary limbic sensory and motor regions (Craig, 2009).

Recently, a specialized class of thin-fibre afferents has been detected in the human skin; C-tactile (CT) fibres (see next section). They respond vigorously to light touch and are thought to contribute to affective aspects of touch. Based on their

projections, CT fibres have been proposed to be part of the described thin fibre network (Craig, 2002).

This thesis studies afferent and efferent aspects of the proposed thin-fibre network, focussing on low-threshold mechano-afferent input (from skin and viscera). Specifically, emphasis is put on the function of tactile C-fibres, and possible cortical involvement in autonomic reactions evoked by low-intensity thin-fibre afferent stimulation.

2 Thin-fibre afferents

Thin-fibre afferents refer to unmyelinated (C) or thinly myelinated (Aδ) nerve fibres. Thin fibre function is generally attributed to pain and thermal sensations and much knowledge has been gained about central representations of these thin-fibre modalities. This thesis however focuses on thin fibres which respond to low-threshold mechano-afferent stimuli. Specifically, two types are studied: 1) CT fibres found in the human hairy skin, responding to light touch and 2) C-fibres innervating the viscera, responding to mechanical deformation of the intestine.

2.1 Tactile C-fibres

In humans, light touch of the skin has traditionally been described as being mediated exclusively by Aβ afferents with mechanosensitive receptors. In the nineties, however, it was discovered that the human hairy skin is additionally equipped with unmyelinated afferents, also responding to light touch (Nordin, 1990). These afferents were first found in the facial skin (Nordin, 1990) and later in the extremities (Vallbo et al., 1993; Vallbo et al., 1999; Edin, 2001; Löken et al., 2007). They were labelled C-tactile (CT) afferents (Vallbo et al., 1999). Afferents of the same type had previously been found in cats (Zotterman, 1939; Iggo, 1960) and other primates (Lynn and Carpenter, 1982; Shea and Perl, 1985). CT afferents respond particularly well to gentle moving touch such as soft brush stroking.

Microneurographic recordings have shown that the firing profile of tactile C afferents is dependent on stimulation velocity. CT afferents fire most vigorously during intermediately slow stroking (1-10cm/s), whereas the firing rate decreases for slower and faster stimulation velocities (Löken et al., 2009). This characteristic inverted u-shape profile is also obtained for psychophysical pleasantness scores for the same stimulation velocities, suggesting that CT afferents encode touch pleasantness (Löken et al., 2009). The inverted u-shape response pattern to varying stimulus velocity has not been observed for other sensory mechanoafferents in the skin; firing of Aβ afferents increase in firing rate as stimulation velocity increases (Löken et al., 2009), making them suitable for encoding stimulus velocity.

Like other cutaneous thin-fibre input (temperature, itch, pain), the CT afferents are thought to project to the spinal cord via the superficial layers (lamina I and II) of the dorsal horn (Kumazawa and Perl, 1977). The lamina I neurons project somatotopically to the thalamus via the contralateral spinothalamic tract. In humans, it

has been suggested that a specific thalamic area, labelled the posterior ventromedial nucleus (VMpo), receives thin-fibre lamina I input (Craig et al., 1994; Dostrovsky and Craig, 1996). For an alternative view, see (Willis et al., 2002). On the cortical level, a previous brain imaging study suggests that CT afferents project to the insular cortex (Olausson et al., 2002), analogous to temperature, itch and pain thin-fibre mediated inputs (Craig, 2002).

2.2 Visceral afferents

Distension of the gastrointestinal (GI) tract provokes a number of reactions in afferent and motor neurons intrinsic to the enteric nervous system (Furness and Costa, 1987), but also in extrinsic afferents carrying information to the CNS. The extrinsic signals give rise to perception from the viscera and are the afferent limb of viscero-autonomic reflexes.

In animal studies, receptor types proposed to be present in the lower GI tract are: high-threshold nociceptors (Cervero, 1994), silent nociceptors activated during inflammation (Jänig and Koltzenburg, 1991) and intensity-coding receptors responding dynamically to a wide range of stimulation intensities, including non-nociceptive ones (Bahns et al., 1987; Cervero and Janig, 1992), for review see (Jänig, 2006). In addition, many of these afferents are polymodal and can also be activated by for example chemical stimuli (Haupt et al., 1983; Coutinho et al., 2000). In the rectum, additional receptors responsive to non-nociceptive input have recently been found in guineapig; so called rectal intraganglionic laminar endings (rIGLEs) (Lynn et al., 2003; Lynn et al., 2005). They are located in the myenteric ganglia in the rectal wall and have been characterized as low-threshold, slowly-adapting mechanoreceptors, activated by stretch or contraction (Lynn et al., 2005). In conclusion, non-painful rectal distension (**Paper IV**) is likely to activate intensity-coding and/or rectal IGLEs.

The primary visceral afferents are unmyelinated (C) or thinly myelinated (Aδ), and the spinal afferents project to secondary neurons in lamina I and V (and deeper lamina VI, VII and X) of the dorsal horn (Willis and Coggeshall, 2004). These secondary dorsal horn neurons are viscero-somatic convergent and can be activated by thin-fibre afferents (C or Aδ) from the skin, deep somatic tissue or the viscera (Craig, 2003a). Furthermore, many primary visceral afferents project to several segments of the spinal cord and compared to the skin, the viscera is less densely innervated (Jänig and Morrison, 1986). Taken together, these factors contribute to the diffuse and non-specific nature of sensations from the viscera, and sometimes result in so called referred sensations (e.g. referred pain).

