The *In Vivo* Functional Neuroanatomy and Neurochemistry of Vibrotactile Processing

PhD Thesis

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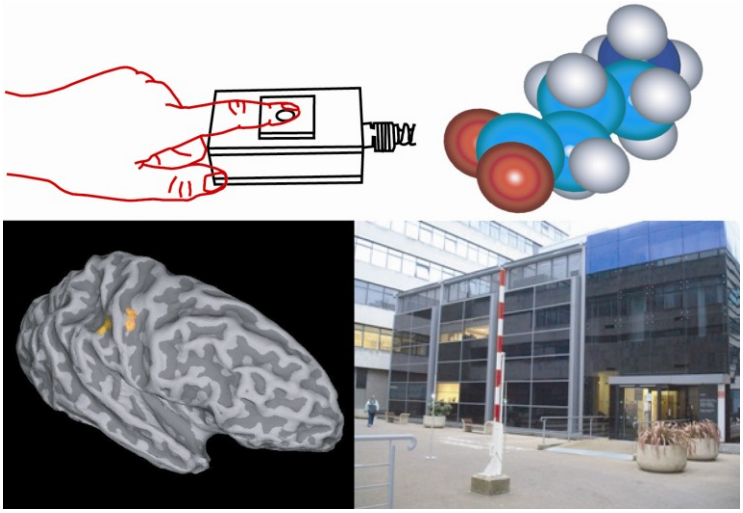

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Summary

Touch is a sense with which humans are able to actively explore the world around them and manipulate objects. Primary somatosensory cortex (S1) processing has been studied to differing degrees at both the macroscopic and microscopic levels in both humans and animals. Both levels of inquiry have their advantages, but attempts to combine the two approaches are still in their infancy. One mechanism that is possibly involved in determining the reponse properties of neurons that are involved in sensory discrimination is inhibition by γ-aminobutyric acid (GABA). Several studies have shown that inhibition is an important mechanism to "tune" the response of neurons. Recently it has become possible to measure the concentration of GABA *in vivo* using edited Magnetic Resonance Spectroscopy (MRS), whereas magnetoencephalography (MEG) offers the possibility to look at changes in neuromagnetic activation with millisecond accuracy. With these methods we aimed to establish whether *in vivo* non-invasive neuroimaging can elucidate the underlying neuronal mechanisms of human tactile behaviour and to determine how such findings can be integrated with what is currently known from invasive methods. Edited GABA-MRS has shown that individual GABA concentration in S1 correlates strongly with tactile frequency discrimination. MEG was used to investigate the neuromagnetic correlates of a frequency discrimination paradigm in which we induced adaptation to a 25 Hz frequency. We showed that S1 is driven by the adapting stimulus and shows that neural rhythms are modulated as a result of adaptation. This is the first time that behavioural psychophysics of tactile adaptation has been investigated using complimentary neuroimaging methods. We combined different methods to complement both physiological and behavioural studies of tactile processing in S1 to investigate the factors involved in the neural dynamics of tactile processing and we show that non-invasive studies on humans can be used to understand physiological underpinnings of somatosensory processing.

Statements

Declaration

This work has not been submitted in substance for any other degree or award at this or any other university or place of learning, nor is being submitted concurrently in candidature for any degree or other award.

Signed ……  …… Date ……7th November 2011…

Statement 1

This thesis is being submitted in partial fulfilment of the requirements for the degree of PhD

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Lay Abstract

Touch is the only sense with which humans are able to interact with the world around them. It is important not only in sensory processing, but also in social and physical interactions and the understanding of the world around us. Touch is processed by a brain-region called the 'somatosensory cortex'. How this brain-region represents information about touch has been studied to differing degrees at both the macroscopic (brain and behaviour) and microscopic (neurons and brain chemicals) levels in both humans and animals. Both levels of inquiry have their advantages, but attempts to combine the two approaches are still in their infancy and how the perception of touch is reflected in brain activity, is less well understood. The inhibitory neurotransmitter (brain chemical) γ-aminobutyric acid (GABA) is known to be involved in the processing of sensory information. For instance, it is involved in regulating how neurons respond to a certain types of touch and it is involved in discrimination between different touch-stimuli. Several studies have shown that inhibition (or suppression) of neuronal activity is an important mechanism to "tune" the response of neurons. Studies investigating human perception have shown that discrimination between two touch-stimuli can improve by 'tuning' or 'adapting' the somatosensory system to a certain stimulus.

Recently, it has become possible to measure the amount of GABA in a particular brain area non-invasively and *in vivo* ('within the living') using an MRI technique called magnetic resonance spectroscopy (MRS) and thus probe human brain

chemistry in living people by scanning the body with MRI. Another non-invasive brain imaging technique called magnetoencephalography (MEG), offers the possibility to look at changes in *in vivo* brain activity with millisecond accuracy. With MEG, different functional brain rhythms can be investigated, each with its' own role in brain function. With these two methods we want to establish whether these brain imaging techniques can elucidate the underlying neuronal mechanisms of human tactile perception and to determine how such findings can be integrated with what is currently known from invasive studies from neuron-to-behavioural levels.

The development and use of GABA-MRS has shown that we can use this method to measure GABA concentration in the somatosensory cortex (Chapter 5). We have shown that GABA concentration in the somatosensory cortex correlates with frequency discrimination (telling touch-stimuli with a certain speed apart; Chapter 6). These findings shows that differences in sensitivity for touch between people can be partially explained by the fact different people have different levels of GABA, showing that GABA is a driving factor in touch discrimination on a behavioural level.

In addition, we used MEG to investigate the neuronal correlates of a frequency discrimination paradigm in which we 'tune' or 'adapt' the system to a 25 Hz frequency. The behavioural results show that the ability to tell frequencies around 25 Hz apart *improves* when people become adapted to 25 Hz (Chapter 7). With MEG we have shown that this behavioural effect is reflected by a change in brain activity (Chapter 7 and Chapter 8). The 25 Hz stimulus attenuates the somatosensory cortex to that particular frequency and results in a change in the

pattern of brain rhythms. These results show that certain types of activity (different bands of brain rhythms) are important in the integration of information from the senses.

To our knowledge, this is the first time that behavioural tests of touch processing have been investigated using complimentary brain imaging methods. In summary, we combined different methods to complement both physiological and behavioural studies of touch processing in the somatosensory cortex. We show that non-invasive studies on humans can be used to understand physiological underpinnings of somatosensory processing. These approaches can be used to gain a better understanding of the micro- and macrolevel brain processes going on during sensory processing. In addition, these findings may provide an understanding of neurological disorders (such as autism, schizophrenia and epilepsy) where tactile processing or the GABAergic system is impaired.

Scientific Abstract

Touch is the only sense with which humans are able to interact with the world around them. Processing of the primary somatosensory cortex has been studied over many decades, but the mechanism by which tactile processing takes place in the cortex is still the cause of many debates and the relation between physiological and behavioural measures is not very well understood. One mechanism that is possibly involved in determining and changing the response properties of neurons that are involved in sensory discrimination is inhibition by γ-aminobutyric acid (GABA) and several studies have shown that inhibition is an important mechanism to "tune" the response of neurons. Somatosensory processing has been studied to differing degrees at both the macroscopic and microscopic levels in both humans and animals. Both levels of inquiry have their advantages, but attempts to combine the two approaches are still in their infancy. Recently it has become possible to measure the concentration of GABA *in vivo* using a technique called Magnetic Resonance Spectroscopy (MRS). *In vivo* MRS offers the possibility of being able to probe *in vivo* human neurochemistry whereas magnetoencephalography (MEG) offers the possibility to look at changes in neuromagnetic activation with millisecond accuracy. With these methods we want to establish whether *in vivo* non-invasive neuroimaging can elucidate the underlying neuronal mechanisms of human tactile behaviour and to determine how such findings can be integrated with what is currently known from invasive methods.

Development and use of edited GABA-MRS has shown that we can measure GABA concentration in the primary somatosensory cortex and that individual GABA concentration in somatosensory cortex correlates strongly with tactile frequency discrimination.

In addition, we used MEG to investigate the neuronal correlates of a frequency discrimination paradigm in which we induce adaptation to a 25 Hz frequency. Our results show that the somatosensory system is driven by the adapting stimulus and shows that the mu-beta rhythm is modulated as a result of adaptation. These results show that the mu-beta rhythm is closely related to integration of sensory signals and that the measures as found by MEG potentially relate to invasive studies.

To our knowledge, this is the first time that behavioural psychophysics of tactile adaptation has been investigated using complimentary neuroimaging methods. In summary, we combined different methods to complement both physiological and behavioural studies of tactile processing in the primary somatosensory cortex to investigate the factors involved in the neural dynamics of tactile processing and we show that non-invasive studies on humans can be used to understand physiological underpinnings of somatosensory processing.

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There are many people without whom this thesis would not have been made possible.

I would first like to thank Dave McGonigle and Richard Edden for supervising me throughout my PhD. Both have been instrumental in training me to become an independent researcher in the world of academia and they have also become good friends.

To me, Dave is a walking encyclopaedia of knowledge and I have learned a great deal from him; from developing experiments to figuring out problems step-by-step and he has been a key-figure in all the experiments described in this thesis. He has encouraged me in understanding and enjoying the combination of biology and neuroimaging and has given me a particular interest in the world of touch. He has always been extremely supportive of my scientific ideas as well as my public engagement activities (see Appendix C, page 269) and I simply enjoyed these 3 years working together. I am sure we will stay in touch.

Richard left Cardiff after the first year of my PhD but I am very lucky and glad that we kept in touch throughout my PhD. Richard got me interested in GABA-MRS and spent a great deal of time explaining it to me and collaborated on development of MRS described in chapter 5. It is because of Richard I have developed myself in the world of GABA and will continue to do so. Our discussions were always lively and engaging. In addition, image-making will never be the same again. I am looking forward to our continuing collaboration.

I want to thank John Evans for lively discussions about new developments in the MRS methods and analysis that formed the basis for Chapter 5. I really enjoyed working with him on a number of MRS projects over the past years and I could not have done this without him. Another CUBRIC member that needs special acknowledgements is Krish Singh, who not only helped with MEG analysis (chapter

3, 7 and 8) but has been instrumental in the development of ideas related to GABA-MRS. I want to thank Ben Dunkley for sharing the PhD experience with me and helping me with understanding MEG; Sonya Bells for being a great lab- and housemate; Luke Dustan, Alan Stone and Kevin Murphy for the banter; Sian Robson for morning chats, whines and being a great friend; Peri Trigkas for sharing Dave *wink*, Ange,Lisa and Becs for chats and gossip and Derek Jones for interesting morning chats, support and sharing the mind-reading experience. I want to thank the rest of CUBRIC, who I wish I could mention separately, for simply making CUBRIC an excellent working environment and keeping it fun. My PhD experience would have been a lot worse without the rest of CUBRIC, the Cardiff Postgraduate Neuroscience Society and the NMHRI public engagement committee. I will miss it very much.

It is very important that I also thank all my participants for helping out so very often. Without you my research would not have been possible.

By deepest thanks is to my dear Gemma Cook for being there for me over the past 2.5 years and being able to share the sorrows of a PhD student. Without her I could not have brought up the courage to finish this piece of work. She has been the most important person during my PhD and we've always been able to share woes, complaints about our PhD, but also to celebrate the accomplishments and the energy and enjoyment of science.

I want to thank my mom and brother for always being supportive and not being surprised whenever I moved again or did not call for a while, although they also should call me once in a while. They have always encouraged me and always shown interest in my work. And finally I want to thank my friends Yanick Aarsen, Luc Brugman and Kim Pieschel for visiting me over the course of these three years and keeping the email conversations with the home country going throughout my time here. I miss you guys!

Academic awards and conference presentations

Academic Awards & Grants

2011: Autism Speak translational post-doctoral fellowship ($98000;2 yrs)
2011: 1st place Poster award ISMRM 2011
2011: Guarantors of BRAIN travel grant (£800)
2011: Graduate School Initiative grant (£1000; with Georgie Powell)
2010: Poster award Young Neuroscientists Day 2010
2010: Maximising Impact Award (gold), School of Biosciences, Cardiff University
2010: Winner 2nd year postgraduate poster evening award, School of Biosciences, Cardiff University
2010: Physiological Society public communication grant (£1000)
2010: "Most Valued Partner Award" by Career Wales Cardiff & Vale Education Business Partnership. With School of Biosciences.
2009: BC-ISMRM student stipend
2008-2011: BBSRC 3 year studentship
2005: 1yr JASSO scholarship, awarded by the Japanese Ministry of Education

Invited Talks

- **2010; Donders Discussions, F.C. Donders Institute, Nijmegen, Netherlands;** "Edited MRS of GABA: Inhibition in functional imaging and behaviour"

Oral conference presentations

- **2011; MEG UK, Glasgow, United Kingdom;** "Neurodynamics of vibrotactile processing in somatosensory cortex"
- **2011; Welsh Institute of Cognitive Neuroscience biannual conference, Bangor United Kingdom;** "Area- specific GABA concentration predicts tactile discrimination threshold in humans"
- **2010; Young Neuroscientists Day, Bristol, United Kingdom;** "Area-specific GABA concentration predicts tactile discrimination threshold in humans"
- **2010; MEG UK, Cardiff, United Kingdom;** "Neuromagnetic correlates of vibrotactile adaptation"
- **2009; Speaking of Science, Cardiff University, 2009;** "Real Brain Training; remoulding the brain "

Conference Poster presentations

- **2011; Human Brain Mapping, Quebec, Canada;** "Area specific GABA concentration predicts tactile discrimination threshold in humans"
- **2010; Donders Discussions, Nijmegen, Netherlands;** "Area specific GABA concentration predicts tactile discrimination threshold in humans"
- **2009; Society for Neuroscience, Chicago, United States; "**Neuromagnetic correlates of vibrotactile adaptation"
- **2009; British Chapter of the ISMRM, Cardiff, United Kingdom;** "Investigation of

long duration edited GABA spectroscopy “,
- **2007**; **Dutch endo-neuro-psych meeting, Doorwerth, Netherlands;** “Artificial grammar learning using high and low predictive relations”,

Conference Proceedings
- **2011, ISMRM, Montreal, Canada:** “Area specific GABA concentration predicts tactile discrimination threshold in humans”
- **2011, HBM, Quebec City, Canada:** “Area specific GABA concentration predicts tactile discrimination threshold in humans”
- **2009, Society for neuroscience, Chicago, USA**: “Neuromagnetic correlates of vibrotactile adaptation”

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Chapter 1 - General Introduction

1.1 Introduction

Humans have five senses: vision, hearing, smell, taste and touch. Of these senses, touch is the only sense that allows us to physically interact with the world around us and manipulate objects and is therefore extremely important. It is thought that forms of touch are one of the oldest senses on the evolutionary time-scale (for instance, non-locomotive aquatic invertebrate animals such as sponges, without any of the other senses, respond to touch and use tactile information to respond to water flow (e.g. Leys et al., 1999) and it is the only sense with which infants can actively explore the world around them and is critical to our social and emotional lives. As with all the senses, our perception of the outside world is processed by the peripheral and central nervous systems.

Touch is processed by the largest organ in the human body: the skin. The skin measures 1.5-2 square meters and physically protects our body against the outside world, but also provides us with information about a range of aspects of our environment such as temperature and pain. In the following thesis we have investigated how the human brain responds to particular aspects of touch and how this relates to our perception of touch.

The human brain consists of about 20 billion cortical neurons, with over 7000 connections each (Drachman, 2005). In studying the brain, the majority of researchers choose between making local invasive recordings of a small number of

neurons (1 - 100) or using neuroimaging or behavioural methods to study the whole brain system-by-system, although recent advances have been made to combine the two (Logothetis, 2010). Bridging the gap between the findings and mindsets of these two different scales of enquiry is a fundamental challenge of modern neuroscience. Invasive studies usually investigate non-human mammals, using a combination of cellular recordings and behavioural measures. While this provides us with data concerning the ongoing neural responses that go along with behaviour, it also poses a large problem in that these studies often relate recordings on a scale of 1-100s of neurons to behavioural measures. Whereas it is assumed that these behavioural measures have neuronal correlates, these often incorporate networks of neurons, although are sometimes visible at the single neuron level (Houweling and Brecht, 2008). On the other hand, non-invasive neuroimaging studies have investigated aspects of tactile processing in humans from a whole brain scale point-of-view, but have not been able to answer questions about the exact neuronal correlates underlying tactile measures of behaviour, although they have been useful in determining the network properties underlying somatosensory processing. In this thesis we will combine behavioural measures with non-invasive neuroimaging techniques that will allow us to probe the underlying neuronal processes (anatomical and chemical) of tactile discrimination and plasticity.

The following thesis will describe a number of studies investigating aspects of somatosensory processing in humans. Studies of the somatosensory system range from the investigation of tactile receptors in the skin to studies investigating the processing of 'higher-order' tactile behaviours involving decision making and

sensory binding in the cortex. The nervous system can be investigated across a range of both spatial –from the level of genetics to whole brain function- and temporal scales –from millisecond neuron-to-neuron firing to behavioural effects that last over minutes and hours. In the following thesis, we have investigated a model of tactile discrimination using a combination of behavioural psychophysics, magnetic resonance spectroscopy (MRS) and magnetoencephalograhy (MEG). The basic biological concepts regarding the somatosensory system as investigated by animal studies will be introduced in this chapter, followed by an introduction to the processing of tactile information from skin to cortex, and a brief discussion of human studies on behaviour and neuroimaging. This is followed by a brief description of how behavioural experiments can investigate cortical dynamics, and the chapter will be concluded by a fuller outline of the aims and purposes of this work.

1.2 Basic Principles

As the aim of this thesis is to combine research across a number of different temporal and spatial scales (despite only using 'macro-level' investigative techniques), it is important to understand the basic underlying structure of a sensory system. A large amount of processing and filtering is necessary before a stimulus becomes a conscious sensation. In the case of touch, information of an indentation on our skin will have to be transferred from our skin to the brain. It is important to understand how information is encoded in the nervous system and secondly, how this is transmitted from the periphery to the brain.

1.2.1 The Basics of Neuronal Function

1.2.1.1 *The neuron*

The basic functional component of the human nervous system is the neuron. Neurons come in many shapes and forms but most exhibit a very particular cytology, with dendrites; a cell body; an axon; and synaptic terminals (as shown in Figure 1.1). A neuron usually receives signals from other neurons at the dendrite (but in some cases through the soma) and through intracellular signal transduction pathways the signal is transferred to the cell body (passively, whereas signal transduction along the axon is active). A typical neuron has many dendrites and may be connected to an average of 7000 other neurons. The axon allows for projections over a long distance (e.g. from the legs to the spinal cord) and the synapses signal electrochemically through the release of chemicals called neurotransmitters. Pre-synaptic *excitatory* connections allow for post-synaptic neurons to become depolarised and results in an excitatory post synaptic potential (or EPSP), exciting the state of a neuron (Figure 1.1A). Enough excitation may lead to the generation of an action potential. Pre-synaptic *inhibitory* connections allow for post-synaptic neurons to become hyperpolarised and results in an inhibitory post synaptic potential (or IPSP) leading to a less active state (Figure 1.1B). Enough inhibition may bias the neurons against producing an action potential. The effect of excitation and inhibition is shown in Figure 1.1. Cell-to-cell interactions primarily occur as a result of action potentials or 'spikes', electrochemical signals that move along the axon and result in release of neurotransmitters at the synaptic terminal. The neurotransmitter release in turn excites or inhibits the postsynaptic neuron. The source of a spike is the axon-hillock, where the axon meets the cell body. When a

certain threshold is met (through integration of signals received by the dendrites), an action potential is generated, and propagates through depolarisation of the axon membrane (primarily through the opening of voltage-gated sodium channels in the neuronal cell membrane). Whether or not a neuron fires, depends on integration of its input signals.

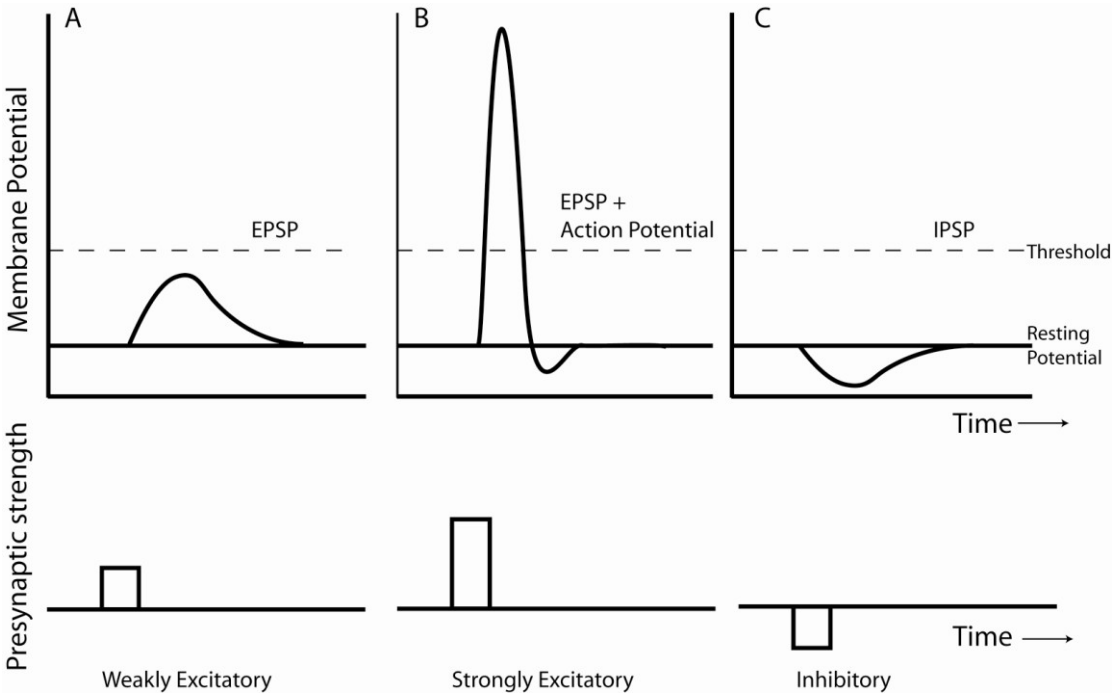

Figure 1.1. Neuronal activity depends on pre-synaptic input. A) Weak excitatory input, which leads to a depolarisation of the membrane (EPSP), which does not reach threshold, does not result in an action potential. B) Strong excitatory input which leads to an EPSP that does reach threshold results in an action potential that transverses the axon. C) Inhibitory input leads to hyperpolarisation of the membrane and biases the postsynaptic neuron against producing an action potential.

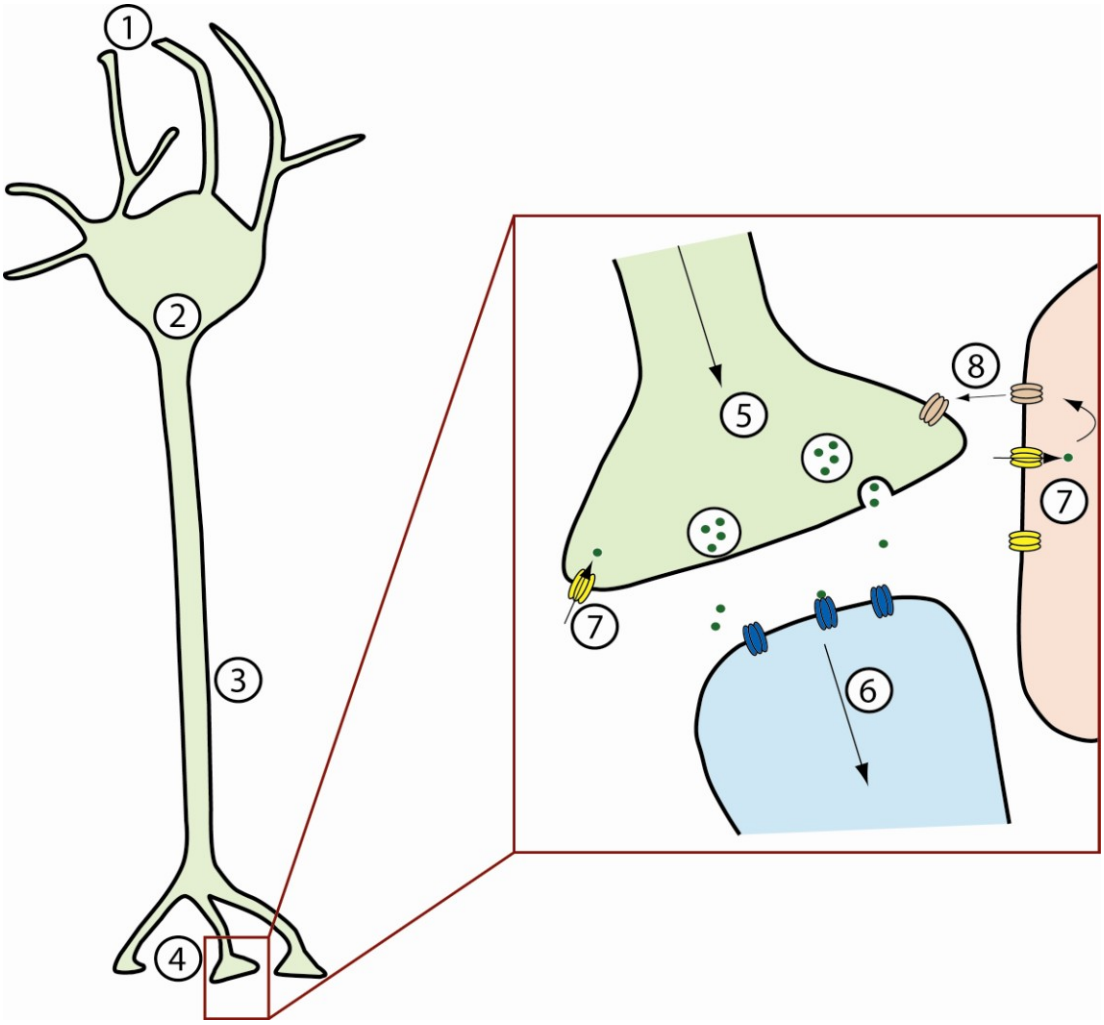

Figure 1.2. Schematic of the cytology of a neuron and the mechanism of GABAergic communication. 1) Information is received through dendrites. 2) Dendritic information is integrated in the cell body and depending on the activation pattern, an action potential is generated. 3) Information transfers along the axon towards the synapses through action potentials 4) An action potential reaches the synapse where it transfers the information to a postsynaptic neuron 5) When an action potential reaches the synapse, calcium influx results in the fusion of vesicles containing neurotransmitter with the cell membrane, releasing the neurotransmitter in the synaptic cleft. 6) Neurotransmitters bind to receptors on the postsynaptic cell and the signal is transduced further. 7) Reuptake of GABA occurs through GAT in both astrocytes and the presynaptic cell and enter the glutamate cycle processed by GABA transaminase. 8) GABA and its precursors are transported back into the presynaptic neuron.

Neuronal responses are extremely diverse. Some neurons respond to a depolarisation with a single spike, whereas other neurons respond by spike trains. Some neurons are spontaneously active. Signal propagation from one neuron to another goes through synapses which can be electrical or chemical (or both). Only the functioning of chemical synapses will be described. Chemical synaptic transmission depends on the release of neurotransmitters from vesicles in the presynaptic terminal that bind to receptors on the postsynaptic neuron. Action potentials lead to a rise in Ca^{+} in the synaptic terminal that leads to fusion of the vesicles with the cell membrane, resulting in the release of neurotransmitter as shown in Figure 1.2. The effect of neurotransmitters depends on the receptors. Receptors are generally neurotransmitter-specific and signal transduction from receptors has an effect on further processing (for instance; opening/closing of ion channels; metabotropic receptors exist as well). Glutamate, the main excitatory neurotransmitter in the adult human brain, mainly acts on receptors that lead to excitation and γ-amino butyric acid (GABA), the main inhibitory neurotransmitter in the adult human brain (it has a role in excitation in development), acts on receptors that lead to inhibition.

1.2.1.2 *The role of inhibition*

A brain that would only consist of excitatory connections would have a tendency towards positive feedback, because neurons would only be able to excite each other on the basis of input signals. As many neuronal representations are based on patterns of activity and inhibition, excitation alone is not sufficient. The regulatory effect of inhibitory connections is vital for proper brain function and is also known to regulate brain metabolism as brain activity is energy-expensive (Buzsaki et al.,

2007). 20-30% of all neurons in the brain are inhibitory interneurons and 70% of these act via GABA, the main cortical inhibitory neurochemical (for a review of GABAergic inhibition, see McCormick, 1989). GABA, once released into the synapse, acts on GABA-A receptors in the postsynaptic terminal and leads to an influx of negatively charged chloride ions which causes an inhibitory postsynaptic potential (IPSP); this is known as *phasic inhibition.* As will be explored further in Chapters 7 and 8, rather than *just* inhibiting further signal transduction, phasic inhibition is centrally involved in the generation of cortical oscillations in neural networks. In contrast, spillover of GABA from the synaptic cleft can activate extrasynaptic receptors on presynaptic terminals or on synapses of neighbouring neurons. Thus, GABA in the *extracellular* space can cause tonic (persistent) activation of GABA-A receptors and is implicated in modulating the sensitivity of neurons (i.e. setting thresholds) and information processing (Lee et al., 2010). These types of inhibition are often distinguished from each other (Farrant and Nusser, 2005). GABA diffuses away from the synaptic cleft and is internalised by high-affinity GABA transporters (GAT) on astrocytes and presynaptic cells (see Figure 1.2). GABA, which is produced from glutamate by glutamic acid decarboxylase (GAD) within GABAergic neurons, is then metabolised to succinic acid semialdehyde by GABA transaminase (GABA-T) and thence to succinate within astrocytic mitochondria and in the presynaptic cell (Chang et al., 2003). In addition, GABA also acts via GABA-B receptors, which are a class of G-protein coupled receptors. GABA-B receptors are involved in mediation of the IPSP and act via changes in potassium current (McCormick, 1989). Furthermore, GABA is known to have an important role in neural plasticity, which will be explained in more detail in section 1.4.2.

1.2.1.3 *Neurons have receptive fields and work in networks*

The range of values within a given stimulus dimension (for example, frequency in audition), to which a neuron responds is called its *receptive field*. Stimulus values lying within this particular range will result in a *change* in the response of that neuron. Imagine for instance, that a stimulus on the skin affects a group of neurons. Each neuron responds maximally to a particular position on the skin (its receptive field). The neuron that is affected most by the stimulus (i.e. has its receptive field centred on the stimulated part of the skin and therefore is most sensitive to stimulation) will fire the 'strongest' (the most action potentials in a given time) and its neighbours will fire less. This will result in an activity *distribution* (as shown in Figure 1.3a). The neuron that becomes most active also inhibits its neighbours by exciting inhibitory neurons connected to the neighbouring neurons, resulting in a much sharper response and enhanced contrast (as shown in Figure 1.3b). This effect is called *centre-surround inhibition*. The concepts of receptive field and centre-surround inhibition are applicable to all levels of sensory processing but become more complex further up in the hierarchy, e.g. receptive fields that respond to a single body part, or respond to stimulus orientation (Warren et al., 1986).

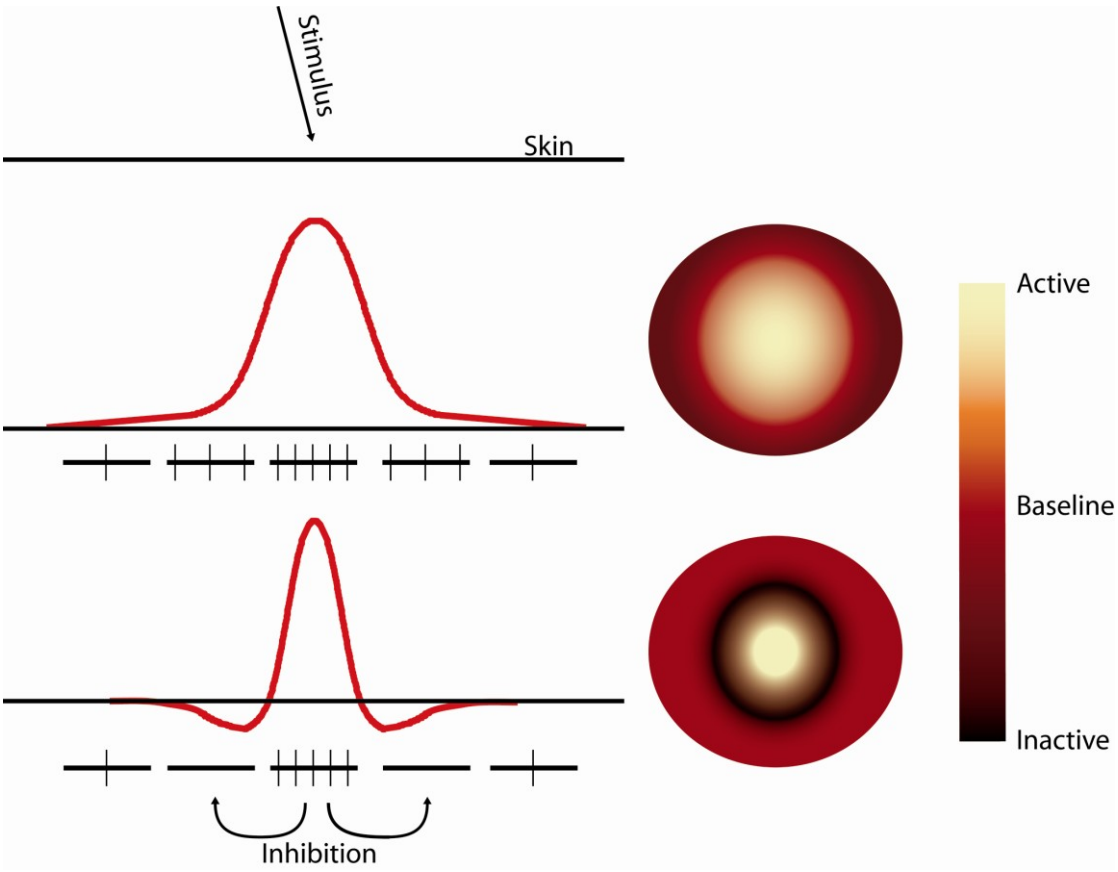

Figure 1.3. The effect of inhibition on sharpening of the neural response after stimulation. Imagine the skin being stimulated. The neuron that is most sensitive to the place of stimulation will fire harder than neighbouring neurons and thus an activity distribution arises. If the "most active" neuron inhibits neighbouring neurons, a sharper more focused activity pattern occurs, increasing the resolution. Based on Kandel et al(2000; figure 21-11)

1.3 The Somatosensory System

The following sections follow the processing of a tactile stimulation from skin to brain.

1.3.1 Cutaneous Processing

1.3.1.1 *Stimulus information is transduced by receptors of primary sensory neurons*

Information enters the body through the skin and is carried by a type of neuron called *primary sensory neurons* which are structurally unique in that they lack dendrites and synaptic inputs but instead terminate in receptors (Gardner, 2000). Primary sensory neurons bridge the gap between the outside world and the *central nervous system* (CNS**)**. In the 19th century (1826), Johannes Müller proposed the "laws of specific sense energies" which state that different types of receptor responds to a different "energy". The human nervous system contains many different types of receptors: cones and rods in the eyes; mechanoreceptors in the skin; chemoreceptors (e.g. olfactory receptors and taste buds in the nose and tongue); and free nerve endings. Each receptor is activated by a specific type of external stimulus. Neurons are "tuned" to specific types of stimulation (a stimulus that results in the optimal and maximal response of a neuron) and the resolution of a response is limited by the receptive fields of neurons.

1.3.1.2 *Stimulus-specific receptors in the skin*

The cutaneous system of the skin processes two main types of stimuli: first, detection of tactile stimulation in all its forms; and secondly, detection of temperature and pain. In the following section, only processing of touch will be

discussed. Touch in humans is processed by four different types of mechanoreceptors that are present in the skin. These mechanoreceptors have been described in a large number of studies and have been given different names, but for consistency the description of Johansson and colleagues will be used (Johansson and Vallbo, 1979a; Johansson and Vallbo, 1979b; Johansson and Löfvenberg, 1984; Vallbo and Johansson, 1984), see figure 1.4 for details.

1) Rapidly adapting units type I (RAI) or Meissner's corpuscles, are mainly found in glabrous skin and can be found just below the epidermis (Figure 1.4, top, a). RAI units respond primarily to moving stimuli or movement of a static object. RAI units have small receptive fields and respond quickly to dynamic tactile stimulation of frequencies lower than 50 Hz, which is referred to as the "flutter" range, and they *adapt* rapidly. This means that RA units respond quickly to stimuli and respond transiently to onset and offset of stimulation, but do not fire task-related for duration of the stimulus. RAI units are responsible for over 40% of sensory innervations in the hands.

(2) Slowly adapting units type I (SAI) or Merkel's discs can be found in the dermis of the skin and respond to sustained pressure and touch (Figure 1.4, top, b). They do not adapt quickly but instead fire for the duration of a stimulus (and sometimes afterwards). SAI units have small receptive fields and respond to vibrotactile stimulation with longer duration. SAI units are thought to underlie processing of perception and form of touch and are more involved in haptic processing (recognition of objects through touch).

(3) Slowly adapting units type II (SAII) (e.g. free nerve endings, Figure 1.4, top, c) have large receptive fields, sometimes as large as an entire finger, and respond to stretching of the skin. Named Ruffini corpuscles in cats (Chambers et al., 1972; Pare et al., 2002), there is no direct evidence that similar corpuscles exists in primate skin (e.g. monkeys; Pare et al., 2002), although SAII-like effects have been found using physiological studies (Rice and Rasmusson, 2000) and may act through patterns of Merkel's discs (Gottschaldt et al., 1972).

Finally, (4) Pacinian corpuscles (PC) lie deeper in the skin (Figure 1.4, top, d) and are responsive to high frequency stimulation above 50 Hz, where the range of RA I units stops (LaMotte and Mountcastle, 1975), also called "vibration". They are rapidly adapting and are thus also referred to as RA II units. PC's have large receptive fields (Vallbo and Johansson, 1984; Gescheider et al., 2004).

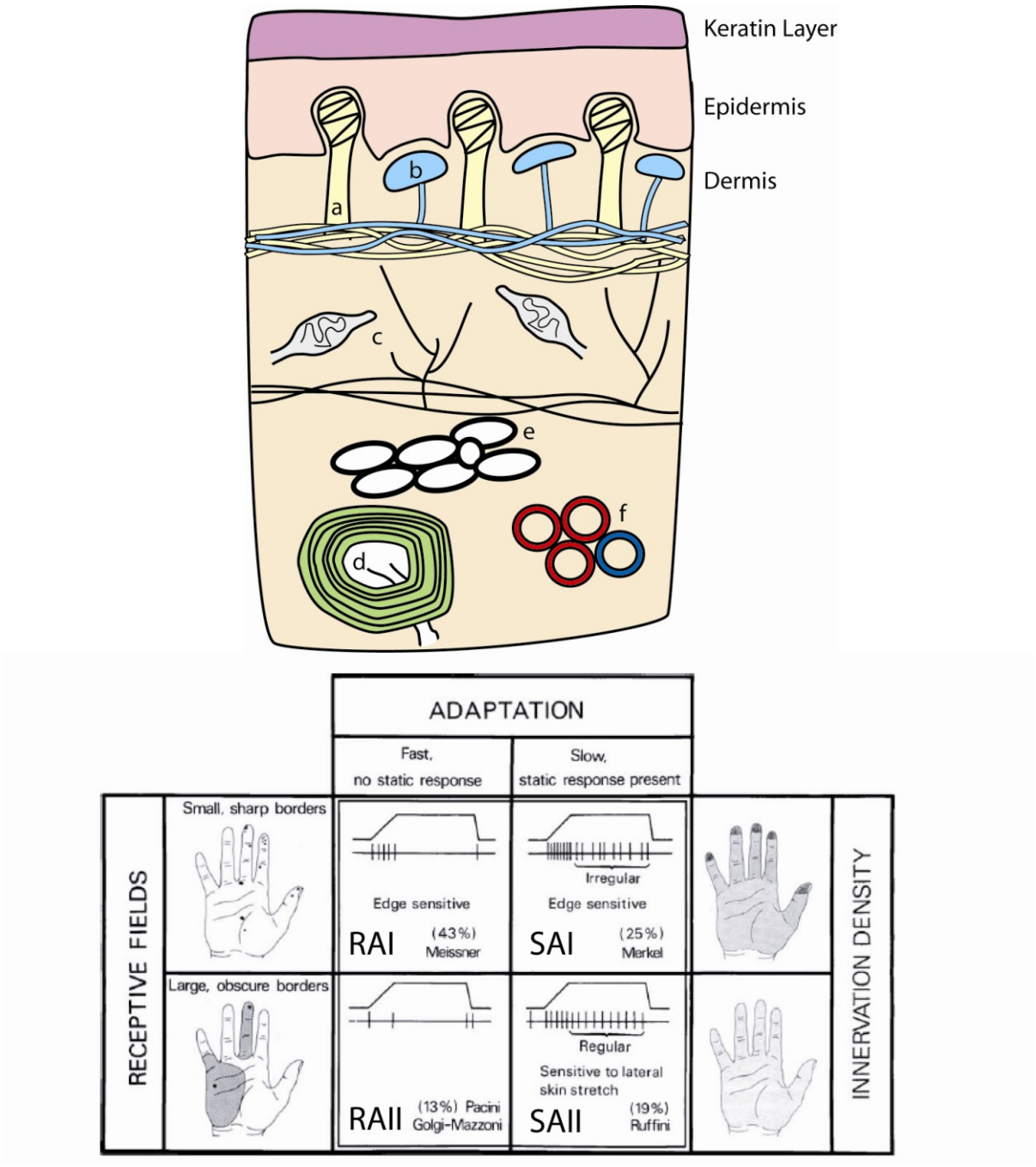

Figure 1.4. The four types of mechanoreceptors in human glabrous skin. Top: Schematic of the skin. RAI or Meissner corpuscles (a) and SAI or Merkels disk (b) units lie just underneath the epidermis. SAII (c) units, both Ruffini corpuscles and free nerve endings lie deeper in the dermis whereas RAII or PC units (d) lie deepest, in the area where binding tissue (e) and vessels (f) lie. Schematic based on Goodwin and Heat (2006). Bottom. Responses of the four mechanoreceptors. In the middle two columns are shown the nerve response to stimulation. RA receptors mainly respond to stimulus onset and then silent whereas SA receptors respond for the entire duration of the stimulus. The left most columns show small receptive fields for type I receptors and large RFs for type II receptors. The right column shows average density for both type of receptors (dark = denser) Adapted from McGlone and Reilly (2010) after Westling (1986)

The characteristics of a stimulus are often mirrored in the response of the receptor to the stimulus (although complex). As noted in Purves "The usefulness of having some receptors that adapt quickly and others that do not is to provide information about both the *dynamic* and *static* qualities of a stimulus." (Purves, 2007, p190). As all characteristics of a stimulus - intensity, frequency, and time course, are encoded in electrochemical signals it is important to be able to pull apart stimulus characteristics in the form of different neuronal responses. For instance, Adrian and Zotterman noted in the 1920s that the frequency with which a neuron fires increases with higher stimulus intensities (Mountcastle et al., 1967; Kandel et al 2000). In addition, a more intense stimulus often affects more receptors, which in turn leads to a larger active neuronal population. Temporal properties are also encoded in changes in neural firing - studies on rat somatosensory cortex have shown that some receptors fire with the frequency of the stimulation and then stop, whereas other types of receptors fire for the duration of the stimulus (e.g. differences between RA and SA receptors) (Mountcastle et al., 1967). Thus different receptors differ in their response duration (e.g. on-off vs continuous).

Whereas single receptors can encode duration, amplitude or frequency of a stimulus, spatial information (position, movement, force) is carried by comparisons *between* the responses of neurons in a population. Studies have shown that the relative timing of spikes between neurons contains information about the relative position of a stimulus. For instance, a difference in timing between two neuronal signals can explain a direction or difference of force. Relative timing can explain how the nervous system quickly responds to dynamic processes (Birznieks et al.,

2001). Differences in spike rates are slower to interpret than differences in timing between the initial spikes (Engel et al., 2001).

Another aspect of neuronal responses is that repetitive stimulation leads to adaptation, a process by which the neuronal response diminishes after sustained stimulation. It has been suggested that adaptation benefits the filtering of (conscious) information (Kandel et al., 2000). However, more recent findings have shown that adaptation is also a beneficial mechanism in sharpening the response of neurons to a particular stimulus and is useful in forms of stimulus-dependent plasticity; this will be discussed in Section 1.4.2.1.

Single receptors encode very specific stimulus characteristics but receptive fields become more complex further up the hierarchy. A more complex representation of a stimulus requires the integration of signals of multiple sensory neurons rather than stimulus-characteristics of a single neuron, as does discrimination between *multiple* stimuli. Differentiation between simultaneous stimulation requires integration of signals between neurons and although discrimination on the basis of a single neuron's response is technically possible, it requires a mechanism by which multiple responses of a single neuron need to be integrated. Dynamic processing of tactile information is further addressed in section 1.4.1.3.

1.3.2 Tactile processing in the central nervous system

Although the peripheral nervous system is anatomically different than the central nervous system, they are functionally related. In both the peripheral and central nervous systems clusters of neurons encoding the same body surface are grouped together and the representations for different body parts are separated in

anatomically distinct areas, this is called somatotopy. This clustering and separation allows for spatiotemporal integration of information to reflect stimulus properties.

1.3.2.1 *From skin to cortex*

In the somatosensory system, ipsilateral primary afferents project to the dorsal horn of the spinal column, where they ascend as the dorsal columns before terminating in the dorsal column nuclei of the medulla (see Figure 1.5). At this point, the axons are already organised in a somatotopic fashion (Brown, 1981; Florence et al., 1989) from medial (foot) to lateral (Marron et al. 1995) dorsal horn (Whitsel et al., 1972). The dorsal column nuclei also contain local connections and 30% of these local connections are thought to be GABAergic and functional in constraining receptive fields (Schwark et al., 1999). Already on this level there is integration/cross-talk between the different tactile pathways (Dykes, 1982) as well as between tactile and pain pathways. These projections are also under influence of descending corticospinal projections that can regulate the activity via both excitatory and inhibitory control to modulate the response properties of neurons in the nuclei (for a review see Willis, 2004).

The axons from the second-order neurons that start in the nuclei in the dorsal column cross to the contralateral side and project to the thalamus following the medial-lemniscus pathway (figure 1.5). Second-order neurons transmitting rapidly and slowly adapting “flutter” information terminate in the ventroposterior lateral nucleus (VPL) in the thalamus. In addition, tactile information from the face joins in the thalamus in the ventral posterior medial nucleus (VPM) through the trigeminal nerve. In contrast, vibratory information transmitted by RA II mechanoreceptors

terminates in the ventroposterior inferior nucleus (VPI) (Kaas et al., 1984) and SAII information in the ventroposterior superior nucleus (VPS) which is more closely connected to proprioception (McCloskey, 1974) although representations of RAII and SAII have been found in the VPL (Dykes and Gabor, 1981; Dykes et al., 1981). At this point, the entire body surface is represented in the VP complex of the thalamus (spatially, the medial to lateral axis corresponds somatotopically to the cranial-caudal direction). SAI and RAI afferents are represented in different clusters that differ in their relative tuning to the different ranges of receptor sensitivity, and so more than one somatosensory body representation already exists in the thalamus (Merzenich et al., 1978; Kaas et al., 1984). The size of the representation of an area in the thalamus is scaled to the receptor density of that particular area (Darian-Smith, 2004). The VP complex also contains local (mainly inhibitory) connections.

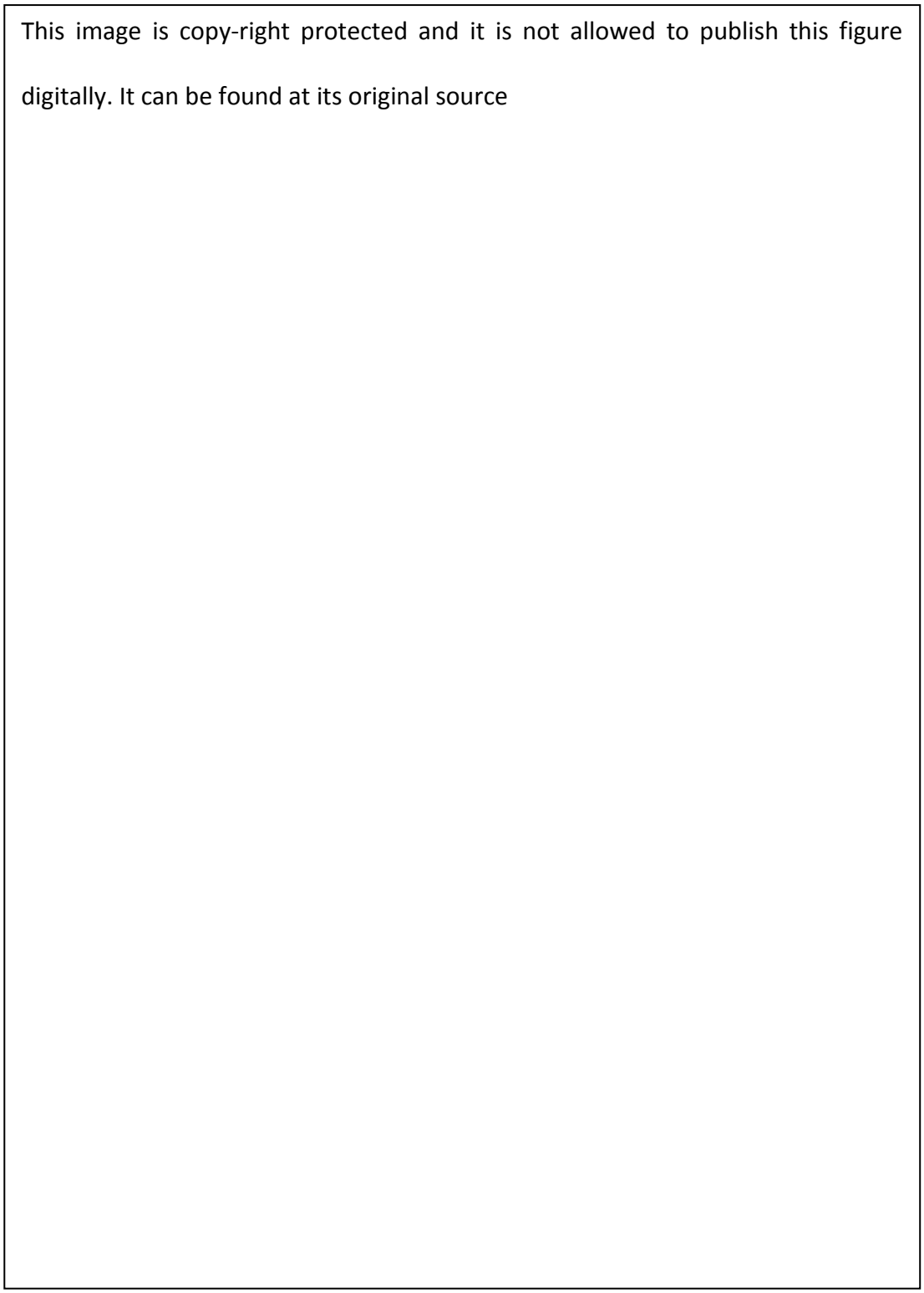

Figure 1.5. Cutaneous pathway. Primary afferent fibers travel to the spinal cord and terminate in nuclei in the brain stem. Second order neurons travel to the ventral posterior nucleus of the thalamus and third-order neurons project to the cortex. From Purves (2004; figure 8.1).