The evoked extrinsic signals are conveyed to the CNS via vagal (mostly for upper GI regions) or spinal pathways (from all GI regions, including the colorectal region) (Berthoud et al., 2004). The spinal afferents from the lower GI tract enter the spinal cord at four levels: 1) lower thoracic cord via the splanchnic nerves, 2) upper lumbar cord via the lumbar splanchnic nerves, 3) lower lumbar cord via the hypogastric nerves and sympathetic trunk, and 4) sacral spinal cord via the pelvic splanchnic nerve (Jänig, 2006). Afferents from the rectum (**Paper IV**) primarily enter at the sacral and lower lumbar levels. At higher levels, inputs from viscera are relayed to the cortex via the nucleus of the solitary tract (NTS), the parabrachial complex and

the thalamus, thereafter projecting primarily to the insular and cingulate cortices (Saper, 2002; Vogt, 2005).

3 Thin-fibre efferents

Thin nerve fibres are also found in the efferent nervous system, mainly conveying signals in the peripheral autonomic nervous system (ANS). The role of the ANS is to maintain a stable internal milieu in the body, a process known as homeostasis (Cannon, 1929, 1939; Bernard, 1957 [1865]). This control is exerted by up or down regulating signals inducing physiological responses in the various target organs depending on internal or external challenges. Adjustments may be induced by for instance physical activity, thermal stimuli or low dietary intake. Examples of adjustments by affecting target organs are regulation of heart rate or blood pressure by control of the heart and vasculature, secretion by control of various glands or thermoregulation by control of vaso- and sudomotor activity in the skin.

3.1 Organisation of the ANS

The autonomic nervous system is by definition *efferent* (Langley, 1903, 1921), although its function closely depends on afferent feedback from the body. Based on the *neuroanatomically* specialized outflow of the ANS, it can be divided into a parasympathetic and a sympathetic branch. The outflow of the sympathetic branch is relayed via the ventral horn of the thoracic and upper lumbar spinal cord, whereas the parasympathetic outflow is conveyed through cranial nerves from the brainstem and via sacral levels of the spinal cord. In both systems, the path to the target organs is organized in two-neuron chains of synaptically connected pre- and postganglionic neurons. In the sympathetic part, the cell bodies of the preganglionic neurons are located in the intermediate zone (between the dorsal and ventral horns) of the spinal cord. Most cell bodies of the postganglionic neurons are assembled into ganglia located along the sides (paravertebral, interconnected by the sympathetic trunk) or in front (prevertebral) of the spinal cord. Thus, the target organs are all remote from the cell bodies of their sympathetic innervation, and the nerve fibres connecting the cell bodies to their target organs are long. In contrast, parasympathetic cell bodies are located near the target organs. The preganglionic neurons are either thinly myelinated or unmyelinated and the postganglionic are unmyelinated, c.f. (Jänig, 2006).

Functionally the sympathetic system is generally regarded to act as up-regulating, preparing the individual for action, whereas the parasympathetic system acts by down-regulating. However, their often described direct antagonistic relation is generally exaggerated; the two systems act in parallel and moreover, several target organs are only innervated by one branch (Jänig, 2006). Within the sympathetic nervous system, neurophysiological studies in both animals and humans have revealed clear-cut differences in nerve traffic between sympathetic subdivisions, such as vigorous baro-reflex control of sympathetic outflow to the muscle vascular bed, in contrast with absent or very limited baro-reflex control of sympathetic activity to

cutaneous effector organs (i.e. skin blood vessels and sweat glands, subcutaneous fat tissue) (Vallbo et al., 1979; Wallin and Elam, 1997).

Autonomic reactions to stimuli (internal or external) with attentional impact are categorized into orienting and startle/defence reflexes by the extent of their peripheral response (Cook and Turpin, 1997). Briefly, both orienting and startle/defence reflexes involve skin vasoconstriction and/or sudomotor activity (evoked by skin sympathetic nerve activity). The orienting reflex may additionally involve heart deceleration, but has no effect on blood pressure. Startle/defence reactions are in contrast characterized by heart rate acceleration and, if significant enough, a transient blood pressure reaction.

3.2 Central control of autonomic function

The basic homeostatic circuits of the ANS are located in the brainstem. However, higher central levels (e.g. hypothalamus, amygdala and the forebrain) are involved by integrating and modulating autonomic function with respect to emotional and sensory input, forming integrative behavioural responses (see Figure 1).

In animal studies, brainstem nuclei of importance for autonomic regulation have been revealed by labelling and tracing techniques (for review see (Loewy, 1990). Some specific pathways and specialized action of certain regions have been distinguished, whereas the mechanisms behind the commonly found integrative function of many sites are unknown. Briefly, nuclei in the brainstem that have been associated with autonomic activity include (from cranial to caudal level; example of function in brackets): periaqueductal gray (PAG; defence reactions, (Bandler and Shipley, 1994), parabrachial nucleus (PB; cardiovascular and respiratory function), nucleus of the solitary tract (NTS; baroreceptor, cardiac and respiratory reflexes) and the ventrolateral medulla (VLM; direct control of sympathetic outflow). Higher in the neuroaxis, the hypothalamus is of major importance for autonomic function. It integrates emotional and autonomic responses and projects to all autonomic centres in the brainstem, and directly or indirectly to most forebrain areas (Swanson, 2000). The above mentioned structures also receive afferent input from the body and interconnect with other structures in the central autonomic network.