1.3.2.2 *The somatosensory cortex*

Sensory information from the thalamus projects towards the cortex through thalamocortical connections in the internal capsule. The somatosensory cortex is organised in six different layers (as are other primary cortical areas) A major function of the cells within the different cortical layers is to organise the input and outputs. Layer IV (or internal granule layer) contains excitatory pyramidal cells that receive projections from the thalamus. These pyramidal cells in layer IV project to the superficial cortical layers I-III. Layer II (or external granule layer) and III consist of pyramidal neurons with shorter axons. Corticothalamic and corticospinal projections emerge in layer VI (multiform layer) and V (internal pyramidal layer) of the primary sensory cortex. Layer VI also contains inhibitory stellate neurons that make connections with neurons ascending from the thalamus. Local inhibitory interneurons (thought to be primarily GABAergic) are present in all cortical layers (Kandel et al., 2000) and different types have been found (for an extensive review, see Ascoli et al., 2008). Cortical neurons generally have vertical projections (connecting with neurons in other layers) and lateral projections (connecting to neighbouring neurons within the same layer) and as described in Kandel et al. (2000) “The profile of inputs to a particular cortical neuron depends more on the distribution of its dendrites than on the location of its cell body.” Studies have shown that neurons are not only organised in layers but that vertical columns traversing these layers are functionally coherent and act as a local small neural network. The columnar organisation of S1 is addressed in section 1.4.

Primary somatosensory cortex

S1 consists of four distinct Brodmann regions (3a, 3b, 1 & 2; see figure 1.6) first described by Brodmann with anatomical analysis and confirmed by modern cytoarchitectonic analysis (Geyer et al., 1997, 1999). Each of these subregions contains a full body map where the body from foot to face is represented from the medial to lateral areas of primary somatosensory cortex (see figure 1.6) and has been shown in rodents (e.g. Beck et al., 1996; Kaas, 2004), monkeys (e.g. Pons et al., 1985) and humans. The somatotopical organisation is well known in humans and was first shown by direct electrical stimulation of the cortex in the 1930's by Walter Penfield (Penfield and Boldrey, 1937) and has been shown to be highly consistent across participants. Later studies using fMRI and MEG have confirmed these findings (Hari et al., 1993; Sakai et al., 1995). The somatotopy is characterised by its "homuncular" organisation where the size of the representation for each body part is dependent on the receptor density of that area (and relates to the sensitivity).

The different Brodmann subregions differ both in anatomy and function. Area 3 is divided into area 3a and 3b, differing in layer IV and in the size of pyramidal cells in the supra- and infragranular layers (Jones and Porter, 1980; Kaas, 1983; Qi et al., 1997; Iwamura, 1998). The different areas also differ in their responses to tactile stimulation. Animal studies have shown that neurons in area 3b and area 1 respond to cutaneous stimulation, whereas area 3a responds mainly to proprioceptive information and that area 2 responds to *both* tactile and proprioceptive stimulation and is likely to be involved in haptic processing. Although the topographic representation of the different Brodmann areas is very similar, the functional properties of the cortical neurons in each region are very distinct. Neurons from VP

project to pyramidal neurons in layer IV of area 3b of S1 (Jones, 1975) and additional spinothalamic projections project via VP to the superficial layers of SI and are thought to be involved in modulation of activity (Jones and Pons, 1998). The receptive fields (RFs) of the neurons in area 3b are relatively small, representing regions on a single digit (Hicks and Dykes, 1983), whereas neurons in Brodmann areas 1 and 2 generally respond to multiple digits. Receptive field size seems to increase from anterior to posterior primary somatosensory cortex (Gardner, 1988). Neurons in area 1 generally respond to a specific spatial direction of stimulation, whereas neurons in area 2 respond only to complex shapes (Kandel et al, 2000; for a review see Qi et al., 1998). Additional evidence that the regions are functionally distinct is that fewer neurons from VP project (and with slower speed) to area 1 than to area 3b and terminate in layer III (Nelson and Kaas, 1981). This shows that even in primary somatosensory cortex, a large differentiation in function and processing already exists, with each area containing at least one body representation.

Other cortical projections

Projections from the thalamus do not solely connect to the primary somatosensory cortex but project in parallel to the posterior and lateral parietal cortices: Area 5 and 7b; (posterior parietal cortex; PPC) are both part of the somatosensory association cortex and involved in e.g. locating objects in space, reaching and proprioception, and are more closely connected with the motor cortex (Kalaska, 1996; Snyder et al., 1998). There are also thalamic projections towards layer IV of the secondary somatosensory cortex S2 (located in the parietal operculum), which is associated with higher cognitive processing. Axons from VPI project to the

superficial layers of S2 and is therefore thought to underlie modulation of S2 activity through activity of S1 (e.g. Pons et al., 1992). S1 projects towards lateral somatosensory areas or S2 via the ventral stream that terminates in layer IV of S2 (for a review see McGlone and Reilly, 2010). Therefore lesions in S1 still result in activation of S2 and PPC. However, these S1-S2 connections seem to be diminished in monkeys and humans compared to other mammals (Friedman and Murray, 1986; Friedman et al., 1986; Krubitzer and Kaas, 1992). It is now widely accepted that the lateral sulcus contains more than one body map, one of which located in S2 and the other in the parietal ventral area (PVA). The body maps in both S2 and PVA are coarser than in S1 (Ruben et al., 2001), possibly due to their larger receptive fields. However, these receptive fields contain information from both sides of the body and are thus, bilateral, and indeed, S2 and PV receive information from bilateral S1 areas. A study by Poranen and Hyvarinen (1982) showed that whereas activity in S1 and thalamic nuclei was independent of attention, S2 activity was dependent on attention of an animal to a tactile stimulus. S2 also exhibits different populations of neurons whose firing rates change with attention. These findings suggest that S2 is involved with more complex detection and discrimination; as well as detection of stimulus patterns (for overviews see: Kandel et al., 2000; Purves, 2004; McGonigle, 2004). In the current thesis our main aim lies in the investigation of early cortical tactile processing and the following sections will focus on processing of tactile information in the primary somatosensory cortex in humans.

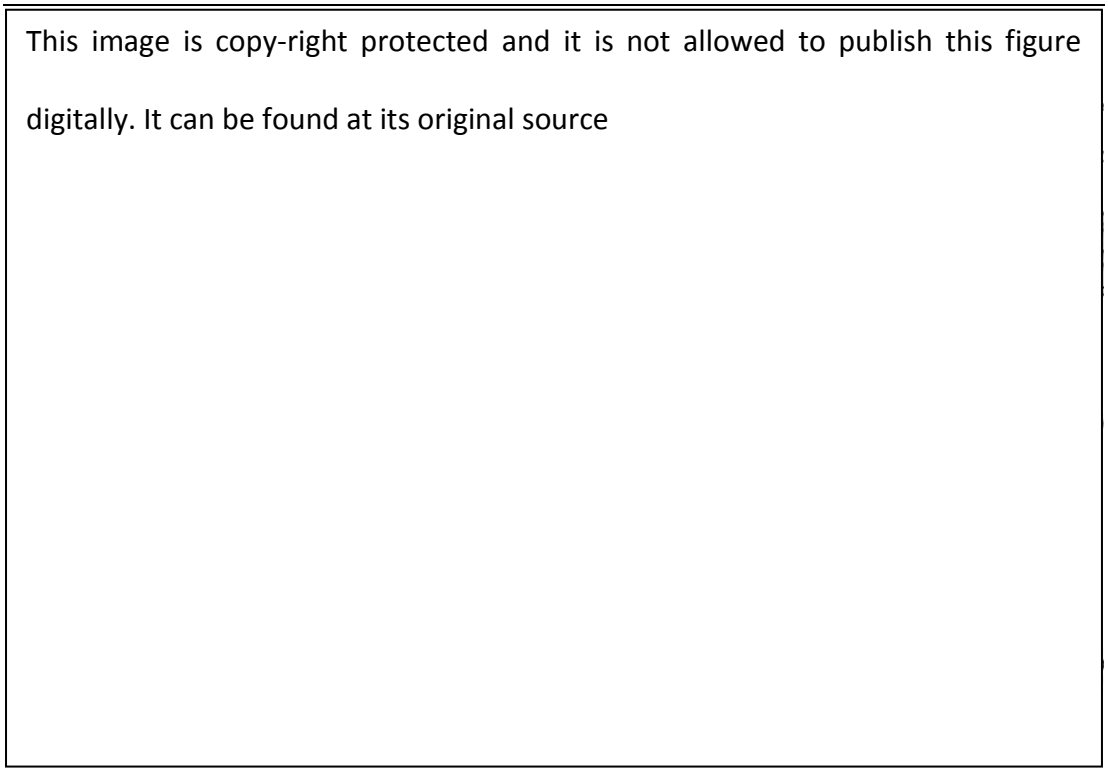

Figure 1.6. Organisation of the primary somatosensory cortex. A shows the organisation of the thalamus, location of the cortex and the differentiation of S1 In areas 1,2, 3a and 3b. B shows the somatotopical organisation of S1. Adapted from Purves (2004; figures 8.7 and 8.8)

1.4 The Neuronal Signature of Tactile Stimulation in S1

Our main interest lies in the dynamics of neuronal processing of cutaneous information in the primary somatosensory cortex. The dynamics of primary somatosensory cortex processing have been studied extensively over the last century and the current literature provides experimental records of somatosensory processing from invasive, non-invasive and psychophysical studies in both non-human mammals and humans. As mentioned in the previous section, vibrotactile stimulation is processed mainly in area 3b of the primary somatosensory cortex (e.g. Purves, 2004; McGonigle, 2004). From this point, the nomenclature primary somatosensory cortex or S1 will refer to area 3b, unless otherwise specified.

1.4.1 Area 3b of the primary somatosensory cortex

Within area 3b of monkeys, cells are clustered in areas that respond to an individual body part and even separation of areas responding to different digits has been shown (Jain et al., 1998; Jain et al., 2001) and a similar anatomical organisation has been suggested in other primates as well. Functionally distinct clusters of cells based on one receptor class have been found in layer IV (where corticothalamic projections terminate) of area 3b of monkeys (Sur et al., 1984) who showed distinct clusters that responded to either RA or SA stimulation. Recent studies have shown that neurons in area 3b are more broadly tuned and reflect both RAI and SAI type stimulation (Pei et al., 2009), partially because SAI neuronal dynamics partially overlap with those of the RAI. In addition, Chen et al. (2003) showed using optical imaging that RAI, RAII and SAI response in area 3b was *not* reflected by different clusters of neurons but rather by differences in spatiotemporal activity across a neuronal population, and Tommerdahl et al (1999b) showed that the same neuronal population is active for both flutter and vibration but responds differently to either type of stimulus. Tommerdahl et al. (2005a) showed that repetitive stimulation affects flutter and vibration differently which also suggests that there exist cross-channel interactions between RAI and RAII (see Chapter 8 for a more detail discussion. In addition to cytoarchitectonic analysis, areas can be distinguished on the basis of receptor density and receptor analysis in rats has shown a larger number of GABAergic receptors in layer IV of the somatosensory cortex than in neighbouring regions (Zilles et al., 2004).

The cortex provides feedback through corticothalamic projections via layer V and VI pyramidal neurons. Projections from layer V are thought to underlie feedforward

mechanisms to VP and spinal targets whereas projections from layer VI seem to underlie modulatory mechanisms in the thalamus. Both act on inhibitory and excitatory neurons in the thalamus and are known to affect receptive field properties and neuronal selectivity (for additional information, see Kaas, 2004).

1.4.1.1 *Anatomical location of area 3b of S1*

The human somatosensory cortex can be found posterior to the central sulcus. A previously used landmark locating area 3b of S1 is the omega-shaped "hand" knob in the precentral gyrus (when viewed in the axial plane, see figure 1.7; A; red = central sulcus; blue arrow = hand knob) which is known to contain the hand area of the primary *motor* cortex (Yousry et al., 1997) and a general assumption is that the somatosensory "hand area" lies directly across the central sulcus (Hari and Forss, 1999), on the postcentral gyrus and thus the motor cortex hand area helps locate S1. Another anatomical landmark that can be used in most participants is a "hook" indicating the primary motor cortex (when viewed in the sagittal plane, see figure 1.7. B; red = central sulcus; green arrow point to "hook").

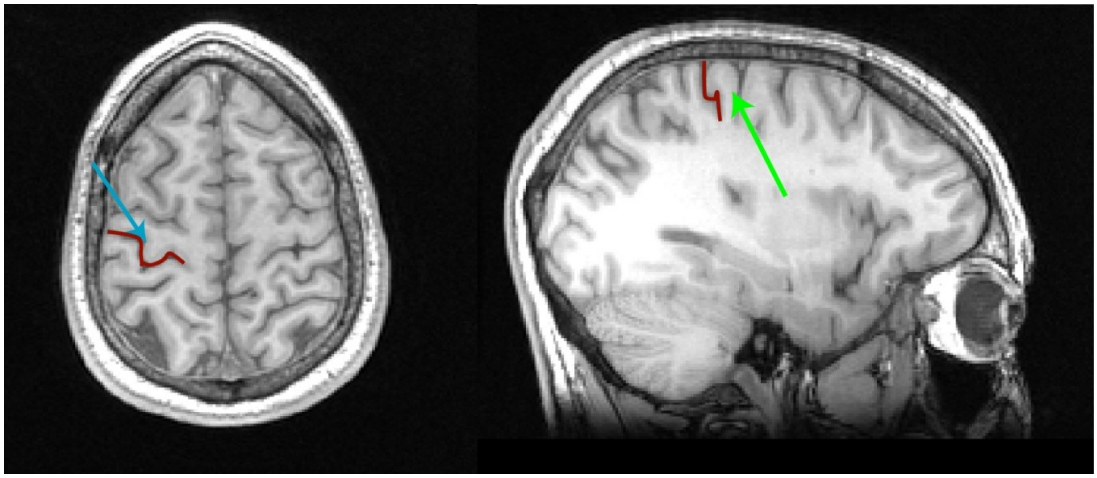

Figure 1.7. **Location of S1 on an anatomical T1 weighted MR image .** The left image (axial) shows the omega-shaped hand-knob in the axial plane. The blue arrow is centred on the omega shaped hand-knob. Red indicates the central sulcus. S1 lies behind the central sulcus. Right image (sagittal): the green arrow indicates the “hook” from the primary motor cortex in the sagittal plane. Red indicates the central sulcus.

1.4.1.2 *Columnar organisation of the primary somatosensory cortex*

In the primary somatosensory cortex, clusters of cells are organised in receptive fields that respond to different regions of the body (Sur et al., 1984; Favorov and Kelly, 1994b; Kaas and Collins, 2001) and as described in section 1.3.2, the primary somatosensory cortex contains a number of full body maps. Such organisation is not limited to the somatosensory cortex. For instance, the visual cortex contains clusters of cells that respond to specific receptive fields that respond to different parts of the visual field (Kandel et al., 2000; Purves, 2004) and the primary auditory cortex is organised in clusters of neurons that respond to different frequencies (Kandel et al, 2000; Purves, 2004).

However, it is less well known how the overlap between different regions of the body is represented in different receptive fields in S1 and how tactile stimulus properties are represented within a receptive field. A number of studies (Mountcastle et al., 1967; Jones et al., 1975; Mountcastle et al., 1990; Lee and Whitsel, 1992a; Lee et al., 1992; Recanzone et al., 1992b; Favorov and Kelly, 1994b, a) have investigated the columnar dynamics of somatosensory processing. Lee & Whitsel (1992a,b) and Favorov and Kelly (1994a,b) proposed the following complimentary models to explain somatosensory processing, originating from *in situ* work, *in vivo* work and computational modelling. According to this model, S1 is organised in distinct “minicolumns”; radial cords of cells with the same RF (originally suggested by Mountcastle, (1957, 1978). Groups of minicolumns are organised in macrocolumns or “segregates” (see figure 1.8). Evidence for this organisation was found by Favorov and Whitsel (1988) and Favorov and Diamond (1990) showing that neurons in the same column had similar receptive fields in the

skin whereas neurons in neighbouring columns differed in receptive field properties and RF field shifted suddenly. Other studies (Tommerdahl et al., 1993, 1987) have shown that minicolumns within a segregate expressed large differences in stimulus-evoked activity. Neurons within a column contained a high level of excitatory connectivity from pyramidal neurons that receive input from the thalamus in layer IV whereas there is mainly lateral inhibitory connectivity *between* minicolumns from GABAergic bouquet cells in the superficial layers of the cortex, so even the simplest tactile stimulation results in an active pattern of inhibition and excitation of multiple cortical columns (Lee & Whitsel, 1992a; Favorov and Kelly, 1994(Mountcastle, 1997). Favorov and Kelly (1994a, b) suggest that such a mechanism would "enhance differences in their stimulus-evoked activities and RF properties". Physiological studies have shown that more neurons are recruited after stimulation and that the latency of activation differs between segregates responding to different receptive fields (Lee & Whitsel, 1992a). This involves more neurons than those that directly represent the stimulated skin. The result of these more complex neuronal responses is the emergence of spatiotemporal representations in the somatic sensory system (Dykes et al., 1984). A recent study by Reed et al., (2008) showed that neurons across the extent of RFs in the hand interact and suggest that a large spatiotemporal pattern of synchronous firing facilitates discrimination. In addition, Chen et al., (2005) and Pei et al., (2009) suggest that a differential response between minicolumns is also dependent on integration of different channels (see also Tommerdahl et al., 1999b; Tommerdahl et al., 2005b).

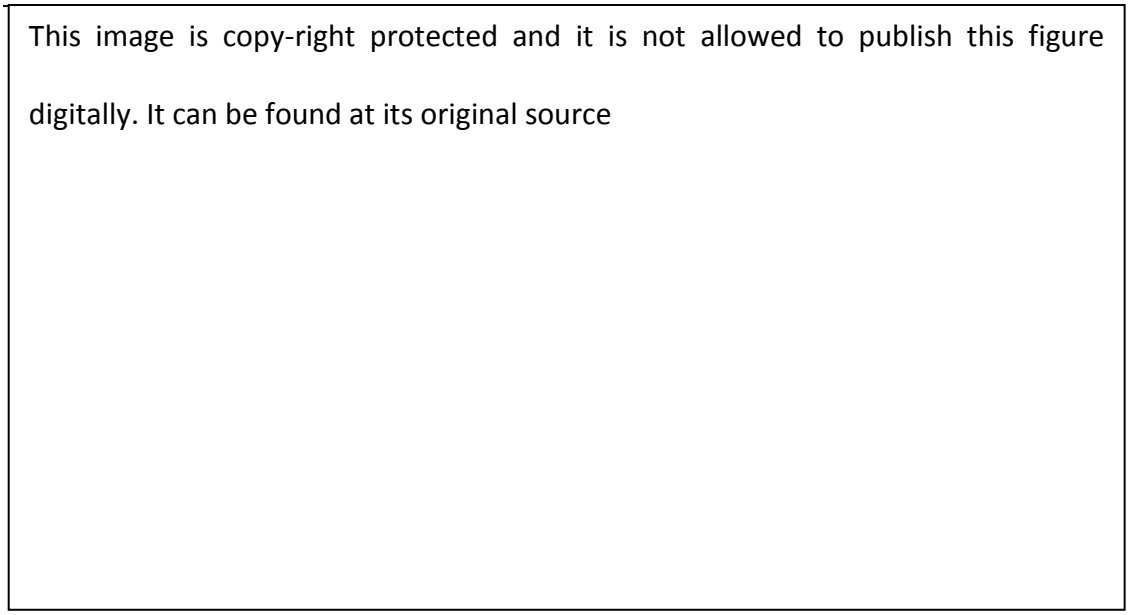

Figure 1.8: (From Tommerdahl et al., 2010). Minicolumnar organisation of primary somatosensory cortex. A-G: neurons within the same minicolumn respond to the same receptive field on the finger, whereas neighbouring minicolumns (1) respond to neighbouring receptive fields (segregate). Different segregates can respond to completely different areas on e.g. different digits (1-30).

1.4.1.3 *Dynamics of neurons in primary somatosensory cortex*

The previous section proposed a model of the organisation of neurons in the primary somatosensory cortex and how this would lead to the emergence of spatiotemporal representations to tactile stimulation. For a better understanding of this organisation as well as an understanding of the *dynamic* and *ongoing* processing of tactile information, it is important to understand how neuronal activity reflects different types of information such as stimulus frequency and intensity. A number of studies have investigated the role of neural activity in correspondence with stimulus characteristics.

Amplitude

Early work by Mountcastle et al., (Mountcastle et al., 1967) and LaMotte and Mountcastle (1975) have shown that in primate somatosensory cortex, different

stimulus amplitudes lead to differences in neural spike frequency as well as the amount of neurons that become active and thus that stimulus intensity is directly reflected in the size of the neural response. This stimulus-amplitude-dependence of the neuronal response in S1 has since been confirmed using optical imaging (absorption increases with higher intensity stimulus) over S1 (Simons et al., 2005; Muniak et al., 2007; Zhang et al., 2007) and human neuroimaging with fMRI; (Arthurs et al., 2000; Backes et al., 2000; Nelson et al., 2004) and MEG; (Iguchi et al., 2002).

Frequency

Neural encoding for frequency is less clear cut and its locations and mechanisms are still the subject of debate in the literature. Human and non-human primate studies (Mountcastle et al., 1990) have demonstrated that the primary somatosensory cortex is involved in coding vibrotactile frequency and other studies have shown that vibrotactile processing is mediated by glutamatergic-excitatory (Tannan et al., 2008) and GABAergic inhibitory (Dykes et al., 1984; Juliano et al., 1989) mechanisms. Many studies have investigated tactile frequency discrimination and the behavioural performance on tactile frequency discrimination has been described in some detail (e.g. Mountcastle et al., 1990; Hollins and Goble, 1994; Tommerdahl et al., 2005b). However, the mechanism by which frequency discrimination is encoded is less obvious.

As described in section 1.3, frequencies can be divided in flutter and vibration, which are processed by different channels (RAI and RAII channels respectively; see section 1.3). Studies by Mountcastle et al., (1969, 1990) and Recanzone et al.

(1992a; 1992b, 1992c) have shown that different frequencies in the flutter range do not show differences in spike frequencies and thus that they are not encoded in absolute spike frequency (whereas amplitude seems to be) (Whitsel et al., 2002). They showed that flutter stimuli are encoded in "highly periodic spike trains" or with "periodicity" encoding, phase-locked to the stimulus, and concluded from this that frequency discrimination cannot be performed on the basis of differences in firing rate but rather that on the basis of differences in the temporal coding of information (also; Mountcastle et al., 1969; Tommerdahl et al., 1987). This has been suggested to be influenced by GABAergic inhibition (McCormick et al., 1985; Whitsel et al., 2002). Recanzone (1992a; 1992b) also shows that specific neurons can change their degree of entrainment to a specific frequency through a perceptual learning paradigm: the temporal response of these "trained" neurons is sharper, due possibly to more cohesive firing between neurons. He proposes a mechanism by which more synchronous firing leads to less variability for frequency encoding, therefore facilitating discrimination.

Several studies have suggested a role for highly distributed cortical processing in frequency discrimination and suggest an important role of spike rate in frequency encoding (Salinas et al., 2000; Harris et al., 2001b; Romo and Salinas, 2001; Romo et al., 2003). A more detailed discussion of this contrast will be given in chapter 6 and chapter 9.

1.4.1.4 *The role of GABA in shaping the response of cortical neurons.*

A number of studies suggest that GABAergic inhibition is directly involved in determining the response properties of neurons that respond to sensory stimuli in

S1, but the exact role of GABA in encoding these response properties is less well understood. Evidence for an important role for GABA in sensory processing is present in both the molecular and the neurophysiological literature (Sillito, 1974, 1984; Alloway and Burton, 1986; McCormick, 1989; Alloway and Burton, 1991). Animal work has shown that GABA is directly involved in determining the response properties of neurons that respond to stimulus orientation (Silito, 1984) in visual cortex. Jones (1993) argues that GABAergic inhibitory transmission might be critical in changing the sensitivity of receptive fields by inhibiting neighbouring RFs, as has been found in cat visual cortex (Sillito, 1974). In motor cortex for instance, inhibition of neighbouring areas might facilitate timing and execution of movement. Rockland (1993) argues that these "context dependent changes in receptive field properties" (Rockland, 1993, p2) depend on the balance between activation (by glutamate) and inhibition (by GABA). The importance of GABA in regulating the neuronal response to tactile frequency has been shown by Juliano et al., (1998) and McLaughlin and Juliano (2005). Both show that modulation of GABAergic processing affects changes in "stimulus-evoked activity" to tactile frequencies. A role of GABA in shaping the neuronal reponses to tactile stimulation has been suggested by Recanconze et al., (1992a) and Kohn and Whitsel (2002) as well. The role of GABA in frequency discrimination is discussed in chapter 6.

1.4.2 Cortical plasticity

The advent of post-mortem analysis of human brains in the 19th Century led to the beginning of the doctrine of localisation of function, with lesions in specific brain areas being related to the loss of aspects of behaviour in individuals. More recently, the functionality of the brain has not just been described in terms of relations

between gross measures of neuroanatomy and function but also in terms of changes in neuronal function. It is evident that the brain is "plastic"; it has the ability to change connections or patterns of activation in the brain, depending on experience. The study of neuronal function has produced relatively well-described mechanisms of how function at a microscopic level can alter due to changes in receptor function and neuronal firing. In addition, there exists an extensive literature demonstrating the ability to improve perceptual, motor or cognitive function. There is, however, less *direct* evidence demonstrating how neuronal changes at the microscopic level relate to changes in plasticity at the behavioural level.

As described in Section 1.3 and Section 1.4, sensory systems in the human brain are organized in a topographic way. While the study of synaptic plasticity at a neuronal level is extensive, less is known on how microlevel changes lead to topographical map reorganization at the macroscopic level. Buonomano and Merzenich (1998) compared studies on plasticity on the neuron level with macroscopic studies involving changes in topographical maps in the cortex and describe the role of inhibitory plasticity on cortical reorganization. They argued that "inhibition may play an important role in shaping neuronal responses" (Buonomano and Merzenich, 1998, p159), suggesting an important role of inhibition on the sharpening of neuronal responses and plastic changes inducing increased sensitivity to particular patterns of input.

1.4.2.1 *Adaptation*

One form of plasticity is adaptation (see chapter 7 for a detailed discussion). Adaptation is defined as the adjustment of a sense to a specific perceptual stimulus

(Kohn, 2007). Adaptation can be thought of as a form of short-term (within milliseconds), stimulus-dependent plasticity. Studies investigating adaptation have one main thing in common; their results all show that an adapting stimulus has the strongest effect when it is closely related to a task (e.g. Kohn, 2007). For instance, an adapting stimulus has the strongest effect on discrimination around a 25 Hz tactile frequency when the adapting stimulus itself is 25Hz. Studies in the visual domain have shown that sensitivity to detect contrast decreases after adaptation (see e.g. Kohn, 2007 for a review). Other studies have shown an increase in sensitivity for motion stimuli after adaptation to motion (Clifford and Wenderoth, 1999; Krekelberg et al., 2006) and effects of adaptation have been found in the auditory system as well (Charron and Botte, 1988). Goble and Hollins (1993, 1994) and Delemos and Hollins (1996) investigated adaptation in the tactile domain and showed that an adapting vibration can *enhance* sensitivity to either amplitude (Goble and Hollins, 1993; Delemos and Hollins, 1996) -or frequency (Goble and Hollins, 1994). A vibrotactile adapting stimulus has shown to enhance the ability to detect differences in amplitude between a standard and a comparison stimulus, only when the adapting stimulus was of the same frequency and amplitude as a "standard stimulus" recurring in every trial. The same effect was found for the effect of adaptation on frequency discrimination (Goble and Hollins, 1994). These data suggests that adaptation of tactile processing is very specific to both the frequency and amplitude of the stimulus.

Several other studies have shown changes in cortical plasticity/tactile sensitivity by manipulating perceptual stimulation in humans (Bliem et al., 2007; Hoffken et al.,

2007; Tommerdahl et al., 2007a; Kalisch et al., 2008) or manipulation of cellular mechanisms (Lee et al., 1992; Lee and Whitsel, 1992b; Folger et al., 2008).

Stimulus-dependent effects on neural activity have also been measured using neuroimaging methods (Hodzic et al., 2004; Maertens and Pollmann, 2005). Lee and Whitsel (1992a) suggest that repetitive stimulation focuses on the adapting stimulus by inhibiting spatially neighbouring receptive fields. They argue (1992b) that this effect is caused by both strengthening and weakening of synaptic connections and by inhibition of surrounding receptive fields, sharpening the response to the adaptive stimulus. Von Bekesy has noted that lateral inhibition, the process by which excitation of an area is accompanied by simultaneous "silencing" of surrounding areas, may be *reduced* for prolonged stimulation. Von Bekesy said that: "It appears that the emergent patterns that are formed by minicolumnar activation from repetitive stimulation could be an important factor in feature extraction" (Cherrick and Cholewiak, 1986). Lee and Whitsel (1992a, b) have shown that the global pattern of activation of receptive fields shifts with repetitive stimulation (Recanzone et al., 1992a; Recanzone et al., 1992b; Simons et al., 2005; Tommerdahl et al., 2005b; Tommerdahl et al., 2007a; Whitsel et al., 2007b). This balance between excitation and inhibition seems to play a generally important role in synaptic dynamics (e.g. Hicks and Dykes, 1983; O'Mara et al., 1988; Lewis and Gonzalez-Burgos, 2000; Brunel and Wang, 2003; Storozhuk et al., 2005; Kohn, 2007) and will be discussed in more detail in Chapters 7 and 8.

Cortical changes after deafferentation suggests that the cortex is able to rapidly decrease rates of inhibition to allow for cortical reorganisation (Merzenich et al.,

1984; Calford and Tweedale, 1991b) and Levy et al., (2002) showed a reduction of GABA as a result of deafferentation and changes in GABA concentration have mainly been linked to long-term plastic changes. Long term plastic changes as a result of cortical reorganisation in S1 on the basis of changes in perceptual input S1 have been shown by several studies (Wang et al., 1995; Dinse et al., 2003; Dinse et al., 2005; Dinse et al., 2008; Ragert et al., 2008; Chow et al., 2009).

1.4.3 Neuroimaging studies of somatosensory processing

As shown in the previous section, there are a large number of studies investigating the neurophysiological correlates of tactile behaviour, but a link between these findings and human studies is limited. However, tactile processing has been studied using neuroimaging and particularly the response in terms of BOLD fMRI and EEG/MEG is well characterised. MEG studies on tactile processing will be extensively described in the next chapters (particularly chapter 7 & 8), and this section is an overview of the work in this field.

The work by Penfield was a major step in understanding the organisation of the somatosensory cortex and a number of studies have investigated the somatotopical organisation of S1 using non-invasive methods (for instance Fox et al. (1988) with PET; Hari et al. (1993) with MEG and Gelnar et al. (1998) and Maldjian et al. (1999) with fMRI) and improvements in the resolution of fMRI have been used to determine finger representations in area 3b specifically (e.g. Gelnar et al., 1998; Francis et al., 2000; van Westen et al., 2004; Sanchez-Panchuelo et al., 2010); comparable to invasive studies such as Wang et al. 1995).

In term of investigating the *functional* role of the primary somatosensory cortex, a number of studies have shown a difference in response to modulation of stimulus characteristics. Several fMRI studies have investigated changes in BOLD signal as a result of stimulus characteristics and have shown e.g. stimulus intensity dependence of the BOLD signal (e.g. Arthurs et al., 2000; see section 1.4.1.3). Other studies have shown changes in BOLD due to perceptual training (Hoffken et al., 2007). As will be described in chapter 5, a number of MEG studies have shown good localisation of S1 as well (also: Suk et al., 1991) and MEG studies have been used to gain an understanding of the role of different bands of cortical oscillations or the role of attention (e.g. Gaetz et al., 2003; Jensen et al., 2005b; Cheyne et al., 2007; Jones et al., 2010). Other advantages of functional neuroimaging are that the network properties of tactile processing can be investigated and several studies have attempted to look at the functional connections underlying tactile processing (e.g. Haegens et al., (2010a) using MEG and Pleger et al., (2006) and Pleger et al., (2008) with fMRI) and complementary MEG/EEG and fMRI have been used to understanding multiple aspects of somatosensory processing (Tuunanen et al., 2003; Parkes et al., 2006). Recent advances in high field MRI techniques at 7 tesla have shown that high resolution structural MRI images can be used to distinguish regions based on their anatomical properties and the laminar layering of the somatosensory cortex has been confirmed using such techniques and using 7T fMRI will provide excellent resolution to investigate S1 specifically (Wacker et al., 2011).

An additional technique that has a central position in this thesis is Magnetic Resonance Spectroscopy (MRS), a technique that uses MR to detect signals from a

large number of metabolites. In a relatively recent adaptation of this technique, MRS can be applied to GABA in particular and allows for the possibility to detect GABA in specific brain regions. MRS of GABA has not yet been used to investigate the somatosensory cortex and we aim to be the first to do this. Chapters 4 and 5 are entirely dedicated to the introduction and development of GABA-MRS.

1.5 Aims and thesis outline

1.5.1 An overall view of somatosensory processing

Somatosensory processing has been studied to differing degrees at both the macroscopic and microscopic levels in both humans and animals. Both levels of enquiry have their advantages, but attempts to combine the two approaches are still in their infancy. Recently it has become possible to measure the concentration of GABA *in vivo* using a technique called Magnetic Resonance Spectroscopy (MRS). *In vivo* MRS offers the possibility of being able to probe *in vivo* human neurochemistry whereas magnetoencephalography (MEG) offers the possibility to look at changes in neuromagnetic activation with millisecond accuracy. With these methods we want to establish whether *in vivo* non-invasive neuroimaging can elucidate the underlying neuronal mechanisms of human tactile behaviour and to determine how such findings can be integrated with what is currently known from invasive methods

GABA has an important role in encoding somatosensory responses to tactile stimuli and in shaping: (1) the sensitivity to tactile discrimination; and (2) stimulus-evoked changes in somatosensory processing. GABA also has a central role in neural plasticity. In this thesis we aim to investigate the neural dynamics that underlie

somatosensory processing using complimentary neuroimaging methods. Frequency discrimination will be used because the behavioural measures are well understood and there is a large literature on measures of tactile frequency discrimination. MEG has a high temporal resolution that can detect changes up to a millisecond timescale and frequency discrimination is a useful measure using MEG because it is not affected by differences in localisation (we stimulate a single digit) or by differences in stimulus amplitude that lead to changes in the power of cortical oscillations. Studies by Goble and Hollins (1994) and Tommerdahl et al. (2005) have shown that adaptation to frequency discrimination leads to changes in behavioural performance. Modulation of this behavioural measure may provide a useful tool in investigating the function of the corresponding neuronal correlates.

We think that the behavioural effect of adaptation as found by Goble and Hollins has clear neuronal correlates in the human brain. We hypothesise that non-invasive neuroimaging can be used to investigate the neuronal correlates of tactile processing, particularly with respect to adaptation. We hypothesise that these *in vivo* measurements reflect processes found using invasive studies described in Section 1.4.1 and that they can be used to understand processes on the micro- and macro-level of tactile processing.

1.5.2 Research questions and thesis outline

To our knowledge, this is the first time that behavioural psychophysics of tactile adaptation has been investigated using complimentary neuroimaging methods. Due to the novelty of these techniques, we first aim to investigate whether the techniques are feasible and we will discuss the following topics:

The details and basic principles of behavioural psychophysics as well as extensive pilot studies on the used psychophysical paradigms are described in Chapter 2. We want to know whether we can adapt the behavioural psychophysics experiments for MEG and induce adaptation to frequency discrimination.

Magnetoencephalography (MEG) will be used to investigate the human somatosensory processing and the effect of stimulus-induced changes in activity that accompanies changes in behavioural performance. The basic principles of MEG will be discussed in Chapter 3.

Magnetic Resonance Spectroscopy (MRS) will be used to investigate the role of GABA in tactile processing and explore the role of inhibition in regulation of tactile sensitivity as measured by behaviour. The basic principles of MRS will be discussed in Chapter 4.

Chapter 5 will discuss the development of edited MRS of GABA as a tool to obtain high quality GABA-MRS spectra over somatosensory regions and investigate what factors affect the acquisition of high quality MRS spectra. This knowledge will be used in further experiments and chapter 6 will discuss whether region-specific GABA concentration predicts individual behavioural differences of tactile frequency discrimination threshold.

In Chapter 7 and 8 two studies investigating adaptation to tactile frequency discrimination as measured by magnetoencephalography (MEG) will be discussed. In these two chapters we investigate the neuromagnetic correlates of tactile

adaptation to see whether a behavioural effect of adaptation is reflected in changes in cortical activity.

These chapters are followed by a general summary, discussion and conclusion of the findings in Chapter 8. The findings will be compared to earlier findings using psychophysics, MEG and invasive studies.

Chapter 2 - Behavioural Psychophysics

2.1 Introduction

The discipline of psychophysics utilises quantitative methods to study sensation and perception (e.g. Gescheider, 1997) and we have used psychophysics of tactile frequency processing to gain an understanding of the physiological processing underlying somatosensory function. As described in Section 1.4.2.1, Goble and Hollins (1994) found that an adapting stimulus of 25 Hz prior to each trial improved frequency discrimination around 25 Hz. As described in Section 1.5, we aim to use and adjust these paradigms for our studies. The tactile paradigms presented in this thesis are based on a number of these established behavioural tasks and will be described in detail in Section 2.3 and these behavioural paradigms have been adapted where needed, for use in MEG, as described in Section 2.4. The basic principles of psychophysics and descriptions and pilot data of the type of tasks we used are outlined between Section 2.2 and Section 2.4.

2.2 Psychophysics of tactile processing

2.2.1 Introduction

First described by Fechner in 1860, psychophysics uses quantitative techniques to measure "mental events". As explained by Gescheider (1997, page ix) "psychophysics is the scientific study of the relation between stimulus [physical domain] and sensation [psychological domain]". A general assumption in psychophysics is that a number of exposures to a stimulus are needed to reliably

measure behavioural responses, because the signal to noise ratio of behavioural measurements is generally low. Psychophysics provides a systematic approach to determining sensory sensitivity.

Using psychophysics, many aspects of sensory processing can be measured and generally four different characteristics are examined: intensity; quality; extension; and duration (Gescheider, 1997). The current thesis is mainly interested in studying detection and discrimination of vibrotactile frequencies. A detection threshold is the smallest stimulus value a participant can consciously perceive, while discrimination threshold is the smallest value *between* stimuli that a participant can distinguish.

Weber discovered that the behavioural response to discrimination does not depend on the absolute difference between two stimuli, but rather on the ratio between two stimuli. For a 25 Hz tactile frequency, a 10 Hz difference is large enough for participants to distinguish from 25 Hz. However, for a 200 Hz tactile frequency, discrimination of a 10 Hz difference can be difficult. This relationship is often expressed as the 'Weber fraction':

$$(\Delta f/f) = \text{constant (Weber fraction)}$$

The Weber fraction is an important measure to compare sensitivity measures across participants, groups and modalities. Often an additional term, representing sensory noise, is taken into consideration, and in Gescheider (1997) it is suggested that this term may represent the increase in neural activity to reach above the noise-level. Many sensory systems have been studied using the Weber fraction as a psychophysical measure of detection and discrimination.

2.2.2 Methods of reaching threshold

There are different approaches to obtaining threshold values. One is the *method of constant stimuli*, in which a range of stimulus values is presented to a participant. By repeating presentation of the same range of stimuli (generally in a random order) a proportion of "yes" responses can be found and plotted for each stimulus, resulting in a psychometric curve for a task. A psychometric curve describes the probability of detection of a stimulus (or discrimination between two stimuli) as a function of changes in the intensity of that stimulus (or differences between the stimuli). As shown in Figure 2.1AB, a psychometric curve generally describes a sigmoid shape, with stimulus parameters on the x-axis and the percentage of detection/discrimination on the y-axis. In a yes-no paradigm (Figure 2.1A), if a participant always detects a stimulus, the participant will always report 'yes' (100%), whereas a participant will always report 'no' for a stimulus that is never perceived. In between, there is a range where a participant sometimes detects a stimulus and sometimes not. A stimulus that is perceived 50% of the time indicates that this value is a measure of the participant's absolute threshold.

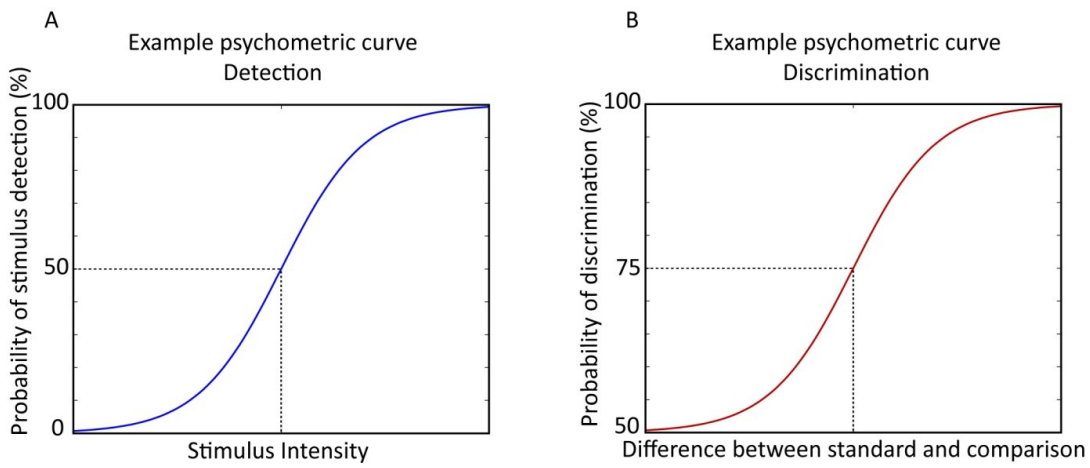

Figure 2.1. Example psychometric curve. A shows a psychometric curve for a *yes-no detection task* in which the stimulus intensity which is perceived 50% of the time is chosen

as threshold. B. In a *discrimination task* (comparison greater than standard?), 50% indicates a level where participants see two stimuli as similar. Therefore 25 or 75% are chosen as threshold values.

Generally a stimulus of fixed value, or *standard stimulus,* is presented together with a *comparison stimulus*, where the comparison stimuli consist of a range of stimuli. The comparison stimulus varies per trial. A participant may be asked whether one stimulus is greater than the other and from these responses, a psychometric curve can be created (see Figure 2.1B). In this case, the proportion of 0.5 does *not* indicate discrimination threshold. A proportion of 0.5 indicates that a participant is unable to tell two stimuli apart and thus performance is at chance level. Generally the value of 75% percent correct is taken as difference threshold.

Another common method is the *method of limits*, where the level of a stimulus is lowered from a suprathreshold point until threshold is reached. An often used implementation of this technique is the staircase method. In a staircase, a stimulus *decreases* stepwise towards threshold when the participant either perceives the stimulus (in case of detection threshold) or perceives it to be different from a standard stimulus (in case of discrimination threshold). When the participant's response changes (i.e. does not perceive the stimulus or does not notice a difference), the direction of stimulus change is reversed (called a reversal) and stimulus level is increased. The threshold can be determined after a number of reversals or by taking the mean of a number of trials. In the case of such a stimulus-tracking method, it is important that an appropriate step size is chosen. Steps that are too large do not allow for precise threshold measurement, but steps that are

too small may cause overestimation of the threshold and may need a large number of trials. An automated tracking method in which step size is relatively fixed allows for less experimenter error as well as experimental matching between participants.

A possible way to overcome the problems with step size is to use an *adaptive tracking* strategy in which the step size is modulated on the basis of a participant's prior performance on a trial. For instance, the step size is large for the start of a task, (and step size may even increase after a number of correct answers to reach a course measure of the threshold value quicker), and step size decreases after each reversal. This allows for more specific approximation of a threshold value (see Figure 2.2).

Several different adaptive tracking approaches are available and two have been used in this thesis. The accelerated stochastic approximation (ASA) increases the stimulus value for misses and decreases it for hits in the first two trials, but is only changed for a reversal in the remainder of the task. Step size is decreased for each reversal. This approach needs fewer trials to reach threshold than conventional 1-up 1-down methods (Kesten, 1958; Treutwein, 1995). Another approach is Parameter Estimation by Sequential Testing (or PEST; Taylor and Creelman, 1967) where step size is halved after each reversal, but doubled after a minimum of three steps in same direction and doubled again for each successive step. It also incorporates a minimum step size.

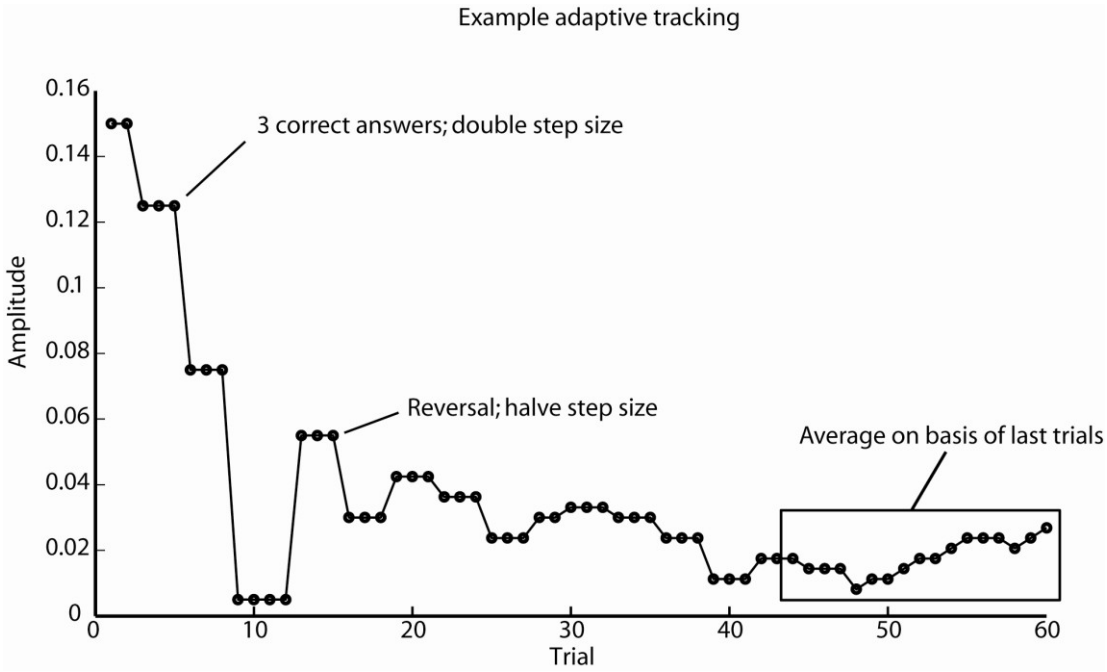

Figure 2.2 An example of adaptive tracking. Step size is increased after 3 correct answers, and halved after a reversal. Adaptive step size allows for large steps to get to a crude estimate of threshold and small steps to determine step size more specifically.

Another implementation of the method of limits is a *forced-choice method* (Blackwell, 1953). The examples above described an experiment where a participant had to report whether a stimulus was perceived or not, or whether a comparison was similar, lesser than, or greater than a standard stimulus. An issue with these approaches is that there may be a response bias. Some participants may report a perceived stimulus more readily than others and the differences between participants may cause inaccuracies in the measurements. A method to overcome this is by having the participant choose between numbers of different stimuli, "only one of which contains the correct stimulus" (Gescheider, 1997, p62). For example, in the case of detection threshold, a participant may be presented with two intervals in sequential order, but only one of the intervals contains a stimulus. In the case of frequency discrimination, a participant may be presented with two

stimuli in a sequential order and is asked which of the two stimuli had a higher frequency.

Experiments often use a combination of forced-choice methods and step-wise tracking, often with randomisation of stimulus occurrence. These are aimed to limit the amount of participant bias. Another possible source of bias in discrimination is that a comparison stimulus may be different from a standard stimulus in more than one aspect. For instance, a large pattern of light will be perceived as more intense compared to a smaller one despite the fact they have a similar luminance. Similarly, a tactile stimulus with a high frequency will be perceived as more intense than a lower frequency, even if they have the same amplitude. The methods described above may be used to match stimuli in intensity (see Section 2.3.4).

2.3 Adaptation to frequency discrimination

'Adaptation' as a neurophysiological process has been described in Section 1.5 and will be described in more detail in Chapter 7. Goble and Hollins (1994) found that adaptation to a 25 Hz tactile frequency improved performance on a tactile frequency discrimination task when one of the stimuli was 25 Hz. For the purposes of this chapter it is important to briefly outline the psychophysical methods Goble and Hollins, used, as they were subsequently adapted for MEG in this thesis. This section will describe the methods as used by Goble and Hollins (1994).

Goble and Hollins used a standard stimulus of 25 Hz - many other studies investigating tactile sensitivity have used 25 Hz (Gescheider et al., 1990; Hollins and Goble, 1993, 1994; Whitsel et al., 2005; Tannan et al., 2006; Simons et al., 2007;

Spitzer et al., 2010). Goble and Hollins (1994) initially performed a detection threshold task to establish a detection threshold for 25 Hz for each of three individual participants using a two-interval forced choice task where a stimulus was only present in one of two intervals. They ran five runs of 70 trials per body site and threshold was taken as the average of the last 50 trials. Adaptive tracking was used with a large step size for the first twenty trials and a smaller step size for the remainder of the task.