In humans, central autonomic networks have more recently been put under investigation by the advent of brain imaging techniques. Studies addressing cortical involvement in cardiovascular regulation and baroreflex function (King et al., 1999; Harper et al., 2000; Williamson et al., 2002; Henderson et al., 2004; Kimmerly et al., 2005; Macefield et al., 2006; Kimmerly et al., 2007a; Kimmerly et al., 2007b; Wong et al., 2007) have outlined a network containing the insula, ACC, amygdala and ventromedial prefrontal cortex (PFC). Other studies, focusing on sympathetic activation as indexed by sudomotor activity (Critchley et al., 2000; Williams et al., 2000; Critchley et al., 2001; Nagai et al., 2004; Williams et al., 2007; Dube et al., 2008; Mobascher et al., 2008) have indicated a similar cortical network. Medullary circuits involved in the generation of vasoconstrictor nerve discharge to the muscle vascular bed have recently been studied, combining fMRI with intraneural sympathetic nerve recording (Macefield and Henderson, 2009). Apart from brain imaging studies,

several studies report deficits in autonomic function following stroke, often in the insular cortex primarily with cardiovascular effects (Sander and Klingelhofer, 1995; Oppenheimer et al., 1996; Rincon et al., 2008). Electrical stimulation of the insular cortex has also elicited cardiovascular responses (Oppenheimer et al., 1992).

The most frequently used brain imaging technique in human studies (functional magnetic resonance imaging, fMRI; see Methods section) suffers from relatively low temporal resolution. Typically, autonomic responses are evoked by exposing the subject to some external stimulus and the subsequent cortical processing thus involves both sensory afferent and autonomic efferent information. The temporal resolution of fMRI (seconds) widely exceed time scales of cortical processing from arrival of afferent activity to efferent signaling (milliseconds). Therefore, it is challenging to design fMRI experiments which allow us to disentangle the autonomic (efferent) from the afferent effects. Furthermore, since responses in the ANS are differentiated with respect to target organ and parasympathetic/sympathetic function, it is also of interest to consider this differentiation when studying central control of autonomic function.

4 Aims of the thesis

The general aim of this thesis was to explore afferent and efferent aspects of the proposed thin-fibre network, focusing on low-threshold mechano-afferent input and autonomic output.

Specifically, the aims were to investigate:

1. Cortical projections of CT afferent stimulation (**Paper I-II**)

2. Functional consequences of a selective CT afferent input, in terms of sensory-discriminative and autonomic impact (**Paper III**)

3. Central control of the autonomic response to a mild C mechano-afferent fibre input (**Paper IV**)

5 Summary of methods

This section begins by giving an introduction to functional magnetic resonance imaging, used to measure cortical activity in **Paper I**, **II** and **IV**. Subsequently, an overview of the objectives and methods for each of the included papers is presented.

5.1 Functional Magnetic Resonance Imaging

Functional magnetic resonance imaging, has over the past decade become a crucial tool in neuroscientific research for understanding how the brain processes and represents information. It is a non-invasive technique, using the oxygenation level of the blood in the brain as an intrinsic contrast. The method is based on magnetic resonance imaging, MRI, and uses a standard MRI scanner with scanning protocols sensitive for changes in oxygenation level.

5.1.1 MRI

For a detailed description of the physics and concepts behind MRI, the reader is referred to (Buxton, 2002). Briefly, the underlying signal in human MRI is from hydrogen in the body. Hydrogen nuclei are slightly magnetic, enough to give rise to a small net magnetization when put into the very strong magnetic field of the MR scanner. This net magnetization starts to rotate in the magnetic field and its dynamics can be manipulated by exposing it to radio frequency pluses in resonance with the rotation. The measured MR signals are small currents induced in pick up coils by the rotating magnetization by means of magnetic induction. Depending on the local magnetic environment in different tissue, the temporal dynamics of the hydrogen and thus the MR signal differs, giving rise to contrast in the images. The MRI technique can be used to produce detailed anatomical images of the human body, but also to study function by measuring physiological changes over time, for example blood flow.

5.1.2 BOLD fMRI

The most commonly used indicator of neuronal activity in fMRI is the blood oxygenation level dependent (BOLD) contrast (Ogawa et al., 1990; Ogawa et al., 1992) The physiological changes during brain activation are complex, but one main factor that affects the MR signal is the concentration of oxygen in the blood.

When neurons are activated, oxygen metabolism and blood flow to provide the oxygenated arterial blood, increase at the site of activation. Oxygen is involved in the process of transforming glucose into ATP, which is the source of energy for the transportation of ions through the cell membranes at neural activity. The oxygen is carried to the site of activation by hemoglobin, which contains (ferromagnetic) iron atoms. In oxygenated hemoglobin, the iron is shielded by the oxygen, whereas in deoxygenated hemoglobin it is not. The effect of the unshielded iron is an increase in the magnetic susceptibility of the blood, which leads to a decreased MR signal. However, during neural activity, the arterial blood flow (carrying oxygenated

hemoglobin) increases more than the oxygen consumption. The consequence is a relatively *lower* concentration of deoxyhemoglobin and hence a slight *increase* in MR signal (Buxton, 2002).

The hemodynamic BOLD response to a short stimulus causing neural activity is modelled to be the difference of two gamma functions (Glover, 1999). The blood flow increase is delayed (for the blood to go from arteries to capillaries and draining veins) and reaches its maximum about 6 seconds after the onset of neural activity (see Figure 2C). The slight undershoot after the peak is hypothesized to be related to a mismatch in blood flow regulation and oxygen consumption (Logothetis and Wandell, 2004; Chen and Pike, 2009). The blood vessel network and the nature of the hemodynamic response are considered to adequately reflect the neural activity down to a few millimetres scale (Kim et al., 2004). The signal changes due to changes in blood dynamics resulting from neural activity are only a few percent, e.g. in the realm of noise.

The details about how different types of neural activity (e.g. synaptic activity, action potentials, inhibition) and their metabolic demands affect the BOLD signal are largely unknown. Electrophysiological recordings from the cortex of monkeys have however shown that the BOLD signal correlates better to local field potentials, thus better reflecting synaptic than actual spiking activity (although both make significant contributions) (Goense and Logothetis, 2008).

5.1.3 Data acquisition and experimental design

Since the signal changes relating to the neural activity are small and noisy, it is not feasible to acquire one "stimulus on" and one "stimuli off" image and simply subtract one from the other. Rather, preprocessing and statistical analysis of the data is necessary to pull out the effects. To get enough statistical power, many samples are needed, i.e. a time series of functional images of the brain acquired continuously and regularly while repeatedly exposing the subject to the stimulus (Figure 2A). The stimuli are commonly presented in blocks of about 15 seconds alternating with blocks of rest (baseline). A typical length of an fMRI time series could be ten minutes, acquiring 3D brain images (volumes) every 3 seconds with a spatial resolution of a few millimetres. Although some structure can be seen in the functional images optimised for BOLD contrast, a high resolution anatomical image is usually also acquired during the experiment for better anatomical identification of activated clusters.

When designing fMRI experiments it is crucial to control that the effects of interest can be truly isolated in the subsequent analysis. This can be accomplished by using for example two experimental conditions where ideally the only quality differing between the two is the effect/process of interest. In the analysis, the conditions may then be statistically contrasted to each other to reveal areas specifically related to the difference, thus controlling for confounding effects.

5.1.4 Data analysis

There are several software packages available for fMRI analysis, some commercial and others freely available. They essentially exhibit similar data preprocessing and analysis procedures. In this thesis, two packages were used: one developed at Montreal Neurological Institute, McGill University, Montreal (**Paper I**, available at http://packages.bic.mni.mcgill.ca/ and http://www.math.mcgill.ca/keith/fmristat/) and one from Functional Imaging Laboratory, University College London, London (SPM5, **Paper II** and **IV**, available at http://www.fil.ion.ucl.ac.uk/spm/).

5.1.4.1 Preprocessing

A number of preprocessing steps are taken to increase signal to noise ratio before analysis of fMRI data. The following describes the general procedure (Figure 2B).

Realignment

Despite stabilization precautions such as foam pads around the head, the subject often inadvertently moves during the course of scanning. While the coordinate system for the spatial encoding is static (in the frame of reference of the scanner), subject head motion will violate the localization correspondence of voxels between volumes in the time series. The realignment algorithm typically aligns each volume to a reference volume in the time series by applying a spatial transformation found by maximizing some similarity function between the images, for example intensity. The magnitude and time course of displacement for each volume is saved as a text-file, allowing for inspection post hoc. Importantly, the realignment is merely a spatial co-registration and movement-related signal changes may still persist after realignment (Friston et al., 1996).

Spatial normalization

Individual brains are different in size and shape. In order to report results in a common coordinate system and make group analysis, the brains are normalized to a standard brain template that conforms to some standard anatomical space (Talairach and Tournoux, 1988; Evans et al., 1993). The normalization usually involves affine rigid body transformation, but various approaches have been demonstrated. For the methods used in this thesis, see (Collins et al., 1994) (**Paper I**) and (Ashburner and Friston, 2005) (**Paper II** and **IV**).

Smoothing

Finally the images are spatially smoothed with a Gaussian kernel; typically with a filter width double the voxel size. The smoothing is performed to validate the assumptions for Gaussian Field Theory during statistical inference (Friston et al., 2000), see *Statistical inference* below).