Previous frequency discrimination studies have shown that perceived intensity varies as a function of frequency as well as intensity (LaMotte and Mountcastle, 1975; Verrillo and Capraro, 1975). When a comparison frequency is of higher frequency than the standard stimulus, but has the same amplitude, participants might be able to detect differences on the basis of intensity rather than frequency, as the higher frequency stimulus feels more intense. To exclude this effect, Goble and Hollins constructed a subjective frequency-intensity matching curve for each individual. The 25 Hz stimulus was matched to a comparison stimulus in each run of 50 trials. In a two-alternative forced choice task participants were asked which of two stimuli felt more intense and adaptive tracking was used for the task. The matching amplitude for the comparison stimulus was taken as the average of the last 25 trials. All comparison stimuli were higher than the standard stimulus, taken in 5 Hz steps and interpolated to create a psychometric curve for subjective frequency-intensity matching. Finally, in the frequency discrimination task, participants were asked to do a two-alternative forced choice paradigm and to judge which of two stimuli had the highest frequency. Starting comparison stimulus

frequency was varied pseudorandomly and intensity was taken from the matched psychometric curve. Comparison frequency varied in a step-wise fashion, increasing by 1 Hz following an error and decreasing after 3 correct answers. Goble and Hollins used runs of 70 trials and frequency discrimination threshold was taken as the average of the last 40 trials. In three of four conditions, each trial was preceded by a 15 second adapting stimulus, differing in amplitude (one condition contained 15 seconds of silence). All three participants were tested for five times per run. Goble and Hollins found that performance increased most (compared to the silent condition) when the adapting stimulus was the same as the standard stimulus in both frequency and amplitude.

2.4 Optimisation of acquisition of tactile psychophysics

The paradigm used by Goble and Hollins (1994) described in Section 2.3 was adapted and optimised for use in MEG. The aim of this section is to determine whether we can replicate the study by Goble and Hollins in an adaptation paradigm adapted for MEG.

2.4.1 Equipment

Vibrotactile stimulation was delivered using a piezoelectric vibrotactile stimulator (McGlone et al., 2002); see Figure 2.3A). This is a single-digit stimulator that is easily controlled from Matlab. Contact with the skin was made via a plastic probe (7mm in diameter). A static surround limited stimulation to the skin region placed on the probe. All stimulation was delivered to the glabrous skin of left digit 2 (index finger). The frequency range used was 10-40 Hz. Stimuli were delivered via the audio output of a laptop computer (Sony Vaio VGN-NS20M, Realtek high definition audio,

volume at 100%) using Matlab2008b (The Mathworks, 2008). Stimulus amplitude was determined on an arbitrary range from 0-1. Amplitude of zero did not cause vibration of the stimulator whereas amplitude of 1 caused maximum displacement of the stimulator. Values above 1 did cause any subsequent changes in the amplitude of the vibration.

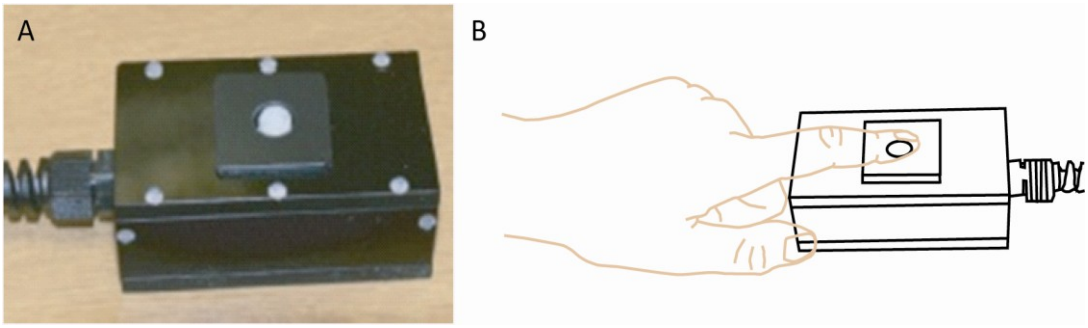

Figure 2.3. The piezoelectric stimulator used throughout this thesis. A. Contact with the skin was made via a plastic probe (7mm in diameter). Stimuli were delivered via the audio output of a laptop computer. B. Finger is placed on the stimulator as shown, and taped into place.

2.4.2 Experimental parameters

Similar to Goble and Hollins, all tasks consisted of a two alternative forced choice task (2AFC) as described in Section 2.2.2. Throughout the experiment the *standard stimulus* consisted of a 25 Hz stimulus which was randomly distributed to occur in either of two trial intervals. If present, the *comparison stimulus* always differed in frequency and/or amplitude from the standard stimulus. Throughout the experiment, inter-trial intervals (Hamalainen et al. 1990) consisted of a 2 second pause with a ~100 ms. jitter to reduce expectation of stimulus occurrence and focus attention. Each trial consisted of two intervals with a pause (ISI; 1s +/- 100 ms) in between, after which participants were asked to respond, indicated by a question mark presented on the screen. Previous studies (e.g. Goble and Hollins, 1993, 1994;

Hollins and Delemos, 1996) used coloured lights to indicate different features of the paradigm. This study did not use coloured indicators to exclude a possible coupled visual activation in MEG, because tactile and visual perception occurs at the same time and neural activation might interfere in the neuroimaging study. However, green or yellow crosshairs were used to indicate correct or incorrect answers respectively in the detection and frequency discrimination tasks.

Initially very low intensity stimuli were used. However, the task was too difficult for participants. It has been shown that the Weber fraction increases non-linearly for stimuli just above detection threshold (Gescheider, 1997), showing that discrimination is hindered by the use of near-threshold stimuli. Therefore it was decided to use stimuli that were easy to detect (as used by Goble and Hollins; Hollins, personal communication). In correspondence with early studies on frequency discrimination (Mountcastle et al., 1990) we used suprathreshold stimuli with an intensity of eight times stimulus detection threshold.

2.4.2.1 *Tracking*

Goble and Hollins used a step-up step-down tracking, but to allow for better modulation and flexibility of the step size and reach threshold quicker, we used adaptive tracking. Initially, adaptive tracking in the form of ASA (Section 2.2.2, Kesten, 1958; see also Treutwein, 1995) was used in all but one experiment.

2.4.3 Preparation

Participants were seated comfortably and their left index finger was placed on the stimulator (see Figure 2.3B) and taped into place to reduce finger movement. Participants received a practice session in which they were exposed to exemplar

vibrotactile stimuli of various frequencies and amplitudes. Once participants reported they were comfortable with discriminating the different stimuli, they received a practice session of each task, ten trials per practice session. The practice sessions were repeated until participants showed a 100% correct level. Participants were instructed to press a response key as fast as possible at the termination of each stimulation period.

2.4.4 Detection Threshold

Participants Nine healthy right-handed participants performed this task once (6 male, avg. age 28.3). One participant repeated the task over five consecutive days (male, 25 years old).

Methods A 2AFC threshold task was used to estimate participants' detection thresholds. Participants had to detect in which of two intervals (each interval was indicated with a yellow crosshair on the screen, the crosshair remained white during the ISI and ITI) the stimulus occurred (see Figure 2.4). 75 trials were used with ASA tracking and the detection threshold was determined as the average of the last 25 trials.

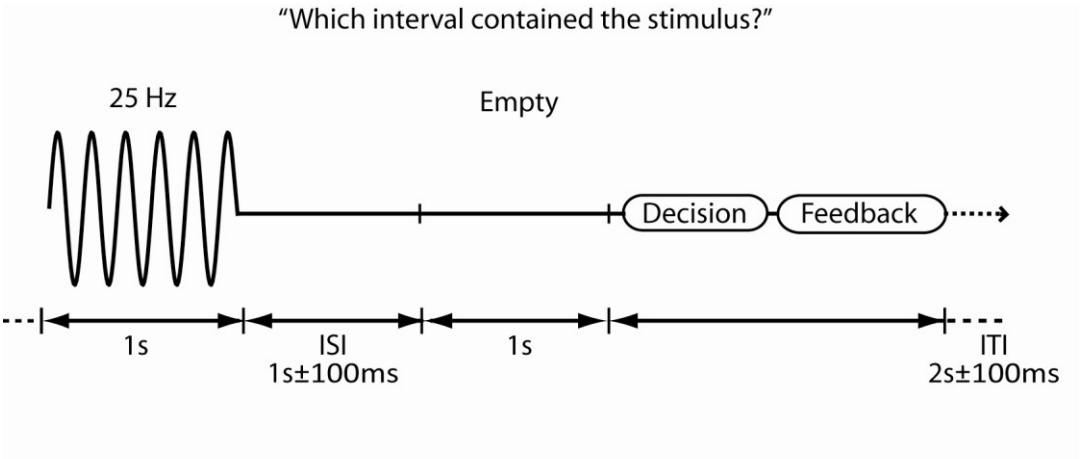

Figure 2.4. Protocol for the detection threshold task. Subjects received the standard stimulus of 25 Hz in one interval while the other interval was empty. Order was counterbalanced. Subjects had to decide in which interval they felt a stimulus. Each interval was indicated by a yellow crosshair.

2.4.4.1 *Results and discussion*

To explore the variability of detection threshold, one participant performed the detection threshold task on five consecutive days (see Figure 2.5). For further studies a single run per participant was used (following a practice session). Detection threshold can be calculated in a number of different ways. Goble and Hollins (1994) determine it as the last 50 trials of a run. Other studies determine it on the basis of number of times the step size is decreased (reversals; see Gescheider et al., 1997). In our studies we used the average amplitude over the last 25 trials. Figure 2.6 shows detection thresholds (mean over last 25 trials ± standard deviation across last 25 trials) for all nine participants.

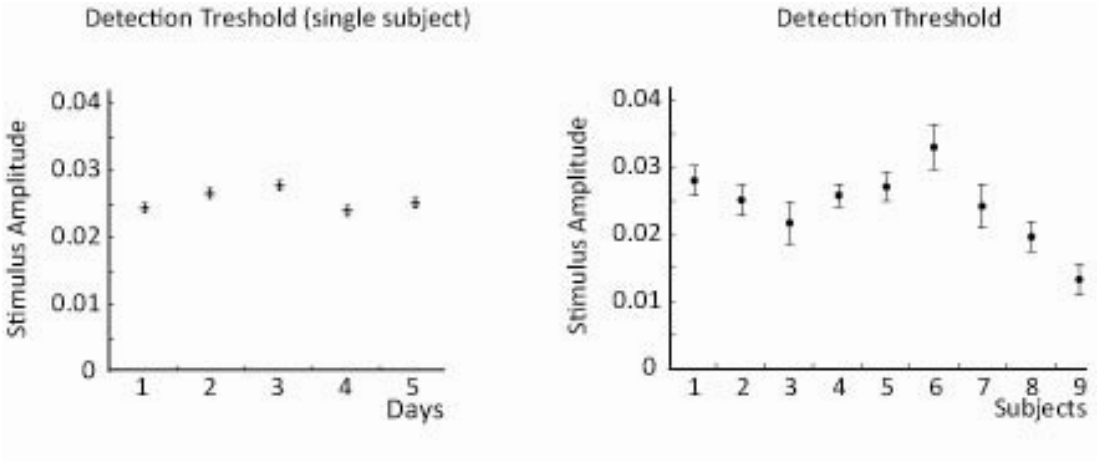

Figure 2.5. Detection threshold measurements. A. A single subject performed the task on five consecutive days. B. Nine subjects performed the detection threshold task. Threshold is taken as the average of the last 25 trials. Error bars indicate the standard deviation of the amplitude over this range.

2.4.5 Subjective frequency-intensity matching

Participants Nine healthy right-handed participants performed this task once (6 male, avg. age 28.3).

Methods To account for the effect of intensity on frequency discrimination (see Section 2.3), a subjective frequency-intensity matching curve was created for

individual participants. A 2AFC amplitude discrimination task was used, in which participants were presented with two intervals in each trial, one of which contained the standard stimulus and the other one containing a comparison frequency (see Figure 2.7). After each trial participants were prompted to respond with a button press indicating "which stimulus felt more intense?" indicated by a question mark. Each run started with a set comparison frequency and the standard stimulus. Starting amplitude for the standard stimulus was chosen as eight times the detection threshold amplitude found using the task described in Section 1.4.3 and amplitude for the comparison stimulus was chosen pseudorandomly. Amplitude of the comparison stimulus decreased when the comparison stimulus was rated as more intense, and increased when the standard stimulus was rated as more intense, following a 1-up 1-down staircase tracking paradigm rather than an adaptive tracking paradigm. A different tracking paradigm was used because the adaptive tracking paradigm is based on levels of reversal and aimed at a 75% correct interval, which is unsuitable for this task as the aim of this task is to obtain specific comparison values, rather than approximations of 75% correct.

In the first 20 trials step size was 0.05, allowing the staircase to theoretically reach each expected subjective intensity value, for the last 30 trials step size was 0.025 to allow for a more precise measurement. There were 50 trials for each run. This task was repeated for 5 frequencies (15, 20, 30, 35 & 40 Hz). For the subjective matching average for the last 15 trials for each of the above frequencies were taken as points on the curve and the corresponding curve was interpolated to create a subjective matching curve with 1 Hz steps. Initially we also compared stimulus amplitude for 10 Hz to the standard stimulus of 25 Hz. However, 10 Hz felt very different from 25

Hz and comparison appeared very difficult and very variable, possibly because 10 Hz did not even feel as flutter. Therefore this frequency was dropped altogether from the remainder of the studies in this thesis.

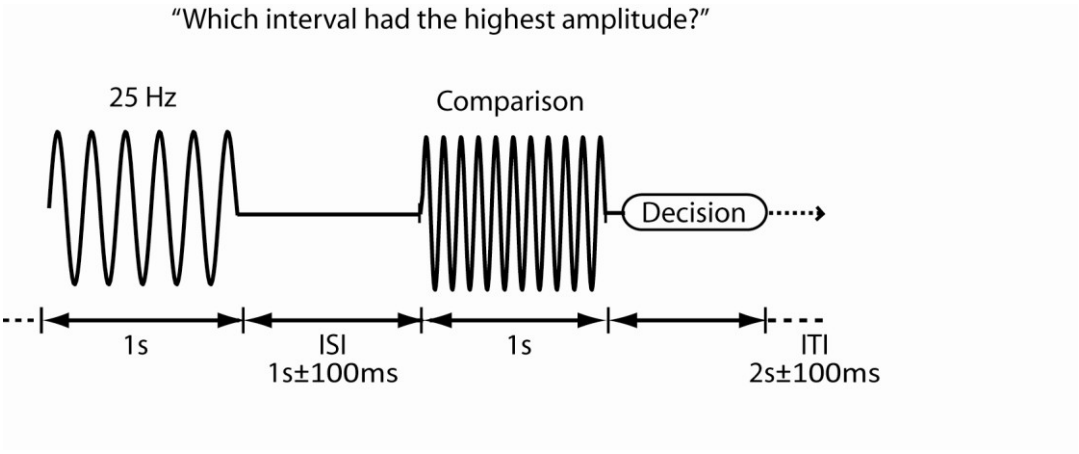

Figure 2.6. Protocol for the subjective frequency-intensity matching task. One stimulus was always the 25 Hz standard stimulus and in each run the comparison stimulus had a fixed frequency (15, 20, 30, 35 and 40 Hz). Subjects were asked to perform an amplitude discrimination task to match the intensity of the different comparison frequencies. There were then interpolated to create a psychometric curve.

2.4.5.1 *Results*

Nine participants performed the task (see Figure 2.7). Previous studies all found a relatively linear decrease in matching intensity with an increase in frequency (Verillo & Capraro, 1975; Goble & Hollins, 1994). The individual effect seemed to replicate itself in one participant who was tested on this task twice. Generally, the individual frequency-intensity curves showed a similar shape and only differed in amplitude due to their different 25 Hz amplitude based on the detection threshold (see Figure 2.7).

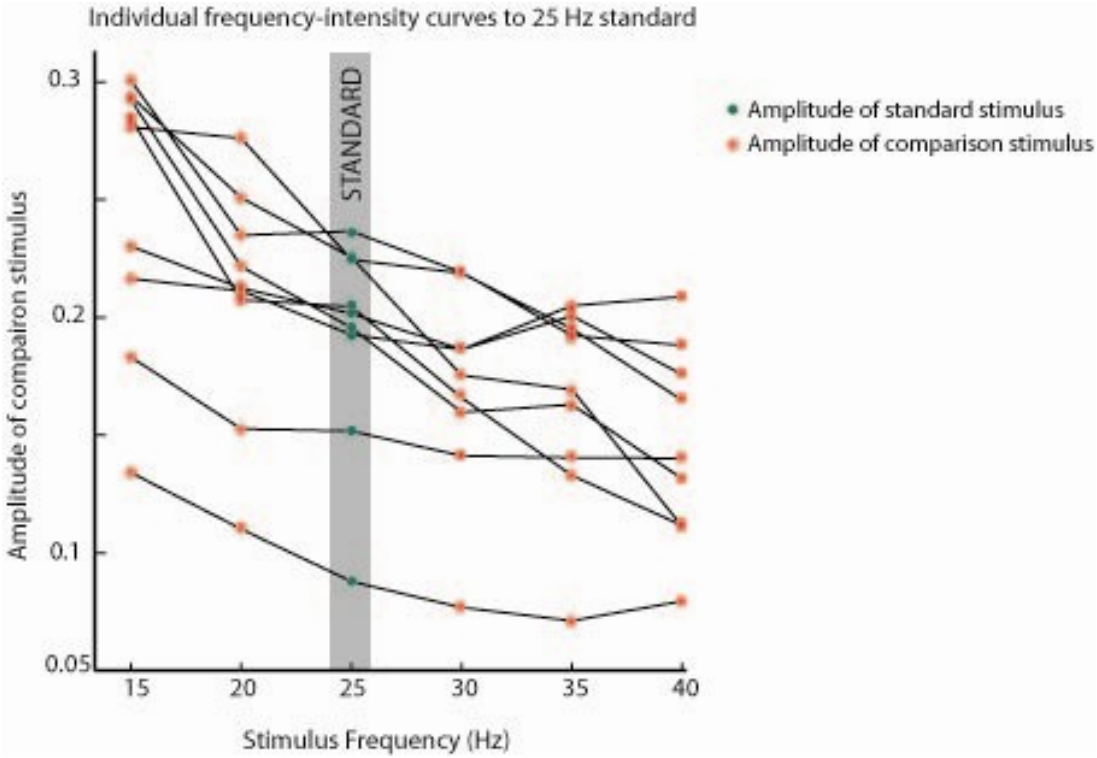

Figure 2.7 Individual frequency-intensity matching curves for nine subjects. The 25 Hz stimulus is the standard stimulus and the amplitude of the other frequencies were matched to the standard stimulus amplitude. Despite a difference in offset (due to differences in detection threshold) the general linear slope is seen for all subjects. The grey area indicates the amplitude of the standard stimulus (eight times detection threshold).

2.4.5.2 *Discussion*

Our results show that despite a difference in offset, the shape of this curve is similar between participants. Both our pilot data and previous findings show a relatively linear decrease in subjective intensity with increased frequency. Therefore a *normalised* frequency-intensity curve was created and for further studies each participant was assigned a specific normalised frequency-intensity curve based on their individual detection threshold, without having to acquire individual frequency-intensity matching curves.

2.4.6 (Adaptation to) Frequency discrimination

Participants Nine healthy right-handed participants performed this task once (6 male, avg. age 28.3).

Methods The length of the adapting stimulus was decreased to 5 seconds as a compromise between experiment length and power in the MEG. In the behavioural tasks described here we investigated whether this decrease in adaptation duration still produced the behavioural effect seen by Goble & Hollins. Two adaptation conditions were used. In one condition the adapting stimulus had the same amplitude *and* frequency as the standard stimulus; in the second condition the adapting stimulus was 40 Hz, which we hypothesised to have a lesser or negative effect on discrimination sensitivity to a 25 Hz standard. Initially we chose a 60 Hz adapting stimulus, as we thought this frequency would have no effect on frequency discrimination of stimuli of a lower frequency. However, this seemed to cause more ambiguity in the participants' perception of the task, possibly because a 60 Hz vibration lies on the edge of flutter and vibration (Goble & Hollins, 1994). Therefore we chose a 40 Hz stimulus.

Each condition (see Figure 2.8) consisted of a frequency discrimination task in which each trial consisted of a 5-second adapting stimulus, followed by a pause, and two 1-second intervals with a 1-second (+/- 100 ms.) pause in between. In each trial, one of two intervals contained the standard stimulus of 25 Hz (amplitude eight times each participant's detection threshold) and the other trial contained the comparison stimulus. Because the 25 Hz standard stimulus was always similar to the adapting stimulus in the 25 Hz adapting stimulus condition, there was a chance that participants simply matched the standard stimulus to the adapting stimulus to

assist in the task. Rather than frequency discrimination this would involve categorical matching (as suggested in Romo and Salinas, 2003) between standard and comparison. Therefore, in comparison to the task used by Goble & Hollins (1994), this task contained *two* tracks, rather than one (Figure 2.8). The starting comparison frequency was 15 Hz in the lower track and 40 Hz in the upper track. The amplitude of the comparison stimulus was determined by the subjective frequency-intensity matching task. The order of tracks was randomized, with a maximum of5 trials of the same track in a given experimental session, and the order of standard and comparison were randomized as well. Each participant was presented with both conditions, creating a within-subject paradigm. Each track contained 50 trials summing up to 100 trials in total. The frequency discrimination threshold was determined by the average of the last 10 trials for each track separately. For each condition the Weber fraction ($\Delta f/f$) was determined by the average of the upper and lower track. To assess whether subjects were biased to a specific stimulus interval, we measured d', a measure of sensitivity bias, for both intervals. The d' is calculated by z-transform of the combined proportion of hits and false alarms (over all responses). A higher d' suggests that a signal can be more easily detected, so for example; a significantly higher d' for the first interval compared to the second interval would suggest subjects were better at discrimination when the higher frequency occurred in the first interval.

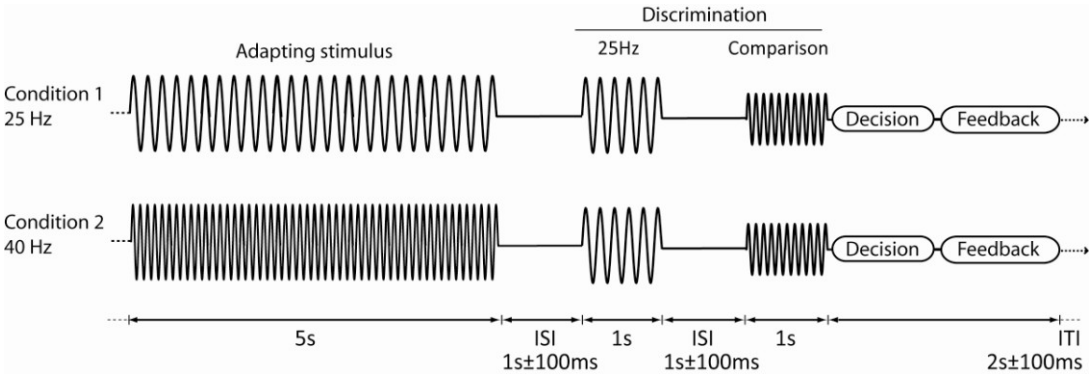

Figure 2.8. **Protocol for the adaptation to frequency discrimination task**. Each frequency discrimination trial consists of a 25 Hz stimulus and a comparison stimulus, which can be either higher or lower in frequency. Subjects are asked which stimulus has the highest frequency. In condition 1, each trial (100 in total) is preceded by a 25 Hz adapting stimulus. In condition 2, each trial is preceded by a 40 Hz adapting stimulus.

2.4.6.1 *Results*

Figure 2.9 shows average Weber fractions for the frequency discrimination threshold for all individuals in both the 25 Hz and 40 Hz adapting stimulus condition. The effect of the 25 Hz adapting stimulus is significantly larger than that of the 40 Hz adapting stimulus (Two-sample T-test, paired, two-tailed, $p < 0.05$) and thus participants were significantly better at frequency discrimination when the adapting stimulus was similar to the standard test stimulus. All participants but one showed the effect of adaptation and Weber fraction between participants ranged between 0.004 and 0.25.

In addition, the threshold curve was steeper for the 25 Hz adapting than for the 40 Hz adapting stimulus (measured as average difference between trial 1:10; Two-sample T-test, paired, $p < 0.05$), but the significance of this for adaptation is unclear. Despite the introduction of a two-track paradigm with randomised order, it may have been possible that participants had a bias towards the first or second interval (particularly when the 25 Hz adapting was followed by the same 25 Hz standard

stimulus. Therefore we calculated the d' (see 2.4.6) which showed no difference in bias between intervals ($p > 0.5$).

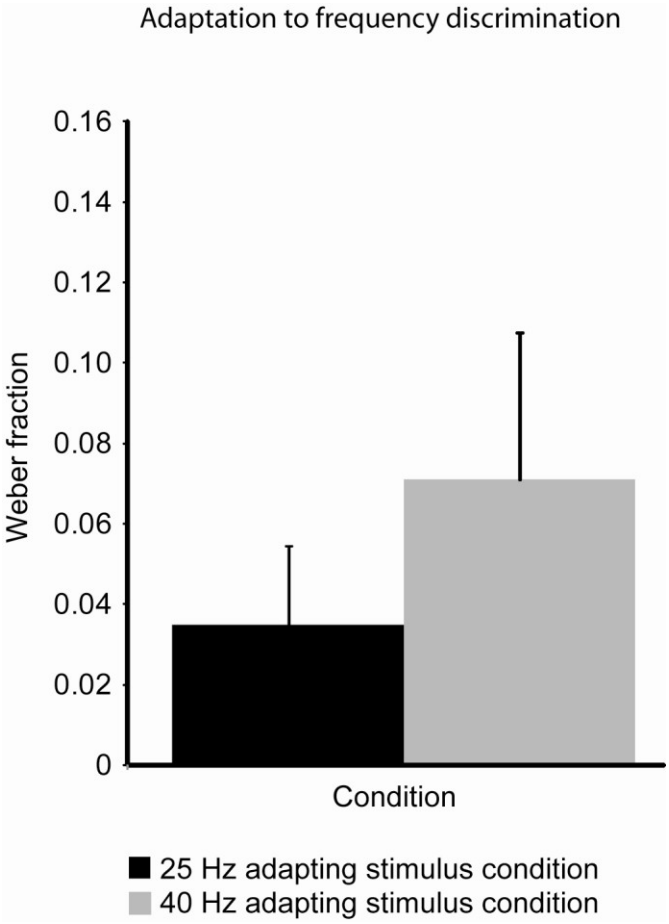

Figure 2.9. Group average Weber fraction (±standard deviation across subjects) for frequency discrimination for the 25 Hz and 40 Hz adapting stimulus conditions. Subjects' frequency discrimination threshold to 25 Hz is significantly lower after adaptation to a 25 Hz adapting stimulus than after adaptation to a 40 Hz adapting stimulus ($p < 0.05$).

2.4.6.2 *Discussion*

The results show that a 5-second adaptation of the same frequency and amplitude as the standard test stimulus increases sensitivity to discriminate frequencies around 25Hz compared to a similar adapting stimulus of 40 Hz. These results confirm and advance previous findings by Goble and Hollins and show that the neuroimaging adapted paradigm is suitable for inducing adaptation.

Comparison of Weber *fractions*

Firstly, some of the individual frequency discrimination thresholds (measured as the Weber fraction) found in our study are far smaller than found in the experiment by Goble and Hollins (1994) and previous work by Mountcastle and colleagues (LaMotte and Mountcastle, 1979; Mountcastle et al., 1990). Generally, Weber fractions between 0.07 and 0.2 have been reported (Rothenberg et al., 1977; Mountcastle et al., 1990), corresponding to a detection difference between 2-5 Hz for a 25 Hz stimulus, and some of the Weber fractions we reported are a factor of 5 smaller and correspond to a detection difference of less than 1 Hz. This effect might be caused by the differences in protocol we introduced. Franzen and Nordmark (1975) also show a far smaller Weber fraction, and they argue that differences in Weber fraction might be caused by differing paradigms in terms of type of stimulation used, skin site, amplitude and psychophysical testing. This makes it difficult to compare Weber fractions across studies. Another suggestion is that the ASA tracking paradigm used may be suboptimal for our population of naive participants: this may result in incorrect answers nearer the end of a task, producing local minima in the threshold estimation. To avoid this in future, we used Parameter-Estimation by Sequential Tracking (PEST; see Section 1.2.2) for all our further frequency detection and discrimination experiments, as its ability to both increase and decrease step sizes may assist participants naive to performing psychophysical paradigms.

Adaptation for MEG

Secondly, despite the changes in stimulus duration, this particular paradigm is suboptimal for MEG as two of the stimuli (the higher and lower comparison stimuli) will continuously change as they adapt throughout the course of the task. As will be explained in detail in section 3.4, MEG analysis generally averages over a number of trials and as stimulus parameters change at each trial, averaging is not possible. A different approach was suggested where frequency discrimination thresholds were acquired *prior* to MEG scanning (a single condition, 100 trials, without adaptation). The frequency discrimination task results in the 75% correct frequency discrimination threshold. Adaptation has been found to increase discrimination threshold. Therefore we explored whether an adapting stimulus improves performance in discrimination between 25 Hz and the frequency discrimination thresholds in a MEG study described in chapter 7.

2.5 General Discussion

Changes to the paradigm used by Goble and Hollins (1994)

Several changes will be made to the paradigm as used by Goble and Hollins (1994).

Experimental setup We are using a different stimulator from the one used by Goble and Hollins (1994). Furthermore, no colour coding was used to indicate intervals to reduce the possibility of visual activity during the MEG experiments (except for during detection threshold measurements, see Section 2.4.4).

Tracking Initially we tried ASA-tracking, compared to step-up step-down, to reach threshold more quickly, but this method did not prove reliable (See Section

2.4.6.2). Therefore we plan to use PEST tracking in our future studies to allow for more flexibility in the stepsize and reduce the likelihood of reaching local minima.

Detection Threshold We have shown that the naive participants are able to perform the detection threshold task (section 2.4.3) and to conserve time, we suggest a single run per participant for our studies compared to multiple runs used by Goble and Hollins (1994).

Subjective frequency intensity matching Despite differences in the standard stimulus amplitude, we have shown that there is little difference in the shape of subjective frequency-intensity curve between participants (Section 2.4.4). The downside of acquiring a frequency-intensity curve for each individual participant is that it takes at least an hour of behavioural testing, even when not acquiring repeated measures per participant. For the benefit of reducing experiment time we suggest creating a frequency-intensity curve for each participant based on a normalised curve rather than obtaining multiple values for each participant for each comparison frequency, as done by Goble and Hollins (1994).

Adaptation task We have shown that adaptation to 25 Hz can be induced with an adapted adaptation paradigm compared to a 40 Hz adapting stimulus and we show similar behavioural effects as Goble and Hollins. In our experiment we used two tracks with a higher and lower comparison frequency to reduce the effect of categorical learning (see Section 2.4.6). We also propose for future work to use PEST-tracking obtain frequency discrimination thresholds (without adaptation) and use these values to investigate the effect of adaptation on sensitivity to frequency discrimination (see 2.4.6.2) the amount of useful signals is increased which will

benefit acquisition in MEG. The basic principles and limitations of MEG as well as the analysis of these tasks in MEG will be discussed in the next Chapter.

Chapter 3 - Magnetoencephalography Methods

3.1 Introduction

Magnetoencephalography (MEG) is a non-invasive neuroimaging technique that allows neuromagnetic activity to be recorded with high temporal resolution of milliseconds. It measures the (weak) magnetic fields generated by neurons using magnetometers. MEG was first applied to measure human brain function in 1968 by Cohen and the first use of superconduction was not until 1972 (Cohen, 1972). The source of the signal acquired with MEG and how MEG is used to measure different aspects of cortical processing will be described in the following sections.

3.2 Basic Principles of MEG

3.2.1 Basis of the neuromagnetic signal

Ionic current flows produce changes in magnetic fields, but at the level of individual neurons this signal is too weak to measure with MEG. MEG requires large numbers – estimated to be in the millions - of neurons to be active synchronously (Hamalainen and Hari, 2002; Singh, 2006). This current is not simply generated by action potentials, but incorporates multiple neuronal signals such as fast electric spiking and slow synaptic potentials. Action potentials are unlikely to be the source of the signal measured with MEG, because they are brief (1ms), can travel in many different directions, and fall off quickly with distance (Hari and Kaukoranta, 1985). Hamalainen et al., (1993) suggested that the source of MEG currents is represented by synchronously firing postsynaptic potentials of pyramidal cells that are aligned

(see also Hari and Forss, 1999; Zhu et al., 2009b) as shown in Figure 3.1Aii. The alignment is important because "a population of closed cells, in which the dendrites are randomly and uniformly distributed, will not generate a net magnetic field" (Singh, 2006) p293, see Figure 3.1Ai). As the apical dendrites of pyramidal neurons are aligned towards the superficial layers of the grey matter, they are a candidate for the generators of the MEG signal acquired from the brain (see Figure 3.1Aii).

To be able to measure the weak magnetic fields generated by the brain, MEG uses Superconducting Quantum Interference Devices (SQUIDS), small superconducting devices, immersed in liquid helium. Many of these SQUIDS together (see Figure 3.2A), around the head, can measure the magnetic field generated by almost the entire cortical surface simultaneously.

MEG is not susceptible to volume conduction by the skull (as EEG is) and therefore acquires signals from the primary source of a current within the brain. However, MEG has its own limitations. Because magnetic field strength drops with distance, MEG is most sensitive to sources that lie relatively close to the surface of the brain although there are ways to measure deeper brain sources as well.

Because MEG measures very weak magnetic currents, it is very susceptible to outside sources. For instance, an elevator moving close to a MEG system will cause currents that are orders of magnitude larger than those generated by the brain and even the beating of the heart is a larger source of magnetic fluctuations than the brain is. To address these issues, a MEG system is generally situated in a shielded room to isolate the system and deflect electromagnetic currents.

In addition, aside from a single coil perpendicular to the surface of the skull (a magnetometer) the MEG uses gradiometers to detect functionally relevant signals over more uniformly distributed noise. Gradiometers use combinations of coils wound in opposite directions and can therefore measure the magnetic field in different directions can be used to "attenuate" signals from distant sources (Vrba and Robinson, 2001) making them more sensitive to the spatial direction of a magnetic field and the difference between gradiometers can also be used to detect sources deeper within the skull as the signal from radial and planar gradiometers (see Singh, 2007) is different for deeper sources. The difference in distance between components of the gradiometer predicts the amount of electronic noise that is rejected, but the smaller the distance, the less sensitive the gradiometer is to deeper sources within the head (Singh, 2007). In addition, MEG is most sensitive to sources that are located in sulci normal to the scalp surface, and due to the curvature of the cortical surface (see Figure 3.1Aiii) it may be less sensitive to activity on the ends of gyri. However, Hillebrand and Barnes (2002) suggest that the cortical surface is generally well localised with MEG. In addition, the direction of the source (see Figure 3.1B) may actually be able to provide information about the alignment of the neurons.

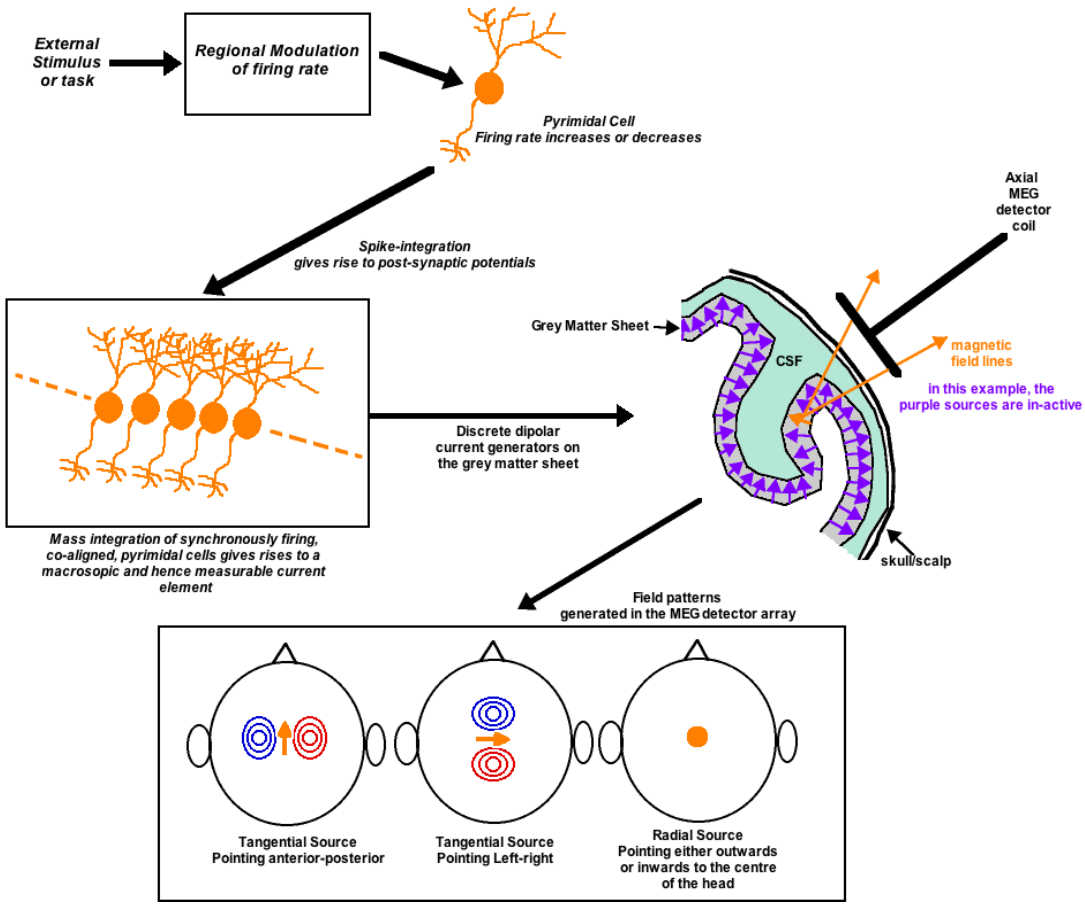

Figure 3.1. Generation of neuromagnetic fields. A. The neuronal processes that lead to measurable neuromagnetic activity. Ai: a tasks leads to changes in local neuronal firing of pyramidal cells. Aii Spiking leads to post-synaptic potentials in networks. Spatially aligned pyramidal cells synchronously fire leading to a measurable current. Aiii: Dipolar currents are generated in the grey matter, but depending on the anatomy of sources, the direction of the dipole is different. B. Different locations and directions of sources lead to a different shape of the dipole. With permission, from Singh, 2006.

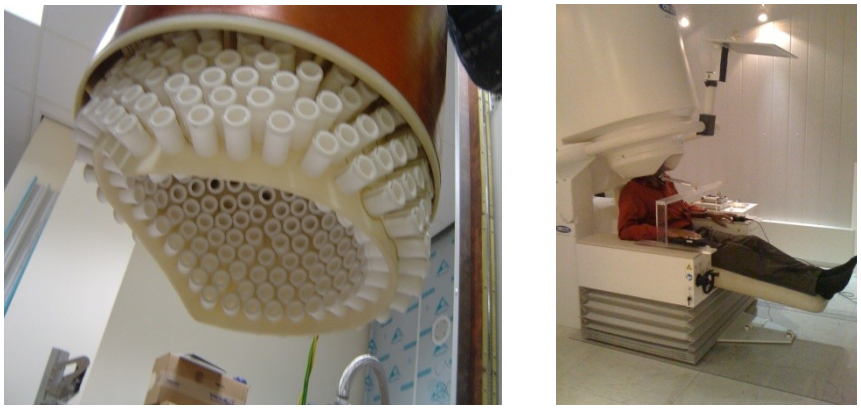

Figure 3.2. The MEG system A shows the 275 gradiometers in the MEG system (prior to install). B shows a subject in the MEG, during a tactile experiment. The subject is raised up into the helmet. A chin-rest is used for additional support

3.2.2 Acquiring MEG data

As there is no clear relationship between scalp anatomy and the position of the brain in the skull, it is necessary to record each participant's head position within the MEG helmet (see Figure 3.2B). Therefore generally three fiducial positions are recorded; two positions indicating the tragus of the ears and one position indicating the nasion (see figure 3.3). Participants are then raised into the MEG helmet with the skull as close to the helmet surface as possible (see Figure 3.2B). To localise the activity on a human brain, a structural MR image is required, and the fiducial positions localised on the MR image.

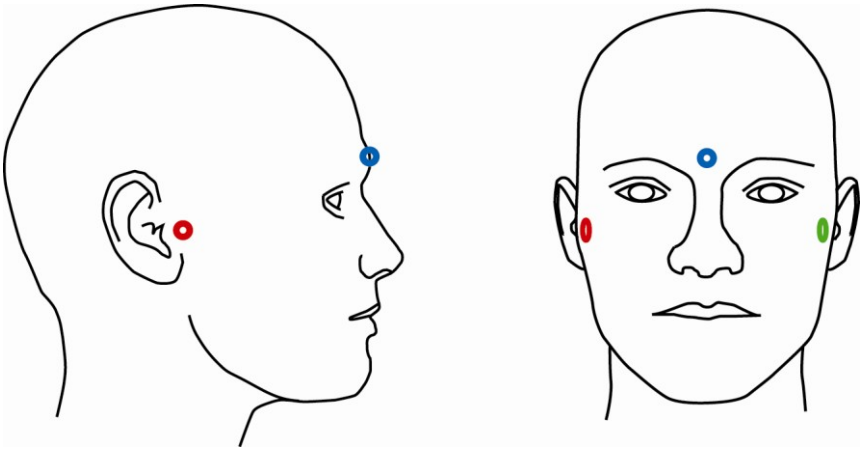

Figure 3.3. Location of the fiducials. To localise a source in the brain, three fiducials are placed on the face of a subject. Two next to left (green) and right (red) tragus and one on the nasion (blue). These are subsequently placed on the subjects anatomical MRI scan. These help localising the source on the brain.

While in some cases it is possible to determine a neuronal response on a single trial, within a single MEG recording session it is typical to deliver a number of trials, with an aim to eventually average the recorded activity. Because electromagnetic noise is generally uncorrelated between different trials, it is usually averaged out.

Because MEG measures the magnetic field with millisecond accuracy, it is important that stimulus triggers are recorded throughout the experiment. This simplifies averaging across trials depending on stimulus.

3.2.3 What can we measure from the data?

MEG data is generally recorded with millisecond-level temporal resolution for each of the gradiometers in the MEG system. By recording triggers and knowing where in the time-course the processes of interest take place (e.g. stimulus occurrence), the data can be divided into epochs*, a priori* specified time regions of interest.

One of the responses that can be measured between epochs is the *average evoked activity.* To obtain the evoked activity, all sensor time series for each epoch are averaged together (see Figure 3.4). This approach allows activity that occurs in every trial, time-locked, to become averaged and visualised. This type of activity is called *phase-locked*, because the phase of the activity is locked to the stimulus onset across trials. This evoked activity is similar conceptually to the evoked potentials approach to EEG, and similarly to EEG, analysis focuses on the amplitude and latency of a number of 'components' (see Section 3.3.4). For example, the M50 is an *event-related field* (ERF, named ERP in EEG research), an increase in magnitude of activity that occurs 50 ms after a given sensory stimulus. In addition, *steady-state* evoked responses can be found, an oscillatory response driven at the stimulus frequency (Nangini et al., 2006).

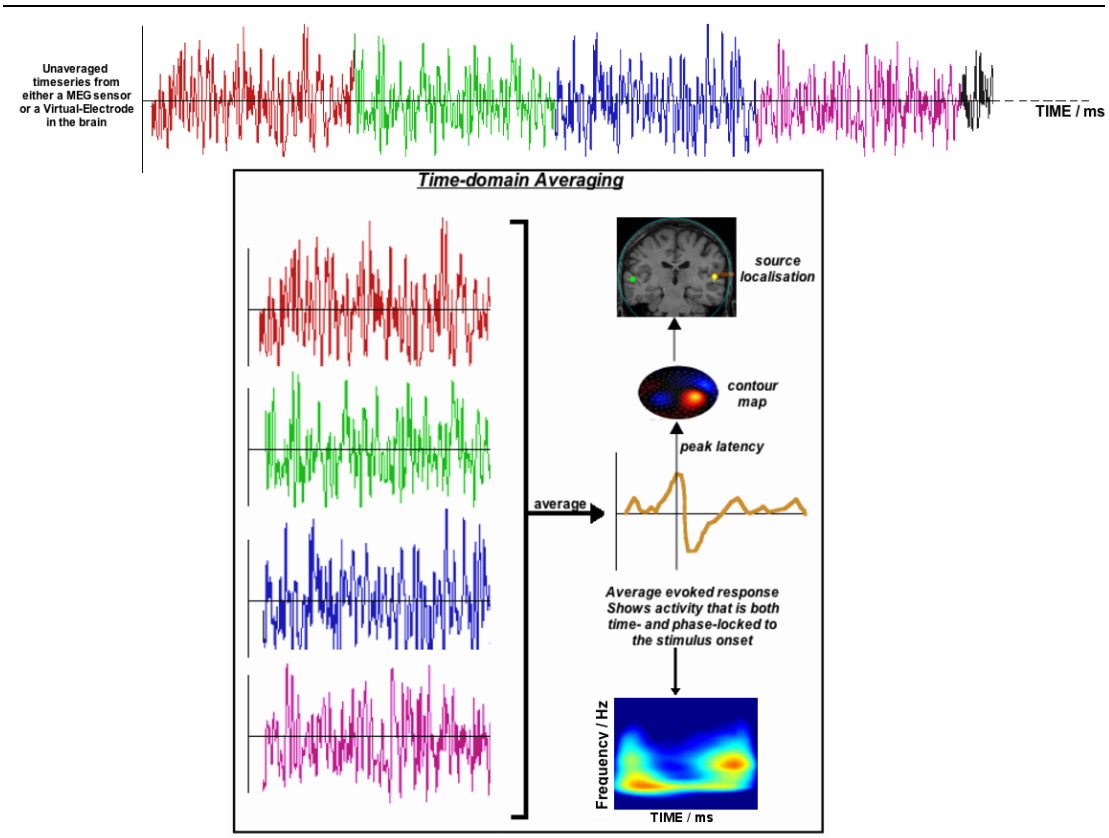

Figure 3.4. Measuring the evoked activity. The average of multiple epochs or trials (coloured traces) are in the time domain, results in an average that shows both phase- and time-locked responses. This is the evoked response. By plotting the magnetic field of separate sensors, the evoked response can also be seen on a contour map (in sensor-space) and can be used for source-localisation). With permission, from Singh, 2006.

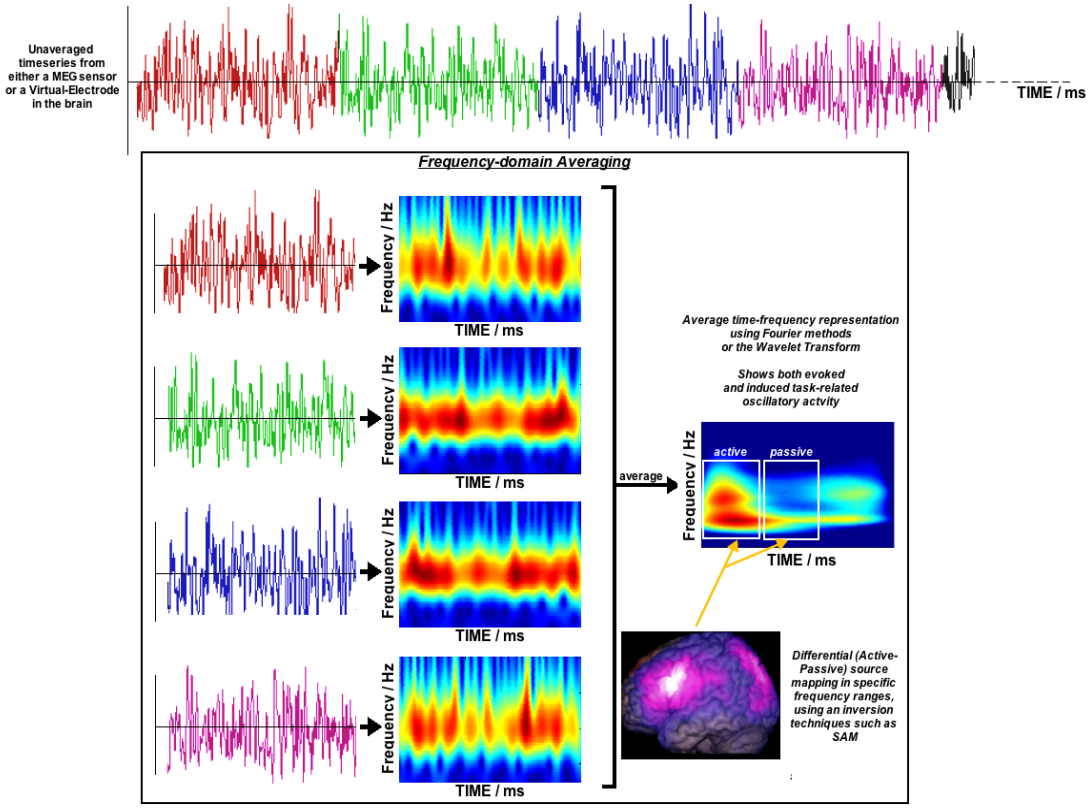

Figure 3.5. Measuring the induced activity. Time-locked but not phase-locked cortical responses (i.e. responses that do not always occur with the same latency/phase) are averaged out by time-averaging (see Figure 3.4). But when trials are first averaged into the time-frequency domain and then averaged, the induced response can be seen. With permission, from Singh, 2006.

If the neural response to a particular stimulus is not phase-locked, it will be averaged out, even if it modulates between trials with only a few milliseconds.

However, the brain also contains functionally relevant oscillatory rhythms that are not phase-locked but *time-locked* to a stimulus. That is, they emerge or change at the presentation of a particular stimulus or during a specific task. This activity is known as the *induced* activity. A number of different functionally relevant frequency bands have been described and; for instance alpha (7 - 15 Hz), beta (15 - 30 Hz) and gamma (30 - 90 Hz), although subdivisions have been described and there is not always consensus on the range of the different frequency bands.

Because the phase of a non-phase locked oscillation can be different for each trial, simply averaging epochs in the time-domain may not show this type of oscillatory activity. However, if the activity is transformed in the frequency domain, oscillatory rhythms can be detected (see Figure 3.5; Tallon-baudry, 1996; Tallon-Baudry et al., 1999). Increases and decreases in power in specific frequency bands are known as, respectively, event-related synchronisation (ERS) and desynchronisation (ERD). The ERS is thought to represent an increase in the synchronous activity or a greater amplitude of activity, of neurons within a local region.