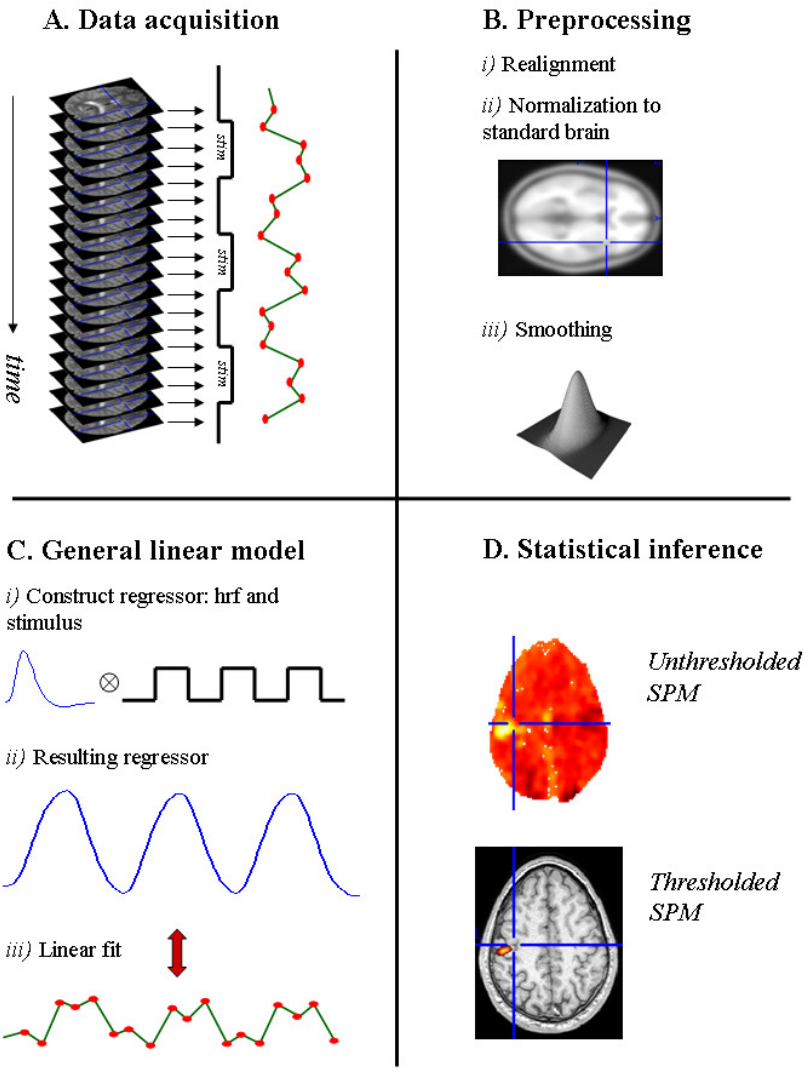

Figure 2. *Acquisition and analysis pipeline for fMRI data. The green line in A and C depicts the signal time course for a selected voxel. Red dots depict every sample in time. The black boxcar indicates stimulation periods. In A, each acquired 3D volume of the brain is here represented by a slice. HRF = hemodynamic response function*

5.1.4.2 The general linear model

The traditional method used to analyze BOLD fMRI data is within the framework of the general linear model, GLM. According to the GLM, the measured signals can be represented as a linear combination of a set of model functions (representing the expected neural responses to some experimental input) plus noise (Buxton, 2002). The

analysis consists of finding the set of amplitudes that scale each model function to provide the best fit of the model in a least-squares fashion by minimizing the sum of squares of the residuals after the estimated signal from the model is subtracted from the data. The model is described by the following equation

$$Y_i = X_{ij}\beta_j + \varepsilon_i \qquad (1)$$

where Y_i is the acquired time series data, X_{ij} the set of model functions (regressors), β_i the amplitudes (parameters) to be estimated and ε_i the residuals. Index *i* denotes the number of time points (volumes) in the times series and index *j* the number of explanatory variables or model functions. The betas are free parameters of the model, however, the model functions are assumed to have a fixed shape. The ordinary least squares solution to the equation system (1) is given by

$$\beta_i = (X_{ij}^T X_{ij})^{-1} X_{ij}^T Y_i \qquad (2)$$

Depending on the number of regressors, the analysis represents simple or multiple linear regression. The analysis is undertaken voxel by voxel independently, i.e. a mass univariate approach. Hence, as an output for each voxel, there will be one parameter estimate (β) per model function/regressor, specifying the effect size for that regressor during the entire functional scanning session.

In practice, the regressors are defined by experimental input and/or other behavioural parameters measured during the experiment. For example, in a simple setting the subject might just be presented with a visual stimulus like a flickering checkerboard in blocks interleaved with a black screen. The regressor would then simply define stimulation periods and thus be ones during checkerboard flickering and zeros during rest. In more advanced setups, the model may include several experimental conditions (e.g. two types of visual stimulation) and possibly behavioural results, like reaction times for subjective judgements relating to the stimulus presentation during scanning. To appropriately mirror the delayed *hemodynamic changes* (which the BOLD signal is a measure of) in response to the neural events, the regressors are also convolved with a canonical hemodynamic response function (HRF, see Figure 2C). In addition, movement parameters are sometimes also included in the model as covariates of no interest attempting to account for variations in signal due to subject motion. There is no unique model for an experimental setup; it can be modelled in different ways depending on effects of interest.

The relative contribution of each regressor (or a combination of them) is assessed with contrasts and t- or F-statistics. A contrast simply specifies an effect of interest, e.g. the β of a regressor vs. baseline or comparing two regressors to each other. The t-statistics for each contrast of interest is computed by dividing the parameter β (or the linear combination of many β:s) by its estimated standard deviation. For details on the variance estimation, see (Kiebel and Holmes, 2007). The results are commonly displayed as a statistical parametric map; SPM, over the whole brain in which each voxel is colour coded according to its t-value (Figure 2D). Subsequently, the SPM is thresholded for significance (see next section). For group

analysis, the un-thresholded contrast files created at the individual level are brought into second level statistical tests (ANOVA, t-tests or F-tests depending on inquiry) and thresholded for inference and display.

As a final remark, the errors in fMRI time series are correlated in time. The general linear model is however extended to allow for temporal correlations, thus justifying the use of t-statistics (Worsley and Friston, 1995).

5.1.4.3 Statistical inference

The ultimate goal of fMRI is to make inferences about which brain areas are involved in the investigated processes. When the model is estimated, the effects (or contrasts between betas) are thus tested statistically under the null hypotheses of the effect being zero. There are many approaches in brain imaging for choosing significance level for topological inference, but preferably the results are somehow corrected for multiple comparisons. Since t-tests are computed usually for a large quantity of voxels, there is a considerable risk of false positives. For example, for a whole brain analysis containing around 100 000 voxels and inference on $p<0.05$ level, around 5000 voxels are expected to be detected as active by chance. Neighbouring voxels are however not independent, hence a traditional Bonferroni correction may be too conservative. Instead, the estimation of independent image elements for multiple comparison correction is typically based on Random Gaussian Field theory (Worsley et al., 1996). Correction can be conducted for the whole brain or for predefined regions of interest (ROIs).

5.2 Ethics and subjects

All studies in this thesis were conducted in accordance with the Declaration of Helsinki and approved by the local ethics committee of the University of Gothenburg. Signed informed consent was obtained from each subject.

In **Paper I** and **III**, two unique neuronopathy patients were studied (see section 5.2.1 for details) together with eight healthy control subjects in **Paper III**. Only healthy subjects were studied in the remaining papers; 12 subjects in **Paper II** and another group of 17 subjects in **Paper IV** (13 in the first session, 11 in the other, seven subjects participated in both sessions). Healthy subjects were recruited by advertising and were financially compensated. For the brain imaging studies, only right handed subjects were included in order to get a more uniform population with respect to hemispheric lateralization of cortical functions. Handedness was checked with a modified inventory (Varney and Benton 1985).

5.2.1 Denervated patients

Since CT afferents are normally activated alongside myelinated Aβ afferents (also responsive to light mechanical stimuli) the functional role of CT afferents is difficult to study in healthy subjects. We have, however, had the opportunity to study two rare patients (GL and IW) who lack functioning Aβ afferents but have remaining thin

afferents; thus offering a possibility to selectively investigate the CT system (**Paper I** and **III**). Their condition is due to a neuronopathy disease which debuted in adulthood (GL age 31 and IW age 19) and resulted in a permanent degeneration of the myelin covering the large primary afferents (Forget and Lamarre, 1995). Thus, they do not receive Aβ mediated mechanical sensory input from their body, disturbing functions relying on Aβ afference such as touch perception, balance and movement. They deny any sensation of touch in daily life and they both have slightly reduced thresholds for pain and/or temperature, indicating some disturbance of the thin-fibre system as well.

5.3 Stimuli and experimental designs

5.3.1 Paper I

In the first study, the cortical activation pattern from selective CT stimulation in neuronopathy patients was addressed. To effectively activate the CT afferents, the skin was stimulated with a handheld soft goat hair artist's brush. In addition, manual caressing by the experimenter's palm was used. The stimuli were delivered on the subjects' forearm of the dominant side, in a block-design while undergoing fMRI.

5.3.2 Paper II

In the second paper, we studied brain activation from CT stimulation in healthy subjects. Aiming to target the CT specific component, 50 Hz vibration (Aβ stimulation) was used as a large-fibre control to soft brush stroking (combined Aβ and CT stimulation) on the skin. Microneurographic recordings have demonstrated that CT afferents respond poorly to rapid vibration (Bessou et al., 1971; Wiklund Fernström et al., 2002), whereas Aβ afferents respond well to both brush stroking and vibration (Bessou et al., 1971; Kumazawa and Perl, 1977; Wiklund Fernström et al., 2002).

fMRI was used to measure cortical activity. Vibration or brush stroking was applied to the right lateral thigh in separate runs within the same scanning session in a counterbalanced order (two runs of vibration, two of brush). The stimuli were delivered in a blocked design as in Paper I. The vibrating device was a piezoelectric element of similar size as the soft brush. After the scanning the subjects were asked to rate the perceived pleasantness as well as intensity for both types of stimuli on a visual analogue scale (VAS).

5.3.3 Paper III

While Papers I, II and IV studied cortical aspects of C-fibre function, this paper concerned peripheral and perceptual consequences of CT stimulation. The denervated subjects have a weak, diffuse perception of touch. In the light of this, we wanted to test whether a selective CT stimulation in these subjects could elicit an autonomic response and study the contribution of CT afferents to discriminative tactile function.

Soft brush strokes were again used as stimuli and delivered to the subjects' forearm of the dominant side. As a measure of autonomic reactions we used galvanic

skin response, to index skin sympathetic nerve activity. The discriminative impact of CT afferents was investigated by testing the subjects' ability to correctly localise brush stimuli to any of the four extremities.

5.3.4 Paper IV

Paper III reports on autonomic activation elicited by a barely perceived selective thin-fibre input in two unique patients. In the fourth study, we wanted to investigate cortical mechanisms involved in a similar thin-fibre afferent-efferent loop in healthy subjects, using low intensity rectal distension as the sensory stimulus. The experiment was specifically aimed at disentangling the cortical correlates to afferent (stimulus intensity) and efferent (degree of autonomic response) processing, in order to reveal central structures directly involved in the generation of the autonomic response.