3.2.4 Analysing MEG data

The type of data we can measure using MEG has been discussed in Section 3.2.3, but a number of steps must be taken to get from raw sensor time-series to estimating the location and amplitude of the neuromagnetic generators within the brain.

3.2.4.1 *Reconstructing the magnetic field*

For any given source, there will be different patterns of activity across different gradiometers (see Figure 3.6A), which can be plotted and (see Figure 3.6B) results in a map of the activity in *sensor-space* (see Figure 3.6C). The source of the magnetic field as shown by the sensors then has to be localised (called the MEG inverse problem). To determine the source of the activity in *source-space*, the signals from all sensors need to be combined to reflect the location of source of the magnetic field within the brain and several approaches to this problem exist (see following sections). This is problematic because a magnetic field distributed across the sensors can be the result of an almost infinite number of different sources. By adding information of prior knowledge (e.g. expected location, latency, correlation between sensors), it might be possible to solve this problem. Many different approaches exist, but only dipole modelling and SAM beamformer approaches will be discussed.

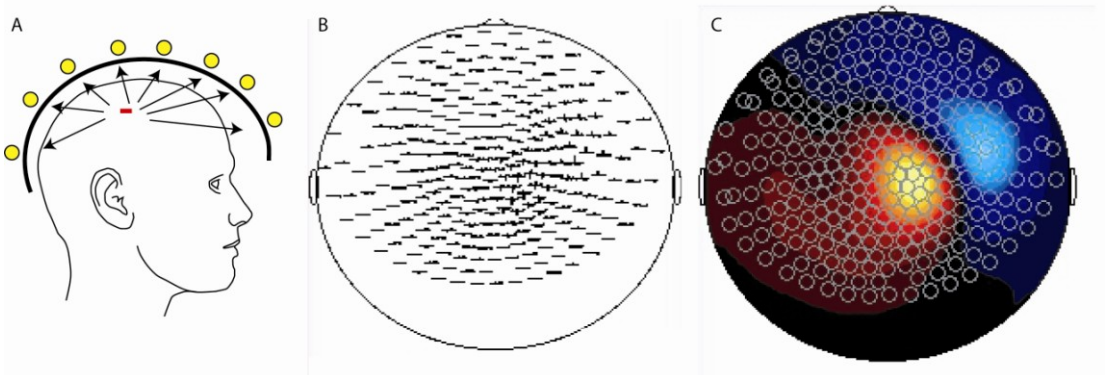

Figure 3.6. Reconstructing the magnetic field. A. a source is seen differently by each sensor. Sensors close to this region are more sensitive to changes in the magnetic field. B. the magnetic field over a certain epoch can be plotted for each sensor. C. From this plot, a map in sensor-space can be created for a single time-point or duration, with the largest changes in magnetic field in the sensors closest to the somatosensory cortex (plot created for 70 ms).

3.2.4.2 Single current dipole

The single current dipole is the simplest model for the observed magnetic field in MEG. This technique assumes there is a single generator, and the source of this current can be determined by measuring the strength of the magnetic field across the different sensors. Usually a simple, spherical head model is used. The dipole is described by five parameters, three of which describe the position of the source within the head, and two defining its orientation and strength. The dipole model is a useful tool in determining the location, direction and strength of current flow for a given latency. Usually, dipoles sources are visualized in tandem with a structural MRI image.

3.2.4.3 *Beamformer approach – Synthetic Aperature Magnetometry (SAM)*

Another technique to estimate the source activity for a particular part in the brain is a beamformer approach called Synthetic Aperature Magnetometry (SAM; see Robinson and Vrba, 1999; Vrba and Robinson, 2001).

SAM partitions the head or brain into a 3D space, effectively 'voxelizing' it, and for each of the points in this space a weighting vector is created. The activity measured by each sensor is weighted for each location in the brain separately, therefore creating a weighted map of activity for the entire brain. To obtain the weighting factor for each location for each gradiometer, information about how much each of the channels in the recording is correlated with each other is used. The SAM algorithm then tries to find a weighting vector that minimises the power/variance for a location. In SAM it is assumed that the magnetic field at a given timepoint is generated by a number of dipoles, and that – crucially - the sources are

uncorrelated. Two sources that do have the same time course (e.g. bilateral, homologous cortical areas such as the primary auditory cortices) may not be detected, and beamforming localisation may fail. The advantage of the beamformer approach is that the data itself provides information about covariations between sensors in time, and provides all the information to create an activity map.

This approach is particularly useful in investigating induced activity. It is possible to determine the time range and frequency *a priori.* The images are thresholded at the single subject level (typically using the pseudo-t statistic) - see Section 3.3.3 for details of the methodological considerations. A similar technique using SAM that is sensitive to multiple active sites reflecting evoked activity, is to determine the average activity over a number of separate time windows compared to a passive period (called SAM event-related field or SAMerf; see Robinson (2004).

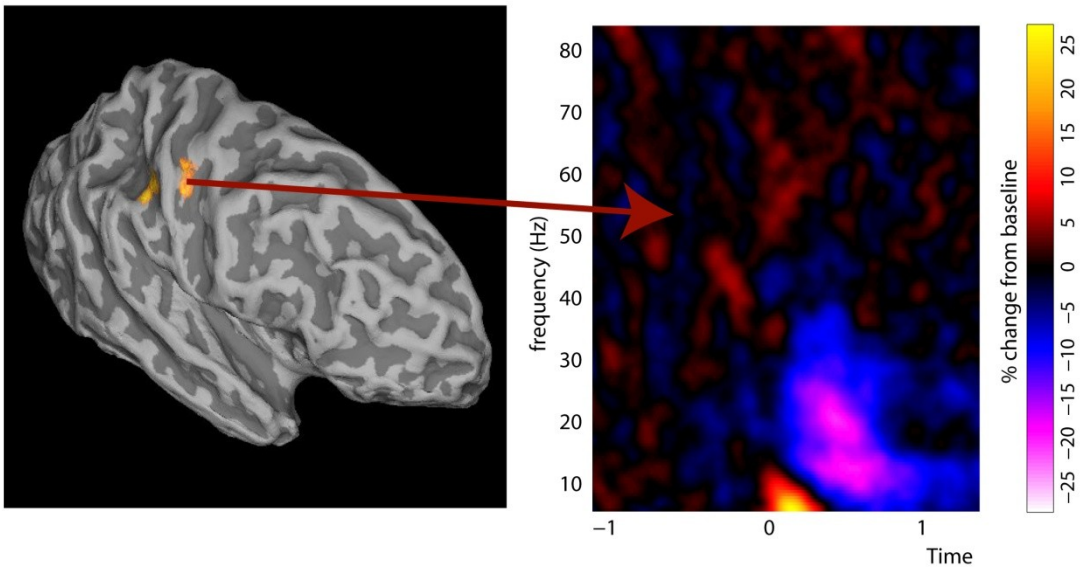

Figure 3.7. Virtual sensor analysis. A. SAMerf analysis for a tactile stimulus gives a peak, for this subject, at 70 ms, localised within primary somatosensory cortex. Time-frequency analysis of the peak voxel, (where the virtual sensor is 'placed') results in a time-frequency plot. The x-axis indicates time with zero being stimulus onset. The spectrum shows a decrease in alpha and beta band (% change from baseline -1-0 seconds) at stimulus onset (0 seconds) and a rebound (ERD) at stimulus offset (1 second)

3.2.4.4 *From SAM to Virtual Sensor analysis*

SAM sensor analysis allows us to localise a source within cortex (Figure 3.7A) on an anatomical scan for a stimulus or condition. To determine the time-frequency course for this particular location for our task, it is possible to do *virtual sensor analysis*. SAM analysis allows us to pick a target location and create a virtual sensor at that location. For a given location, the covariance matrix of the sensor data (a matrix that reflects how each channel is correlated with each other for a given time-window) is used to determine the power of time-frequency data for both the time- and phase-locked activity (see Figure 3.7B for an example of time-locked but not phase-locked (or induced) activity). An example for tactile processing is shown in Figure 3.6. Details of virtual sensor analysis are discussed in Section 3.3.4.

3.3 Methods - Tactile processing and MEG

3.3.1 Scanner and preprocessing parameters

For tactile processing, a large number of approaches to analyse MEG data have been used. Simple dipole modelling has been done to investigate different locations of tactile activity (e.g. Hari and Kaukoranta, 1985; Hari et al., 1993), ERF analysis has been done to investigate the phase-locked neural responses to tactile stimulation (e.g. Nangini et al., 2006) and a number of ERFs similar to somatosensory ERPs in EEG have been described (e.g. Jones et al., 2007). In addition, a number of studies have investigated the role of cortical oscillations in tactile processing (e.g Gaetz et al., 2003; Jensen et al., 2005b; Gaetz and Cheyne, 2006; Cheyne et al., 2007; Haegens et al., 2010b; Haegens et al., 2011a). For reasons that will be outlined in Chapter 7 & 8 in particular, we are mainly interested in the role of cortical oscillations in processing of tactile stimuli and in particular what role these functionally relevant oscillations play in adaptation.

3.3.1.1 *Acquisition*

All MEG recordings in this thesis were acquired using a CTF-Omega 275-channel system at CUBRIC sampled at 1.2 kHz (0 – 300 Hz bandpass) in third-order gradiometer mode and recorded continuously for the entire duration. Three of the 275 channels were turned off due to excessive sensor noise. For all MEG acquisitions, three fiducial locations were placed according to Figure 3.3 and digital photographs taken. For all tactile tasks, participants wore ear plugs to mask any stimulator noise. Participants were seated comfortably in the MEG chair and slowly raised until the top of their scalp touched the MEG helmet (see Figure 3.1B). The stimulator

described in Section 2.4.1 was placed underneath the finger and taped into place. When responses were required, participants used a LUMItouch trigger box (LUMItouch, Photon Control Inc., Burnaby, Canada).

3.3.1.2 *Preprocessing*

Continuously acquired data was epoched depending on the nature of the task and visually checked for artefacts. Fiducial locations were manually positioned (on the basis of the digital photographs of fiducial position on the participants face) on the participants structural MRI (axial FSPGR; 1mm isotropic resolution) acquired on a GE SignaHDx 3 Tesla MRI scanner (General Electric Healthcare, Chalfont St. Giles, UK), using an 8-element head coil for receive and the body coil for transmit. Brain-shape was extracted using a multiple local-spheres forward model (Huang et al., 1999) by fitting spheres to the brain surface as extracted by FSL's Brain Extraction Tool (BET; Smith, 2002). Prior to virtual sensor analysis, data was reconstructed within the frequencies of interest.

3.3.2 Localisation

3.3.2.1 *Parameters for S1 localisation*

As described in Section 1.4 and Section 1.5, we are mainly interested in tactile processing in the primary somatosensory cortex. Therefore it is important that we are able to localise the activity induced by our tactile stimulation using MEG. Previous studies have used a multitude of different techniques to stimulate the somatosensory system, from simple tactile flutter and vibration (e.g. Hamalainen et al., 1990; Suk et al., 1991; Gaetz et al., 2003) to median nerve stimulation (e.g Okada et al., 1984; Hari and Kaukoranta, 1985; Baumgartner et al., 1991a; Forss and

Lin, 2002; Pfurtscheller et al., 2002). Median nerve stimulation has been mainly used to elicit a strong enough signal to aid localisation of S1 but single-digit stimulation can also be used (see Figure 3.6 and e.g. Hamalainen et al., 1990; Hari and Forss, 1999; Nangini et al., 2006).

Because our main interest lies in analysing activity within S1, localisation will be performed for latencies around 70 ms, representing the first response in S1 to naturalistic stimulation; this peak occurs earlier for median nerve stimulation (see also; Cheyne et al.; Hamalainen et al., 1990; Fukuda et al., 2008). Pilot data has shown that the latency of this response varies between 60-110 ms (Figure 3.5; see also Nangini et al., 2006) for flutter stimuli, and vibration may result in earlier deflections of the same kind (Jousmaki and Hari, 1999). It should be noted that earlier SEFs have been shown between 20 and 50ms after stimulus onset (e.g. Hari and Kaukoranta, 1985; Hari and Forss, 1999) of median nerve stimulation, but Jones et al., (2007) suggest that tactile detection correlates with modulation of the peak amplitude around 70ms.

3.3.2.2 *Analysis of S1 localisation*

MEG activity after tactile stimulation can be seen in S1 between 50-110 ms (Elbert et al., 1995; Hari and Forss, 1999; Jousmaki and Hari, 1999; Jousmaki, 2000; Tuunanen et al., 2003; Nangini et al., 2006). Synthetic Aperature Magnetometry event-related fields (SAMerf) (Vrba and Robinson, 2001) are useful for locating S1 and tracking of the cortical response to a tactile stimulus.

Single subject Synthetic Aperature Magnetometry event-related fields (SAMerf) was used to create three-dimensional differential images of source power

(pseudo-t statistics) for 1 second of baseline (-1 – 0 seconds) compared to 10ms bins spanning between 0 - 150 ms post-stimulus for each participant to track the cortical pattern of activity over time. SAM images were constructed for frequencies between 2 – 90 Hz and used to detect peak location in S1 (expected between 60-70 ms, see Figure 3.7A). To acquire pseudo-t weighted values for each voxel, the contributed noise for the power in a sensor was first computed to acquire a normalised voxel value (pseudo-Z). Because source-activity is related to the task, the sensor-value was also corrected against the power in the passive period and because the difference between active and passive period also suffers from the contributed noise, the sensor value was once again normalised, resulting in a pseudo-t value for each sensor (Vrba and Robinson, 2001). Pilot data has confirmed that initial S1 activity appears around 70 ms (see Figure 3.7A). In addition, activity in bilateral S1 and S2 was also detected. This latter finding was not visible in all participants possible due to sources that are temporally correlated and low SNR.

Group analysis To look at group differences between conditions, a group SAM analysis was performed between an active period and passive period, which latency is dependent on the nature of the task and described in each respective chapter. Individual SAM images were normalised using FSL FLIRT (Jenkinson and Smith, 2001) into an MNI template (T1 of 152 individuals). A voxel-wise t-statistic image was then calculated using the inter-subject variability and a non-parametric permutation performed, using 2^n (n = number of subjects) permutations for each condition. This created an estimate of the t-value distribution for the null

hypothesis (no significant group activation). The significance of voxelwise t-values was then tested against this distribution.

To account for multiple comparisons, the largest t-value in each permutation was used to create a probability map for the range of largest t-values and only unpermuted t-values larger than this threshold were significant (at $p < 0.05$) (Nichols and Holmes, 2002; Singh et al., 2003).

3.3.3 Evoked activity

3.3.3.1 *Evoked time course of somatosensory activity*

As mentioned in the previous paragraph, we looked for the evoked activity around the postcentral gyrus between 50-110 ms to localise specific digit foci in S1 for each subject. It is well known that the interstimulus-interval has a large effect on the size of the response in S1 (Burton and Sinclair, 2000). Because the ISI is of importance (it is unclear how long the effect of adaptation is retained) it was important that the ISI was optimised. Nangini et al., (2006) and Zhu et al., (2009) both show that there is little difference between a 1- and 2 second ISI, but that the response of a tactile stimulus after a short ISI (for instance 0.33 second) is severely diminished. Similar results have been found using invasive recordings (McLaughlin and Kelly, 1993). Therefore we used a 1 second ISI as used by Goble and Hollins (1994).

Response to a tactile stimulus is typically reflected by dipolar deflections in the evoked activity. The latency of these peaks is strongly dependent on the method of stimulation as discussed in 3.3.2.1. Jones et al. (2010) show a typical MEG response to median nerve stimulation with an upward peak around 50 ms after stimulus

onset (the M50), followed by a negative deflection (M70) and an upward deflection (M100-135; see also Pfurtscheller et al., 2002). Because we use single digit naturalistic stimulation and a small number of averages compared to extensive ERF studies, we expect to see an initial peak around 70 (M70), 150 (M150) and between 200-300 (M200-300) milliseconds post stimulus onset (Hamalainen et al., 1990; Hari and Forss, 1999; Hoechstetter et al., 2001; Inoue et al., 2005; Pihko et al., 2010).

It is known that repetitive tactile stimulation leads to steady-state responses oscillating at the frequency (and harmonics) of stimulation, in addition to a transient evoked response. Nangini et al. (2006) investigated the steady-state response (SSR) to a tactile stimulus and found that the SSR is less affected by ISI length. Furthermore, SSRs can be observed over the entire duration of the stimulus at the frequency of the driving stimulus. Nangini et al suggest a role of the SSR in neuronal population tuning. To investigate SSRs we will measure whether an adapting stimulus indeed results in an SSR response at the driving frequency (25 Hz and 40 Hz) and whether this may have an effect on the processing of the subsequent stimuli. Nangini et al. used a latency of 200 ms after stimulus onset to stimulus offset to avoid influence of the transient response.

3.3.3.2 *Analysis of the evoked activity*

To look at the evoked activity, time-frequency analysis was performed on the peak S1 location (as described in Section 3.3.2.2) using the Hilbert transform between 2 and 90 Hz in 0.5 Hz frequency steps (against 1 second prior to stimulus onset as baseline) and averaged across participants between conditions. From these time–frequency spectra, evoked activity will be plotted, expressed as percentage change

from baseline. Differences between conditions will measured as significant differences in power averaged over a given time interval, and analysed with a two-sample paired t-test between conditions.

3.3.4 Induced activity

3.3.4.1 *Induced time-course of somatosensory activity*

After localisation with SAM, the peak voxel location representing digit stimulation in S1 will be used to create a virtual sensor for the duration of the stimuli within a trial (task-dependent) for both evoked (3.3.4) and induced activity. Several studies have shown a characteristic induced response of S1 to a tactile stimulus in the alpha (7-14 Hz) and beta (15-30 Hz) band. These studies have shown a characteristic alpha and beta ERD at stimulus onset and an ERS at stimulus offset (see Figure 3.7B; Cheyne et al., 2003; Gaetz et al., 2003; Gaetz and Cheyne, 2006; Gaetz et al., 2008; Cebolla et al., 2009; Reinacher et al., 2009; Dockstader et al., 2010; Jones et al., 2010). The beta band seems to be modulated by attention (Bauer et al., 2006b; Dockstader et al., 2010) and under influence of GABAergic mechanisms (Jensen et al., 2005b; Gaetz et al., 2011) and will therefore be the frequency-bands of interest. The functional role of these oscillations will be discussed in detail Chapter 7 and 8.

It should be noted that some studies also show increases in oscillatory power in the gamma band after stimulation (Bauer et al., 2006b; Cebolla et al., 2009; Haegens et al., 2010b). The majority of these studies, however, used median nerve stimulation which is known to result in higher level of stimulation to the entire nerve. Median nerve stimulation results in a larger and stronger pattern of activity and gamma may be measured. The functional significance of gamma-band activity measured by

MEG after tactile stimulation is not currently clear, and in this thesis we will concentrate on analyses of both mu-alpha and mu-beta band activity.

3.3.4.2 *Analysis of the induced activity*

Time-frequency analysis will performed using the Hilbert transform between 2 and 90 Hz in 0.5 Hz frequency steps for the entire trial (1.2 kHz temporal resolution). Previous studies have used a prestimulus duration as baseline and look at changes in power compared to the baseline duration. Baseline duration has varied in the literature from 50 ms to 1s and we chose a baseline of 1s duration. Data is presented as percentage change from the average baseline (-1 to 0 seconds) and will be averaged across participants between conditions. To investigate changes in oscillatory activity, we subsequently extracted average frequency, power and latency data for the different frequency bands of interest (mu-alpha: 7-15 Hz, and mu-beta: 15-30 Hz) also expressed as percentage change from baseline. Differences between conditions will measured as significant differences in power for a predetermined time-power envelope for separate frequency bands. Statistical analysis is dependent on the nature of the task and will be described in each chapter separately.

3.4 General Discussion

To investigate the role of adaptation using MEG, the study by Goble and Hollins (2004) was adapted. The optimisation of these methods has been discussed in Section 2.3. Our modulated paradigm has shown to be stable and our changes in the adaptation task have shown to induce adaptation. By modulating certain

aspects of the tasks such as number of tracks, the number of possible biases has been reduced.

MEG will be used to investigate the effect of adaptation on cortical processing. Because a more detailed discussion of the source of the different MEG signals is largely related to cortical functioning, these will be described in more detail in the following chapters, but the principles by which MEG is measured are discussed in Section 3.2. MEG is a useful tool to measure cortical dynamics on a millisecond time-scale. Different aspects of cortical processing can be investigated which will provide useful information about the mechanisms involved in the processing of adaptation. As the investigation of the somatosensory cortex using MEG has been done in the past, established approaches (as described in Section 3.3) will be used to analyse the data.

Chapter 4 - MRS Basic Principles and Methods

4.1 Introduction

Magnetic resonance spectroscopy (MRS) is a methodology that allows for *in vivo* detection of endogenous metabolites in the human body. Although historically most often applied within a clinical context, recent developments have shown an increased use of MRS in studying the healthy brain. Edited magnetic resonance spectroscopy of GABA has proved to be a useful tool in investigating the relationship between the concentration of the inhibitory neurotransmitter GABA and disease (e.g. Schizophrenia: Tayoshi et al., (2010); Epilepsy: Petroff et al., (1996), behaviour and individual variability (Edden et al., 2009; Boy et al., 2010; Sumner et al., 2010; Boy et al., 2011, Puts et al., in press) underlying neurophysiology of neuronal activity (Muthukumaraswamy et al., 2009) and cortical plasticity (Levy et al., 2002; Stagg et al., 2009; Stagg et al., 2011).

Although GABA is only present in the human brain at millimolar levels, it is possible to measure GABA concentration with MRS, usually by tailoring the MRS experiment specifically to isolate GABA signals from the spectrum. In studying the role of GABA in cortical processing of tactile stimuli, MRS may prove to be an important tool because it non-invasively detects GABA *in vivo* in a predefined region of the brain. The application of GABA-MRS and the combination of GABA-MRS with functional neuroimaging and behavioural psychophysics is a new area and the following two

chapters will contain data acquired during the development of GABA-MRS, mainly focused on the acquisition of GABA-MRS over somatosensory cortex.

4.2 MRS & MRI

Magnetic resonance spectroscopy is the *in vivo* application of nuclear magnetic resonance (NMR) spectroscopy, as applied on magnetic resonance imaging (MRI) scanners. MRI uses a static magnetic field to induce a net magnetization among the hydrogen proton spins in the body (see Figure 4.1A) in which precession occurs. Radiofrequency (RF) pulses can be applied to bring all these different spins into phase (see Figure 4.1B). This precessing magnetisation can be detected by the MRI scanner. Since the size of this magnetisation and the dynamics of its return to equilibrium differ for different parts of the body, magnetic field gradients which encode spatial information in the spins can be used to produce a 3D image with contrast between different tissues. Alternative imaging contrasts can also represent a number of factors from blood flow to water diffusion, and it is a great strength of MR that sequences can be designed to encode these numerous contrast into quantitative images.

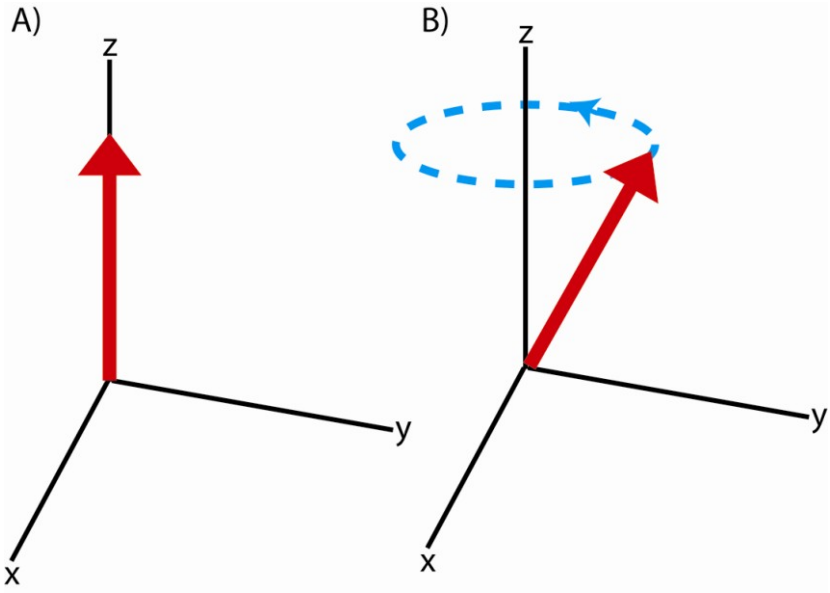

Figure 4.1. Magnetization. A shows the net equilibrium magnetization within a static field. B) After an RF pulse, the magnetization precesses in phase round the z-axis and slowly relaxes towards equilibrium.

4.2.1 Imaging contrasts based on relaxation

Relaxation is the process by which magnetization returns to equilibrium after excitation by an RF pulse. In order to return to equilibrium, magnetization must restore its z-component, and also lose its transverse component. These two processes are independent and are referred to as *longitudinal relaxation* and *transverse relaxation*, respectively (see Figure 4.3). They are both approximately exponential and can be described by the time constants T1 and T2. Contrast between different tissues in an MR image can be generated beause different tissues have different characteristic values of T1 and T2 (see Figure 4.3).

In order to describe how T1-weighted and T2-weighted contrast can be generated, it is helpful to describe two key parameters of MR experiments – the relaxation

time TR and the echo time TE (see Figure 4.2). The relaxation time is the time from one excitation to another, or to put it a different way, the time for which T1 relaxation will occur in between excitations. The echo time is the time from excitation to acquisition of signal, and can be thought of as the time for which transverse relaxation will occur.

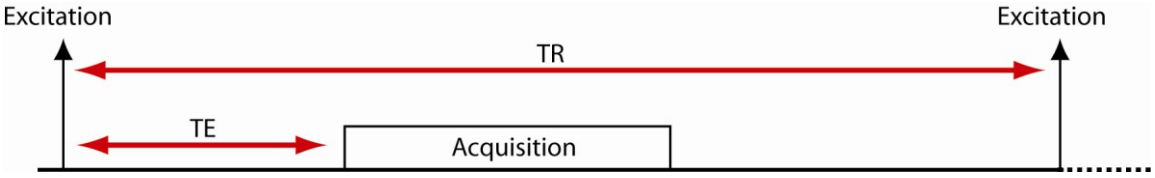

Figure 4.2. TR and TE. TR is the relaxation time, the time from one excitation to another. The echo time TE is the time from one excitation to the acquisition of signal

Therefore, to generate an image with T1-weighted contrast, imaging is performed with a short TR (on the order of T1 of the tissues), so that the magnetization of all tissues is incompletely relaxed (and to differing degrees) when excitation occurs. It is also important to image with a short TE, so as not to mix in any T2-based contrast to the image. Conversely, a T2-weighted image can be generated by using a long TE (to inject T2-based contrast) and a long TR (to ensure that longitudinal relaxation is complete and there is no T1-based contrast in the image).

T1-weighting is the most often used contrast for basic structural MRI. In a T1-weighted image, grey matter appears darker than white matter because it has a longer T1.

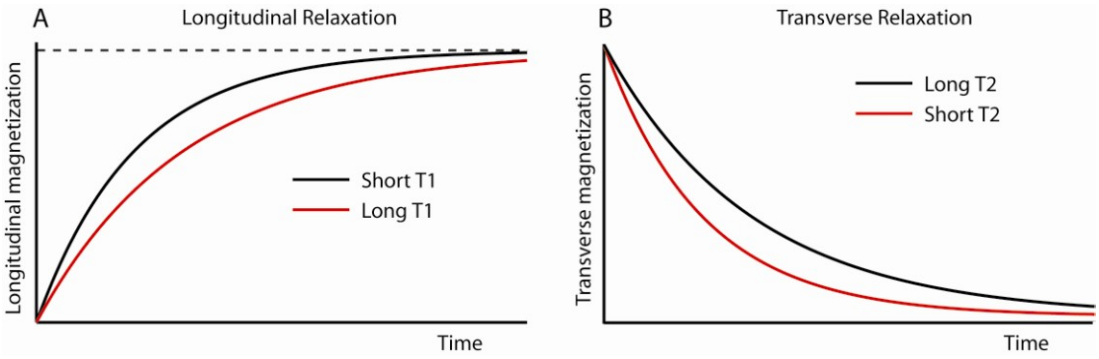

Figure 4.3. T1 and T2 relaxation. A) After any change in the z-component, the magnetization relaxes exponentially back towards equilibrium (dashed line); this is known as longitudinal (or T1) relaxation. B) After excitation, magnetization loses its transverse component, this decay is known as transverse (or T2) relaxation. T1 and T2 relaxation times are different for different tissue types.

4.3 Chemical Shift

Water is the most abundant molecule in the brain. However if the strong water signal is suppressed, a collection of other metabolites (with much lower concentrations) can be detected (Rothman et al., 1993; Mescher et al., 1998). Due to their chemical environment, which is mainly determined by local electron density, hydrogen spins of different metabolites precess with slightly different frequencies. This metabolite-dependent difference in resonant frequency is called *chemical shift*. Thus different metabolites give rise to different frequency signals which can be plotted as the MR spectrum. Figure 4.4 shows the MR spectrum acquired from an occipital region of the human brain, showing signals associated with a number of metabolites, most prominent among them being N-acetyl aspartate (NAA), creatine- (Cr) and choline-containing compounds (Cho). The spectrum is a plot of signal intensity (roughly proportional to metabolite concentration) against chemical shift. Chemical shift is reported in field-independent frequency units, ppm (or parts per million of the proton frequency), so that NAA, for example, always gives a signal

at 2.0 ppm even though signals are acquired at close to 64 MHz in a 1.5T scanner and 128 MHz in a 3T scanner. It should be noted that in this spectrum the water signal has been suppressed - the unsuppressed water signal is over 10000 times as large as the signals from other metabolites.

In order to perform useful MRS experiments in the brain, it is necessary to localize the MR signal acquired to a particular region, either by only exciting signals within a specific volume (or voxel - known as *single-voxel MRS*) or by performing a hybrid MRS and imaging experiment, known as *magnetic resonance spectroscopic imaging* (MRSI, also referred to as chemical shift imaging, CSI). Single-voxel spectra are usually acquired using either the PRESS (Point-RESolved Spectroscopy (Bottomley, 1987) or STEAM (STimulated Echo Acquisition Mode) method. Several approaches have been made to the spectroscopic imaging of GABA: DQF-MRSI (Shen et al., 1999a; Shen et al., 1999b; Jensen et al., 2005a Brown, & Renshaw, 2005; Choi et al., 2006). Only the single-voxel technique PRESS will be discussed.

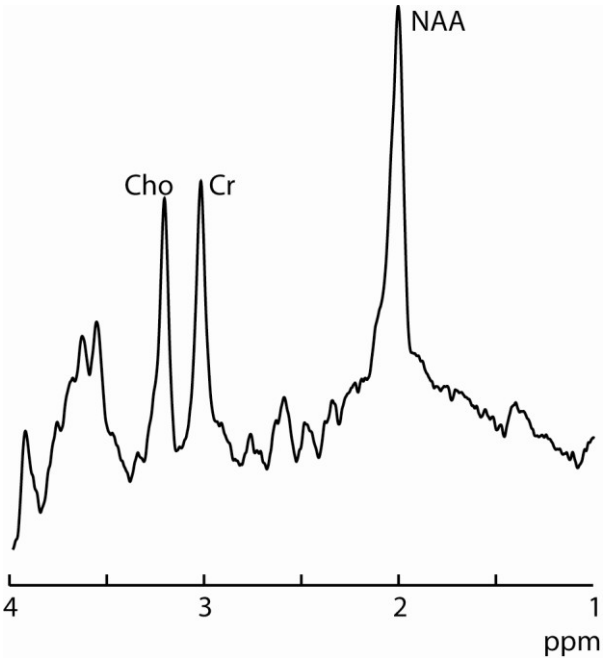

Figure 4.4. MRS PRESS spectrum at 3T. The main visible peaks are Creatine (Cr), Choline (Cho) and NAA. GABA is not visible in this spectrum due to its low concentration.

4.4 Point-Resolved Spectroscopy

In single voxel spectroscopy, a volume-of-interest is defined by three orthogonal slice-selective pulses. The most commonly used experiment is called *point-resolved spectroscopy* (PRESS; Bottomley, 1987). The spectrum in Figure 4.4 exhibits a fundamental problem of *in vivo* ^{1}H-MRS, especially as applied to GABA – the dispersion of different signals along the chemical shift axis is limited in comparison with the line width of signals (even with good field homogeneity). Therefore signals from different metabolites often overlap, and signals from more abundant metabolites often obscure those from less abundant metabolites such as GABA (Puts and Edden; de Graaf and Rothman, 2001).

4.4.1 PRESS and the spin echo

PRESS is a localised measurement in a predetermined part of the brain and PRESS voxels are determined by slice-selective pulses in the x-, y- and z- direction, as shown in Figure 4.5 and de Graaf and Rothman (2001). The first slice-selective pulse is an excitation pulse which rotates the equilibrium magnetization (aligned with the z-axis) through 90^{o} into the transverse plane. The further two dimensions are defined by slice-selective spin echoes – a spin echo is an extremely important pulse sequence element that will now be explained.

Figure 4.5. The PRESS sequence. Slice selective pulses are given in three orthogonal directions (labelled X, Y, Z). Two spin echoes are used, the first of duration 2δ and the second 2δ'. Time delta indicates the time magnetisation is allowed to precess.

Due to the chemical shift, transverse magnetization from different metabolites precesses at different frequencies. Since it is necessary to acquire signals when they have the same phase, a mechanism is needed to refocus the evolution of different frequencies during the experiment; this is the role of a spin echo. Transverse magnetization that precesses for a time δ, will develop a different phase depending on its resonant frequency (figure 4.6B). If a 180 degree pulse is applied, for example about the y-axis, the position of each magnetization element in the transverse plane (Figure 4.6C) will be set so that after precessing with the same frequency for another time delta, the magnetization will be aligned along same axis (*cf* Figure 4.6a and 4.6D). This is a spin echo; the delay-180-delay structure is central to many MRS experiments. PRESS has two consecutive spin echoes. If the 180^{o} pulses are applied slice-selectively, only signals within the volume of interest will be refocused by the spin echoes and therefore be detected.

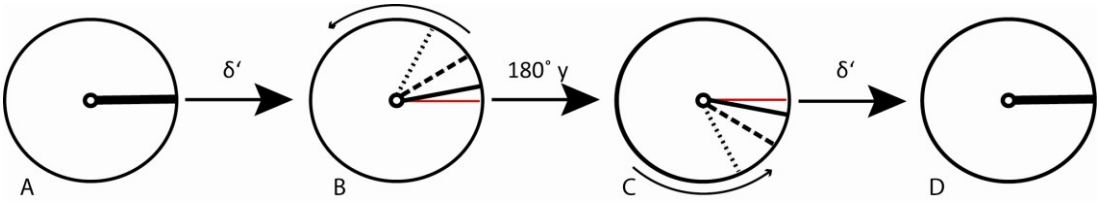

Figure 4.6: Spin Echo. Transverse magnetization (A) precesses around the z-axis (perpendicular to the page), at different frequencies (solid/large-dotted/small-dotted line) and thus acquires different phases (B). After an 180^{o} pulse about the y-axis (C), if the magnetization precesses around the z-axis for a further time delta, it will be refocused (D).

4.5 Coupling

One common feature of the MR spectrum is the appearance of multiplets, signals associated with a single hydrogen environment that are split into a number of sub-peaks (e.g Figure 4.7B. Within a molecule, the field experienced by a spin is affected by adjacent spins of other hydrogen nuclei within the molecule. This spin-to-spin interaction is called *coupling* (e.g. de Graaf and Rothman, 2001) and affects the appearance of signals in the MR spectrum, splitting the peaks up into *multiplets.* The magnetic moment of adjacent spins will have a small additive or subtractive effect on the magnetic field experienced by a spin, depending on whether they are either up or down. Averaging over all the cases where a neighbouring spin is spin-up, this will create a peak to the left of the usual frequency, whereas when neighbouring spins are down, this will create a peak to the right of the usual frequency. In the case of the γ and α methylene groups of GABA (see Figure 4.7B), each signal is coupled to the β methylene group. These two neighbouring spins will be either both spin-up, one spin-up and one spin-down, or both spin-down (with 25%, 50% and 25% probability), leading to a signal that is split into a 1:2:1 triplet.

Splittings due to coupling result in signals that have lower peak intensity and a broader footprint along the chemical shift axis, both of which make coupled species such as GABA and glutamate harder to detect and quantify. Faced with the limited dispersion of signals along the chemical shift axis, there are three possible approaches to reduce signal overlap in order to resolve GABA signals. Firstly, it is possible to reduce the amount of signals that appear in a spectrum by applying one of a series of *spectral editing* methods. Alternatively, overlap between signals can be alleviated by spreading signals out into a second frequency dimension in *two-dimensional* MRS methods. The third approach is to move to higher field strength, since the relative width of multiplets (in ppm) scales inversely with field strength.

Coupling also impacts the evolution of signals during the PRESS experiment. A spin echo refocuses the evolution of the chemical shift, but it does *not* refocus the evolution of coupling. However additional frequency-selective 'editing' pulses can be added to the PRESS experiment in order to refocus the evolution of specific couplings, which opens up the possibility of *editing* the information content of the spectrum to alleviate crowding and resolve signals that are not otherwise quantifiable. In this thesis, editing is used to separate the weak GABA signals from the MR spectrum.

4.6 Edited detection of GABA

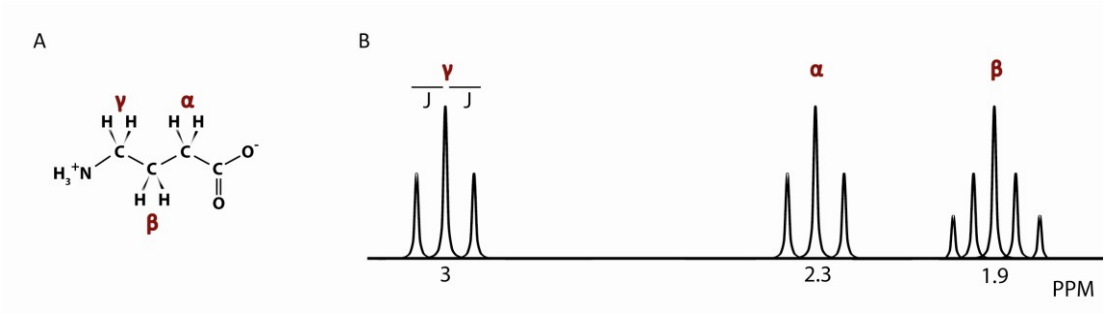

Figure 4.7. The chemical structure and schematic MR spectrum of GABA. A) shows the chemical structure of GABA. B) The gamma and alpha methylene groups are expected to appear as triplets and the beta methylene group as a quintuplet, assuming that all coupling interactions have the same strength.

GABA is present in the human brain at a concentration of approximately 1.3 - 1.9 mM/kg (Govindaraju et al., 2000), which is an order of magnitude lower than the more concentrated metabolites and ~40,000 times lower than water. The GABA concentration is so low that it is difficult to quantify GABA from the PRESS spectrum with specificity. The chemical structure and MR spectrum of GABA is shown in Figure 4.7; the three different multiplets in figure 4.7B correspond to the three methylene (CH_2) groups in the molecule as shown in Figure 4.7A . The β-methylene group gives a signal at 1.9ppm, the methylene group closest to the carboxylate group at 2.3 ppm, and the methylene group closest to the amino-group at 3ppm (determined by high-resolution NMR; Govindaraju et al., 2000). The different groups differ due to their slightly different chemical environment (see Section 4.3). The GABA peaks at 2.3 and 3ppm (corresponding to the outer methylene groups) appear in the spectrum as triplets, as the β-methylene spins can have three possible configurations – up-up; up-down and down-down. The signal at 1.9 ppm is

more complex as the β-methylene spins are coupled to four neighbouring spins and theoretically visualises as a quintuplet (see figure 4.7B)

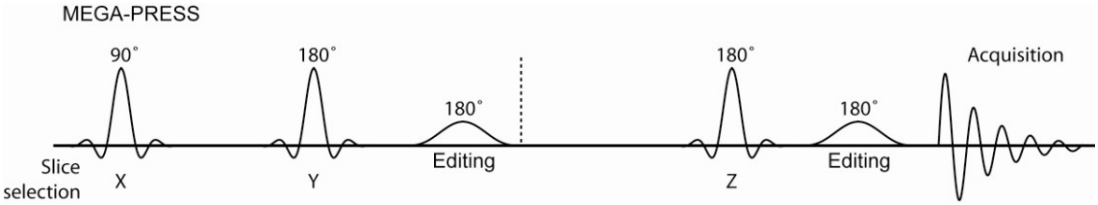

Figure 4.8. The MEGA-PRESS sequence. The three slice-selective pulses used in PRESS (see Figure 4.5) are used in combination with two editing pulses that selectively refocus the coupling between signals at 1.9 ppm and 3ppm. By doing scans with and without these editing pulses and taking the difference spectrum, GABA signal can be visualised.

The pioneering work of Rothman et al. (Rothman et al., 1993) first made possible the unambiguous detection of GABA *in vivo*. The MEGA-PRESS experiment (Mescher et al., 1998), which we use to detect GABA, combines PRESS (see Figure 4.5) localisation with two frequency-selective editing pulses (Figure 4.8), and is relatively simple to implement as a development of the PRESS single-voxel experiment. MEGA-PRESS is a difference experiment; a spectrum acquired with editing pulses is subtracted from one acquired without, removing peaks that are the same in both spectra (i.e. those that are unaffected by the editing pulse), leaving only the peaks affected by the editing pulse.

In performing MEGA-PRESS for GABA, frequency-selective editing pulses are applied to the GABA spins at 1.9 ppm (see figure 4.8). The editing pulse inverts the spins at 1.9 ppm (but not at other frequencies), which are coupled to the spins at 3ppm, selectively refocusing the evolution of coupling between these spins and changing the appearance of the 3 ppm multiplet. Such a pulse will have no effect on other signals at 3 ppm, because they are *not* coupled to spins close to 1.9 ppm. If two

experiments are performed, with and without this frequency-selective *editing* pulse, the difference will give a spectrum that *only* contains those signals that are affected by the pulse (the *edited* spectrum) and peaks that are unaffected by the editing pulse will be suppressed. Because the centre peak of the 3 ppm triplet is unaffected by evolution of the coupling, GABA is visualised in the difference spectrum as a pseudo-doublet (see Figure 4.9) which generally appears in vivo as a broad Gaussian-shaped peak (see Figure 4.9). In addition to GABA, one can see co-edited Glx (a combination of glutamate, glutamine and glutathione) at 3.75 ppm, a negative NAA peak at 2 ppm (due to the direct effect of the editing pulse at 1.9 ppm) and lipid between 0 and 2 ppm. The 1.9 and 2.3 ppm peaks do contribute to edited spectrum and are present as shown in a phantom spectrum as shown in figure 4.7, but are not successfully isolated from other signals in an edited *in vivo* spectrum, such as glutamate and NAA. One disadvantage of such J-difference methods is their reliance upon subtraction to remove the strong overlapping signals from the spectrum. Any instability in the experiment, whether caused by instrumental factors or participant movement, will result in subtraction artefacts that can obscure the intended edited GABA signals. Difference methods are usually acquired in an interleaved fashion and an MRS experiment is typically repeated many times and the acquired signals averaged to obtain a spectrum with improved signal-to-noise ratio (SNR).

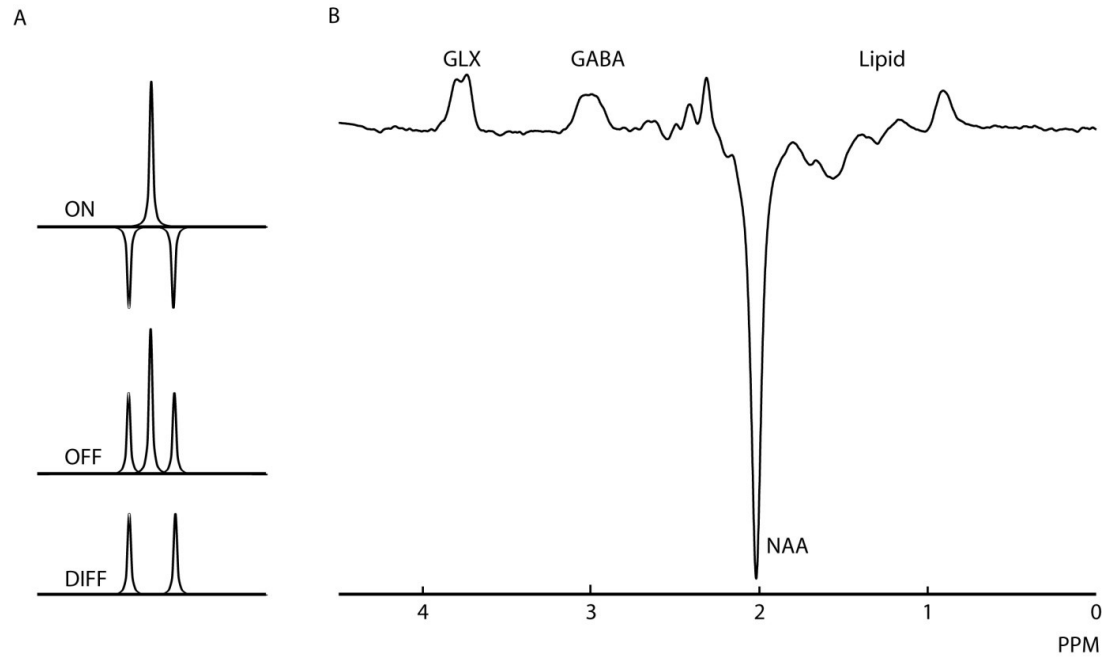

Figure 4.9. Difference editing of the GABA signal. A. In the 'editing pulse off' case, coupling evolves for the duration of the echo time to give a 'W-triplet'. When the editing pulse is applied, the evolution of coupling is refocused, giving an in-phase triplet. The difference between these (which is taken to generate the difference spectrum) is a pseudo-doublet. Subtraction also removes any overlying signals that are not coupled to spins at 1.9 ppm, such as Cr. Typical in vivo GABA-edited spectrum. This is an example difference spectrum for GABA at 3T. B. The approximately Gaussian-shaped GABA signal can be seen at 3 ppm. At 3.75 ppm is the co-edited Glx peak. The 1.9 and 2.3 ppm GABA peaks do contribute to the in vivo spectrum, but are not successfully isolated from other signals in the spectrum, such as glutamate and NAA.

4.6.1.1 Macro-molecules

It is important to note that that the edited GABA signal contains signals from macromolecules (MM's) which can make up to 40% of the GABA peak. Despite the fact that findings correlating behavioural or neuronal measures with MRS are often better explained by functional metabolites such as GABA (referred to as GABA+ in the context of MM), the existence of this MM contamination should be taken into consideration. There are techniques available that adapt MEGA-PRESS to control for MM contamination (Henry et al., 2001; Near et al., 2011).

4.6.1.2 Signal quantification

It is not yet possible to quantify GABA in real concentrations. Because tissues have different T1 and T2 values and these values are difficult to obtain for GABA, GABA is generally quantified according to reference metabolites. Quantification of MRS signals can be done using different reference metabolites. Quantification relative to creatine, NAA and water has all been used (Puts and Edden). Each methods has its advantages: the creatine signal has a chemical shift of 3.0 ppm and therefore the signal comes from the identical volume as GABA, with no chemical shift displacement artefact; NAA can be quantified directly from the edited difference spectrum, in which it appears as a strong negative signal at 2 ppm; and water quantification has excellent signal-to-noise and brings GABA measurements into line with most other MRS measurements. So far, none of the metabolites seems to have a justified advantage over the other.

4.7 Summary

J-edited PRESS-based methods, such as MEGA-PRESS, have been applied in a number of different brain regions – occipital, parietal and frontal regions (including the anterior cingulate). A very recent development has been the application of MRS (and MEGA-PRESS specifically) to investigate correlates between neuronal activity and neurochemistry as a basis for individual variability, to determine the effect of pharmacological modulation, and to investigate GABAergic deficits in pathology *in vivo* (see section 4.1; for an extensive review see Puts and Edden, 2011). These studies have contributed to understanding neuronal function in normal and disease-state as well as understanding the effect of pharmacological treatment on brain function at the chemical level.

As the rest of this thesis focuses on studies of the human primary somatosensory cortex, it is important to understand acquisition of MRS over that region. As the next Chapter will outline, many factors have an effect on the quality of acquired spectra.

Chapter 5 - MRS Development

5.1 Aims - Factors influencing the quality of GABA spectra

In this thesis we are interested in using MRS to study GABA in primary somatosensory cortex. In order to do this, it is important that we use methods that allow us to acquire good quality spectra from a well-defined location within a limited scan time. The process of deciding on the appropriate size and standardized position of the voxel is an important step in developing a robust and repeatable MRS study. It is also important to understand the limitations of the MEGA-PRESS sequence and factors that influence spectral quality. In this chapter I will describe a number of experiments that were performed to investigate different factors that determine the quality of GABA-MRS spectra and repeatability of measurements, in order to optimise acquisition of GABA-MRS over somatosensory cortex. The factors discussed are ones that have the most direct possible effect on spectral quality and have been discussed *a priori* to be optimised for somatosensory MRS-acquisition. We investigated what the optimal voxel location for acquisition over S1 is (Section 4.3) and whether a standard scan time of 10 minutes would provide us with good quality spectra and what effect changing the scan duration and voxel size have on the SNR (Section 4.4). In addition, scanner parameters such as the directions of gradients may have an effect on the chemical shift of water and lipid and therefore may have an effect on signal quality, so the optimal gradient direction was

investigated (Section 4.5). As there is no standard way of analysis GABA-MRS spectra, several approaches were compared (Section 4.6)

5.2 General scanner and analysis Parameters

In the following sections, several parameters will be changed individually for experimental purposes. However, since the majority of scan parameters are common to all these experiments, it is useful to describe the typical parameters before addressing the individual experiments.

5.2.1 Basic Scanner parameters

All scanning was carried out on a GE Signa HDx 3 Tesla MRI scanner (General Electric Healthcare, Chalfont St. Giles, UK), using an 8-element head coil for receive and the body coil for transmit. Prior to each MRS acquisition, a $1mm^3$ isotropic-resolution T1-weighted anatomical scan (FSPGR), and a sagittal and coronal Fast Spin Echo (FSE) were acquired to determine anatomical voxel placement. A short oblique imaging scan was often performed to permit voxel rotation. GABA-edited MR spectra were acquired using the MEGA-PRESS method (Mescher et al., 1998; Edden and Barker, 2007). The following acquisition parameters were used: TR = 1800 ms; TE = 68ms; 332 scans of 2048 datapoints per 10-minute acquisition; 16 ms Gaussian editing pulse applied at 1.9 ppm (ON) and 7.46 ppm (OFF) in interleaved scans.

5.2.2 Participants

Detailed information on participants is given per subsection. All participants gave informed consent to participate in the study and ethics were approved by the local

ethics committee at Cardiff University School of Psychology. During all sessions, participants were asked to lie in the MRI scanner and were told to either watch a movie or close their eyes. An alarm bell was provided in case of emergency. All participants scanned in this chapter were MR-habituated volunteers.

It has been suggested that the menstrual cycle may affect GABA concentration in the female brain (Epperson et al., 2002; Epperson et al., 2005; Epperson et al., 2006; Harada et al., 2010; O'Gorman et al., 2011). For this reason, it was decided to only scan male participants for some of the experiments described here.

5.2.3 General Analysis

All data was analysed using our in-house software Gannet, programmed in Matlab. Phased array coil data were combined by using the first point of the water scan FID to determine the relative amplitude and phase of the signal from the different coil elements. Time domain data were apodized by exponential window function (usually 4 Hz) prior to time averaging. Fourier transformation allowed frequency correction to be applied based on the Cr signal in individual shots. Time averaging allowed the difference (edited), sum, and off spectra to be calculated. The difference spectrum was plotted and the GABA peak between 2.9ppm and 3.16 ppm, centred at 3.026 ppm, was fitted using a Gaussian curve with 5 parameters (amplitude, offset, centre frequency, linear baseline gradient and amplitude offset) using Matlab. The water-peak was fitted using a Voigt (Lorentz-Gaussian; Marshall et al., 1997) curve fit between 4.6 ppm and 4.8 ppm. GABA concentration was quantified in institutional units from the ratio of the integral of the fit of the GABA

signal and the unsuppressed water signal from the same volume. Further analysis of the data is described per subsection.

5.2.3.1 *Data quality assessment*

There are several ways to determine the quality of MRS spectra. Dependent on the analysis method, fit errors, residuals or other diagnostics may be calculated. However, it is valuable to determine the quality of a spectrum by visual inspection. Figure 5.1 (which is a cohort-mean spectrum and therefore appears relatively artefact-free) shows the three main regions of the spectrum that are important for assessing the spectral quality. The most important are the shape of the GABA peak and the baseline (A). The GABA peak should appear symmetrical (and have either Gaussian or doublet character, see Section 4.6 and Section 5.7). Asymmetry is unlikely to arise from true GABA signal, and can cause imperfect fitting. The baseline should be linear either side of the GABA peak (A). Disturbance of the baseline region can significantly affect the fitting performance, and therefore the measured GABA concentration. Ideally, the Glx peak (B) should also be well-resolved and artefact-free. Finally, a large signal in the lipid region (C) may indicate poor localisation of signal to the MRS voxel or participant movement, and a large amount of lipid contamination may impact fitting of the GABA signal, particularly if lipid signals extend beyond the NAA signal.

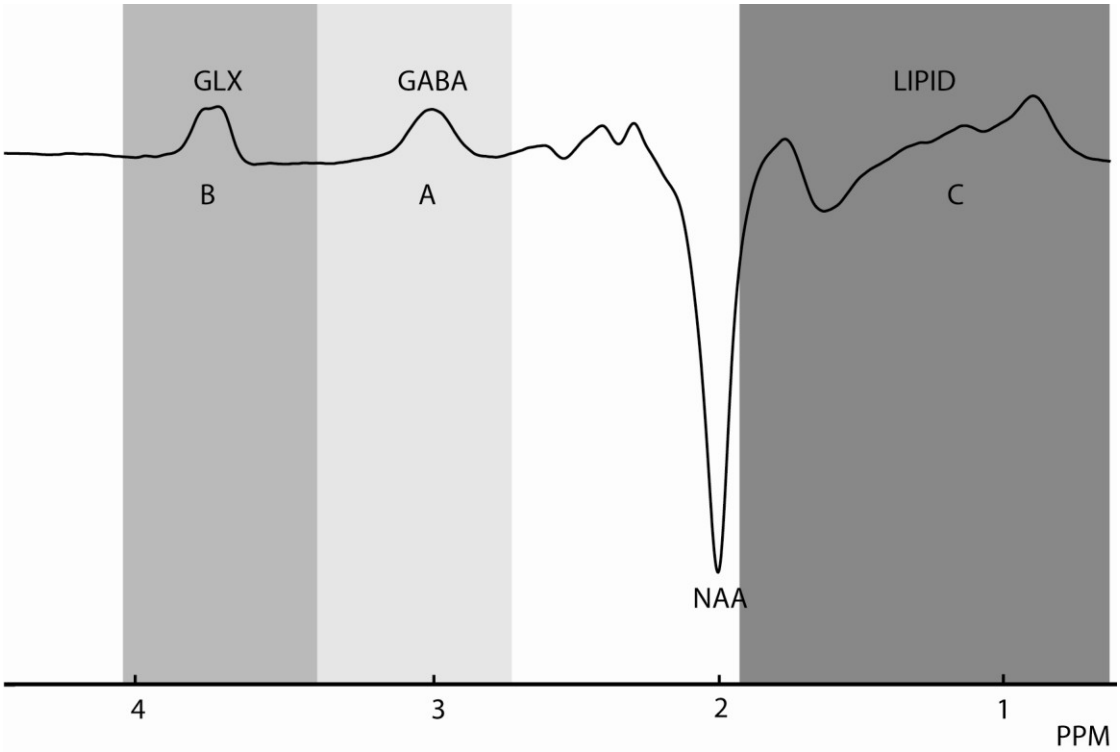

Figure 5.1. Regions that are important for visual inspection. A) Imperfections in the GABA peak (e.g. asymmetry) can cause imperfect fitting. In addition, noise in the baseline region may lead to large differences in fitting. B) Noise in the Glx peak and C) a large amount of lipid may indicate poor localisation performance.

More recent versions of our analysis pipeline calculate residuals for the fitting as well as fit error diagnostics. In addition, post hoc frequency correction can be performed on the basis of the Cr peak to reduce subtraction artefacts in the difference spectra. As these steps are more recent developments, their use will be specifically mentioned.

5.3 Experiment 1 - Voxel location

5.3.1 Introduction

Occipital cortex has been most widely studied in the literature (Puts and Edden, 2011) primarily due to experimental limitations – measurements that use a surface

receive-coil are most conveniently carried out in this location, and those that use a volume coil often have best SNR in this location due to the proximity of occipital brain to the coil elements. The location of the voxel and its distance to the receiver coils may affect the signal-to-noise ratio (SNR) of MR spectra. Sources close to the cortical surface will lie closer to the receivers whereas deep-brain sources such as the thalamus lie further away and will therefore have a lower potential SNR. Localisation of voxels is usually dependent on anatomical (e.g. occipital: Edden et al., (2009) or functional landmarks (FEF; Sumner et al., 2010) and is therefore subject to large individual differences in cortical anatomy and function as well as physical orientation in the scanner (the latter is particularly important in studies with multiple scan sessions). The primary somatosensory cortex can either be localized using anatomical landmarks or functional localization (i.e. using fMRI to show activation after tactile stimulation). The specific anatomical landmarks (the "handknob" in the axial plane and the "hook" in the sagittal plane) that indicate the hand-area of the primary motor cortex (see Section 1.4) can be used to locate S1 on a high-resolution anatomical MRI scan. Because MRS voxels have a cuboid shape, the aim here was to optimise voxel orientation so that it follows the edge of the skull, staying inside the brain, whilst staying centred at the omega-shaped hand knob that indicates the hand area of the motor cortex (Yousry et al., 1997).

5.3.2 Methods

Acquisition The voxel was rotated in both the sagittal and coronal planes so that the voxel was aligned with the cortical surface (see figure 5.2). The centre of the voxel was placed on the central sulcus posterior to the hand area of the right

primary motor cortex. Voxel dimensions of $(3cm)^3$ are known to provide high quality spectra and a single participant (male, 32 yrs old) was scanned for 10 minutes.

Analysis Spectra were plotted and inspected for quality. SNR was measured as the height of the GABA peak at 3ppm over the standard deviation of the baseline between -4 & -5 ppm.

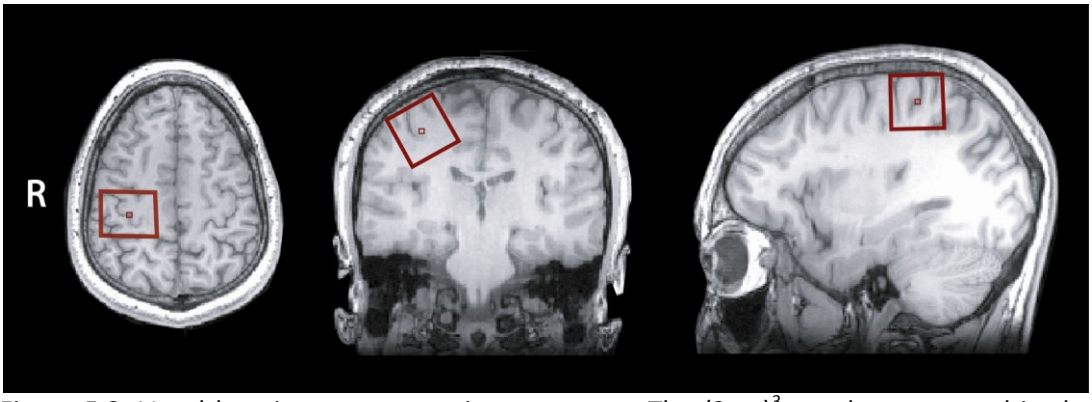

Figure 5.2. Voxel location over sensorimotor cortex. The $(3cm)^3$ voxel was rotated in the sagittal and coronal planes to align with the cortical surface. The centre of the voxel was placed on the central sulcus, posterior from the 'handknob', indicating the hand-area of the primary motor cortex.

5.3.3 Results

The acquired spectrum is shown in Figure 5.3. The spectrum exhibits an identifiable GABA peak at 3 ppm and lacks large peaks over the water and lipid regions of the spectrum. This particular GABA peak has a SNR of 23.1. The data show that MEGA-PRESS over sensorimotor cortex with these parameters results in high-quality spectra with good SNR, comparable to acquisition over occipital cortex as shown by prior studies in CUBRIC, see also Section 5.4. This voxel will be used and adapted in following experiments.

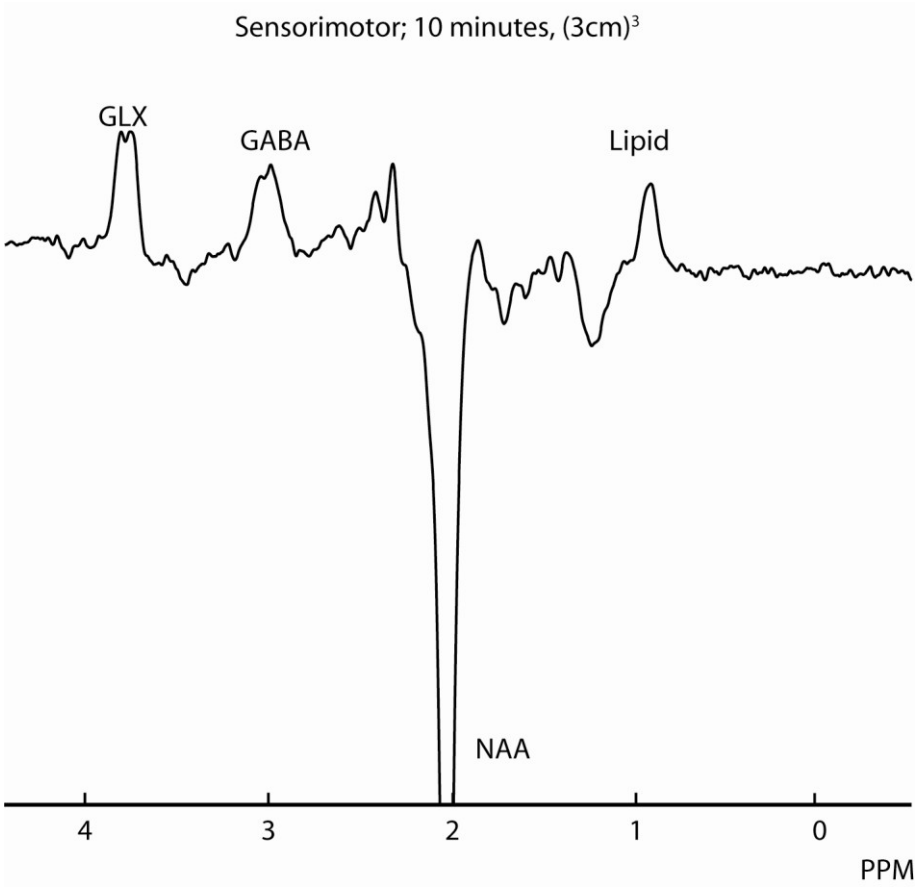

Figure 5.3. GABA-edited spectrum for a $(3cm)^3$ volume over sensorimotor cortex. GABA peak at 3 ppm, Glx peak at 3.8 ppm, NAA peak at 1.9 ppm (direct effect of editing pulse shown as negative peak), incomplete suppressed water signal at 5 ppm and lipid at 0.9 ppm

5.3.4 Discussion

The results described in this experiment show that we are able to detect GABA in the sensorimotor region using anatomical landmarks and that we can obtain good quality MRS spectra from this region. By rotating the voxel in two different directions it is possible to follow the anatomy of the brain surface and this allows us to place the voxel over the cortical and grey matter regions of the sensorimotor cortex more specifically. Prior studies have used $(3cm)^3$ voxels for other regions (Puts and Edden, 2011) and this initial assessment shows that the same voxel size provides us with good quality spectra over the sensorimotor cortex. Unfortunately, this voxel size is too large to incorporate only primary somatosensory cortex. However, for a more specific localisation over somatosensory cortex we are limited by scan duration and voxel size, as well as the experimental restriction to a cuboidal volume. In the following section voxel size and scan time will be modulated to investigate the possibility of smaller voxel size to investigate the primary somatosensory cortex more specifically.

5.4 Experiment 2 – Voxel Size, Scan Time and SNR

5.4.1 Introduction

In the design of MRS studies in general, and especially those studying the low concentration metabolites such as GABA, there is a tension between needing to minimise volume in order to increase anatomical specificity, to maximise SNR and measurement precision, and to minimise experiment time to increase participant comfort (and thereby data quality). A typical MEGA-PRESS experiment is equivalent to a 10-minute measurement of a $(3cm)^3$ volume at 3T (Puts & Edden, 2011).

Aside from the physical location of the MRS voxel relative to coil elements, two additional factors that determine the SNR of a spectrum are the size of voxel studied and the duration of acquisition, or scan time. A smaller voxel volume gains anatomical specificity at the expense of linearly decreased SNR. Theory predicts SNR is proportional to the square root of scan time (√n) (Puts and Edden; De Graaf, 2007). Theoretically, the signal acquired in each scan is the same, so the total signal is proportional to the number of scans, but in contrast, the noise is random and so does not add linearly but the amplitude of the noise increases as the square root of the number of scans added together. Concerns exist as to whether reduced participant compliance during long scans will significantly diminish SNR gains from long experiments. Additionally in the case of editing, B0 field drift (magnetic field instability) may lead to degraded editing efficiency during a long experiment. To be able to apply GABA-MRS to functionally relevant experiments, the aim is to detect the primary somatosensory cortex as specifically as possible. SNR is proportional to volume (as the volume gets doubles, the acquired signal doubles as well). Typically MEGA-PRESS MRS studies use voxels with a size of 3x3x3 cm^3 to achieve good SNR. Since S1 does not have dimensions 3 cm x 3 cm x 3 cm, some compromise between anatomical specificity and SNR must be struck.

Initially, a comparison is made between a voxel over occipital cortex and a voxel over sensorimotor cortex and between a standard $(3cm)^3$ voxel over sensorimotor cortex and a voxel with decreased size. Secondly, we investigate the relationship between SNR, scan time and voxel volume to determine what benefit smaller voxel size has with respect to changes in scan time and thus SNR.

5.4.2 Methods

5.4.2.1 *Voxel size*

Acquisition Optimisation of the size and dimensions of the voxel was carried out. A 10 min scan using a (3cm^3) in the occipital lobe (which is known to result in good quality spectra in terms of SNR and visual inspection; see Section 5.2.3.1) was compared to a 10 minute scan using a (3cm^3) voxel (see figure 5.4a) with its centre located posterior to the omega-shaped hand knob as described in Section 5.3, and to a *30* minute scan using a 2.2x2.2x2.2 cm^3 voxel (10.7 cm^3) (see figure 5.4b); a reduction of 60%, also centred at the hand knob. This voxel is rotated in the sagittal and coronal plane.

Analysis SNR was calculated as ratio of the peak amplitude of the GABA peak at 3ppm to the standard deviation of the signal between -4 to -5 ppm.

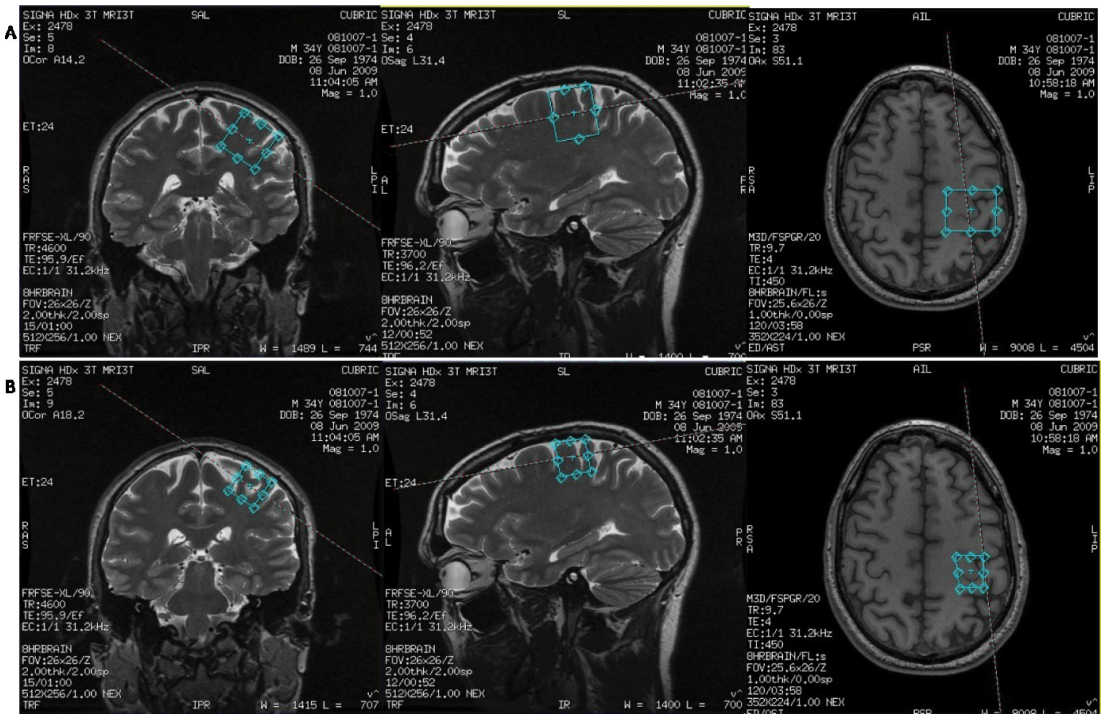

Figure 5.4. Voxel Size. Three voxels were compared. A 3x3x3 cm^3 voxel in the occipital lobe (not shown), A. A 3x3x3 cm^3 voxel over sensorimotor cortex (above; 10 min scan) and B. A 2.2x2.2x2.2 cm^3 voxel over sensorimotor cortex (below; 30 min scan).

5.4.2.2 *SNR; Scan Duration& Voxel Size*

Acquisition To investigate the relationship between SNR and scan time, GABA-MRS scans were acquired with 1444 scans of 2k datapoints per 45-minute acquisition on 3 healthy participants (2 male, aged 31 ± 2.6 yrs) on a standard $(3cm)^3$ voxel localised over sensorimotor cortex. The relationship between SNR and voxel size was investigated by using GABA-MRS scans with 332 scans of 2k datapoints per 10-minute MEGA-PRESS acquisition in $(2\ cm)^3$, $(2.5\ cm)^3$, $(3\ cm)^3$ and $(3.5\ cm)^3$ volumes on one participant only (aged 27). Centre of the voxel placed on sensorimotor cortex as described in previous sections (See Section 5.3 and Figure 5.2).

Analysis Subspectra were calculated from the sum of the first *n* excitations, where *n* covers the even square numbers (4, 16, 36...1444). The processed spectrum as acquired over the full 45 min was used as a model to fit the amplitude of signal for subsets of the data (across a range from 2.7 to 4.2 ppm). The signal-to-noise ratio (SNR) was determined from the amplitude of the fit of the GABA signal and noise from standard deviation between -4 & -5 ppm. The B0 drift was calculated for the subspectra from the maximum value of NAA peak (from the MEGA-PRESS sum spectra).

5.4.3 Results

5.4.3.1 *Voxel size*

Figure 5.5 shows the three spectra acquired. Figure 5.5a shows the spectrum for a $(3\ cm)^3$ voxel in the occipital lobe after a 10 minute MRS scan (GABA SNR occipital:

36.7). The occipital lobe voxel is used as a standard against which to compare our S1 data, as occipital lobe GABA has been well studied. Figure 5.5b shows the spectrum for the $(3\ cm)^3$ voxel in S1 after a 10 min MRS scan (GABA SNR: 23.1). A lower SNR is expected due to S1 lying farther away from the receiver coils. Figure 5.5c shows the spectrum for the $(2.2\ cm)^3$ voxel in S1 after a 30 minute MRS scan. Because the SNR changes linearly with the voxel size and the square root of the scan time, a decline in SNR of about 30% for the smaller volume is expected. The SNR of GABA was measured as 18.9, which is an acceptable decrease in SNR, for such a significant improvement in anatomical specificity. The results indicate that a 30-minute experiment provides a good-quality spectrum and thus that decreasing the size of the voxel, but lengthening the scan, is a reasonable step to take.

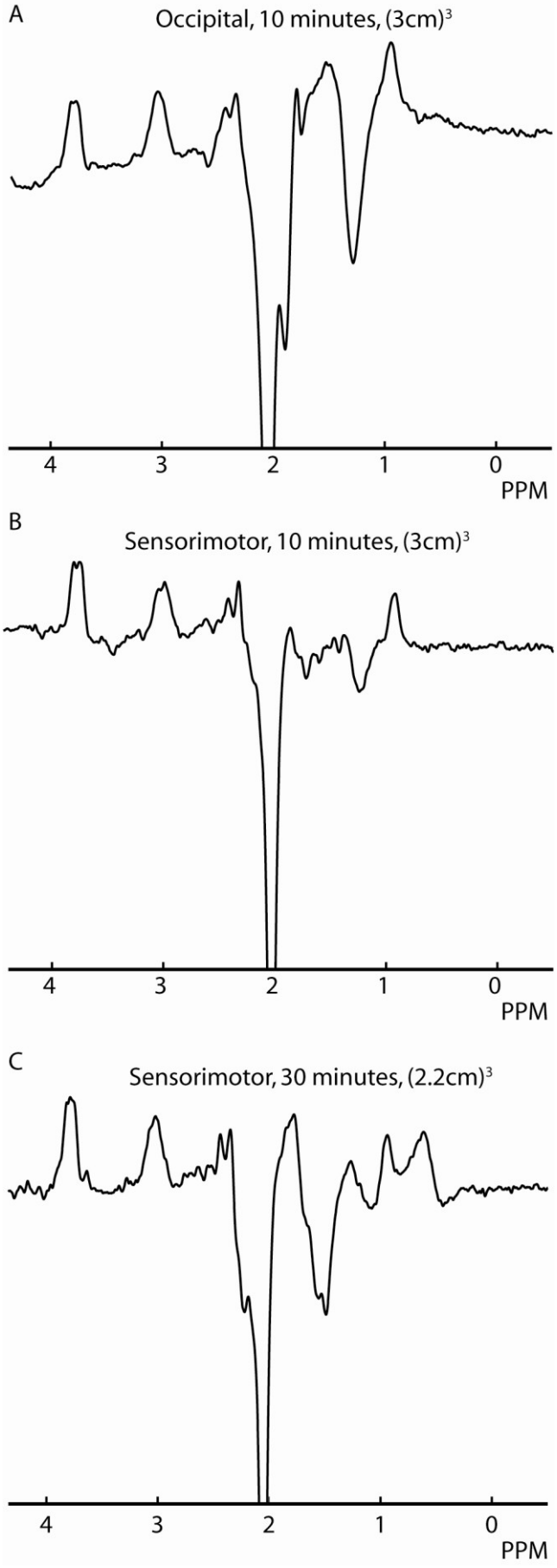

Figure 5.5. Spectra from three different voxels. A shows the spectrum for a $(3cm)^3$ voxel in the occipital lobe (10 min scan). SNR for GABA (peak at 3ppm) = 36.7. B shows the spectrum for a $(3cm)^3$ voxel over sensorimotor (10 min scan). SNR for GABA = 23.1. C shows the the spectrum for a $(2.2cm)^3$voxel in sensorimotor (30 min scan). SNR for GABA = 18.7.

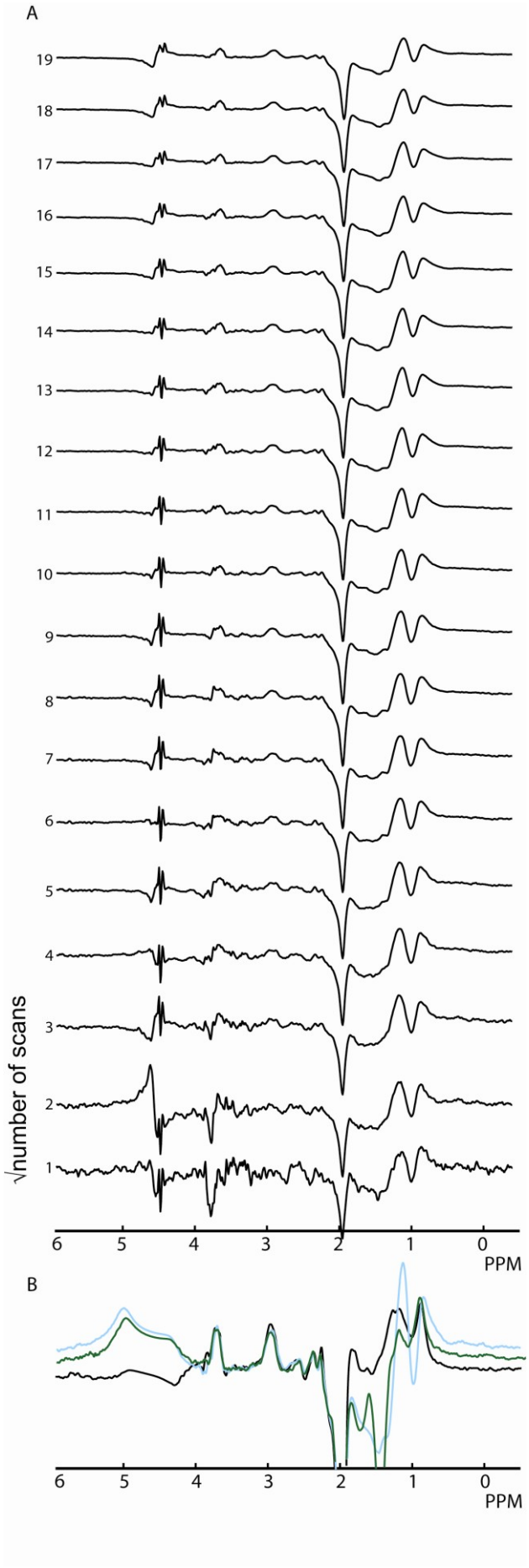

Figure 5.6. Subspectra and averaged spectrum for a 45 min scan over sensorimotor cortex.A) shows subspectra for the √ of the number of scans for a single participant and shows improvement of spectral quality over time. B) shows the averaged spectrum after 45 min for all three participants.

5.4.3.2 *SNR, Scan Duration& Voxel Size*

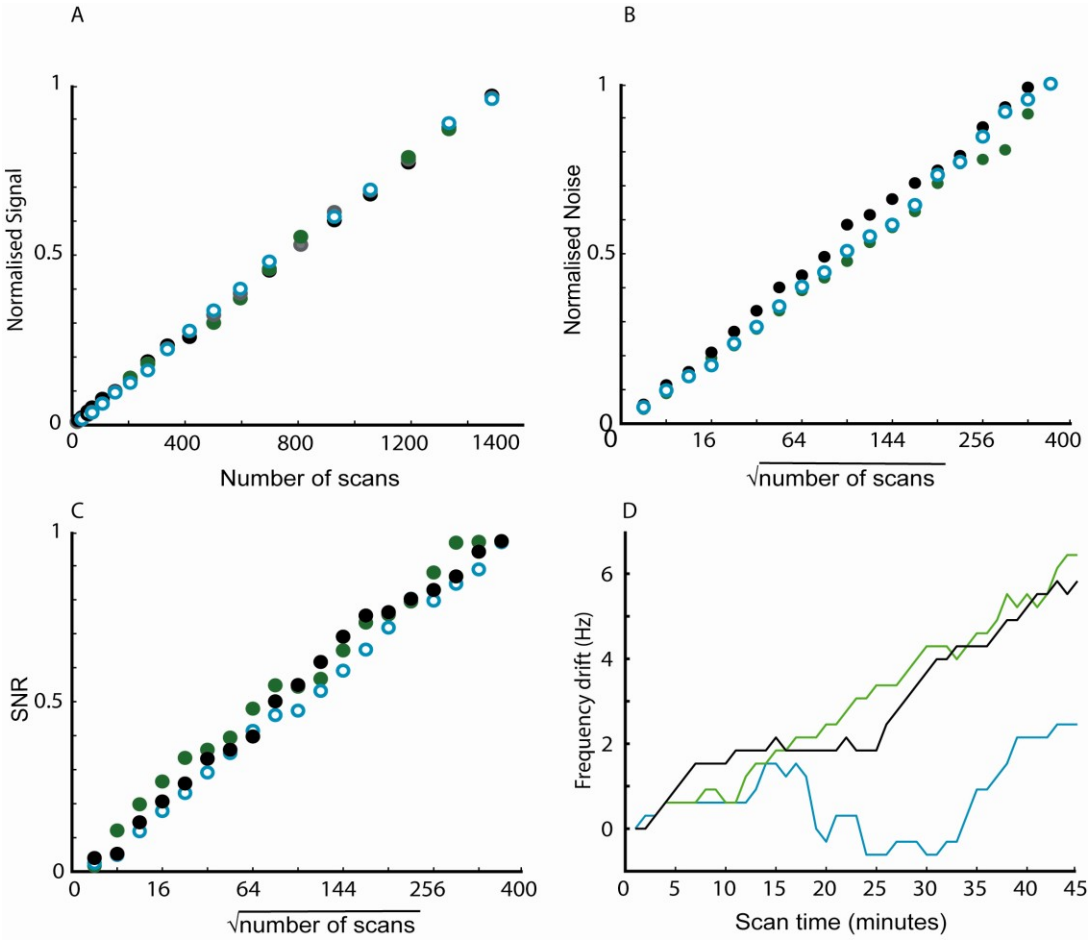

Figure 5.7: Plots of normalised signal against scan number (a), of normalised noise against √n (b) and of normalised SNR against √n (c) for all three participants. (d) shows frequency drift over a 45 min scan.

The GABA SNRs for the 45-minute experiments for the three individuals was 58, 67 and 76 respectively. Figure 5.6 shows the gradual improvement of the spectra incorporating n scans and the full difference spectra for all three participants (5.6B). Figure 5.7A-C shows plots of: the normalised GABA signal against scan time (5.7A); the normalised noise standard deviation against the square root of the scan time (5.7B); and the normalised signal-to-noise against the square root of the scan time to (5.7C), for all three individuals demonstrating the predicted linear improvement of SNR with square root of number of scans. Figure 4.7D shows the linear frequency drift in two of the participants with an average drift of 4.9 Hz ± 2.1 over 45 minutes, (less than the line width), corresponding to 0.11 Hz/min. Participant 3 shows a non-linear frequency drift resulting in a sum frequency drift less than the other 2 participants.

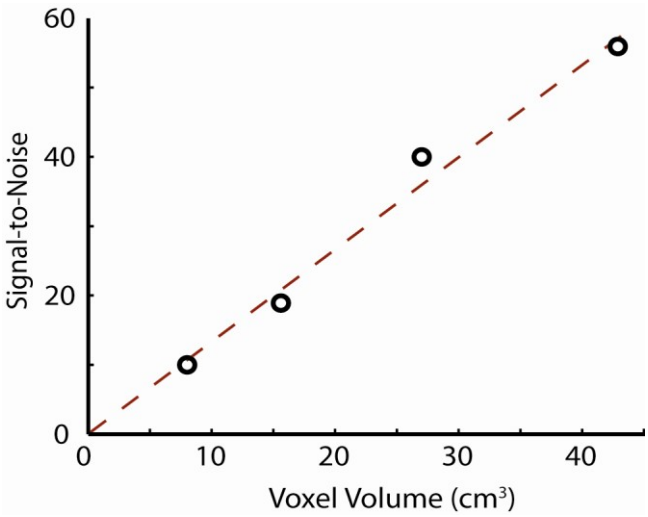

Figure 5.8: SNR against voxel volume

The results in Figure 5.8 show a linear relation between voxel size and signal-to-noise ratio, as expected. Thus to obtain a twofold increase in SNR with longer scan time, the scan needs to be four times as long.

5.4.4 Discussion

These experiments showed that the experimental results largely follow theoretical predictions that the SNR increases with the square root of the number of scans and the voxel size. Finally, the frequency drift observed throughout the scans is small (compared with the inversion bandwidth of the editing pulses) and should have no effect on editing efficiency of the GABA signal. However, MR-habituated volunteers may be more compliant than naive participants. This study is also limited in using only three participants. The results show that small voxels are possible and that the theoretical relationship between voxel sizes and scan time is experimentally valid and does not suffer from physiological variability.

These data suggest that the theoretical relationship between scan time and SNR can be relied upon for MEGA-PRESS studies of GABA. In practice, more specific localisation over somatosensory cortex is still problematic. Scan time is limited and in many imaging experiments it is not feasible to scan for longer than 10-15 minutes per voxel location and due to the anatomy of the somatosensory cortex, reducing the voxel's size may still not result in significantly improved matching to somatosensory cortex due to its cuboidal shape.

5.5 Experiment 3 – Gradient direction

5.5.1 Introduction

One undesirable feature of the slice-selective localisation used in MEGA-PRESS is chemical shift displacement, which causes the excited volume to differ slightly for signals with differing chemical shift. The spectrum of interesting metabolites is

bounded on one side by lipid signals at 1.3 ppm and on the other by the water signal at 4.7 ppm. The excited volume for water and lipid will be shifted in opposite directions from that excited for GABA signals.

If the direction of the fat-water chemical shift displacement were chosen to be away from the scalp, (the primary location of MR-visible lipid), this would minimise the appearance of lipid signals in the spectrum, a desirable outcome. However, this would result in an excited volume for water that is shifted towards the edge of the brain; possibly reducing the quality of water suppression. It is therefore not immediately clear which orientation is preferable.

It is important in placing the voxel to minimise the amount of non-brain volume, i.e ventricles, skull, sinus etc. For example, the voxel location that we have developed over sensorimotor cortex described in section 5.3 (see Figure 5.2 for voxel location) lies with one face along the edge of the brain and close to the skull. Such a scan is problematic, as imperfect localisation can lead to excitation of excessive signals from subcutaneous lipid. In addition, magnetic field homogeneity deteriorates at tissue/air boundaries, which can lead to incomplete water suppression. A large fat signal or poorly suppressed water signal can have a significant impact on the reproducibility of GABA measurements from the spectrum. As a voxel is created by slices in three orthogonal planes, the direction of the x- y- and z-gradients can have a significant effect on the water and lipid suppression of the spectra due to the difference in chemical shift of water. We know that acquisition with default gradient direction for right sensorimotor cortex (Section 5.3 and 5.4) shows good quality spectra and does not contain poor lipid- and water suppression. This would suggest that for left sensorimotor cortex, negative

gradients would be optimal. Pilot testing was done to test this theory. Due to the rotation of the voxel it is unclear which direction the different gradients are; but it is assumed that x-direction refers to anterior-posterior (AP), the y-direction to left-right (LR) and the z-direction to superior-inferior (Marshall et al. 2000). Because the voxel is aligned with the skull, it would be most affected by AP and SF direction gradients and it was assumed that changing the y- (LR) and z-(SI) gradients to negative would make the largest difference whereas changing the x-gradient should not lead to qualitative differences.

5.5.2 Methods

Acquisition Initially, two scans were performed on a $(3cm)^3$ volume over *left* sensorimotor cortex of one participant (male, 26yrs old). In the first scan, gradient direction was positive for all slice-selection gradients; in the second scan, the direction of the LR- and SI-gradients was reversed. For further confirmation that this configuration was optimal, two participants (male; avg. age 30.4yrs ± 3.5) were scanned to compare the reversal of LR- and SI-gradients only with the reversal of *all* gradients. The order of scans was counterbalanced, so that diminishing participant compliance toward the end of the session did not bias interpretation of the data.

Analysis GABA concentration was calculated as described in section 5.2.3. Analysis of spectra was performed including frequency realignment and phase correction of individual timepoints which is known to improve quality (Waddell et al., 2007)

5.5.3 Results

Figure 5.9 shows the spectra acquired after a 10 min scan in left sensorimotor region with the slice-selective gradients positive, the default (black line) and with both LR- and SI-gradients reversed (red line). Reversal of the gradients lead to an improvement in water suppression (A) and a large decrease is the amount of lipid signal (B). These results show that we were successful in reversing the gradients in order to reduce the effect of lipid in the signal. Surprisingly, the suppression of the water signal improved as well, allowing for a better quality spectrum for GABA.

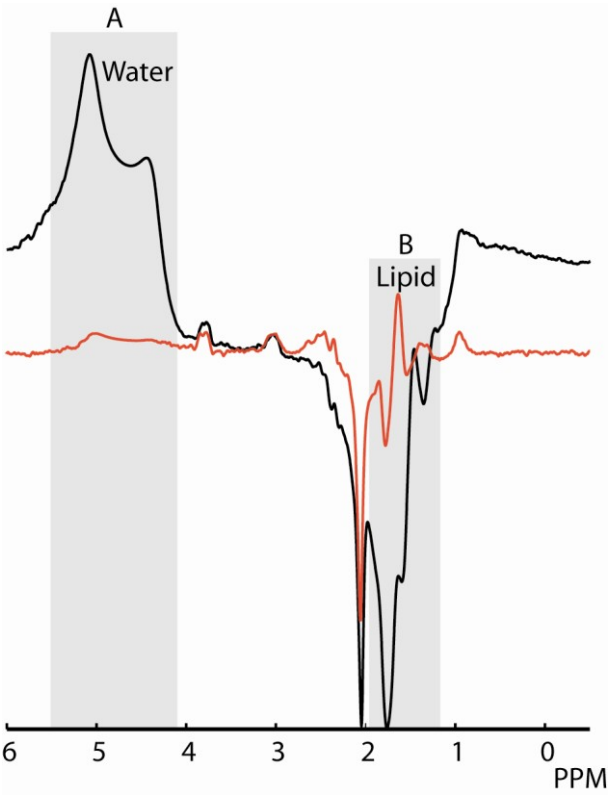

Figure 5.9. Two spectra for a voxel in left sensorimotor (10 min scan) with different gradients. The black line shows the spectrum for positive gradients. The red line shows the spectrum for the experiment with the LR- and SI-gradients reversed. A shows that reversing the gradients leads to a better water suppression. B shows that reversing the gradients decreases the amount of lipid in the signal

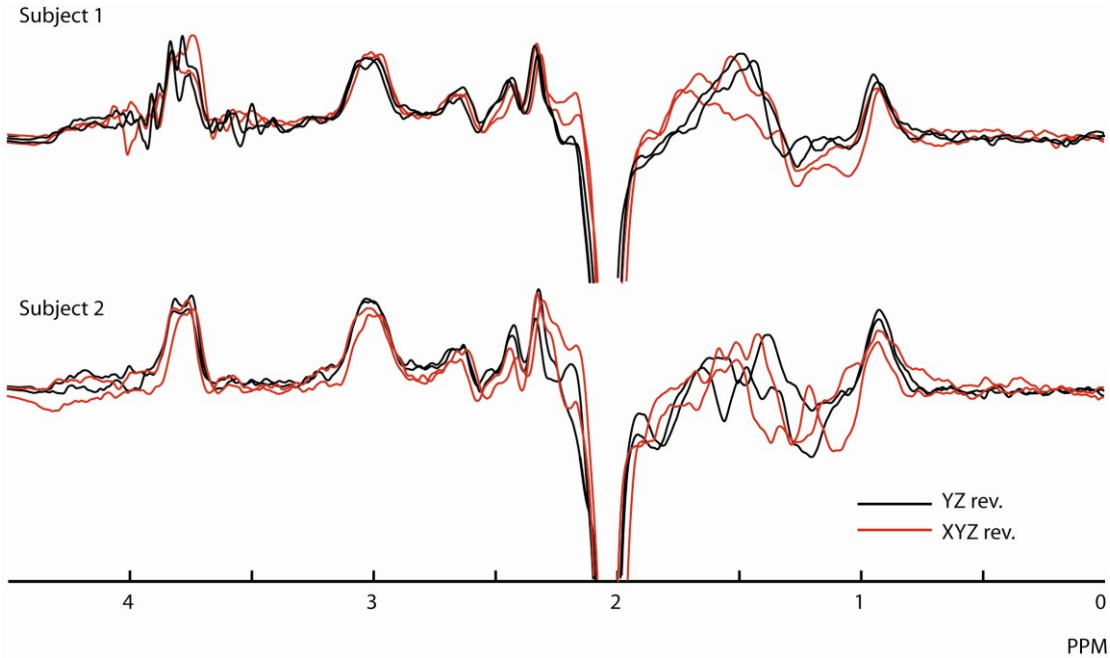

Figure 5.10. The effect of different gradient reversal on spectra over left sensorimotor cortex for two participants. The black line indicates spectra that were acquired with the LR and SI gradients reversed. The red line indicates spectra that were acquired with all gradients reversed. Visual inspections shows no significant differences in lipid, baseline or GABA signal between either acquisition.

As Figure 5.10 shows, either configuration shows good quality spectra with a clearly visible GABA peak at 3 ppm and limited lipid signal. Average GABA concentration was slightly different between conditions (participant 1: LR-SI negative = 1.126; All negative = 1.071. Participant 2: LR-SI negative = 1.085; all negative = 1.31), but not in any particular direction. There was no difference in the variability between the two scans for the two conditions.

5.5.4 Discussion

These results suggest that reversing the LR- and SI-gradient direction has a positive effect on the spectral quality over left sensorimotor cortex. However, due to the limited number of participants, a clear comparison between the two configurations cannot be made. These measures also confirm that changing the AP-gradient does not affect spectral quality. The use of only one configuration is suggested because there are differences in GABA concentration between configurations.

These results show that the chemical shift direction of slice-selective pulses is an important issue in determining the quality of MRS spectra and the reproducibility of GABA measurements. It must be noted that this issue is very likely to be scanner-specific as this seems to only be apparent in GE scanners (for instance, Philips MRI scanners allow the experimenter to visualise fat-water shift during voxel placement and therefore position the voxel accordingly).

It is important to understand the effects of lipid contamination on the quality and reproducibility of MRS data. In this section we have shown the effect of gradient direction and we have shown how optimisation of the acquisition may benefit data quality.

5.6 Experiment 4 - Analysis methods and fitting parameters

5.6.1 Introduction

Traditionally, MRS spectra are quantified by manual integration of metabolite peaks. This approach is time consuming and has the potential for user error and bias to impact results (Marshall et al., 2000; Gillies et al., 2006). Therefore, it is generally accepted that automated analysis is preferable, using tools such as LCModel (Provencher, 2001). Several groups have applied the LCModel to quantify J-edited spectra, but great care must be taken to optimise control parameters for appropriate fitting. One potential pitfall is to assign almost the entire edited signal at 3 ppm to the macromolecular baseline e.g. (Tayoshi et al., 2010). It is widely felt (including by Dr Provencher) that LCModel is not optimised to analysing difference-edited spectra, and so we have processed data using local Matlab code.

Within this code, the GABA peak at 3ppm has usually been fit using a Gaussian function. However, in theory, the edited GABA peak should appear as a pseudo-doublet (as discussed in Section 4.6 and although the GABA signal in our GE acquisitions frequently appears as a single peak, studies performed with other MEGA-PRESS implementations do show a pseudo-doublet edited signal (Wadell et al., 2007). Therefore additional work has been performed to investigate whether a more sophisticated model should be used to determine the GABA peak area. We investigated the variability that arises from fitting the GABA-peak at 3ppm with four different models: a Gaussian; a Lorentzian doublet; a Gaussian doublet; and a model based on the group-mean spectrum (GMS).

5.6.2 Methods

Data Data from a separate study was used. 12 participants (avg. age 30.1), all male were scanned in two sessions with two scans for each voxel per session. Three voxels were scanned: Occipital cortex (Occ), sensorimotor cortex (SM) and dorsolateral prefrontal cortex (DLPFC), all with $(3cm)^3$ voxel dimensions. Order was randomised across voxel locations. The sensorimotor voxel was placed in line with section 4.3. This study resulted in 144 spectra. In addition, the analysis was replicated using 73 spectra from a voxel over the limbic region, acquired in a study by my collaborator Paul Keedwell.

Analysis Data was fitted using four different models: (1) A single Gaussian, as described in section 5.2, as the general method of analysis for our studies; (2) a Gaussian doublet (with fixed splitting based on the GABA coupling values); (3) a Lorentzian doublet (again of fixed splitting based on the GABA coupling values); and (4) a template spectrum based on the GMS (n = 144; n = 73). Data was integrated for the entire fit. Pearson correlations were used to examine the reliability between different methods of fitting.

5.6.3 Results

Correlations between different methods can be seen in Table 3.1 and Figure 5.11 shows the spectra as fit by a each model. It can clearly be seen that the integrals determined are strongly preserved across different models. The correlation between GMS and other fitting methods is less than for the other fitting methods; this can be explained by the fact that the GMS has been compiled from three different regions which differ slightly in GABA spectrum, so rather than comparing a

single scan with different fitting methods; the GMS does not allow for a sloping baseline and is an average over three regions, thereby introducing noise. A correlation between different fitting methods of 73 additional spectra from a single region confirm this (range between fitting methods; R^2 = 0.95-097).

Table 3.1. Pearson correlation coefficients (r^2) between different fitting methods. Different fitting methods correlate strongly

Fitting model	Single Gauss	Double Gauss	Double Lorentz	GMS
Single Gaussian	-	0.991	0.993	0.871
Gaussian Doublet	0.991	-	0.985	0.9015
Lorentzian Doublet	0.993	0.985	-	0.8464

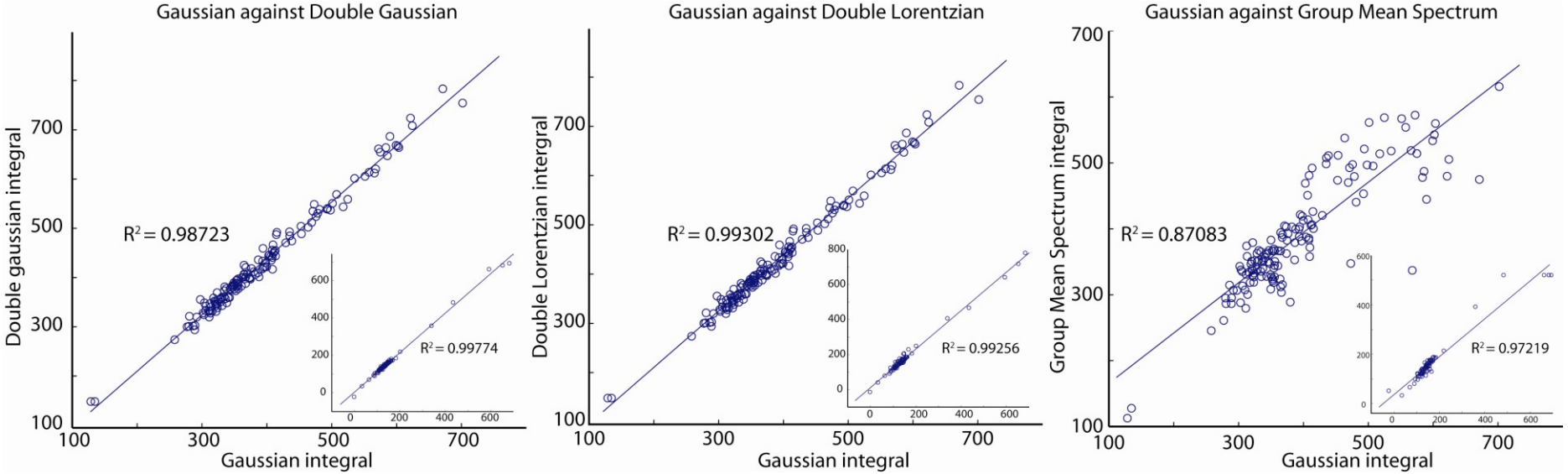

Figure 5.11. Correlations between different fitting models. Some studies show a pseudo-doublet for GABA whereas others show a single peak. To investigate whether fitting GABA with a doublet of singlet has an effect of approximation of the GABA concentration, 144 (large graphs) and 73 (small inset) were fitted with a single Gaussian, a Gaussian doublet, a Lorentzian doublet and a template based on the Group Mean Spectrum (GMS). Pearson correlations between the measures were calculated. These results show strong correlations between the different fitting methods for both datasets. The GMS fit for the 144 spectra is correlated less, possibly due to the fact that the GMS is calculated from three different locations. GMS based on a single location (inset) shows a strong correlation.

5.7 General Discussion

5.7.1 Acquisition of GABA-MRS over somatosensory cortex

MRS has the potential to become a powerful tool for the investigation of the inhibitory neurotransmitter GABA *in vivo*. This is a significant success, given the significant methodological hurdles involved: low concentration; a coupled spin system; and overlapping metabolite peaks.