Three low-intensity rectal distension levels were established for each subject; at perception threshold, 50% below and 50% above. In a first session the autonomic responses to the stimulations were characterized. During a second session, the same stimulation protocol was repeated during fMRI. Importantly, the subsequent analysis modelled both sensory afference (as defined by the distension levels) and autonomic efference (as defined by stimulus induced skin sympathetic reactions) in the same GLM analysis. Inferences for central activity related to skin sympathetic reactions were thus controlled for sensory input.

6 Results and discussion

6.1 Cortical activation by CT fibre stimulation (Paper I and II)

The cortical processing of CT afferent input was investigated in two studies. In **Paper I**, the cortical response to selective CT stimulation was studied in two patients lacking Aβ afferents. In **Paper II**, vibration on the skin was compared to soft brush stroking, aiming to study the CT specific component in healthy subjects. The results of **Paper I** confirmed previous findings based on one of these patients, showing that CT stimulation activates the insular cortex (Olausson et al., 2002). Specifically, gentle caressing activated contralateral posterior and ipsilateral mid-insular cortices. Interestingly, CT stimulation deactivated (compared to resting baseline) somatosensory cortices in both patients. In **Paper II**, the posterior contralateral insular cortex was activated by brushing also in healthy subjects. An additional small cluster was detected in ipsilateral mid-insula, in agreement with Paper I. Both vibration and brushing activated somatosensory cortices. In a direct comparison between the two stimuli, the ventromedial prefrontal cortex (vmPFC) was significantly more activated during brushing. The VAS rating revealed that brushing was perceived as more pleasant but less intense than vibration.

Somatosensory cortices receive Aβ projections and are known to play crucial roles in discriminative touch (Kandel, 2000). In the patients lacking Aβ afferent input, a deactivation of S1 was detected in response to CT stimulation, in line with the

notion that CT is not a system for discriminative touch. Ventromedial prefrontal cortex has previously been implied to code for positive emotional value (Knutson et al., 2001; O'Doherty et al., 2003; van den Bos et al., 2007). The insular activation elicited by brushing or vibration, respectively, did not significantly differ. Several explanations for this finding are possible:
1) *Aβ afferents also activate the insular cortex*, directly or by tight interactions with somatosensory cortices (Schneider et al., 1993, Olausson, 2002 #12).
2) *Vibration activates CT afferents to some extent.* Some CT afferents seem to initially respond to 50 Hz vibration whereas they generally adapt and stop firing after a few seconds (Bessou et al., 1971; Wiklund Fernström et al., 2002). While this behaviour could potentially be used as an agency to further study cortical projections of CT afferents (since vibration then may function as a control to itself regarding non-CT components), more microneurography recordings are required to characterize the weak responsiveness of CT fibres to vibration.
3) *The study lacked sufficient statistical power* to identify a putative differential effect in insula. Larger power may have been achieved by mixing vibration and brush stroking within the same runs. However, due to technical considerations (hand-held piezo electric vibrator which could potentially induce noise when moved around), the current experimental design was preferred.

We acknowledge that there may be components unrelated to CT that differs between vibration and brushing. For example, vibration was perceived as more intense than brushing. The Aβ firing pattern also differs between brushing and vibration conditions.

In conclusion, the results of **Paper I** and **II** suggest that selective stimulation of CT afferents activates the insular cortex while deactivating S1, supporting a role in affective rather than discriminative aspects of touch.

6.2 CT fibres and autonomic responses (Paper III)

The patients' capacity for localizing soft brush stroking to the correct limb was poor but above chance. Plain detection of stimulus occurrence (yes/no) did not imply correct localization. Despite difficulties localizing the tactile stimulus, it gave (on average) rise to a skin sympathetic reaction as indicated by a galvanic skin response (GSR). Healthy subjects of course had no difficulties localizing the stimulus to the correct extremity and brush strokes typically evoked a GSR. Mean GSR onset latency was similar in healthy subjects and GL (1 s) but considerably longer in IW (3 s).

The findings from this study demonstrate that stimulation of CT fibres may instigate autonomic responses. This may be driven as a reflex on brainstem level, or involve higher levels such as the hypothalamus or limbic/paralimbic structures (c.f. hypothalamus as an integrative area fundamental for eliciting autonomic reactions to emotional stimuli, (Smith et al., 1980). Despite single subject inference and some uncertainty in timing due to manual indication of the stimulus onset, the marked delay in GSR onset latency for IW compared to GL and healthy subjects may reveal mechanistic information. Microneurographic recordings have shown that the average

latency for a GSR in the palm elicited by a transcutaneous electrical stimulus is roughly 0.6 s, out of which the central delay time was estimated to 0.3 seconds (Fagius and Wallin, 1980). Taking the less distinct stimulus onset during a brush stroke into account, these latencies agree well with our results for GL and healthy subjects, while the mean onset latency for IW was almost 2 s longer. Since IW had a much weaker perception of the stimulus than GL (see Table 2, **Paper III**), we suggest that the prolonged latency reflects increased central processing for IW until he could perceive the stimulus. Thus, we propose that the GSR requires perception and that higher areas are likely to be involved in the generation of the response, directly or indirectly.