In this Chapter we have shown different approaches to optimise the use of MRS to detect GABA in S1. By specifically placing the voxel on the basis of anatomical landmarks, and rotating the voxel to align with the cortical surface, MRS of a $(3cm)^3$ voxel results in good quality spectra. Voxel size and scan time are limiting factors in the acquisition of GABA-MRS. Reduction of voxel size possibly allows for more specific voxel placement, but reduces the SNR of the measurement. Increasing noise decreases the ability to detect significant differences in GABA concentration. However, by increasing the scan duration, SNR can be increased. Specificity of single voxel localised MRS is still an issue and although we have shown that we can theoretically obtain high quality GABA measurements from a small volume, longer scan time is made necessary which is something that is not always possible. Despite higher specificity due to a decrease in the voxel volume, anatomical considerations may cause proper localisation to be problematic, in specific for somatosensory cortex where neither a single, double or triple rotated cuboid voxel can align with the underlying cortical anatomy. Therefore a $(3cm)^3$ over sensorimotor cortex is suggested as the optimal voxel volume at 3T.

The quality of the acquisition can be measured by investigating how repeatable the measures are within and between participants. Within- and between-session reproducibility was tested in a separate study and showed that for a standard sensorimotor voxel the average coefficients of variations are 8.1% within two scans and 10.1% across two sessions, suggesting reasonable reproducibility (see also Bogner et al., 2010; O'Gorman et al., 2011). These values may improve with new and better analysis pathways.

5.7.2 Analysis of GABA-MRS data

5.7.2.1 *Fitting the pseudo-doublet*

It is fair to say that there currently exists no perfect method for analysing edited MRS data, so it is important that the effect of different analysis parameters is understood, and in the future that some consensus is reached. Previous studies have suggested that the pseudo-doublet of GABA at 3 ppm is fitted with two peaks rather than one; the results presented above suggest that for our data this is not.

5.7.2.2 *Post-processing prior to fitting*

Further analysis steps have been suggested in the literature. As mentioned in Section 4.6, MEGA-PRESS consists of a large number of acquisitions that are averaged to give a final spectrum. When one or more of these scans are problematic (whether due to movement or other factors) the entire combined spectrum may be affected. More recent findings have been analysed using improved software that allows for automatic realignment and rephasing of the data as described in (Waddell et al., 2007). Because spectra acquired via MEGA-PRESS experiments are taken as the average from a number of scans, it is beneficial to realign these separate scans. This will provide both a better data quality, while the

realignment parameters may serve as well as a diagnostic tool for determining scan quality and outliers.

5.7.3 Summary

In summary, we are able to obtain good quality, reproducible GABA-MRS data from sensorimotor cortex. Despite exploring the possibility of measurements from a smaller volume, a decision was made to use a standard $(3cm)^3$ volume is used for further studies as this allows excellent SNR in scan times of the order of ten minutes.

A particularly interesting recent field is the application of MRS to study individual differences in GABA concentration as they relate to inhibition-dependent behavioural processes. In the next chapter these optimised methods will be used to investigate whether measurements of GABA in sensorimotor cortex have behavioural correlates with measures of tactile sensitivity as measured by behavioural psychophysics.

Chapter 6 - GABA and Tactile Frequency Discrimination

6.1 Introduction

GABAergic inhibition plays an important role in defining the selectivity of cortical responses to behaviorally relevant stimuli. The application of GABAergic antagonists, such as bicuculline, has been shown to modulate the responses of individual neurons to visual (Sillito, 1975; Tsumoto et al., 1979; Sillito et al., 1980; Wolf et al., 1986), auditory (Muller and Scheich, 1988; Fuzessery and Hall, 1996) and somatosensory (Dykes et al., 1984; Alloway and Burton, 1986; Juliano et al., 1989) stimuli.

Recent work in non-invasive MRS has demonstrated that it is possible to link measures of GABAergic function to psychophysical measurements (Edden et al., 2009; Boy et al., 2010; Sumner et al., 2010). For example, in the visual system it has been shown that orientation discrimination thresholds correlate with GABA concentration in visual cortex (Edden et al., 2009) in the sense that those participants who perform well at the orientation discrimination task have more GABA in their occipital regions.

In the somatosensory system, vibrotactile frequency discrimination in the flutter range (5 - 50Hz) is regularly used as a behavioral paradigm to investigate the neuronal correlates of sensory encoding and decision making (Mountcastle et al., 1967; LaMotte and Mountcastle, 1975; Mountcastle, 1997; Hernandez et al., 2000; Romo et al., 2000; Salinas et al., 2000; Hernandez et al., 2002; Romo et al., 2002).

Non-human primate studies (Mountcastle et al., 1990) have demonstrated that the primary somatosensory cortex is involved in coding vibrotactile frequency. At the level of individual neurotransmitters, work has demonstrated that vibrotactile processing is driven by NMDA (Tannan et al., 2008) excitatory and GABAergic (Dykes et al., 1984; Juliano et al., 1989) inhibitory mechanisms. Several studies have shown that frequency encoding is represented by highly periodic spike trains (Mountcastle et al., 1990; Recanzone et al., 1992b; Recanzone et al., 1992a) and that different frequencies are represented by differences in the periodic encoding of information (also; Tommerdahl et al., 1987). And although a role of spike rate is suggested as well (Romo et al., 1990; Salinas et al., 2000), LFP recordings also show a role of periodic encoding to tactile frequencies (Haegens et al., 2011b). This discrepancy will be discussed in chapter 9. The role of GABA in frequency encoding is discussed by McLaughlin & Juliano (2005) showing that disruption of GABAergic signaling leads to a deficit in temporal encoding of frequencies in ferret somatosensory cortex, suggesting an important role for GABA in coding of frequencies and discrimination.

While much is currently known about how representations of vibrotactile frequency are gradually transformed into motor outputs by successive cortical regions (Romo and Salinas, 2003; Luna et al., 2005; Hernandez et al., 2010), it is important to realize that individuals differ in their functional neuroanatomy and behavioural performance (Myasnikov et al., 1997) and it remains unclear how differences in somatosensory processing between individuals can explain differences in performance on a frequency discrimination task. Investigation on inter-individual

differences in non-human primates is problematic due to small cohort sizes and the large amount of training required, but learning studies have shown that increases in individual tactile performance through training can be linked directly to changes in neuronal responses in primary somatosensory cortex (S1; (Recanzone et al., 1992b). GABAergic mechanisms are thought to underlie these functional alterations as a result of training (Kohn and Whitsel, 2002). These studies show that GABA is important in shaping the response of neurons sensory stimulation, but more importantly for the investigation of the somatosensory system, that GABA may play an important role in the encoding of tactile frequencies (Mclaughlin and Juliano, 2005).

Here we directly test the hypothesis that differences in GABA *between* individuals can account for differences in perceptual performance. We obtained individual frequency discrimination thresholds with a paradigm similar to the one used in 2.4. We used magnetic resonance spectroscopy (MRS; see Chapter 4 and 5) to determine GABA concentration over sensorimotor cortex. We further tested the hypothesis that the relationship between brain GABA concentration and behavioural performance is regionally specific, by comparing GABA measurements in the sensorimotor region with similar measurements in an occipital (control) region.

6.2 Materials and Methods

6.2.1 Participants

Sixteen right-handed participants (mean age 27.3; SD 4.3; range 22-34; 10 male) were recruited for this study. None had a history of neurological disease. All

participants gave informed consent and all procedures were approved by the local ethics committee at Cardiff University's School of Psychology. One participant was excluded from further analysis due to poor execution of the behavioral task, which prevented the determination of threshold in this individual.

6.2.2 Equipment

The vibrotactile stimulator is described in Chapter 2.4. The PEST algorithm (Taylor and Creelman, 1967; see also Section 2.2) was used to determine all threshold measurements.

6.2.3 Behavioural psychophysics

6.2.3.1 Preparation

Participants were seated comfortably and their left index finger was placed on the stimulator and taped into place to reduce finger movement. Participants received a practice session in which they were exposed to exemplar vibrotactile stimuli of various frequencies and amplitudes. Once participants reported they were comfortable with discriminating the different stimuli, they received a practice session of each task. The practice session for the detection threshold measurement consisted of ten trials of detection of a suprathreshold stimulus in one of two intervals. The practice session for the frequency discrimination task consisted of ten trials of two intervals in which one stimulus was always higher (supra-discrimination threshold) than the other and participants were asked to choose the interval with the highest frequency. The practice sessions were repeated until participants showed a 100% correct level. Participants were instructed to maintain fixation for

the entire experiment and to press a response key as fast as possible at the termination of each stimulation period.

6.2.3.2 Detection Threshold

Prior to the frequency discrimination task, individual detection threshold values were determined using a 60 trial 2-interval forced choice (2IFC; interval duration 1s; 1±100ms ISI) paradigm in which the amplitude of the target was altered in a step-wise fashion to find participants' 75% correct threshold. The resulting amplitude was increased by 8 dB for the discrimination task (after LaMotte and Mountcastle, 1975) as described in Section 2.4.

6.2.3.3 Subjective frequency-intensity matching

Previous frequency discrimination studies have shown that perceived intensity varies as a function of frequency (LaMotte and Mountcastle, 1975; Verrillo and Capraro, 1975), so the amplitude of the comparison stimulus was adjusted as before (see Section 2.4)

6.2.3.4 Frequency Discrimination

Vibrotactile discrimination thresholds around the standard were measured using a 100 trial two-track interleaved 2 alternative forced-choice design (2AFC; 1s±100ms ISI) with comparison stimulus frequency as the adaptive variable (Figure 6.1). The order of the tracks was pseudo-randomized, so that a maximum of 5 trials of the same track occurred sequentially. The order of standard and comparison stimuli was randomized between trials. Frequency discrimination thresholds were calculated by taking the average of the last 15 trials across the two tracks. For the task, inter-trial intervals (ITIs) consisted of a 2-second pause with ±100 ms jitter.

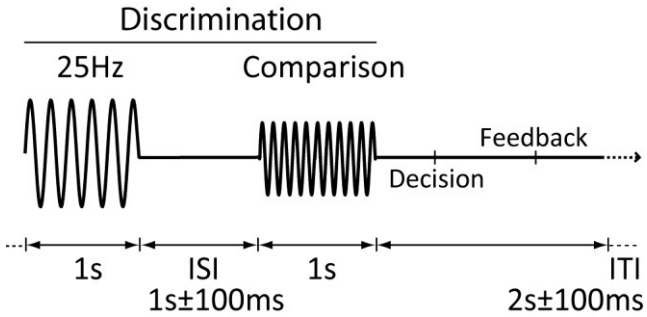

Figure 6.1. Frequency discrimination paradigm. Frequency discrimination thresholds to a 25Hz stimulus were determined for frequencies higher and lower than 25Hz in a 2 alternate forced choice (2AFC) paradigm. The order of presentation of the standard (25Hz) and comparison stimuli were randomized.

6.2.4 MR experiment

After vibrotactile thresholds were determined, participants proceeded to the MR scanning suite at CUBRIC. All scanning was carried out on a GE SignaHDx 3 Tesla MRI scanner (General Electric Healthcare, Chalfont St. Giles, UK), using an 8-element head coil for receive and the body coil for transmit. Prior to MRS acquisition, a 1mm^3 isotropic-resolution T1-weighted anatomical scan (FSPGR) was acquired to determine voxel placement. GABA-edited MR spectra were acquired using the MEGA-PRESS method (Mescher et al., 1998; Edden and Barker, 2007) in two (3 cm)^3 volumes in the right sensorimotor and midline occipital regions as shown in Figure 6.2A and 6.2B. Sensorimotor voxel localization was determined as described previously in Section 5.2 and was rotated in both the sagittal and coronal planes so that one face of the voxel was parallel to the cortical surface as shown in Figure 6.2A. The visual voxel was placed on the midline and aligned with the

cerebellar tentorium as described in Muthukumaraswamy et al. (2009) and shown in Figure 6.2B. The following acquisition parameters were used: TR/TE 1800/68 ms; 332 scans of 2k datapoints per 10-minute acquisition; 16 ms Gaussian editing pulse applied at 1.9 ppm (ON) and 7.46 ppm (OFF) in interleaved scans.

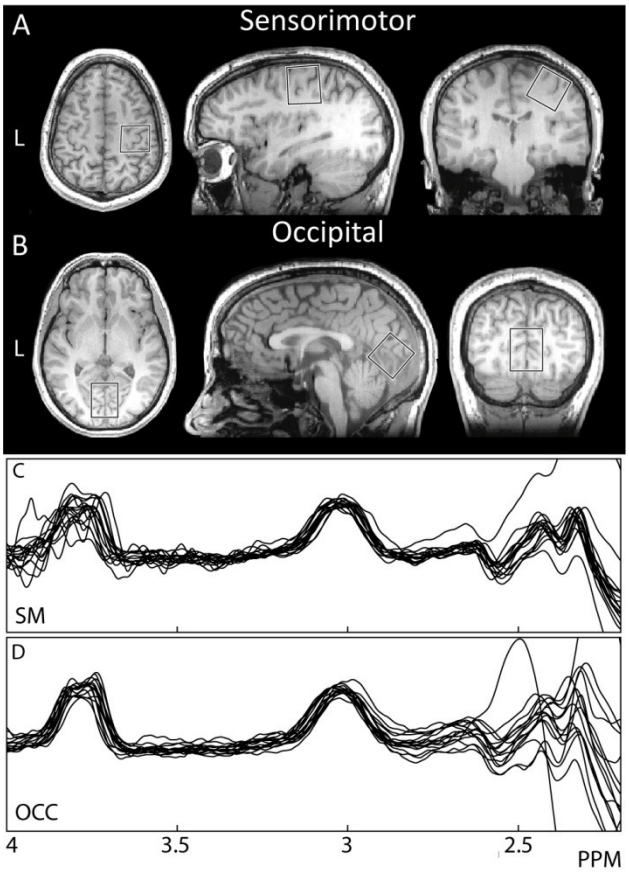

Figure 6.2. Voxel locations and individual spectra. (a) shows a single-participant example voxel over the right sensorimotor cortex. The centre of the voxel is placed on the "hand knob", an anatomical landmark indicating the hand area of the primary motor cortex, with the hand area of primary somatosensory cortex, directly posterior across the central sulcus, also included (Yousry et al., 1997). (b) shows an example voxel over occipital cortex. (c and d) show high-quality spectra from all participants for the sensorimotor (SM) and occipital cortex (OCC) voxels respectively, with a clearly distinguishable GABA peak at 3ppm for all participants.

GABA concentration was quantified in institutional units as described in Section 5.2.3. All processing of MRS data was carried out using in-house software written in MATLAB.

To account for differences in voxel tissue composition, GABA concentrations were corrected for the voxel tissue fraction (grey matter + white matter). The brain was extracted from individual structural MRI images with FSL Brain Extraction Tool (Smith, 2002) and the brain structures were segmented in grey matter, white matter and CSF fraction using FAST (Zhang et al., 2001). FreeSurfer was used to obtain cortical thickness measurements of the pre- and post-central gyrus, with each gyrus defined by an automated parcellation technique (Dale et al., 1999; Fischl and Dale, 2000) Estimates of cortical thickness and gray matter volume were also computed for the part of each gyrus that was contained within our sensorimotor MRS voxel for each participant.

6.2.5 Statistical analysis

All results are quoted as mean ± standard deviation. Correlations between GABA concentration, frequency discrimination threshold, and structural anatomical parameters were tested using the Pearson correlation coefficients (r). Group differences between male and female participants were tested using Student's t-test.

6.3 Results

6.3.1 Behavioural psychophysics

Average frequency discrimination thresholds to the 25 Hz standard were 4.9 ± 1.23 Hz as shown in Figure 6.3. These values are consistent with previous studies measuring frequency discrimination thresholds in healthy adults to stimuli in the flutter range (Goble and Hollins, 1994; Tommerdahl et al., 2005).

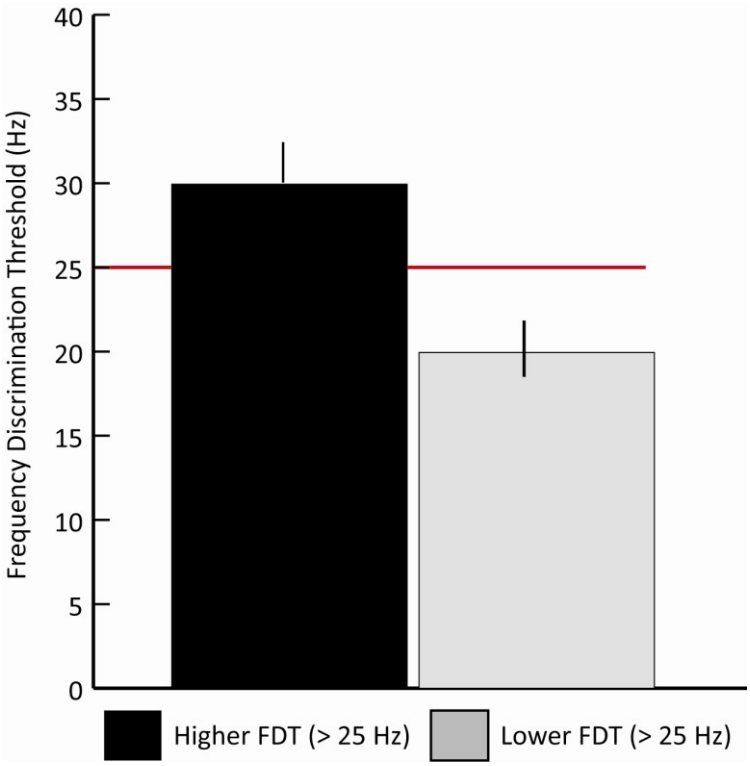

Figure 6.3. Average frequency discrimination thresholds (in Hz) for both the upper and lower track. Frequency discrimination threshold was not significantly different between the higher and lower track (paired two Sample T-Test, P > 0.5).

6.3.2 GABA-MRS and behaviour

High-quality MRS spectra were obtained in all participants. The GABA-spectrum between 2 and 4 ppm for the sensorimotor and occipital voxels is shown for each individual in Figure 6.2C & D), showing a clear edited signal at 3 ppm. GABA

concentration in sensorimotor cortex was found to correlate significantly ($r = -0.58$, $p < 0.05$) with frequency discrimination threshold (Figure 6.4) .

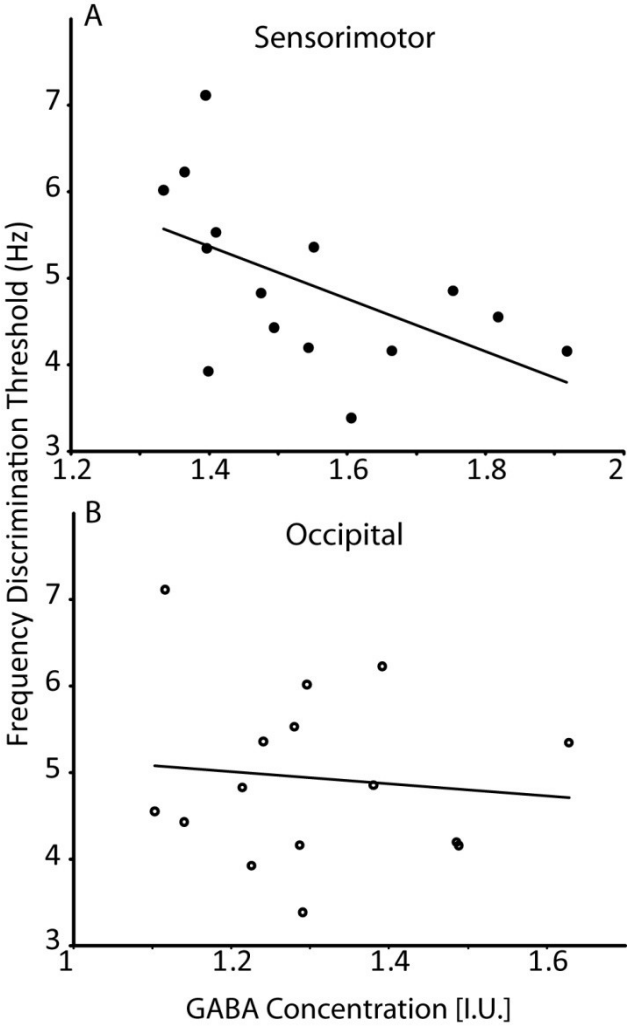

Figure 6.4. (a) Individual GABA concentration in sensorimotor cortex correlates significantly with frequency discrimination threshold ($r = -0.58$, $p < 0.05$). (b) GABA concentration in occipital cortex does not correlate with tactile frequency discrimination threshold ($r = -0.04$, NS). GABA concentrations are corrected for tissue volume (grey matter + white matter).

Participants with a higher level of GABA performed better at the task, as shown in Figure 6.4. To test the effects of outliers on our correlations, we performed a jackknife analysis: the data were resampled n times (n = number of participants), excluding one participant each time, and the correlation was computed. For the

correlation between GABA concentration and discrimination threshold, this produced a range of r values between -0.53 to -0.68, all of which were significant at $p < 0.05$. No correlation was seen between GABA concentration in occipital cortex and discrimination threshold ($r = -0.04$; $p > 0.5$; jackknifed r range -0.2175 to 0.1123, all of which were NS). There was no correlation between GABA in sensorimotor and occipital voxels ($r = 0.06$, p = n.s.)

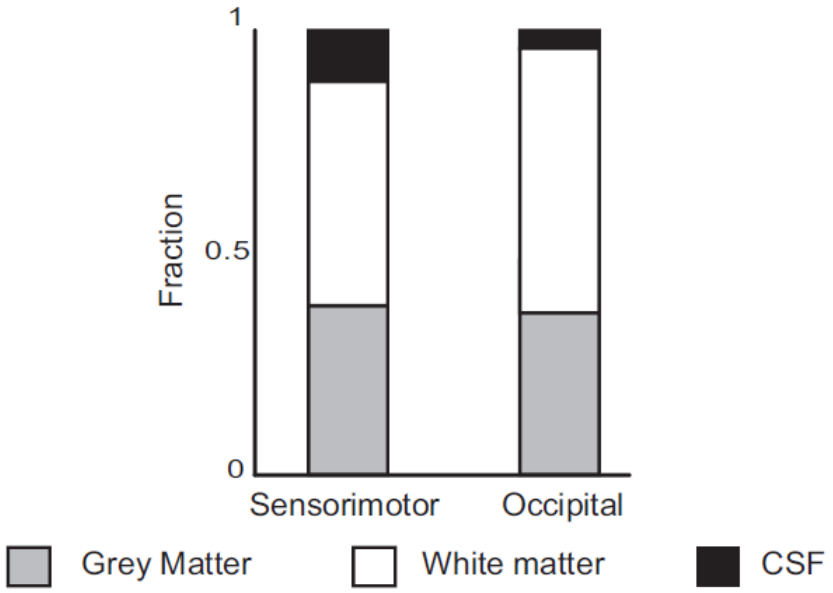

Figure 6.5. Mean fraction of CSF, grey matter and white matter in sensorimotor and occipital voxels. Due to differences in segmentation between the two locations (mainly in white matter and CSF); GABA concentration is corrected for amount of tissue (grey matter + white matter)

There were differences in segmentation between the two voxels (see Figure 6.5). Due to these segmentation differences the GABA concentration was corrected for tissue fraction per participant. As studies have suggested links between the anatomical structure of the sensorimotor cortical area and aspects of motor and sensory abilities in individuals (e.g. Gaser and Schlaug, 2003, ADD), the cortical thickness and total gray matter volume of both pre- and post-central gyri in each

participant was measured. In addition, the cortical thickness and gray matter volume of those areas of either gyri that were contained within our MRS voxel was calculated, allowing us to separate the anatomy within the MRS voxel into primarily motor (precentral) and tactile (postcentral) regions. No significant correlations were found between any of these measures of anatomy and either tactile discrimination thresholds or GABA concentration in the sensorimotor voxel.

No significant correlation was seen between GABA concentration and age within this relatively homogeneous cohort ($r = -0.08$). There was no significant difference in concentration between male and female participants for either region ($p > 0.3$ for both regions).

6.4 Discussion

Our findings show a significant correlation between GABA concentration and tactile frequency discrimination threshold only in the sensorimotor cortex voxel. Higher GABA concentrations in this region predict lower discrimination thresholds, consistent with our previous study linking orientation discrimination and GABA in the visual cortex (Edden et al., 2009).

6.4.1 GABAergic influences on tactile discrimination

How might differences in GABA concentration, measured at the coarse spatial scale of our MRS data, determine individual differences in tactile function? The mechanisms linking GABAergic inhibition to tactile processing are not well understood at the cellular level, and while this study has focused on the mechanism by which individual differences in tactile sensitivity are reflected by individual GABA levels, our results do not provide further insights into how frequency discrimination

is encoded in human participants with no previous experience on the task (see Chapter 9 for a discussion). However, they do suggest that the GABAergic system plays a crucial role in determining individual differences in frequency discrimination in healthy adults.

Studies have shown that GABAergic inhibition acts via the lateral inhibition of neighboring neurons or columns in somatosensory cortex to influence spatial receptive fields in S1 (Lee and Whitsel, 1992). Favorov and Kelly (1994a), among others, suggested that inhibition of neighbouring minicolumns (as discussed in section 1.2.2.) by GABAergic neurons sharpens sensitivity of neurons to specific spatial patterns of incoming information. How GABA is involved in spatial inhibition, may also be paralleled by a similar mechanism in the temporal domain.

Neurons in area 3b explicitly encode information about flutter frequency, firing in phase with each stimulus cycle of the mechanical vibrations (periodicity; Mountcastle et al., 1969; Hernández et al., 2000a; see Section 1.4). Perceptual learning studies (Recanzone et al., 1992; Wang et al., 1995), have suggested that improvements in tactile discrimination may be driven by a 'sharpening' in the tuning of neuronal responses to the indentation cycles of vibrotactile stimuli (see also; O' Mara et al., 1999). This would enhance the signal-to-noise ratio (SNR) of the sensory response, and in doing so improve the constancy of the stimulus representation (as suggested in Harris et al., 2001). Similarly, reductions in the efficacy of GABAergic transmission are known to reduce the SNR of tactile frequency encoding in S1 (McLaughlin and Juliano, 2005), and age-related changes in the SNR of visual cortical neurons are affected by GABA levels (Leventhal et al.,

2003). Harris et al., (2006) showed that adding noise to a tactile stimulus reduces the ability to discriminate and Lak et al., (2010) have suggested that perceptual noise within a stimulus "affects the interaction between inhibitory and excitatory neurons". Knoblich et al. (2010) suggested that there is a temporal 'window of opportunity' (also, Mountcastle et al., 1990) that determines whether a sensory stimulus is encoded efficiently and suggest that synchronous inhibition of fast-spiking interneurons leads to more robust sensory-driven spiking (also; Bacci and Huguenard, 2006).

6.4.2 MRS measurements of baseline GABA concentration

It is unclear what aspect of GABAergic functioning we measure using MRS (see Chapter 4 and 5). MRS does not distinguish between intracellular and extracellular GABA and we can therefore not distinguish between GABA in vesicles and synaptic GABA. However, we believe that the correlation between tactile processing and GABA concentration we found is best explained by considering that the individual differences on the discrimination task are primarily driven by individual differences in the GABA signal from S1. As suggested by histological measurements in non-human primates (Hendry et al., 1987), our measurements may instead reflect the proportion of GABAergic inhibitory interneurons in a given cortical area. As signal transmission is based on the relative contributions of excitatory and inhibitory strength within a cortical circuit (Kohn and Whitsel, 2002), we propose here that our measurements of baseline GABA concentration provide a way to sample the efficacy of normal cortical function in a given brain region. Furthermore, a recent study by Marenco et al. (2010) showed a correlation between GABA as measured with MRS and GAD67 (which produces GABA from glutamate).

In addition, our study shows that GABA concentration in occipital cortex, a sensory region that is not thought to play a role in tactile frequency encoding, does not correlate with the frequency discrimination thresholds. This suggests that although many studies have shown similarities in sensory functioning between domains (Mountcastle, 1997), the effect of GABA is local and does not reflect whole-brain sensory processing. Interestingly, PET studies investigating GABA-receptors have shown the largest density of GABA-receptors in V1 (Zilles et al., 2002), whereas immunohistochemical studies show that S1 has a larger proportion of GABAergic neurons than V1 (Morin and Beaulieu, 1994) and even within the different areas of S1 the number of GABAergic interneurons differ (Zilles et al., 1995). This shows that cortical thickness or laminar organisation may not reflect absolute numbers of neurons (Clemo et al., 2003). These findings reflect another important aspects of GABAergic processing; that not only the balance between excitation and inhibition is important in shaping the response to sensory stimulation (Kohn and Whitsel, 2002), but that this efficacy is also dependent on the ratio of interneurons and its' connections.

We do not think that our measures of GABA merely reflect a non-specific increase in neuronal density or surface cortical thickness that could underlie the differences in tactile discrimination ability between participants. Because previous work has suggested links between anatomical structure and behaviour in the cortical regions within our sensorimotor voxel and a recent study by (Schwarzkopf et al., 2011) showed that object size perception is correlated with each individual's overall size of V1 surface, we tested whether the size of S1 (both the entire post-central region

as well as the area of S1 within our voxel) predicted the behavioural performance. Furthermore we tested whether the individual variability in GABA concentration or tactile discrimination thresholds could be explained by cortical thickness or grey matter volume. None of these analyses were significant, suggesting that the correlation between GABA and behaviour in our data is unlikely to be driven by gross anatomical differences between participants. It might be possible that our results is driven by individual differences in the size of the area encoding the stimulated region (LD2) but it is not possible to extract such anatomical measures from our anatomical MRI images.

6.4.3 Limits of MEGA-PRESS measurements of GABA

The MEGA-PRESS technique used to measure GABA still has several limitations as discussed in detail in Chapter 5. As discussed, the size of the voxel used is relatively large (3 x 3 x 3 cm^3) and it is therefore not possible to place a voxel specifically on the primary somatosensory cortex. Although other studies of GABA have used smaller volumes (for review see Puts and Edden, 2011), correlative studies of individual differences require optimal signal-to-noise, and the reliance on PRESS localization gives a cuboidal excitation volume that is fundamentally different from the curved geometry of S1.

6.4.4 Conclusions

In conclusion, the present study is the first to link somatosensory discrimination performance to GABAergic inhibition among healthy individuals. Combined with previous studies in visual cortex, this suggests a general role for GABAergic inhibition in behavioral discrimination in healthy participants.

To further understand the mechanisms involved in processing of frequency discrimination a possibility is to use neuroimaging techniques such as MEG which can be used to explore the underlying neural dynamics of frequency discrimination in the primary somatosensory cortex. In addition to looking at individual differences in performance, another way to gain an understanding of mechanisms involved in sensory processing is to modulate the behavioural performance to look at cortical correlates of this modulation. In the following chapters sensitivity to frequency discrimination will be modulated and the dynamical processes underlying this modulation will be investigated using MEG.

Chapter 7 - Magnetoencephalograhy of Tactile Adaptation

7.1 Introduction

The role of GABA, as measured by edited MRS, in frequency discrimination has been discussed in chapter 6. The effect of adaptation to a 25Hz frequency on tactile frequency discrimination thresholds around 25Hz was first reported by Goble and Hollins in 1994 and the effect of adaptation on tactile processing has been extensively discussed in the Introduction (Section 1.4) and the Behavioural methods chapters' (Section 2.3 and 2.4). In Section 2.4 we showed that we are able to induce adaptation to frequency discrimination around 25 Hz in a paradigm adapted for MEG as well. However, it is unclear how this behavioural effect of adaptation can be explained by cortical dynamics. As discussed in Section 1.4; adaptation is a form of short-term stimulus-induced plasticity, and it is unlikely that anatomical changes take place. A more reasonable assumption is that immediate millisecond-level changes in neuronal dynamics (ie. direct changes in firing rate or amplitude as a result of a stimulus) underlie the effect of adaptation. As the effect of adaptation can be seen after only a short exposure, the changes in neural dynamics immediately follow exposure to an adapting stimulus (e.g. O'Mara et al., 1988). Several studies have shown an effect of adaptation on tactile processing and Simons et al., (2007) has demonstrated the effect of repetitive stimulation in the primary somatosensory cortex of cats with optical imaging. With its high temporal resolution, magnetoencephalograhy (MEG) is a useful technique to investigate

changes in the temporal dynamics of neuromagnetic signals as well being able to distinguish between different functional frequency bands (see chapter 3). In the following chapter the current understanding of tactile adaptation will be discussed, followed by a discussion of the possible MEG measures underlying tactile processing as well as providing an understanding of the benefits and limitations of using MEG to investigate tactile adaptation.

7.1.1 Adaptation

In the introduction, adaptation is described as a form of short-term, stimulus-dependent plasticity. Studies of adaptation have demonstrated rises in absolute threshold values of a stimulus (e.g. a tactile frequency) from normal, or the reduction of sensory magnitude of a stimulus from normal, following the presentation of some finite suprathreshold value of that stimulus and this effect has been investigated in a number of different domains (see 1.4.2.1). However, as discussed in detail in Section 2.3, Goble and Hollins (1993, 1994) and Delemos and Hollins (1996) investigated adaptation in the tactile domain, and showed that an adapting vibration can enhance sensitivity to either amplitude or frequency. A vibrotactile adapting stimulus has shown to enhance the ability to detect differences in frequency, only when the adapting stimulus was of the same frequency and amplitude as a "standard stimulus" recurring in every trial. In our own version of this study we managed to replicate their results (see Section 2.4). Other studies have found adaptation to spatial discrimination in the tactile domain as well (Tannan et al., 2006). However, as also discussed in the introduction, adaptation in the visual field may *reduce* sensitivity for stimuli similar to the

adapting stimulus (for a review, see Kohn, 2007) and it is important to note that adaptation may not be reflected by the same mechanisms across modalities.

7.1.1.1 *The Neuronal Correlates of Adaptation*

As discussed in the introduction, early work by Von Bekesy noted that lateral inhibition, the process by which excitation of an area is accompanied by simultaneous "silencing" of surrounding areas, may be *reduced* for prolonged stimulation. Von Bekesy said that: "It appears that the emergent patterns that are formed by minicolumnar activation from repetitive stimulation could be an important factor in feature extraction". The pattern of neuronal excitatory activity is thought to sharpen the response to the adapting stimulus (see; Merzenich et al., 1984; Calford and Tweedale, 1991a; Lee and Whitsel, 1992b; Kohn, 2007). Lee and Whitsel (1992a, 1992b) have shown that the global pattern of activation of receptive fields shifts with repetitive stimulation in both the spatial and temporal domain. Several studies have shown that repetitive stimulation leads to a more "funnelled" response in S1 (Tommerdahl et al., 1993; Whitsel et al., 2003). Simons et al., (2007) showed a shift in activity by investigating the effect of long duration stimulation (5 seconds) on monkey somatosensory cortex with optical imaging. They found that the active region S1 becomes *more* active for the entire duration of 5 second stimulation whereas the surrounding areas become *less* active. Cannestra et al., (1998a) found a similar effect using optical imaging, and showed that the response to a subsequent adapting stimulus was reduced whereas the response to a novel stimulus was not. Bensmaia et al. (2005) have reported a change in the pattern of activity as a result of repetitive stimulation in the periphery, showing

that receptors near the area of stimulation become less active, although how these findings relate to cortical dynamics is unclear. Other studies have reported an effect of adaptation in subcortical regions where ipsilateral cortex becomes less active and in cortical areas where repetitive stimulation leads to a smaller active area (see also; Tommerdahl et al., 1996 in cat; Moore et al., 1999 in rat). On the other hand however, it is important to note that adaptation in the visual cortex, for instance after repetitive contrast stimulation, leads to a reduction in the activity in the stimulated region and an increase in the activity in the surrounding region (e.g. Kohn, 2007) but even this effect can be different for different areas of the visual cortex. For instance, adaptation reduces responsiveness of direction-specific neurons in V1 but adaptors increase the responsiveness of direction-matched neurons in MT but not other neurons and 'tune' the system to the adaptor, not dissimilar to S1 processing,. In summary, repetitive stimulation leads to change in the cortical response to sensory stimulation, from both a temporal and spatial point of view (for a review, see McLaughlin and Kelly, 1993; Kohn, 2007) and may be modality and area-specific.

It is suggested that the neuronal changes in S1 due to repetitive stimulation (of long duration) are linked to the processing of tactile stimuli and are reflected in the behavioural response. These findings further confirm suggestions that S1 activity contributes to tactile discrimination.

Cellular mechanisms

A number of studies investigated the mechanisms underlying these changes in cortical activity as a result of repetitive stimulation. As repetitive stimulation causes

increases of activity in the stimulated region but progressive decreases in activity in neighbouring regions, GABAergic mechanisms may play a role in adaptation in S1. Juliano et al., (1989) showed that blocking of GABA with bicuculline (a GABA antagonist) lead to a broader and less focal cortical response to repetitive stimulation. In addition, studies investigating autism have shown that the behavioural effect of adaptation in not present in participants with autism. Tommerdahl et al., (2007a), discusses the suggestion that a deficit in GABA mediated neurotransmission, particularly inhibition between cortical minicolumns, underlies the behavioural effect. Finally, Folger et al. (2008) investigated the role of NMDA (excitatory) processes on inhibition and showed that adaptation is impaired when NMDA is blocked by dextromethorphan. However, Vidyasagar (1990) investigated the effect of bicuculline on visual adaptation and found that adaptation remains unimpaired even after administration of this GABA antagonist and thus that GABA is not involved in adaptation. Although the similarities and differences between visual and tactile adaptation remain unclear, the differences between these studies show that there are a number of different mechanisms that may underlie changes due to repetitive stimulation. Repetitive contrast stimulation is known to lead to afterhyperpolarisation due to sodium influx, while there is little synaptic input, which may result in the reduction in a cells responsiveness. In addition, depletion of synaptic vessels due to repetitive in increased stimulation may also add to this effect.On the other hand, studies in visual area MT have shown that adaptation has an effect on inhibition (Kohn and Mosvon, 2000; Kohn, 2007) suggesting a role of GABA and Yang et al., (2003) showed that GABA-b agonists increase effects of adaptation in the LGN. Finally, Teich and Qian (2003) have

suggested that whereas an increase in inhibition (or reduction in excitatory input) 'tunes' neurons to an adaptive stimulus (as seen in MT) whereas an overall decrease in both excitation and inhibition may shift the activity and preference of a system (as seen in V1). It therefore seems apparent that a multitude of different mechanisms underlie (different aspects of) adaptation which may be modality, area, and stimulus specific.

7.1.2 MEG in somatosensory cortex

To investigate the changes in the pattern of activation in S1 as a result of repetitive stimulation/adaptation in the tactile domain in humans, we used magnetoencephalography (MEG) to investigate whether these changes in the pattern of activity are reflected in brain-scale neuromagnetic responses. In addition, we aim to measure the (changes in) the cortical response as a result of adaptation *during* the adaptation task described in Section 2.4 to investigate the cortical correlates of behavioural adaptation directly.

Chapter 3 contains a brief discussion of the location and time-course of cortical activity to tactile stimulation. However, in this experiment we are interested in investigating the function of these cortical responses in a tactile adaptation paradigm.

7.1.2.1 *The functional role of MEG signals*

As described in Chapter 3, MEG is capable of localising a source in S1 with relatively good accuracy and it is possible to discriminate the location of activation foci for different digits using MEG (Baumgartner et al., 1991c). It has been described that each finger representation measures about 15 – 20 mm in area 3b of S1

(Baumgartner et al., 1991b; Hari et al., 1993) and agree with intracranial studies (see Hari and Forss, 1999). According to Hari and Forss (1999), the current of the first peak of activity after tactile stimulation comes from the deep layers, directed towards the superficial layers of the cortex, followed by a negative current reflecting feedback from the superficial layers to deeper cortical layers, possibly reflecting cortical inhibition. It is still a debate how much different areas of S1 contribute to the MEG signal and as discussed in Hari and Forss (1999) some studies suggest that different peaks have different origins. In addition, Zhu et al. (2009a) investigated the source of the MEG signal as found by tactile stimulation in macaque monkeys by comparing it to local field potentials (LFPs) and multi-unit activity (MUA). LFP's have been linked to MEG activity in earlier studies (see Singh, 2006). According to Zhu et al., the latencies acquired via LFP recordings reflect the latency of stellate cells in layer 4 of the cortex, but that the MEG signal is unlikely to occur from layer 4 because it contains mainly stellate cells which have a weak current and lack apical dendrites. Zhu et al., suggest that pyramidal cells in layer 2/3 and 5 are more likely to be the source of the MEG signal, possibly adding to the delay in latency compared to invasive recordings. Jones et al. (2007) investigated the possible neuromagnetic source of MEG signals using both experimental data and modelling, and suggest that the source of the S1 evoked response lies in the intracellular currents of pyramidal neurons in layer 5 driven by excitatory input from layer 4, and input into layer 2/3.

In addition, a number of studies found frequency-dependent steady-state responses (SSR) to tactile stimulation at the frequency of stimulation (Kelly and

Folger, 1999; Nangini et al., 2006; Giabbiconi et al., 2007; Spitzer et al., 2010). These are localised over primary somatosensory cortex and drive the neurons at the stimulating frequency. These SSRs are thought to reflect RAI specific driving of the system, and might be a target for selectively tuning of the system towards a specific frequency (Kelly and Folger, 1999; Tobimatsu et al., 1999). Kelly and Folger have further shown with EEG that the driving response undergoes changes in location and amplitude after repetitive stimulation (4 seconds).

7.1.2.2 *The role of cortical oscillations*

The sources of cortical oscillations are by no means fully understood, but a number of studies have attempted to gain an understanding of the function of activity in different frequency bands. As described briefly in Chapter 3, a neuromagnetic response to a tactile stimulus is characterised by two distinct frequency bands, alpha- and beta- oscillations. As also discussed briefly in Chapter 3; there is no current consensus on frequency band nomenclature. In tactile processing, elements of both the alpha and beta rhythms have been incorporated into a rhythm called *mu*, which seems to be particular to sensorimotor cortex. In this thesis, we will refer to oscillations between 7 – 15 Hz as mu-alpha band oscillations and 15 - 30Hz as mu-beta band oscillations. A tactile stimulus leads to mu-alpha and mu-beta event-related-desynchronisation (ERD) after stimulus onset (as shown by (Salenius et al., 1997; Gaetz et al., 2003; Gaetz and Cheyne, 2006), and Gaetz and Cheyne (2006) show sustained suppression in the mu-alpha and mu-beta band for the duration of a stimulus. This ERD is followed by a re-synchronisation (ERS) or "rebound". The rebound has been shown to occur on stimulus offset Gaetz and

Cheyne (2006), but appears slightly prolonged in the mu-alpha band (Gaetz et al., 2003). Gaetz and Cheyne (2006) suggested that a *desynchronisation* of mu-beta oscillations is associated with ongoing activity, processing and coordination of tactile stimulation as well as possible inhibition of motor cortex (see also; Neuper et al., 2006). In addition, mu-beta band oscillations have been associated with the GABAergic inhibitory network in S1 (Jensen et al., 2005b; Hall et al., 2010; Jones et al., 2010; Gaetz et al., 2011).

The role of these oscillations is still relatively unclear, but several studies have suggested a role of beta as an 'idling' rhythm and more specifically in the suppression of distracting sensory input or focusing of a sensory system to incoming stimulation (Hari and Salmelin, 1997; Jensen et al., 2005b; Jones et al., 2009). Dockstader et al., (2010) showed a modulation of the mu-beta rhythm by attention in somatosensory cortex (also; Wrobel, 2000; Bauer et al., 2006a). Spitzer et al., (2010) have shown with EEG that the beta-rhythm has a role in discrimination, as the power in the beta-band differs between correct and incorrect trials on a frequency discrimination task and Haegens et al., (2011) show a differential response in the beta-band for two different frequencies in a discrimination task. In addition to these possible roles of the beta rhythm, a number of studies suggest a role of the beta-rhythm in sensory integration of subsequent information (Lalo et al., 2007; Donner et al., 2009; Engel and Fries, 2010; Kopell et al., 2010; Haegens et al., 2011b) because it integrates both feedforward and feedback connections.

The possible sources of the mu-rhythm were investigated and modelled by Jones et al. (2009). They suggested that the source of the mu-alpha rhythm lies in

feedforward thalamocortical connections, with the source of the mu-beta rhythm lying in the inhibitory interneuron network of S1, receiving both thalamic *and* cortical signals (Jones et al. 2009).

7.1.3 Aims and goals

In this study we aim to induce adaptation to frequency discrimination in a version of the paradigm by Goble & Hollins (1994) adapted for MEG. Invasive studies have shown a shift in the neuronal activity in S1 to repetitive stimulation, leading to an increase in the neuronal activity of a more spatially restricted area (see Section 7.1.1.1). In our task we have adaptation in both conditions, and thus do not predict any differences in spatial distribution.

Previous studies have suggested that sharpening of a spatiotemporal pattern of activity as a result of adaptation underlie the improvement in performance (section 7.1.1.1). In this experiment, we measure the cortical response in S1 during the adaptation task. By measuring the cortical activity as a result of adaptation, we aim to investigate how changes in spatiotemporal activity relate to changes in tactile sensitivity. We will measure the effects of adaptation on frequency discrimination and will compare a condition where the adapting stimulus is the *same* as the standard stimulus (25 Hz) to a condition where the adapting stimulus is *different*. We hypothesise to see differences in the mu-alpha and mu-beta frequency bands. As both mechanisms of adaptation and the mu-band have been associated with the inhibitory GABA network we investigated the role of GABA concentration on individual differences in adaptation.

7.2 Methods

7.2.1 Participants

The same participants were used as in Section 6.2.1.

7.2.2 Equipment

Vibrotactile stimulation was delivered using a piezoelectric vibrotactile stimulator similar to the one used by McGlone et al. (2002). Contact with the skin was made via a plastic probe (7mm in diameter). A static surround limited stimulation to the skin region placed on the probe. All stimulation was delivered to the glabrous skin of left digit 2 (index finger). The frequency range used was 15-40 Hz. During the behavioural testing prior to MEG acquisition, stimuli were delivered via the audio output of a laptop computer (Sony Vaio VGN-NS20M, Realtek high definition audio, volume at 100%) using Matlab2008b (The Mathworks, 2008). During MEG scanning, stimuli were delivered via a standard PC using Matlab 2007. Stimulus amplitude was matched between the two stimulus computers.

7.2.3 Preparation

Before the experiment, detection threshold and frequency discrimination thresholds were obtained as described in Section 2.4 and Section 6.2. For the MEG experiment, participants were seated comfortably and their left index finger was placed on the stimulator and taped into place to reduce finger movement. Participants received a practice session in which they were exposed to exemplar vibrotactile stimuli of various frequencies and amplitudes. Once participants reported they were comfortable with discriminating the different stimuli, they received a practice session of the adaptation task. The practice session consisted of

ten trials consisting of a 5-second stimulus, followed by discrimination between a 25 Hz stimulus and a supra-discrimination threshold stimulus. The practice session was repeated until participants showed a 100% correct level. Participants were then raised into the MEG helmet and were provided with a chin-rest to provide comfort. Participants were instructed to maintain fixation for the entire experiment and to press a response key as fast as possible at the termination of each stimulation period.

7.2.4 Behavioural Task - Adaptation to frequency discrimination

The behavioural task performed in the MEG system consisted of a two alternative forced choice task (2AFC), similar to the task described in Section 2.4.3, but with the difference that the comparison stimuli frequencies were chosen to be either the 75% higher and lower *thresholds* for frequency discrimination around the 25Hz standard. In two separate conditions, each trial consisted of a 5 second long stimulus of either 25Hz (condition 1) or 40Hz (condition 2) followed by a 1s pause, followed by two stimuli (standard and comparison stimulus; randomised) with a pause (1s +/- 100 ms) in between. After each trial participants were asked to respond which interval contained the stimulus with the highest frequency, indicated by a question mark presented on the screen. After this, feedback was given. Throughout the experiment, inter-trial intervals consisted of a 2 second pause with a ~100 ms. jitter to reduce expectation of stimulus occurrence and focus attention. The task consisted of 50 trials per track. All participants performed both conditions. The task was programmed using Matlab2008a. Performance was

measured as the percentage of correct answers in either condition, collapsed over

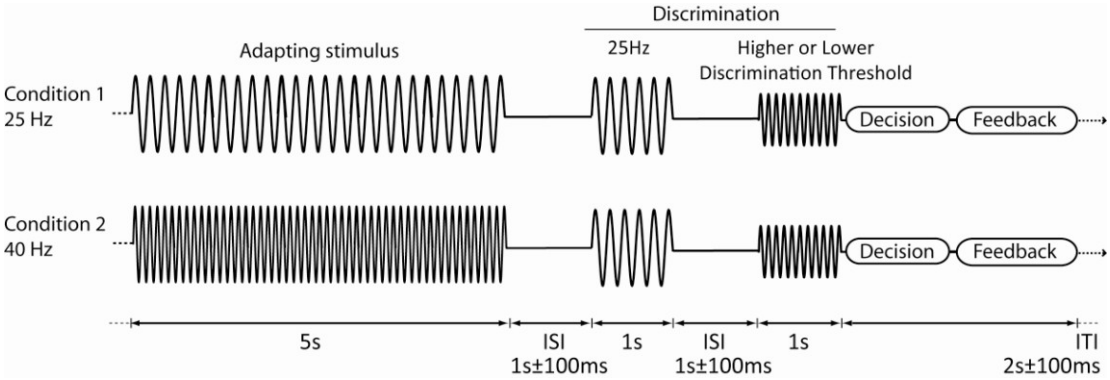

Figure 7.1. Protocol for the adaptation to frequency discrimination task. Each trial was preceded by a 25Hz 5s stimulus (condition 1) or 40Hz 5s stimulus (condition 2). This was followed by a discrimination task between the standard 25Hz stimulus and either the higher or lower frequency discrimination *threshold*. Order was randomised. Participants were given feedback.

the upper and lower frequency discrimination threshold.

7.2.5 MEG methods

Acquisition MEG recordings were made as discussed in Section 3.3.3. A Mitsubishi Diamond Pro 2070 monitor controlled by Matlab software was used to present the crosshair and feedback (1024 x 768 resolution, 100 Hz refresh rate). Data was recorded continuously. Triggers were used for each of the different stimuli (adapting stimulus; standard stimulus as first trial stimulus; standard stimulus as second stimulus; higher threshold stimulus as first stimulus; higher threshold stimulus as second stimulus; lower threshold stimulus as first stimulus; lower threshold stimulus as second stimulus).

Analysis Prior to data analysis the data was visually checked and corrected for artefacts and noisy trials. Because we are interested in the peak location of the

initial S1 activity, Synthetic Aperature Magnetometry event-related fields (SAMerf) (Vrba and Robinson, 2001) was used to create three-dimensional differential images of source power (pseudo-t statistics) for 1 second of baseline (-1 – 0 seconds) compared to 10ms bins spanning between 0 - 150 ms post-stimulus for the adapting stimulus for each participant. SAM images were constructed for frequencies between 2 – 90 Hz. The peak location of activity in the primary somatosensory cortex was localised in the volumetric images (generally between 60-70ms) for both adapting stimuli and SAM virtual electrodes reconstructions were generated for these locations using covariance matrices bandpass filtered between 0 and 90 Hz for the entire trial duration.

To contrast the first peak of S1 activity between the two conditions, a group SAM analysis was performed between the active periods between 50 – 100 ms of the adapting stimulus in both conditions. SAM images were normalised using FSL FLIRT (Jenkinson and Smith, 2001) into an MNI template (T1 of 152 individuals). Non-parametric permutation was performed using 4096 permutations for each condition and thresholded using the omnibus test statistic at $p < 0.05$ (Nichols and Holmes, 2002; Singh et al., 2003). Output is a t-weighted map with voxel-based corrections for multiple comparisons.

To look at specific frequency bands, time-frequency analysis was performed using the Hilbert transform between 1 and 90 Hz in 0.5 Hz frequency steps and averaged across participants between conditions. From these time– frequency spectra, evoked activity was plotted between 0-90 Hz, expressed as percentage change from baseline. Peak mu-alpha (7-15Hz) and mu-beta (15-30Hz) band frequency and

amplitudes, expressed as percentage change from baseline, were obtained for the induced (non-phase locked) activity. Differences between conditions were measured as significant differences in power for the mu-alpha and mu-beta band separately, as measured by Student-T tests.

7.2.6 GABA-MRS

GABA values acquired in the previous experiment were used (see Section 6.2).

7.3 Results

7.3.1 Adaptation to frequency discrimination

The results show that performance in the 25Hz adapting stimulus condition is significantly better than performance in the 40Hz adapting stimulus condition (Two-sample T-test, paired, $p<0.001$, see Figure 7.2). The effect of the 25 Hz adapting stimulus on performance is significantly larger than the 40 Hz adapting stimulus and thus participants were significantly better at frequency discrimination when the adapting stimulus was the same as the standard test stimulus of 25Hz. We investigated whether participants had a bias to the first or second stimulus between different conditions (see section 2.4.6) due to the similarity between the 25Hz adaptor and standard stimulus but no significant difference was found (avg. d'(25Hz) against avg. d'(40Hz): p = n.s) (Gescheider, 1997). Reaction times were not different between conditions (p = n.s.).

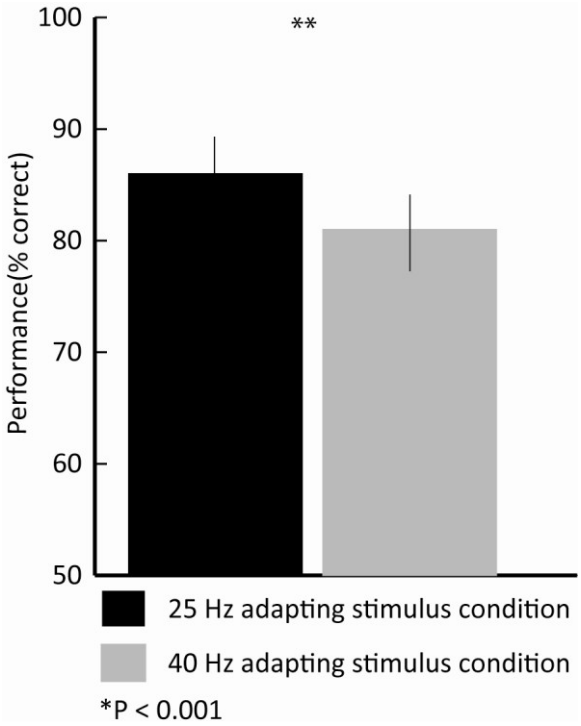

Figure 7.2. Participants performance at frequency discrimination around 25Hz becomes significantly better when they are exposed to a 5s long 25Hz stimulus than when they are exposed to a 5s long 40 Hz stimulus.

7.3.2 Magnetoencephalography of vibrotactile adaptation

One participant was excluded from the analysis due to large number of artefacts in the MEG recordings. Due to a small number of occurrences of each trial-stimulus and large numbers of artefacts between participants, the trial numbers for the comparison threshold stimuli were limited and not used for further analysis. In the following analysis, only the adapting stimulus and standard stimulus when directly following the adapting stimulus were investigated.

7.3.2.1 *SAMerf and GroupSAM*

Figure 7.3 shows the SAMerf localisation 60-70 ms after tactile stimulation located over the primary somatosensory cortex in the postcentral gyrus as expected, for one individual. Peak activity for each individual was chosen for localisation of the virtual sensor. We also noted subsequent activity in ipsilateral S1, bilateral S2 and

prefrontal activity; but the presence of this activity was variable across participants and if activity was present in these areas, the time-course varied much as well. Such differences have been noticed in the literature (Gaetz and Cheyne, 2006; Tommerdahl et al., 2010). Group SAM analysis between the initial 50 – 100 ms activity of the adapting stimulus did not show any significant differences in S1 location between the 25 Hz and 40 Hz condition. Figure 7.4 shows group SAM averaged locations for both stimuli. The peak activity is located post-centrally.

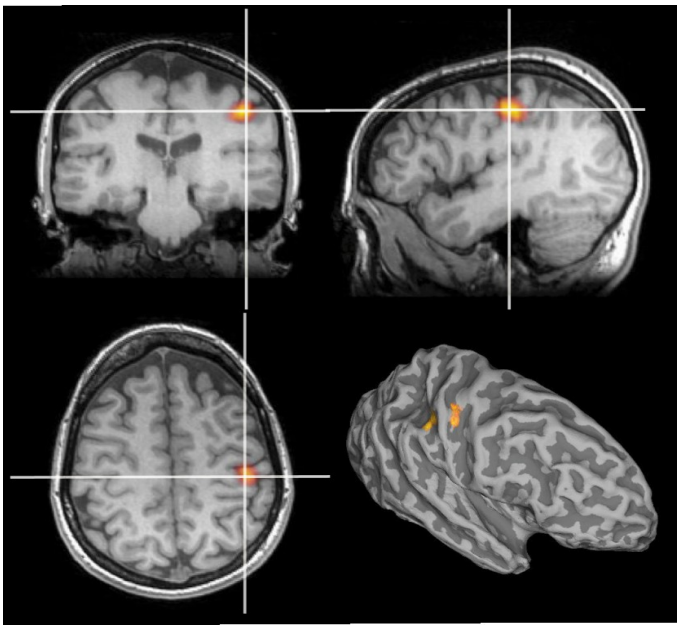

Figure 7.3. Peak activity 60-70 ms after tactile stimulation as found using SAMerf between 2-90 Hz. The figure shows activity in the postcentral gyrus, just behind the handknob. Figure a-c show activity on the anatomical MRI scan, figure d shows activity on a 3D mesh brain. Location of activity was chosen for virtual sensor placement for subsequent time-frequency analysis.

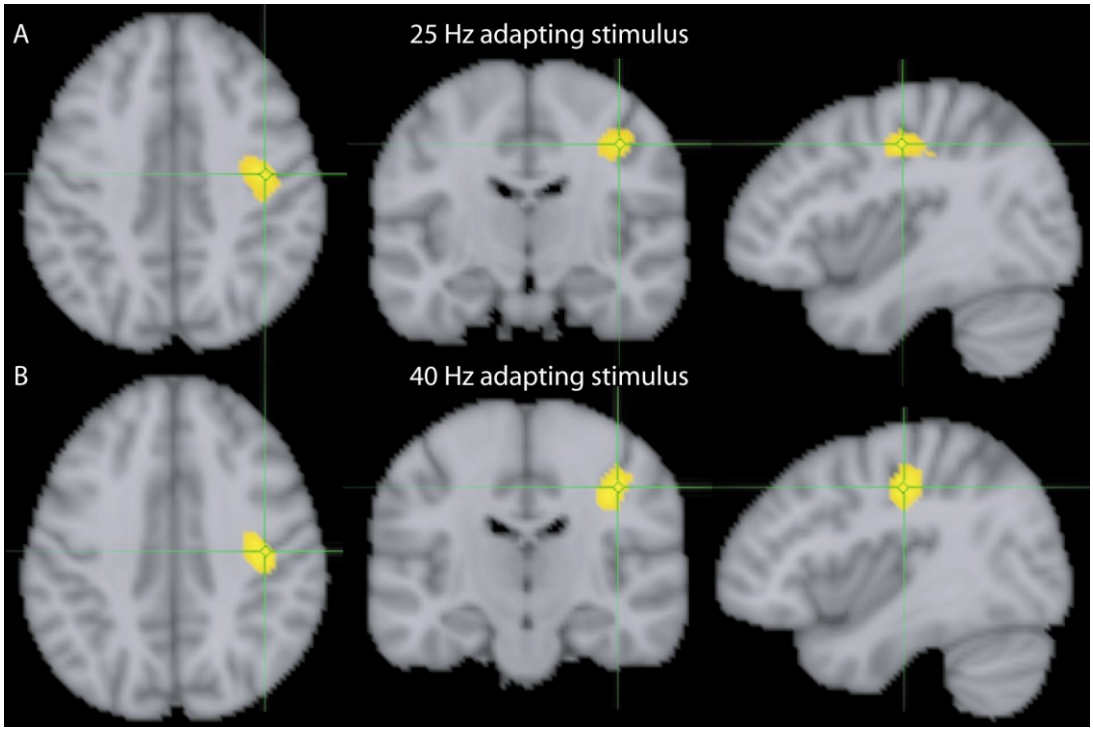

Figure 7.4. Group SAM analysis for the 25 Hz adapting stimulus (A) and (B) 40 Hz adapting stimulus between 50-100 ms on a template brain (FSL T1 average of 152 individuals). Activity can be seen posterior to the central sulcus. In the sagittal section the 'hook', described in Section 1.5, can be seen. Group SAM statistical analysis did not show differences in location between the two stimuli.

7.3.2.2 *Virtual sensor analysis*

Evoked activity Subsequently, virtual electrode analysis was performed at the cortical location showing the peak amplitude for each participant. Figure 7.5 shows the group average evoked activity for the 25 Hz and 40 Hz adapting stimulus from -2 - 6 seconds. The adapting stimulus runs from 0 – 5 seconds. Consistent with previous findings these evoked results show a sustained steady state response (SSR) for the duration of the stimulus at the frequency of stimulation. The SSR for the 25 Hz adapting stimulus is driven at 25 Hz (with a harmonic visible at 50 Hz) and the SSR for the 40 Hz adapting stimulus is driven at 40 Hz (with less visible harmonic seen at 80 Hz). Further analysis of the evoked activity between 0-90 Hz did not show characteristic somatosensory evoked potentials (see Jones et al., 2007 and Section 3.3) due to large individual differences due to poor data quality.

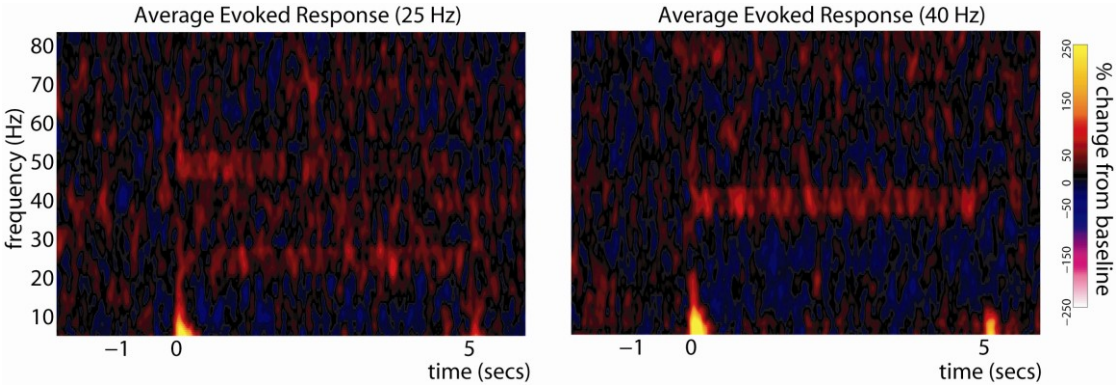

Figure 7.5 Group average time-frequency spectrograms for theevoked activity for the 25 Hz (left panel) and 40 Hz (right panel) adapting stimulus expressed in percentage change from baseline. Both the 25 Hz and 40 Hz stimuli show a SSR for the duration of the stimulus at the frequency of stimulation. The plots also show a trace of a harmonic at 50 Hz and 80 Hz respectively.

Induced activity Figure 7.6 shows the induced activity spectrograms for the 25 Hz (a) and 40 Hz (b) adapting stimulus from -2 – 6 seconds. The adapting stimulus runs from 0-5s. Both plots show a large mu-alpha and mu-beta desynchronisation at stimulus onset, which is characteristic for somatosensory stimulation. This is followed by sustained mu-alpha and mu-beta desynchronisation for the entire duration of the stimulus which can be seen as two distinct bands of activity. The time-frequency spectrum was further processed in a time-power plot for both mu-alpha and mu-beta band activity separately. These results are shown in figure 7.8. Figure 7.8A shows mu-alpha band activity for the 25Hz (blue line) and 40Hz (red line) adapting stimulus. Figure 7.8B shows the same plot for the mu-beta band. Both plots show a sustained desynchronisation for the duration of the adapting stimulus and also show that desynchronisation occurs until after stimulus-offset (5 seconds). Neither of the plots shows significant differences between the two adapting stimuli for the entire duration of the stimulus. The initial upward peak in the mu-alpha band is a trace from the evoked response.

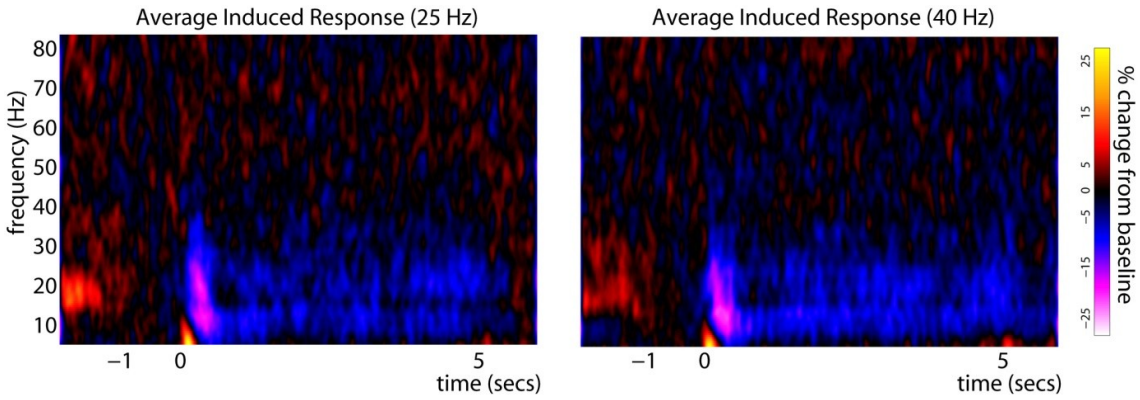

Figure 7.6. Group average time-frequency spectrograms for the induced activity for the 25Hz (left panel) and 40Hz (right panel) adapting stimulus. Duration of the stimulus is from 0 - 5 s. Both stimuli show similar activity.An initial mu-alpha and mu-beta desynchronisation after stimulus onset, followed by sustained desynchronisation for the duration of the stimulus.

One would expect to see the SSR in the induced response as well, as they are both phase- and time-locked to the stimulus (see chapter 2). However, we do not see any 25 Hz or 40 Hz SSR in the induced spectrograms. One possibility is that frequency smoothing reduces the frequency specific SSR activity. A bertogram is created by sliding windows of a Gaussian function and a compromise has to be struck between temporal and spatial resolution. In our analysis we applied frequency smoothing to increase the temporal resolution in the spectrogram it is possible that the very specific SSR frequency is smoothed out. Another possibility is that the SSR has very low SNR but becomes visible through averaging across trials as is done in for the evoked activity, but is averaged out by taking the frequency information from each individual trial and then averaging this information together as is done for the induced activity.

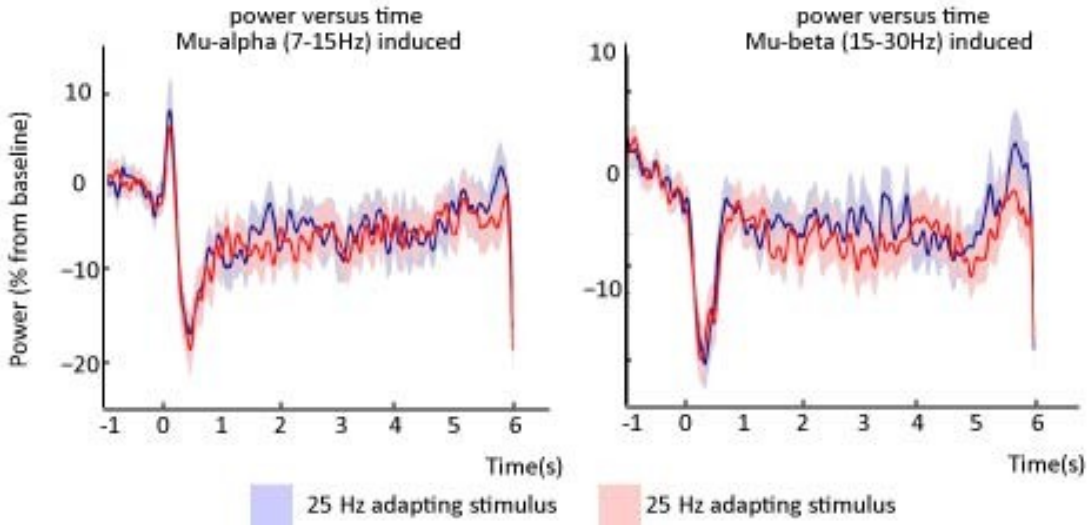

Figure 7.7. Group average frequency-power plots for the 25 Hz (blue line) and 40 Hz (red line) adapting stimulus (0-5sec). A) shows the ERD at stimulus offset, followed by a sustained decrease in power for the mu-alpha band throughout the adapting stimulus duration (0 – 5 seconds) and shows no difference between conditions. B) shows the ERD at stimulus offset followed by a decrease in power for the mu-beta band throughout the adapting stimulus duration (0 – 5 seconds) and shows no difference between conditions. The initial mu-alpha and mu-beta desynchronisation is clearly visible. Power seems to recover to baseline +/- 500 ms after stimulus offset. Shaded lines indicate standard error.

The next step was to investigate the subsequent 25Hz stimulus. This stimulus could have either been preceded by the 25Hz adapting stimulus (or 'same') or the 40Hz adapting stimulus (or 'different'). We hypothesised to see an effect of the adapting stimulus on this first trial stimulus when they were similar. The plots are shown in figure 7.8 where the blue line means the 25Hz stimulus was preceded by a 25Hz adapting stimulus ('same') and the red line indicates that the 25Hz stimulus was preceded by a 40Hz adapting stimulus ('different'). Figure 7.8 shows the distinctive mu-alpha (figure 7.8A) and mu-beta desynchronisation (figure 7.8B) at stimulus-onset, followed by a sustained desynchronisation until after stimulus offset. Neither of the frequency bands shows a significant difference between the two conditions.

As can be seen in Figure 7.8, stimulus level prior to stimulus offset is characterised by a state of desynchronisation, suggesting that there is sustained desynchronisation throughout the entire duration of the trial.

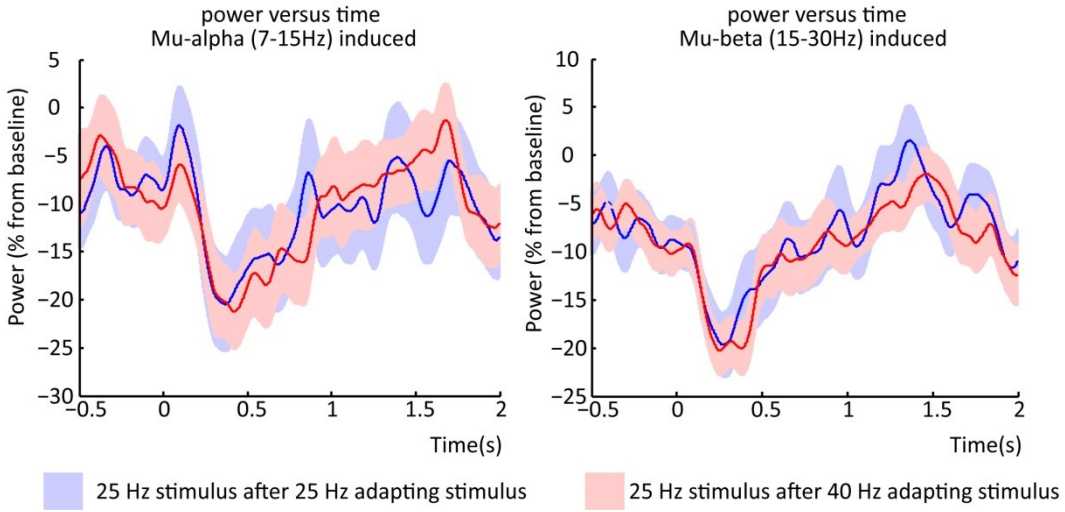

Figure 7.8. Group average frequency-power plots for the 1 second 25Hz stimulus after a 25Hz adapting stimulus (blue line) and a 40Hz adapting stimulus (red line). A) shows the power changes for the mu-alpha band for the duration of the stimulus (0 – 1 second) and shows no difference between conditions. B) shows the decrease in power for the mu-beta band for the stimulus duration (0 – 1 second) and shows no difference between conditions.

7.3.2.3 *Correlations with GABA*

We did not find a significant correlation between the difference in performance between the two conditions and individual GABA concentration ($R = 0.19$; $p > 0.2$). The data was not of sufficient quality to determine individual values of MEG activity in the different frequency bands.

7.4 Discussion

7.4.1 Adaptation to frequency discrimination threshold

In our MEG study we replicated the results of Goble & Hollins (1994) showing that adaptation to a 25Hz adapting stimulus, but not a 40Hz adapting stimulus, leads to an increase in sensitivity to frequency discrimination around 25Hz.

7.4.2 MEG correlates of vibrotactile adaptation

Adapting stimulus Initial analysis of the MEG data is in line with previous studies investigating S1 with MEG. Initial evoked responses occurred around 70 ms after stimulus onset and via SAMerf analysis it was possible to localise S1 for the adapting stimulus. Group SAM analysis did not show any difference in the response between the 25 Hz or 40 Hz stimulus. This was not expected because all stimuli were within the flutter range and project to the same area of S1. Kelly and Folger (1999) did find changes in location and amplitude in EEG after repetitive stimulation, but in our experiment repetitive stimulation occurred in both conditions and their finding was the result of “extensive and longitudinal analysis”, for which our power was not strong enough.

As expected, aside from the transient evoked response ±70 ms after stimulus onset we measured an SSR for the entire duration of the stimulus which was strongest for the frequency of stimulation. It is possible that the SSR reflects entrainment of the neurons to a specific frequency and that this entrainment contributes to tuning of the neurons to that particular frequency (Kelly and Folger, 1999).

In the induced response our results show a sustained desynchronisation for the entire duration of the adapting stimulus in both mu-alpha and mu-beta bands, followed by a re-synchronisation at stimulus offset, which is in line with earlier findings using shorter stimulus duration (e.g. Gaetz and Cheyne, 2006).

The duration-dependent response of both the evoked and induced activity shown in both conditions is in line with previous findings, e.g. using optical imaging in squirrel monkey (Simons et al., 2007). Whereas the evoked response shows a frequency-dependent SSR, the induced response for both the mu-alpha and mu-beta band is the same for both adapting stimuli, suggesting that the induced neuronal dynamics as measured by MEG are similar for 25Hz and 40Hz, as recently suggested by Spitzer et al. (2010) in EEG.

One difference between our current results and earlier studies is the lack of a resynchronisation at stimulus-offset as the power seems to remain desynchronised after the rebound. Our results show a sustained desynchronisation for the entire trial duration, suggesting that the adapting stimulus affects S1 dynamics, shaping the ongoing network state for subsequent processing, as suggested by Kopbell et al. (2010). Kopell et al. suggest that the beta-rhythm is also useful for "manipulating signals that are in place, to facilitate further processing" and a recent study by Haegens et al. (2011b) suggest a role of the beta-rhythm in the processing of stimuli leading up to a decision outcome. Jones et al., (2009) suggest that the mu-rhythm consists of thalamo-cortical (mu-alpha) and cortico-cortical (mu-beta) activity and several studies suggest a role of the mu-rhythm in integration of network activity. A mechanism by which the response to incoming sensory input is compared to prior

activity would aid temporal integration and the sustained desynchronisation possibly reflects ongoing monitoring of sensory input. A study by Lu et al., (1992) on auditory adaptation showed that an adapting stimulus reduced the amplitude of the N100 evoked response of a probe stimulus, but only when they occurred with a certain ISI, and conclude that a trace of the adapting stimulus remains in the short-term memory after stimulus offset. It is however, possible that this effect is due to reconstruction of the data in the time-frequency domain, as lower frequencies have poor temporal resolution, but smearing due to reconstruction would have also appeared prior to stimulus onset which it does not.

Test-stimuli We expected to find an effect of adaptation on the mu-alpha and mu-beta band for the standard 25 Hz stimulus. Time-frequency power analysis of the first 25 Hz frequency stimulus showed a mu-alpha and mu-beta desynchronisation followed by an immediate rebound, although activity stays desynchronised after the rebound. The cause of this phenomenon is unclear and might reflect an effect of repetitive stimulation on the state of the network in the sense that repetitive stimulation leads to a continuously desynchronised state in which ERD and ERS occur, facilitating sensory processing. However, no differences were found between the two conditions.

7.4.3 Possible role of GABA

Finally, we did not find a correlation between GABA and measures of adaptation whereas we did find a correlation between GABA and measures of tactile discrimination. For experimental reasons participants discriminated between 25 Hz and previously obtained threshold values and a difference in performance between

conditions shows an effect of adaptation but may not reflect absolute improvements in tactile sensitivity over baseline frequency discrimination values. It is unclear what the role of GABA is in adaptation. It is likely that changes in GABAergic inhibitory efficacy underlie the changes in neuronal activity due to repetitive stimulation (Kohn and Whitsel, 2002) and it is possible that this may results in changes in GABA concentration. However, as mentioned in Chapter 5 and 6, MRS measures GABA concentration over the course of 10 minutes, regardless of the location of GABA within the voxel, and changes in overall GABA concentration are unlikely within the millisecond time-scale of adaptation.

From invasive studies it is likely that repetitive stimulation the efficacy of excitatory pyramidal cells is reduced but the efficacy of inhibitory interneurons increases (Markram et al., 1998; Kohn and Whitsel, 2002) and the "relative strength of excitation and inhibition in a cortical circuit would be expected to change" (Kohn and Whitsel, 2002). It is possible that baseline GABA concentration is an indication of the ability of S1 neurons to 'tune' its' responses to a certain frequency by inhibiting irrelevant input. Inhibition of irrelevant information would lead to a sharper effect of adaptation on the spatiotemporal activity pattern and therefore better discrimination (O'Mara et al., 1988; Mclaughlin and Juliano, 2005). Repetitive stimulation may also lead to spill-over of GABA in the synaptic cleft, leading to activation of GABA-B receptors which may modulate subsequent activity (Kohn and Whitsel, 2002).

On the other hand one could argue that GABA concentration is an indicator of the flexibility of neurons to change its' dynamics depending on sensory input. If

neurons are under a lot of inhibitory influence, they might be less likely to change its' characteristics and therefore more GABA leads to *less* flexibility of a system and thus leads to less of an effect of adaptation. Our measures of GABA concentration are coarse and may reflect the efficacy of normal cortical function as discussed in Section 6.4. Other studies have shown a correlation between GABAergic mechanisms and gamma-frequency (e.g. Muthukumaraswamy et al., 2009) and beta-power (e.g. Jensen et al., 2005; Gaetz et al., 2011) as found by MEG. Unfortunately our data was insufficient in quality to obtain reasonable values of individual beta-power, possibly due to the complexity of the task.

7.4.4 Conclusion

The results found using MEG are inconclusive and show no direct difference to account for the behavioural effects of adaptation between the two conditions. As discussed in Chapter 1, work by Mountcastle (1990) and Recanzone (1992a, 1992b) has shown that firing rate changes little within frequencies in the flutter range, although Romo et al., (2003)show frequency specific firing in single neurons, but also show a periodic response in LFP recordings (Haegens et al., 2011b). A recent paper by Spitzer et al. (2010) showed in an EEG study that there is no difference in induced activity over S1 between different frequencies in the flutter range either.

So what might our MEG findings reflect? It is unclear how findings on the single-neuron level are reflected by MEG signals. Lee and Whitsel (1992a) demonstrated that repetitive tactile stimulation decreases the firing rate of neurons and Whitsel (2003) showed that S1 neurons show better entrainment after long duration stimulation (2-30 s). However, as discussed in section 3.2, MEG signals are thought

to reflect the synchronous firing of aligned post-synaptic potential on a population dynamics level and therefore changes in neuronal firing are not thought to be reflected in MEG. Kelly and Folger (1999) suggest that their EEG recordings (a change in location and amplitude of evoked activity as a result of repetitive stimulation) do not reflect shifts from one cortical area to another, but rather reflect the shift in activity as a result of repetitive stimulation as shown by optical imaging (Simons et al., 2007). Similarly, we have also shown a sustained SSR for the duration of the stimulus, possibly reflecting the results found by Simons et al. as well. As Reed et al., (2008) showed with electrode recordings that single digit stimulation influences synchronous firing among a large population of neurons in S1, encompassing the whole hand, and "Because spike synchrony potentiates the activation of commonly targeted neurons, synchronous neural activity in primary somatosensory cortex can contribute to discrimination of complex tactile stimuli". With MEG we may probe the mechanism by which large numbers of neurons synchronously fire to funnel the response to a tactile stimulus more specifically. Buonomano and Merzenich (1995) showed in a modelling study that indeed, temporal coding might be transformed into a spatial representation in the cortex and that discrimination between temporal stimuli may occur on the basis of spatial patterns. Furthermore, the induced response possibly reflects complex ongoing stimulus monitoring by integration of thalamocortical and corticocortical feedback loops and might under influence of extensive excitatory and inhibitory interactions.

However, it is of course possible that due to the nature of our psychophysical task, changes are present further up the pathway in e.g. S2 or even posterior parietal or

prefrontal areas that are thought to be involved in tactile discrimination and decision making (e.g. Salinas et al., 2000; Romo et al., 2003; Chow et al., 2009).

7.4.5 Future studies

To minimise the number of different trial occurrences and increase the number of useful trials as well as tapping into different mechanisms than just flutter, we propose a follow-up experiment comparing the effect of adaptation between a flutter stimulus (25Hz) and a vibration stimulus (200Hz) because previous studies have shown a cross-channel (RAI vs RAII; see section 1.3) effect of the two different types of tactile stimulation leading to a decrease in behavioural performance (for a review, see Tommerdahl et al., 2010) in a paradigm where participants are not asked to perform a complex task, but are rather exposed to adaptation to look at the cortical response when the system is driven to a 25 Hz frequency.

Chapter 8 - Passive Adaptation

8.1 Introduction

In the previous chapter we showed that an adapting stimulus of 5 seconds results in a frequency-specific evoked SSR for the entire duration of the adapting stimulus. As suggested by earlier studies, this entrainment of a neuronal population at a specific frequency might aid 'tuning' of neuronal responses to a certain stimulus (O' Mara et al., 1988; Mountcastle et al., 1990), but the role of these SSRs is relatively unknown. In addition, our results show a sustained desynchronisation in the induced mu-alpha and mu-beta band for the entire duration of the adapting stimulus, possibly reflecting actively integrating prior and ongoing network activity (e.g. Engel and Fries, 2010; Kopbell et al., 2010). Previous studies have shown a modulation of the beta-rhythm with attention (Dockstader et al., 2010) and discrimination (Spitzer et al., 2010; Haegens et al., 2011b). These studies suggest that the mu-beta rhythm is important in task-related events and might be involved in the process of comparing prior to subsequent events. No effect of this entrainment on the subsequent stimuli was visible in the subsequent task (see Section 7.4), but from the behavioural results it is clear that the adapting stimulus of 25 Hz facilitates frequency discrimination around 25 Hz.

To further investigate the effect of delivering 'probe' stimuli after a period of adaptation; we developed a further paradigm for MEG designed to optimally measure these interactions, based on the adaptation task described in Section 2.4

and Section 7.2. Participants were exposed to an adapting stimulus followed by a probe, to measure the effect of the adapting stimulus on the probe. Secondly, in the previous chapter we did not find an effect of different frequencies on the induced activity or of different adapting stimuli on the 25 Hz standard stimulus. As discussed in Section 7.4, by using flutter stimuli, we tap into a single transduction mechanism (RAI-units) and it might be possible that we were not sensitive enough to detect differences between 25 Hz and 40 Hz with MEG. Therefore for this next experiment we use a comparison between a 25 Hz flutter stimulus and a 180 Hz vibration.

8.1.1 Flutter and vibration; cross-channel interactions

As described in Section 1.4, it is well known that different mechanisms underlie the processing of flutter and vibration. Flutter (0 – 60 Hz) is primarily processed by RAI units in the skin, whereas vibration (60 Hz upwards) is processed by RAII units. Perception of flutter is consistent with the duration of stimulation and as shown in the previous chapter, repetitive stimulation at 25 Hz prior to each frequency discrimination trial increases discrimination performance around 25 Hz. The perception of vibration however, decreases after 500 ms of stimulus onset (Berglund and Berglund, 1970). Tommerdahl et al., (1999b) suggested that difference in cortical processing in S1 underlie the differences in perception between flutter and vibration. With optical imaging they showed that whereas flutter stimulation leads to an increase in absorbance (and therefore pattern of activity) in area 3b, vibration leads to an initial weaker increase in absorbance, followed by a decrease in absorbance for the duration of the stimulus when

stimulus length is increased to over 3 seconds (Tommerdahl et al., 1999a; Tommerdahl et al., 1999b). These findings suggest that increased duration vibratory stimulation leads to inhibition of S1 (Lebedev et al., 1994) and that vibration and flutter are processed by two distinct mechanisms in the periphery but by the same neuronal population in the cortex. Pertovaara and Hamailanen (1981) investigated the effect of these cross-channel interactions on behavioural performance and found that long duration (+ 5 seconds) high-frequency stimulation inhibited the detection of lower frequencies, possibly by co-activation of vibration channels. Tommerdahl et al., (2005b) investigated these cross-channel interactions with respect to adaptation and found that adaptation to a differential stimulus (i.e. adaptation to 200 Hz for discrimination around 25 Hz) does not improve behavioural performance, confirming the existence of cross-channel interactions. In addition, several studies have shown that RAII channels are more specifically processed by S2 in other primates (Ferrington and Rowe, 1980; Rowe et al., 1996; Tommerdahl et al., 1999b), but the role of S2 in humans is more likely to be higher-order stimulus processing (e.g. Romo and Salinas, 2001; Blatow et al., 2007). However, S1-S2 connections seem to be diminished in monkeys and humans compared to other mammals (Friedman and Murray, 1986; Friedman et al., 1986; Krubitzer and Kaas, 1992) and McGlone et al., (2002) have suggested a role of parallel thalamocortical processing between S1 and S2 as found with fMRI.

8.1.2 Aims

In the current experiment, we are interested in comparing the cortical response of adaptation when a test stimulus or 'probe' is the same or differs to the adaptor. In

chapter 7 we did not find a cortical effect of adaptation on subsequent stimuli, possible due to the fact that all stimuli were in the flutter range. In the following experiment we investigate the effect of 25 Hz adaptation on a subsequent 'probe' of 25 Hz, compared to the effect of 25 Hz adaptation on a 180 Hz probe. The reason for choosing a stimulus in the vibration range is that a 180 Hz stimulus is different from 25 Hz but thought to be processed by the same neuronal population in S1 (for a review see; Tommerdahl et al., 2010). A 180 Hz stimulus will be used instead of 200 Hz (Tommerdahl et al., 1999ab). Because a 25 Hz adapting stimulus results in increased discrimination around 25 Hz but has no effect (or an impaired effect) on processing of 180 Hz, we expect to see an effect of 25 Hz adaptation on a 25 Hz stimulus compared to a 180 Hz stimulus. As described in Section 7.1, the mu-beta rhythm is thought to underlie temporal integration of sequential stimuli and we expect to see differences in this band between the two conditions.

In addition, this experiment will result in a comparison of vibration versus flutter processing in S1 as measured by MEG, providing an addition to the known animal literature. The effect of adaptation in the flutter range is well known (see Sections 2.3 and 7.1) but the effect of adaptation in the vibration range is less well understood and the results on repetitive stimulation in the vibration range are limited. O'Mara et al., (1988) have shown that repetitive stimulation in the vibration range leads to depression of neuronal responsiveness which lasts up to several minutes, suggesting a different effect from flutter adaptation. Because we are interested in the effect of flutter adaptation, only a single 25 Hz adapting stimulus will be used.

Due to this experimental setup, a difference in cortical response for the probes could emerge due to the 25 Hz probe being the same as the adapting stimulus or due to differential processing of vibration compared to flutter. Analysis of the cortical response of the probes compared to the adapting stimuli might aid in the understanding of the role of the neuromagnetic signal in tactile stimulus processing.

8.2 Methods

8.2.1 Participants

12 participants (6 male; average age 30 yrs old, std: 4.8) participated in this task. All participants provided consent in line with the ethics regulation at Cardiff University School of Psychology and were told about the goal of the task afterwards. None of the participants had a history of neurological disease.

8.2.2 Equipment

The vibrotactile stimulator is described in Section 2.4 and Section 7.2.2.

8.2.3 Preparation

Participants were seated comfortably and their left index finger was placed on the stimulator and taped into place to reduce finger movement. Participants received a practice session in which they were exposed to exemplar vibrotactile stimuli of various frequencies and amplitudes in both the flutter and vibration range. Once participants reported they were comfortable with discriminating the different stimuli, they received a practice session of the passive adaptation task. The practice session consisted of ten trials consisting of a 5-second stimulus, followed by a single probe. Participants were instructed to maintain fixation for the entire experiment

and to press a key on a button box as fast as possible at the termination of each trial for both the practice and experimental tasks. Participants were then raised into the MEG helmet and were provided with a chin-rest to provide comfort.

8.2.4 Experimental task

The behavioural task performed in the MEG scanner consisted of two separate conditions in two separate runs. In both conditions, each trial consisted of a 5 second long stimulus of 25 Hz followed by a 1s pause, followed by a single stimulus or *probe* (see Figure 8.1). In one condition the probe consisted of a 25 Hz stimulus. In the other condition the probe consisted of a 180 Hz stimulus. All stimuli were presented at suprathreshold level. The 25 Hz adapting stimulus and the 25 Hz probe had the same amplitude. Because perception of flutter and frequency is different and the perceived magnitude for vibration is higher than for flutter, the amplitude for the 180 Hz probe was set as 10% of the 25 Hz stimulus, which was reported as similar intensity (see also Mountcastle et al., 1967). Throughout the experiment, inter-trial intervals consisted of a 2 second pause with a ±100 ms. jitter to reduce expectation of stimulus occurrence and focus attention. During the task, participants were asked to look at a crosshair on the screen. After each probe stimulus, a question mark appeared on the screen and participants were asked to press a button as quickly as possible to focus attention. The task consisted of 100 trials per condition. All participants performed both conditions and order was counterbalanced. The task was programmed using Matlab2008b.

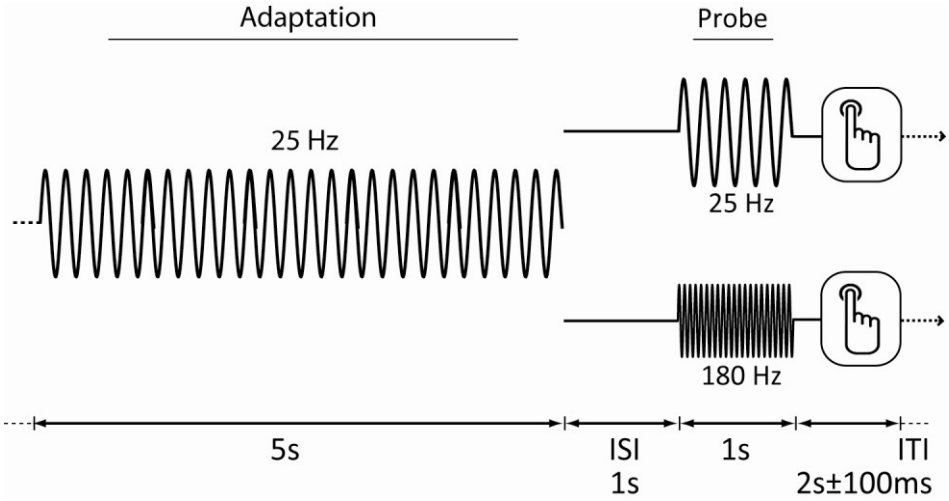

Figure 8.1. Protocol for the passive adaptation task. Each trial was preceded by a 25 Hz 5s stimulus in both conditions. In one condition the 25 Hz stimulus was followed by a 1 second 25 Hz probe (condition 1) with the same amplitude as the adapting stimulus. In the other conditions it was followed by a 1 second 180 Hz probe with an amplitude 10% of the adapting stimulus. This was followed by a question mark on the screen and participants had to press a button as quickly as possible.

8.2.5 MEG methods

Acquisition The details of the MEG scanner and acquisition parameters are described in Section 3.3.3 and 7.2.5

Analysis Prior to data analysis the data was visually checked and corrected for artefacts and noisy trials. Because we are interested in the peak location of early S1 activity, Synthetic Aperature Magnetometry event-related fields (SAMerf) (Vrba and Robinson, 2001) was used to create three-dimensional differential images of source power (pseudo-t statistics) for 1 second of baseline (-1 – 0 seconds) compared to 10ms bins spanning between 0 - 150 ms post-stimulus for the adapting stimulus for each participant. SAM images were constructed for frequencies between 2 – 90 Hz for the adapting stimulus and both probes separately. Peak location of activity in each primary somatosensory cortex was located in the volumetric images for each

stimulus. SAM virtual electrodes reconstructions were generated for these locations using covariance matrices (see Section 3.4) band pass filtered between 0 and 90 Hz for the stimuli separately.

To contrast the first peak of S1 activity between flutter and vibration, a group SAM analysis was performed between the active periods (30 – 100 ms after stimulus onset) of the probe in both conditions. SAM images were spatially normalised using FLIRT (Jenkinson and Smith, 2001) to the MNI 152 template. Non-parametric permutation testing for statistical significance was performed using 4096 permutations for each condition, and thresholded using the omnibus test statistic at $p < 0.05$ (Nichols and Holmes, 2002; Singh et al., 2003). Output is a t-statistic weighted map with voxel-based corrections for multiple comparisons.

Time-frequency analysis was performed on the peak voxel location of the adapting stimulus over the entire trial duration. For the evoked (phase-locked) activity, time-frequency analysis was performed using the Hilbert transform between 1 and 200 Hz in 0.5 Hz frequency steps, and averaged across participants between conditions. For further investigation of power changes, evoked activity was plotted between 0-90 Hz for the duration of the trial, expressed as percentage change from baseline (1 second prior to stimulus onset for the adapting stimulus).

To look at specific frequency bands in the induced (non-phase locked) activity, time-frequency analysis was performed on both adapting stimulus and probe separately as with the evoked analysis. Peak mu-alpha (7-15Hz) and mu-beta (15 - 30Hz) band frequency and amplitudes, expressed as percentage change from baseline, were

extracted for the induced activity. Differences between conditions were measured as significant differences in power for the mu-alpha and mu-beta ERD/ERS separately as measured from 100-1000 ms after stimulus onset, and assessed statistically by one-way ANOVA using Bonferonni correction for multiple comparisons. Finally, peak mu-band frequency (7 - 30 Hz) was extracted for the probe duration and compared against mu-band frequency for the adapting stimulus (one-way ANOVA with Bonferonni correction).

8.3 Results

One participant was excluded due to excessive noise and artefacts in the MEG data.

8.3.1 Location

SAMerf analysis demonstrated significant activity in S1 for all participants (single subject, see Figure 8.2), however, participants varied in the latency of their first S1 response between 30 ms and 100 ms and therefore group SAM analysis was performed between 30 – 100 ms. Figure 8.3 shows group SAM analysis for the 25 Hz and 180 Hz probe between 30 - 100 ms. The peak for the 25 Hz probe is slightly more lateral in location (a difference of 4.2 in Talaraich coordinates. 25 Hz; 48.2—28.1-44; 180 Hz; 44.2-28.1-44) by comparing group SAM analysis in both conditions, but t-weighted comparison analysis between the two group SAM images did not show significant differences between the two locations.

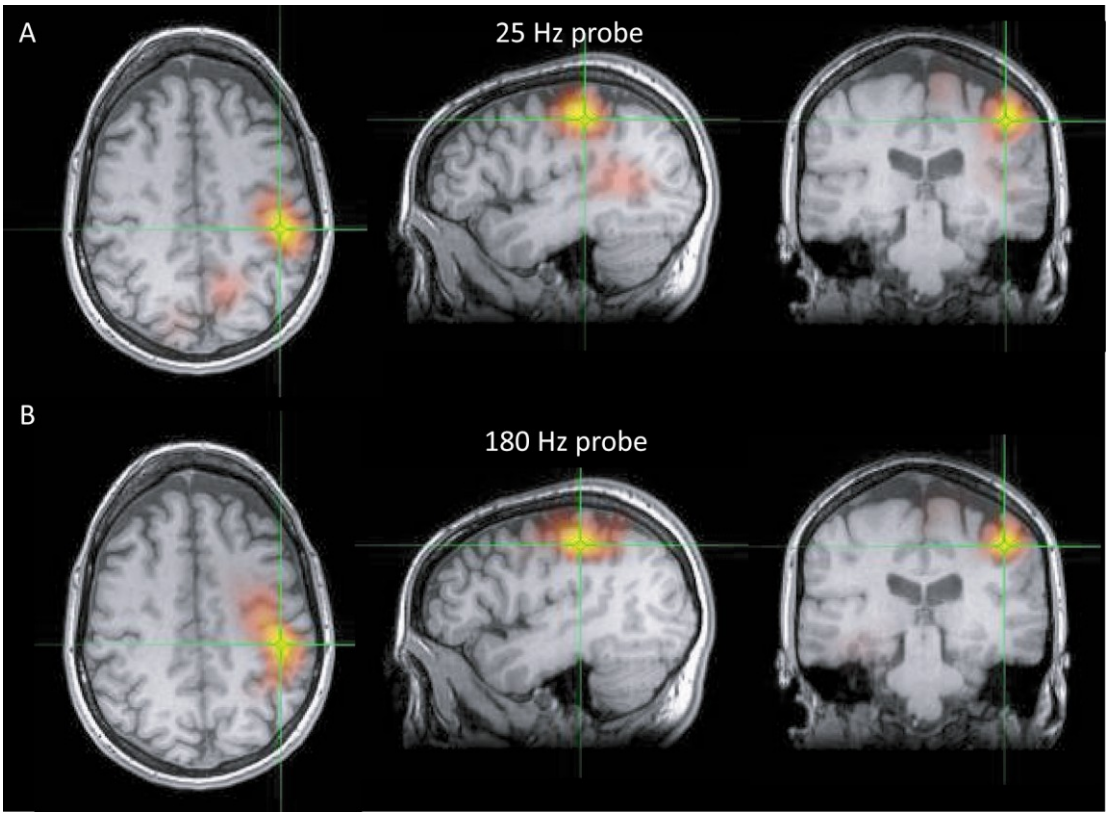

Figure 8.2. Peak location for S1 for a single subject using SAMerf analysis. Both 25 Hz and 180 Hz probe occurred at the same latency for this subject, although other subjects showed variation in the latency.

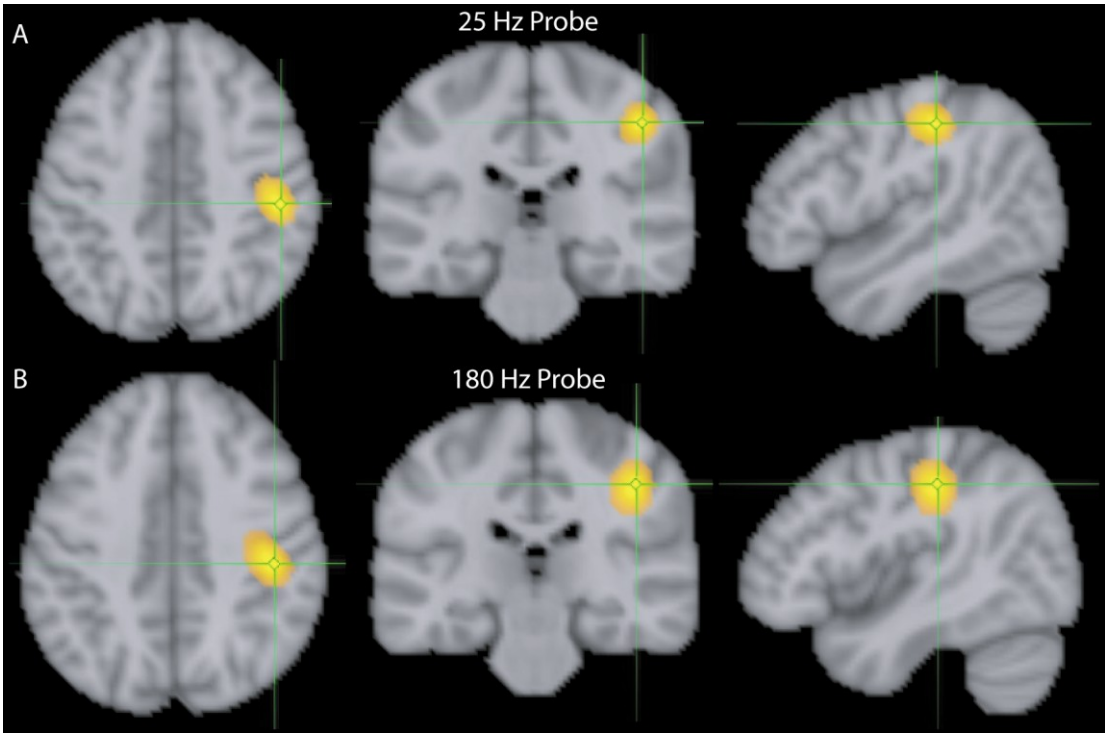

Figure 8.3. Group SAM volumetric analysis for the 25 Hz and 180 Hz probe. The peak localisation for the 25 Hz stimulus is 2 voxels more lateral than for the 180 Hz stimulus. However, statistical analysis did not show significant differences between the two stimuli.