In conclusion, these observations demonstrate that the CT system is suboptimal for discriminative purposes but selective CT stimulation nevertheless gives rise to a vague percept. Such selective CT stimulation can also evoke a sympathetic response. The latter finding is in line with the hypothesis that thin afferent and efferent activity is tightly coupled (Craig, 2002). However, the crucial aspect for a skin sympathetic reaction to occur may be that the stimulus is perceived, irrespective of the type of afferent pathway generating the percept.

6.3 Central control of autonomic responses (Paper IV)

Mild rectal distension evoked a cutaneous sympathetic but no cardiac response and activated, in accordance with earlier studies (Mertz et al., 2000; Lotze et al., 2001; Hobday et al., 2002; Kern and Shaker, 2002; Sidhu et al., 2004; Andresen et al., 2005; Eickhoff et al., 2006; Lawal et al., 2006), insular and cingulate cortices. Central activation specifically related to the skin sympathetic response was, in addition to the brainstem, limited to the right inferior frontal gyrus (IFG). Neither brain activation, nor autonomic responses, was detected for rectal distension at levels below psychophysical detection.

Right inferior frontal gyrus was the only cortical region with activity found to correlate to the skin sympathetic response. This region has previously been associated with sudomotor activity (Critchley et al., 2000; Williams et al., 2007; Dube et al., 2008), and has also been implied as part of the ventral attention network (Corbetta et al., 2008). Our results indicate that in the processing related to mild rectal distension and its autonomic consequences, insular activation mainly reflects afferent input whereas IFG lies functionally closer to the autonomic output. This suggests that IFG is involved in the direct generation of the sympathetic reaction. However, since IFG exhibited covariation with rAIC (involved in interoception and stimulus awareness, (Craig, 2003b; Critchley et al., 2004) but not with the brainstem, the activation may alternatively express the perceptual impact of the stimulus.

7 General discussion

Findings from studies in this thesis extend and support the current view that the C tactile system carries emotional rather than discriminative aspects of touch. CT stimulation activates limbic related structures such as insular and prefrontal cortex.

CT afferents are complicated to study in healthy subjects since they can not be selectively activated. The Aβ deafferented patients thus offer a unique possibility for examination of the system. However, considering possible neural plasticity changes, it is problematic to make inferences to healthy subjects. Further, in healthy subjects, the perception of pleasantness most likely depends on Aβ afferent input in addition to CT input. The concept of pleasant touch also embodies top-down influences modulated by qualities such as context and emotional state (Morrison et al., 2009). Exploiting the recently demonstrated velocity dependence of the firing of CT afferents (Löken et al., 2009) may provide further insight into the cortical processing of CT input in healthy subjects.

Our studies using near perception threshold C-fibre inputs suggest that perception of the stimulus, and thus cortical involvement, is a prerequisite to elicit a cutaneous sympathetic reaction (**Paper III** and **IV**). In **Paper IV**, IFG emerged as the only cortical area closely related to stimulus induced skin sympathetic reactions. This finding may reflect that the cortical role in this situation is mainly permissive, allowing a response generated in sub-cortical structures.

It should also be emphasized that we used perceptually weak stimuli, eliciting selective autonomic responses. In other experimental conditions using more intense (emotionally or sensory) stimuli, a more extensive cortical modulation may be expected. In other words, more generalized stimulus-induced autonomic responses may recruit other cortical areas directly modulating their generation.

The exploration of higher central control of autonomic function in humans has only just begun. Much of the knowledge gained from brain imaging in this field so far lacks a clear distinction between afferent and efferent processing (Cechetto and Shoemaker, 2009). The relatively low temporal resolution of fMRI further complicates the dissociation. Rather, the entire loop from input to output is investigated as a whole. This may be a relevant approach in the sense that the emotional "state" of an individual is an integrative process, involving many components: sensory (and/or cognitive) input, autonomic reactions or regulations in response to the input and possibly also second level feedback from the elicited bodily changes (Damasio, 1993; Craig, 2009). This is potentially reflected as typical overall patterns of brain activation. However, it is of great bearing to assess more specific components in this integrative process. For this purpose, it is crucial to apply specific experimental approaches, e.g. modelling both input and output, varying and controlling for different contextual and emotional states and investigating many modalities of processing (skin vs. viscera, pain vs. pleasure, sympathetic vs. parasympathetic). The distinction is crucial for our ability to predict autonomic consequences of CNS lesions and to better understand central mechanisms behind psychosomatic disorders (with autonomic influence).

8 Conclusions

The main conclusions from this thesis are that our findings

1) support the concept that CT fibres underpin affective rather than discriminative aspects of touch

2) indicate that insular activation via low-threshold mechano-afferent thin fibres predominantly reflects afferent processing

3) suggest that stimulus perception is a prerequisite for autonomic responses to a low-threshold mechano-afferent thin-fibre stimulus

4) suggest that the right inferior frontal gyrus may subserve such a permissive role, linking perception and the generation of autonomic responses

These findings set the stage for future studies of thin nerve fibre function, including neural mechanisms of hedonic processing as well as pathophysiological studies of conditions such as irritable bowel syndrome, which may tease out putative contributions from afferent input, cognitive processing and autonomic consequences.

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