8.3.2 Virtual sensor analysis

8.3.2.1 Evoked activity

The average evoked activity between 0 - 200 Hz was plotted for the entire duration of the trial, as seen in Figure 8.4. Time-frequency analysis of the evoked response between 0 - 200 Hz reveals an interesting phenomenon. Figure 8.3 shows a characteristic SSR at 25 Hz and harmonics at 50 Hz for the adapting stimulus and 25 Hz probe. No SSR at 180 Hz is visible. However, in the ISI (5 - 6 seconds) an evoked response at 150 Hz can be seen. In the 'same' condition this response is only visible for the duration of the ISI, whereas it is clearly present before and after the ISI in the 'different' condition.

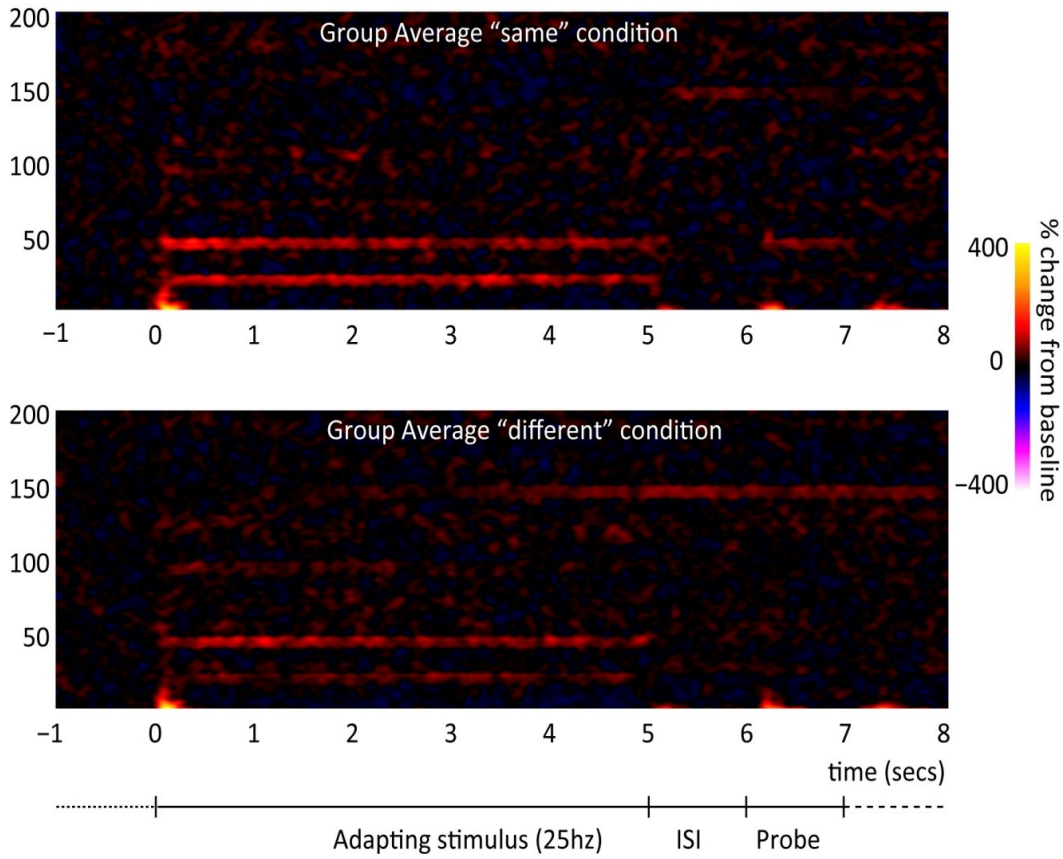

Figure 8.4. Group average time-frequency spectrograms for the evoked activity between 0 – 200 Hz for the entire trial duration for both conditions expressed in percentage change from baseline. A characteristic SSR can be seen for the 25 Hz adapting stimulus and probe at 25 Hz and its harmonics. No clear SSR is visible for the 180 Hz stimulus. In the 'same' condition a 150 Hz SSR can be seen for the duration of the ISI, whereas the SSR at 150 Hz is also visible before and after the ISI in the 'different' condition.

Analysis of the normalised (by the mean response) evoked trace between 0 – 90 Hz shows a similar trace for both conditions as shown in Figure 8.5A. Characteristic positive deflection (M70) is followed by a negative deflection (M100) followed by an upward positive deflection (M200 - M300) for both adapting stimuli and probes (see Pfurthscheller et al., 2002; Jones et al., 2007 and Section 3.3). Activity for the M200-M300 for the probe (see green box in Figure and Figure 8.5B) shows a stronger component for the 180 Hz probe than for the 25 Hz probe, but this is not significant (Two-sample paired T-test of evoked power between 200 – 600 ms after onset of the probes, p = 0.068).

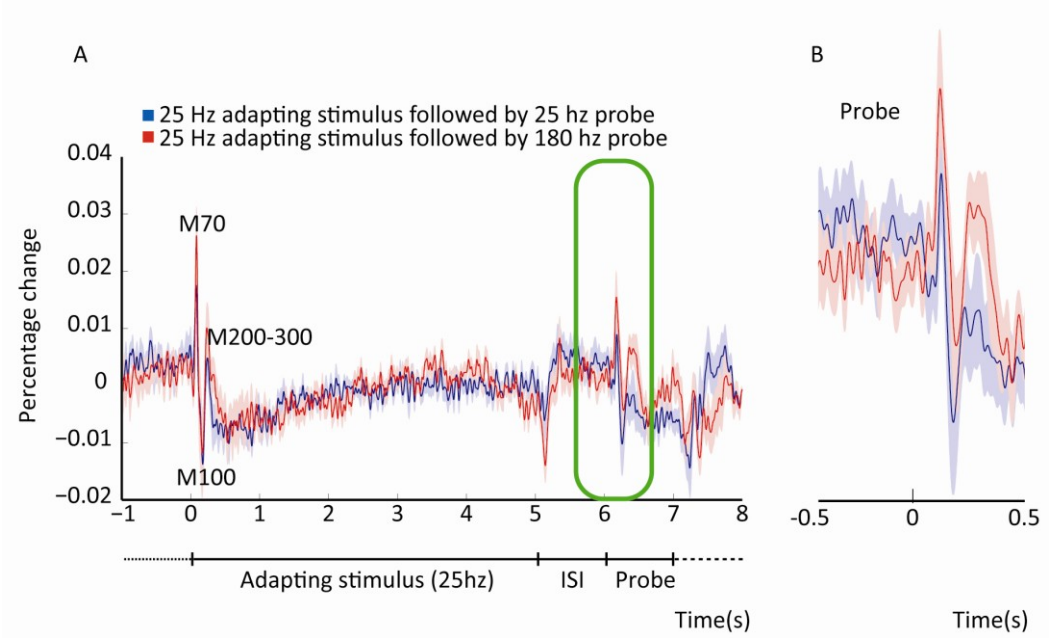

Figure 8.5. A. Normalised evoked trace between 0 – 90 Hz for the whole trial in both conditions. Analysis shows characteristic M70-M100-M200/300 deflections for both stimuli. The area within the green box is enlarged in B and shows a larger M200/300 component when the adapting stimulus and probe are different (not significant).

8.3.2.2 Induced activity

Figure 8.6 shows the induced activity spectrogram between -1 and 7 seconds for both the adapting stimulus (0 – 5s) and probes (6 - 7s). Consistent with our previous findings (see Section 7.3) the results show an initial mu-alpha and mu-beta ERD for the adapting stimulus, followed by a sustained desynchronisation for the duration of the stimulus. Similarly, the probe is characterised by a mu-alpha and mu-beta ERD followed by an ERS. There are no significant differences in the mu-beta power between the initial ERD/ERS duration (within 1 second from stimulus onset) of the adapting stimulus between both conditions (Two Sample paired T-Test; p = 0.22).

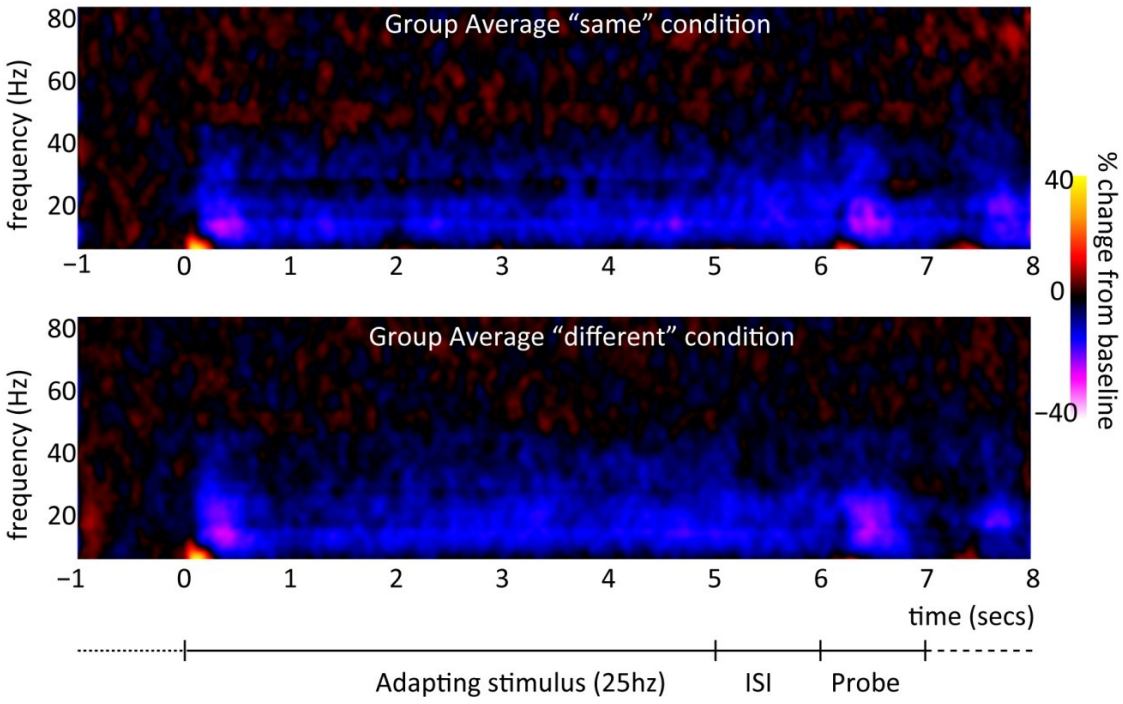

Figure 8.6. Group average time-frequency spectrograms for the induced activity between 0 – 90 Hz for the same and different conditions. Duration of the adapting stimulus is from 0 - 5 s, duration of the probe is from 6 – 7 seconds. Both stimuli show similar activity. In both conditions the adapting stimulus shows initial mu-alpha and mu-beta ERD after stimulus onset, followed by sustained desynchronisation for the duration of the stimulus. Both probes show a similar mu-alpha and mu-beta ERD followed by an ERS.

We investigated the power of the mu-alpha and mu-beta ERD/ERS for the 25 Hz and 180 Hz probe. As can be seen in Figure 8.7A, the traces for both probes in the mu-alpha band are similar, whereas Figure 8.7B shows a difference in mu-beta power between the 25 Hz and 180 Hz probe. To investigate whether these differences in mu-beta ERD/ERS power are significant, we tested whether the average ERD/ERS power for the 25 Hz probe was significantly different from the 180 Hz probe. To ensure that a difference in power is not due to the different stimulus characteristics (‘flutter’ vs. ‘vibration'), the probe mu-beta ERD/ERS power was also compared against the average adapting stimulus mu-beta ERD/ERS over the same time period (One-Way ANOVA) and showed a significant difference between conditions; df = 2, F = 4.928, p = 0.014). Further post-hoc analysis with Bonferonni correction shows that the average power for the mu-beta ERD/ERS complex is significantly less for the 25 Hz probe than for the 25 Hz adapting stimulus ($p = 0.012$), whereas the 180 Hz probe was not ($p > 0.5$). Although there was a trend for a higher ERD/ERS desynchronisation for the 25 Hz probe than for the 180 Hz probe, and all individual participants showed this effect, this was not significant ($p = 0.1$) as shown in figure 8.8.

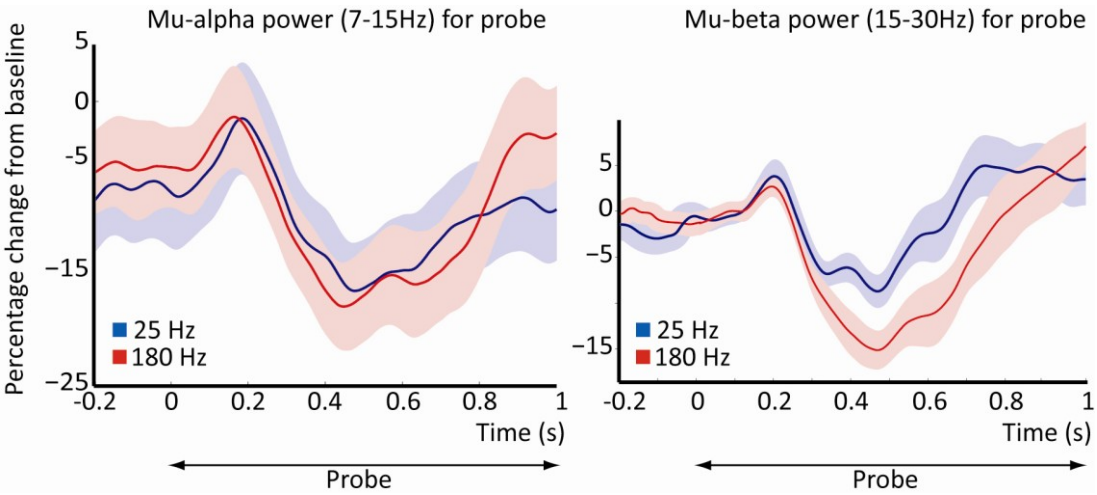

Figure 8.7. Mu-alpha and mu-beta power for the probe stimulus. Blue indicates the 25 Hz probe, red indicates the 180 Hz probe. Both mu-alpha and mu-beta show a typical ERD/ERS complex. Mu-alpha power is the same in both conditions. Mu-beta power is different between conditions.

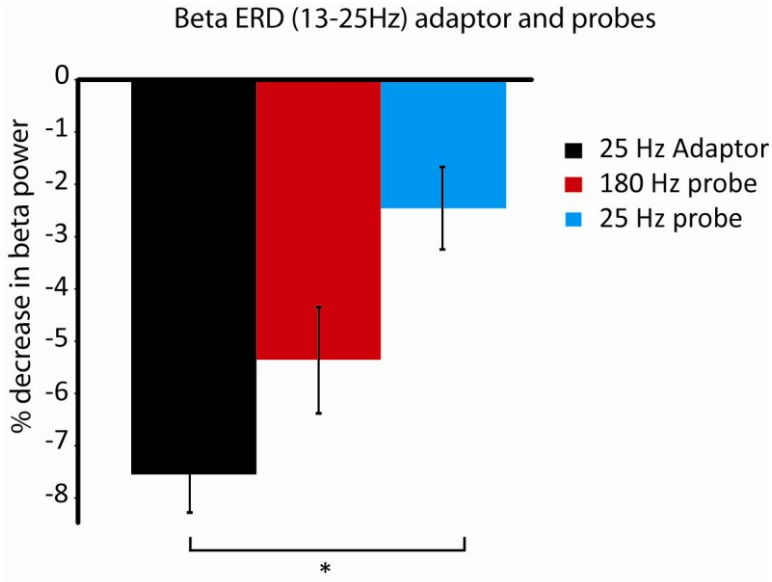

Figure 8.8. Mu-beta power over the ERD/ERS complex. Power is significantly smaller for the 25 Hz probe than the adapting stimulus ($p = 0.012$). The ERD/ERS power for the 180 Hz probe is not different from the adapting stimulus. Lines indicate standard error.

We also investigated whether there were differences in mu- *frequency* between the 25 Hz and 180 Hz condition. The results show that the average mu-frequency for the ERD/ERS was significantly lower for the 25 Hz probe than for the 180 Hz probe ($p = 0.044$) but not compared to the adapting stimulus. The 180 Hz probe was also not significantly different from the adapting stimulus ($p > 0.2$).

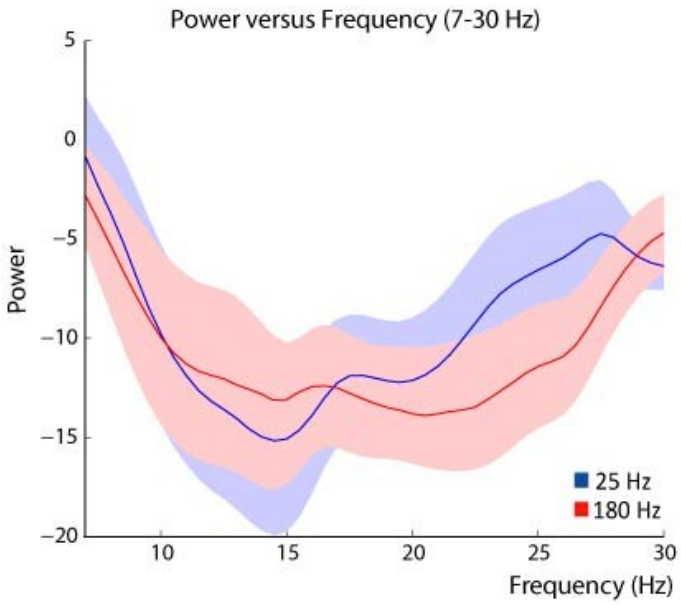

Figure 8.9. Peak mu frequency. Peak mu-frequency is significantly smaller for the 25 Hz probe (15 Hz) than for the 180 Hz probe 20 Hz, ($p = 0.044$). Lines indicate standard error.

8.4 Discussion

8.4.1 Whole brain analysis

Group analysis showed no significant difference in peak location between the 25 Hz and 180 Hz stimulus. We cannot conclude that the same neurons are activated by both 25 Hz and 180 Hz as there may be different clusters of cells responding to flutter and vibration, but the findings are consistent with invasive studies (Tommerdahl et al., 1999a, 1999b) showing that the same neuronal population becomes activated after stimulation with either 25 Hz or 200 Hz.

8.4.2 Evoked activity

Consistent with the previous experiment, we showed a SSR at 25 Hz and its harmonics for the 25 Hz adapting stimulus and 25 Hz probe, showing that the neuronal population is driven at stimulus frequency. However, we were not able to distinguish an SSR at 180 Hz. Tommerdahl et al., (1999ab) have shown that a vibration leads to a weaker optical imaging response in S1 compared to a flutter stimulus and given the smaller amplitude for the 180 Hz stimulus in our task, it may be responsible for the lack of 180 Hz SSR. In addition, our results show a 150 Hz SSR centred on the ISI. In the 'same' condition, the 150 Hz SSR was only present during the ISI, but in the 'different' condition, the 150 Hz SSR was also present before and after the ISI. No stimulus was given during the ISI and the 150 Hz SSR seems to be a phenomenon emerging from the adaptation task as it is a harmonic of 25 Hz. The role of SSRs in general is not well understood and the literature does not mention SSRs during stimulus intervals. Because the 150 Hz SSR is longer in the 'different' condition, it might be a result of a memory-effect induced by the adapting stimulus. However, the role of this SSR is not understood.

The trace of the evoked activity shows characteristic peak latencies (see Section 7.1, see also Jones et al., 2007). The M70 and M100 are typical for tactile stimulation and are thought to underlie feedforward and feedback interactions. Both conditions also show a typical M200-300 component. This late component of the evoked activity is thought to underlie higher-level cognitive processing and could potentially involve a memory-component. Although the difference in this component is not significant between the 25 Hz and 180 Hz probe, a higher evoked

activity may indicate that a greater memory component as the result of the stimulus being 'different'. Jones et al., (2007) showed a larger late component for perceived stimuli compared to non-perceived stimuli, suggesting that this component is involved in attention and our results may show that the brain is more attentive or responsive when stimuli are different.

8.4.3 The role of cortical oscillations in adaptation

Our results show a significant difference in mu-beta power between the 'same' and 'different' conditions. The mu-beta ERD/ERS is lower than the adapting stimulus when the probe is the same frequency as the adapting stimulus but not when the probe is *different* from the adapting stimulus, confirming our hypothesis that an effect in the power of the mu-beta band would be seen. Further analysis confirmed that the difference in probe mu-beta power does not reflect differences in stimulus frequency, because the mu-beta ERD/ERS for the 180 Hz probe is not significantly different from the 25 Hz adapting stimulus. These results show that the mu-beta response to tactile stimulation is the same irrespective of whether vibrotactile stimulation is delivered in the flutter or vibration ranges, which has not been shown before. In addition, the results confirm that power in the mu-beta band does not directly reflect the characteristics of afferent stimulation, but is indicative of an integration of prior activity with afferent input.

The smaller mu-beta ERD/ERS when the stimuli are the same seems to reflect the effects of adaptation on subsequent stimulation. These results show that the network state within S1 affects subsequent processing of the 'probe' in the mu-beta band range but not in the mu-alpha range. These indicate the functional role

of the beta-rhythm in shaping the response of S1 to afferent input, whereas the mu-alpha rhythm is more concerned with suppression of inactivation during a task, irrespective of stimulus characteristics.

It remains unclear what underlying neurobiological mechanism generates the differences in mu-beta ERD/ERS produced when adapting stimulus and probe are the same. It is likely that GABAergic inhibition plays a role in the change in activity due to adaptation as seen with MEG (see Section 7.4.3). Invasive studies have shown that the efficacy of excitatory pyramidal cells is reduced but the efficacy of inhibitory interneurons increases (Markram et al., 1998; Kohn and Whitsel, 2002) as a result of adaptation and the "relative strength of excitation and inhibition in a cortical circuit would be expected to change" (Kohn and Whitsel, 2002). Several studies have shown a link between mu-beta power and GABAergic mechanisms (Jensen et al., 2005; Gaetz at al., 2011; Hall et al., 2010) showing that more GABA is correlated with a larger increase in ERS power in the mu-beta range. As we suggest that baseline MRS measures of GABA reflect inhibitory efficacy and that increased activity of GABAergic neurons may underlie adaptation effects, it would be expected that the mu-beta rhythm would increase as GABAergic activity increases: indeed we find a smaller desynchronisation of the mu-beta rhythm after adaptation. An increase in GABAergic activity might aid in tuning the neuronal response to 25 Hz and inhibition of irrelevant information and thus lead to a shift in activity. As suggested by Kelly and Folger (1999), it is likely that our data reflect the sharpening of the area affected by repetitive stimulation as shown by Simons et al. (2007) with optical imaging. Furthermore, Cannestra et al., (1998a) found a similar effect, using

optical imaging, and showed that the neuronal response to a subsequent adapting stimulus after the same adapting stimulus was reduced, whereas the response to a novel stimulus was not. It is more likely that our data reflect a general 'sharpening' in activity due to adaptation controlled by inhibitory interneurons.

Interestingly, we also found a significant difference in peak mu-frequency between the two probes. Both in our previous experiment (Section 7.4) and previous studies (Spitzer et al., 2010), no frequency-dependent effects have been shown for the mu-rhythm in the flutter range. Because there is no significant difference in mu-band frequency between the 180 Hz probe and the adapting stimulus, the difference in mu-frequency is unlikely to arise due to stimulus-characteristics and is possibly a result of adaptation. The role of the mu-rhythm is yet unclear and whilst some studies have focused on the role of mu-alpha and beta *power*, very few studies have investigated the role of the *frequency* of the mu-rhythm. In addition, peak mu-frequency was investigated between 7 – 30 Hz because participants did not always show a distinct mu-alpha and mu-beta band. This only became apparent during averaging.

Given that the mu-beta rhythm is thought to be generated by both thalamocortical and corticocortical interactions and there are thought to be cross-channel interactions between RAI and RAII afferents both in the periphery and central nervous system (Tommerdahl et al., 1999a, 1999b, 2005, 2010), it is possible that the difference in beta-frequency reflects incorporations of multiple channels of tactile stimulation. Finally, previous studies have suggested a role of the mu-beta rhythm in discrimination (Spitzer et al., 2010; Haegens et al., 2011b), but whilst not

excluding the suggestion that mu-beta is involved in discrimination, we show more specifically that the mu-beta rhythm is affected by subsequent stimulation in a non-discrimination task.

8.4.4 Limitations

A limitation in this task lies in the fact that we did not use a 180 Hz adapting stimulus condition. Aside from an increased scan-time, affecting data quality, we were not interested in adaptation to 180 Hz. As discussed in 8.1.1; a repetitive stimulation with a vibratory stimulus leads to deactivation and inhibition of S1 and a decrease in perception (O'Mara et al., 1988; Tommerdahl et al., 1999b) and therefore the mechanism by which repetitive vibration is processed seems to be different from repetitive flutter stimulation. A 180 Hz stimulus was used was to provide a tactile stimulus different enough from the 25 Hz stimulus as the flutter stimuli used in the previous experiment did not result in different responses. The findings that both mu-beta power and mu-beta frequency were different for the 25 Hz probe compared to the adapting stimulus but not for the 25 Hz, suggest that the effect seen is not due to the 180 Hz probe being processed by a different pathway. In addition, this study focused on group average differences and the role of individual differences in mu-alpha and mu-beta power is not well understood.

8.4.5 Summary

Firstly, the effect of adaptation seen in the neuromagnetic activity of S1 results in a change in mu-beta power. Given that mu-beta is thought to be involved in actively integrating prior and ongoing network activity, it is likely that less of a mu-beta desynchronisation is necessary for two ‘similar’ stimuli because the stimulus

characteristics are the same. This suggests that the mu-beta rhythm is involved in functional coding of task-related stimuli, shaping the response of the S1 to prior stimulation by modulating activity via corticocortical circuits, and adds to the possible role of mu-beta as discussed in Section 7.4.2. It is apparent that mu-beta therefore plays an important role of adaptation of the network. In addition, our results show that despite acting via different channels, processing of vibration and flutter act via the same neuronal population in S1 and that the cortical oscillations underlying this processing are the same regardless of stimulus-frequency, confirming findings by Spitzer et al., (2010) and the previous chapter, but also showing this is irrespective of receptor channel.

Chapter 9 - General Discussion

The experiments described in this thesis have been designed to better understand basic somatosensory processing in humans, and to investigate the links between behavioural measures and neuromagnetic and neurochemical metrics in tactile processing.

9.1 Summary of the experimental results

Chapter 2 introduced the behavioural experiments performed in this thesis. Frequency discrimination has been investigated since the 1950's and the psychophysical procedures have been described in detail and these have been used in combination with invasive recordings. Goble and Hollins (1994) found that an adapting stimulus can modulate the sensitivity to vibrotactile frequency discrimination. We proposed that we would be able to detect changes in cortical activity as a result of this modulation. In an adaptation task adjusted for MEG, we showed that we can induce adaptation to frequency discrimination around 25 Hz with a shorter duration adapting stimulus and in a task that removes some aspects of subject bias, confirming and improving upon the findings by Goble and Hollins (1994).

Chapter 5 discussed our development of edited GABA-MRS to detect GABA concentration in the sensorimotor cortex, and to investigate and optimise the quality of the GABA signal obtained. The results discussed in this chapter show that GABA can be measured from the sensorimotor cortex. A smaller voxel size can be

used and longer scan times result in better data quality, but due to experimental and anatomical limitations a $(3cm)^3$ voxel is proposed for standard voxel acquisition. For future studies it is important that standardised analysis methods are used.

Chapter 6 discussed our finding that the GABA concentration in sensorimotor cortex correlates significantly with a behavioural measure of frequency discrimination performance. We have shown that this effect of GABA is region-specific because GABA concentration in visual cortex does not correlate with tactile discrimination. This experiment showed that GABA is correlated with frequency discrimination thresholds in humans. The possible role of GABA in somatosensory dynamics will be discussed in Section 9.2.2 and 9.3.2 .

Finally, chapter 7 and chapter 8 discussed our MEG studies of tactile adaptation. Using a similar paradigm to the adaptation paradigm used by Goble and Hollins (1994) and our own paradigm described in Section 2.4, we have replicated previous studies showing that tactile adaptation to frequency discrimination – improving discrimination thresholds - can be induced by a stimulus with the same frequency and amplitude as the test-stimulus. We measured the accompanying neuromagnetic activity during the task. The results showed a sustained evoked steady-state response oscillating at the frequency of the adapting stimulus and sustained desynchronisation in the mu-alpha and mu-beta band, for the duration of the stimulus. However, no effect of adaptation was found on the neuromagnetic signature of the test stimuli in the frequency discrimination task. In a subsequent task described in chapter 8, a passive adaptation task was used to probe the effect of an adapting stimulus on processing of subsequent stimuli. In this task the effect

of a 25 Hz adapting stimulus on either 25Hz or 180 Hz was measured. Consistent with the results found in Chapter 7, the adapting stimulus was reflected by an evoked SSR at stimulus frequency and sustained desynchronisation for the duration of the stimulus. As a result of the 25 Hz adapting stimulus, the mu-beta power ERD/ERS for the 25 Hz probe (or 'same' condition) was significantly lower in power than for the 180 Hz probe (or 'different' condition). Furthermore, mu-frequency was significantly lower for the 25 Hz probe. The role of cortical oscillations in somatosensory dynamics will be discussed in Section 9.2 and 9.3.

9.2 Dynamics of tactile discrimination

We have made progress in combining findings from the invasive neurophysiology with findings using neuroimaging techniques.

Both invasive studies and our own studies (see Section 9.2.2 and 9.3.2) have shown an involvement of GABA in sensory processing and a number of invasive studies have suggested a role of GABA on dynamical changes as a result of adaptation. In a study investigating adaptation to vibrotactile frequency discrimination, we did not find a correlation between behavioural measures of adaptation, neuromagnetic activity and GABA concentration (chapter 7), but the effect of adaptation on neuronal rhythms might reflect how GABAergic mechanisms are involved.

9.2.1 Neuronal dynamics of frequency encoding and discrimination

As briefly discussed in Section 1.4 and Section 6.4, the underlying mechanism by which frequency is encoded is still a subject of debate. A number of studies (e.g. Mountcastle et al., 1990; Recanzone et al., 1992a) have suggested that frequency is

encoded as highly synchronous spike trains rather than average firing rate, but other studies have suggested a role of spike-rate (e.g. Salinas et al., 2000; Romo and Salinas, 2003; Pleger et al., 2006).

Mountcastle and colleagues (Mountcastle et al., 1967; Mountcastle et al., 1969; LaMotte and Mountcastle, 1975; Mountcastle et al., 1990) investigated the coding of vibrotactile frequency in S1. They found that the periodicities in the neuronal activity represented the vibrotactile stimulus frequency, rather than the exact stimulus rate as discharge rate was virtually identical for all frequencies. Recanzone et al. (1992) showed an increase in the temporal acuity of periodic spike trains after training. Both Mountcastle et al (1975, 1990) and Recanzone et al., (1992) linked their results to a frequency discrimination paradigm and showed that performance in this task correlates with periodic interspike intervals. McLaughlin and Juliano (2005) have shown that an increase in temporal noise affects periodic encoding and that discrimination is impaired.

In support of theories suggesting that firing rate is involved in frequency encoding, Salinas et al., (2000) and Hernandez et al., (2000) found that the firing rate of RA neurons in S1 does change with stimulus frequency, and that the results by Mountcastle and colleagues were based on few neurons within a limited frequency range. Salinas et al. found that firing rate varies as a function of stimulus frequency and found that differences in discrimination ability correlated with fluctuations in the firing rate. Hernandez et al., (2000) and Luna et al., (2005) proposed that frequency discrimination is possible solely on the basis of firing rate, although Luna et al. found that frequencies are encoded by firing rate and periodicity. Salinas et al.,

(2000) also found that periodicity was more phase-locked to the stimulus during active discrimination than during passive discrimination. According to Salinas et al., (2000) frequency encoding purely on the basis of periodicity would be too rigid, and they suggest a mechanism by which both periodicity and firing rate contribute to discrimination, particularly with respect to population dynamics. In support of this, some S1 neurons demonstrate periodicity encoding and others display firing rate encoding (Hernandez et al., 2000).

From our MEG data on adaptation to frequency discrimination described in chapters 7 and 8, we cannot determine whether frequencies are encoded by spike rate, periodicity or a combination of the two. Tommerdahl et al., (2007b) discussed that there is a growing body of evidence suggesting a role of synchronised neuronal firing to encode sensory stimuli. A number of studies have suggested a role of a spatiotemporal pattern of activity in discrimination (Whittington et al., 2000; Tommerdahl et al., 2007b) and periodicity would aid periodic, synchronous time-dependent firing across a neuronal population. LFP recordings and MEG recordings are able to measure these population level dynamics. LFP recordings have shown that peak frequencies reflect stimulus frequency in vibrotactile discrimination, and therefore that stimulus frequency is encoded at a population level with periodic synchronous activity (Haegens et al., 2011b).

Single cell recordings probe the response of single neurons to a stimulus and can measure variability across e.g. firing rate, amplitude and timing. However, any systematic activity seen within a single cell may not be reflected in population recordings (such as multi-unit recordings, local field potentials or EEG/MEG),

because they incorporate and integrate multitude of neurons over a distinct time-window (Belitski et al., 2010). Population level dynamics reflect integration of single cells into a pattern of activity, incorporating local inhibitory and excitatory connections but may also include feedback from other regions. Whereas these population level recordings are useful to gain an understanding of cognitive processes underlying stimulus processing, the exact contribution of different single-cell processes is poorly understood (Whittingstall and Logothetis, 2009). As explained in the introduction, both levels of inquiry have their advantage, and by understanding both levels, the effect of single-cell recordings, and the contribution of separate processing, on a neuronal population can be understood (e.g. Logothetis and Wandell, 2004; Nir et al., 2007; Denker et al., 2010; Liebe et al., 2011).

The evoked response in our MEG data shows frequency-dependent activity, suggesting that different frequencies are reflected in the evoked activity of the S1 network. The power of the induced mu-beta oscillations appear to be independent of stimulus frequency.

9.2.2 GABA and frequency discrimination

From the animal literature it is known that GABA is important in shaping the neuronal response to sensory stimulation (eg. Dykes et al., 1984; Sillito, 1984; Juliano et al., 1989). Many of these studies have suggested a role of GABA in the inhibition of neighbouring minicolumns to enhance spatial discriminative ability, but the involvement of GABAergic inhibition in temporal coding has been shown as well (McLaughlin and Juliano, 2005). Our studies have not provided additional

evidence as to how frequencies are encoded or as to *how* GABA affects the signal-to-noise at the level of changing temporal patterns of neuronal activity. We have shown how non-invasive neuroimaging methods can be used to provide additional evidence to understanding the general processes of tactile discrimination Our findings show that individuals with more GABA in their sensorimotor cortex are better at frequency discrimination. McLaughlin and Juliano showed that GABA is important in regulating the temporal noise in stimulus activity to a particular frequency and it therefore seems likely that more GABA and thus, more inhibition, results in a decrease in temporal noise and therefore in a better signal. In addition, Harris (2006) showed that adding noise to vibrotactile frequencies results in worse discriminative capacity and a role of GABAergic neurons in neuronal synchrony is well known (Haider et al., 2006; Gentet et al., 2010; Buzsaki, 2011). Furthermore, Moore et al. (2010) suggest that GABAergic inhibition through fast-spiking interneurons is crucial for effective encoding of sensory stimuli (ie. the 'window of opportunity' described in Section 6.4), whereas somastatin-positive interneurons act slower and might have a more graded control of excitatory neurons. A possible contribution of several inhibitory mechanisms is discussed in 9.3.2.

9.2.3 The role of S1 in frequency discrimination

Vibrotactile frequency discrimination relies upon a distributed network of cortical areas (Salinas et al., 2000; Romo and Salinas, 2001; Preuschhof et al., 2006; Chow et al., 2009) network. However, Lamotte and Mountcastle (1972; 1975; 1979) have shown that destruction of S1 removes the ability of monkeys to *discriminate* and identify frequencies in the flutter range, although *detection* of flutter stimulation

remains intact. The finding that S1 GABA concentration predicts frequency discrimination threshold suggest that *local* GABA concentration is a driving factor in determining the sensitivity in tactile frequency discrimination, suggesting that local S1 GABAergic mechanisms underlie frequency discrimination. These findings are in correspondence with earlier studies showing that GABA is involved in the temporal response of neurons and therefore the ability to discriminate different frequencies based on the neuronal response in S1.

9.3 Dynamics of tactile adaptation

9.3.1 Cortical rhythms and adaptation

As discussed throughout this thesis, a number of studies have investigated mechanisms of tactile adaptation and have described dynamic mechanisms underlying repetitive stimulation. As discussed by Kohn and Whitsel (2002), it is unlikely that a change in behavioural performance as a result of adaptation is due to integration of the adapting stimulus signal over the entire task. It is more likely that due to adaptation, the properties of a 'hard-wired' network change dynamically. By temporally integrating the neuronal signal, dynamic changes can be made based on a single stimulus. This also allows for a single mechanism to be broadly-tuned but change its response characteristics by quickly adapting to external stimuli (also; Moore et al., 2010). These 'stimulus-driven' changes in cortical functioning lead to more efficient neuronal activity; diminished responses and better separation between stimulus representations (Moore et al., 1999). A mechanism by which neuronal populations effectively encode stimuli is by sparse coding (Foldiak, 1990), in which only a subset of the neuronal population encodes

for the input to reduce the number of 'reduntant' active neurons (Kohn and Whitsel, 2002). Keeping the brain active costs energy and reducing the amount of the population activated by a stimulus has a beneficial effect on the energy metabolism. Inhibition has been described to be important not only in homeostatic regulation of activity, but also as an energy-conserving mechanism (Buzsaki et al., 2007) and although there is no direct evidence for sparse coding in the human somatosensory cortex, Gentet et al. (2010) suggest that sparse coding in rat barrel cortex is partially under influence of inhibition by GABAergic neurons.

These suggestions are consistent with experimental findings showing a shift and decrease in response pattern as a result of repetitive stimulation (Simons et al., 2007). In addition, Kelly and Folger (1999) showed with EEG that repetitive stimulation leads to a shift in peak activity location and discuss that a shift in location as found with EEG, reflect the optical imaging findings (earlier found by Tommerdahl et al., (1996). We have shown that repetitive stimulation results in a sustained evoked response at stimulus frequency, suggesting that the S1 neurons are driven at stimulus frequency. As a result of repetitive stimulation at a certain frequency, the network response also shows a sustained induced response in the mu-alpha and mu-beta band. The source of the mu-beta is thought to lie in cortico-cortical interactions (Jones et al., 2010). Modelling studies have shown that the mu-beta rhythm might originate through interactions between inhibitory neurons (Whittington et al., 2000; Jensen et al., 2005) whereas invasive studies have shown that the mu-beta rhythm may be generated by neuronal interactions through m-type potassium currents (a subthreshold potassium current) of deep- and

superficial layer excitatory and inhibitory neurons (Roopun et al., 2006; Kopell et al., 2010). The m-type potassium current is known to be modulated by repetitive stimulation and is thought to underlie changes in the responsivity of neuronal populations (O'Mara et al., 1988; Kohn and Whitsel, 2002) by changing the efficacy of afferent input to a neuron.

Kelly and Folger (1999) showed a shift in peak location after repetitive stimulation, but we used repetitive stimulation (in the form of an adapting stimulus in every trial) in both conditions. Therefore we were not able to compare an 'unadapted' to an 'adapted' condition and show such a shift in peak location. It is possible that the decrease in mu-beta power reflect a reduction in the active neuronal population. In addition to the response to an adapting stimulus, Cannestra et al., (1998b) showed with optical imaging that the response to the same stimulus ,after adaptation to that stimulus, is reduced from normal whereas a "novel" stimulus results in a normal pattern of activity. Desynchronisation in the mu-beta band reflects neuronal activity as a result of task-engagement and is thought to reflect increased pyramidal neuron excitability (Pfurtscheller et al., 2002; Neuper et al., 2006b). Our results described in Chapter 8 show that repetitive stimulation leads to less of a mu-beta ERD/ERS for the subsequent stimulus and we may conclude that the neuronal population in S1 becomes less active as a result of adaptation. Although the functional role of the mu-beta is still unclear, it is thought to be involved in integrating prior and ongoing network activity and recent studies have suggested a role of mu-beta activity in temporal integration via cortico-cortical loops (Jones et al., 2010; Kopbell et al., 2010). We suggest that repetitive stimulation leads to

temporal integration of the adapting stimulus, resulting in dynamical changes in the properties of S1 neurons, focusing on the adapting stimulus frequency. As suggested by previous studies (Kohn and Whitsel, 2007; Moore et al., 2010) this could result in a reduction in the amount of active neurons and more efficient encoding of stimuli.

In addition, we also showed that the mu-beta band is modulated due to transient and sustained stimulation. This suggests that the mu-beta band is not just a mechanism involved in 'online monitoring' of sensory input, but that its dynamics are able to change depending on the input. In our experiment, the mu-beta band seems to reflect a state of network activity involved in the temporal integration of sensory input during and following stimulus-drive, and might involve monitoring and integration components.

Finally, Romo and Salinas (2003) suggest that in a frequency discrimination task, the first stimulus leaves a 'memory trace' within S1 that is used for comparison against the second stimulus (also suggested by Burton and Sinclair, 2000). Harris et al., (2001a; 2002) have shown that transcranial magnetic stimulation in S1, delivered during the pause between the stimuli in a frequency discrimination task, impairs performance, suggesting that there is a memory of the first stimulus present in S1. Despite not being a discrimination task, in chapter 8 we showed a sustained mu-beta desynchronisation, which may reflect a memory component of the adapting stimulus. Previous studies have suggested a role of the mu-beta rhythm in discrimination (Spitzer et al., 2010; Haegens et al., 2011b), but whilst not excluding that mu-beta is involved in discrimination, we suggest that the mu-beta rhythm is

affected by subsequent stimulation in a non-discrimination task, and therefore likely to have a major role in temporal integration, shaping the neuromagnetic response to incoming sensory stimuli.

9.3.2 GABA and tactile adaptation

In Chapter 7 we did not find a correlation between GABA and measures of adaptation. However, despite an absence of a correlation between GABA and measures of adaptation, it is likely that GABA is involved in adaptation. A role of GABA in the sharpening of neurons as a result of adaptation has been suggested by Favorov and Kelly (Favorov and Kelly, 1994b; Favorov and Kelly, 1996) and indeed, the response properties of some S1 neurons depend on GABA (McLaughlin and Juliano, 2005). In addition, a modelling study by Norman et al. (2006) showed that inhibition facilitates the selective focusing of neural activity. In addition, a GABAergic deficit is thought to underlie certain aspects of ASD and the effect of adaptation is absent in these subjects.

It remains unclear how *baseline* GABA concentration affects adaptation. As suggested in Chapter 7, it is likely that changes in GABAergic inhibitory efficacy underlie the changes in neuronal activity in S1 neurons due to repetitive stimulation (Kohn and Whitsel, 2002). As discussed in Chapter 7 & 8, whether more GABA leads to more inhibition and therefore a more focused response, or whether more GABA leads to a more rigid system leading to less ability to dynamically respond is currently unknown.

Invasive studies have shown that the efficacy of excitatory pyramidal cells is reduced but the efficacy of inhibitory interneurons increases after repetitive

stimulation (Markram et al., 1998; Kohn and Whitsel, 2002). Kohn and Whitsel (2002) suggest that the "relative strength of excitation and inhibition in a cortical circuit would be expected to change" and a number of studies have demonstrated the importance of the balance between excitation and inhibition in the production of neuronal synchrony (Haider et al, 2006; Gentet et al., 2010; Moore et al. 2010). It is possible that baseline GABA concentration is an indication of the ability of S1 neurons to 'tune' their responses to a certain frequency by increasing coding efficiency to that stimulus and inhibiting the efficiency of encoding 'other' stimuli. As proposed above, repetitive stimulation may result in a dynamical change in cortical activity. By inhibiting the efficiency of neuronal activity that does not encode for the adapting stimulus, a decrease in activity and an increase in sensitivity is expected. Inhibition of redundant activity would lead to a sharper effect of adaptation on the spatiotemporal activity pattern and therefore better discrimination (O'Mara et al., 1988; Mclaughlin and Juliano, 2005) and spill-over of GABA in the synaptic cleft, leading to activation of GABA-B receptors, could modulate subsequent activity (Kohn and Whitsel, 2002).

Moore et al. (2010) discuss how GABAergic inhibition works to affect the efficiency of sensory encoding by creating a window of opportunity – a time-window that determines whether a sensory stimulus is encoded efficiently (Bacci and Huguenard, 2006). As adaptation changes the balance between excitation and inhibition, it might be possible that an increase in GABAergic efficacy affects this window and alters the encoding efficiency by allowing only encoding for the 'tuned' response (25 Hz).

Our measures of GABA concentration are coarse and may reflect the efficacy of normal cortical function as discussed in Section 6.4. Other studies have shown a correlation between GABAergic mechanisms and gamma-frequency (e.g. Muthukumaraswamy et al., 2009) and beta-power (e.g. Jensen et al., 2005; Gaetz et al., 2011) as found by MEG. Whereas a relation between GABA and mu-beta power has been shown, it remains unclear how the efficacy of GABAergic inhibition can explain a smaller mu-beta ERD/ERS due to adaptation. The mu-beta rhythm has been linked to GABAergic mechanisms by both experimental (Roopun et al., 2006; Hall et al., 2010; Gaetz et al., 2011) and modelling work (Jensen et al., 2005; Jones et al., 2010, Vierling-Klaasen et al., 2010). Jensen et al., (2005) showed that administration of Diapam (a benzodiazepine) results in an increase in mu-beta power. Gaetz et al., (2011) showed a correlation between GABA and the increase in power in the mu-beta rebound (ERS). As the rebound is thought to be an indication of cortical inhibition after a period of activity, one could argue that more inhibition would also lead to a smaller desynchronisation in power *during* a stimulus by increased inhibition on excitatory neurons (also, e.g. Gentet et al., 2010). We have suggested that the GABA concentration found with MRS reflects a general but local inhibitory efficacy, and one would indeed expect a decrease in mu-beta ERD/ERS if GABAergic efficacy increases due to adaptation. This would lead to a shift in activity as shown by optical imaging and a reduction in the amount of activity in S1 (also suggested by Moore et al., 2010) so a decrease in beta ERD/ERS power as a result of repetitive stimulation might reflect a shift in cortical activity under influence of GABAergic mechanisms.

The mu-beta rhythm does not solely reflect inhibitory functioning and is thought to reflect excitatory and inhibitory interactions between thalamo-cortical and cortico-cortical processing. The probable mechanism by which GABA is involved in adaptation is complex, because GABA affects local connections and is involved in sharpening of a response but inhibition across a neural network limits the amount of flexibility (e.g. Calford and Tweedale, 1991b; Levy et al., 2002). Interestingly, Hall et al. (2010) showed that administration of zolpidem results in a *decrease* in mu-beta power, but that zolpiclone *increases* mu-beta power. They explain that zolpidem affects the GABA-A receptor α1 sub-unit specifically, whereas drugs such as zolpiclone and lorazepam (as used by Jensen et al., 2005) are non-selective GABA-A receptor drugs. These show that differences in inhibitory processing (i.e. by different GABAergic neurons; see; Thomson et al., 2000) can differentially affect the mu-beta rhythm and studies combining MEG with pharmacology, may provide a better look at the source of the mu-beta rhythm as well as understanding of the different levels of dynamic neuronal processing. And indeed, Gentet et al., (2010) and Moore et al., (2010) suggest that differences between inhibitory neurons (fast spiking versus non-fast spiking) might have functionally different roles in sensory processing and perception.

Interestingly, we did not find gamma activity in our experiments, possibly due to low signal-to-noise and single-digit stimulation. The role of the gamma rhythm encoding tactile information is unclear but is thought to have a role in sensory binding and higher-order processing in other modalities. Haegens et al. (2011) found a weak effect of gamma which was not visible in every trial, and gamma did

not reflect stimulus parameters as the mu-beta rhythm did. Gamma is thought to be under influence of GABAergic mechanisms as found by invasive studies (e.g. Whittington et al., 2000; Moore et al., 2010; Carlén et al., 2011) and MRS (Muthukumaraswamy et al., 2009; Edden et al., 2009). Moore et al. (2010) suggest that the gamma rhythm reflects selective sensory gating through FS-neuronal inhibition.

9.3.3 GABA and plasticity

The role of GABA in synaptic plasticity has been investigated in more detail. Dinse et al. (2003) showed that administration of the GABA-A receptor agonist Lorazepam, reduced tactile perceptual learning in a co-activation paradigm which is known to increase two-point discrimination under normal conditions, suggesting that more inhibition leads to a reduction in plastic changes. In addition, Froemke et al., (2007) showed that synaptic plasticity lead to a reduction in inhibition followed by an activity dependent increase in inhibition to rebalance inhibition and excitation (see also; Weiss et al., 2004; Floyer-Lea et al., 2006) for similar findings; see Buonomano and Merzenich, (1998), for a review). And it should be noted that contrast adaptation in the visual cortex does not seem to be dependent on GABA (DeBruyn and Bonds, 1986; Vidyasagar 1990). However, as discussed in Section 1.4, adaptation in this thesis is particularly interesting because it shows an enhancement of sensitivity and visual adaptation as described in deBruyn and Bonds may reflect a different mechanism. As described in Section 7.1.1.1, different mechanisms may underlie different aspects of adaptation in different cortical areas and in some synaptic depression or a general reduction in excitatory activity may

lead to non-responsiveness and a reduction in sensitivity as seen in V1, whereas input of GABAergic mechanism may contribute to an increase in sensitivity for the adapting stimulus, as seen both in tactile S1 and visual MT. Given the suggestion that flexibility of a system is more consistent with physical plastic changes rather than changes in network properties, and the evidence that more GABA leads to less temporal noise on a small-network level, we suggest that more GABA allows for more inhibition of non-essential information and a more focused response as a result of adaptation.

9.4 A summary of tactile frequency processing in S1

One of the aims of this thesis was to gain an understanding of the micro- and macro levels of somatosensory processing using different methods. Therefore an understanding of the results is difficult without additional evidence from both an invasive and behavioural nature. Our results suggest the following general mechanism for the sequence of events taking place in somatosensory processing: Sensory input is projected towards the contralateral somatosensory cortex through thalamocortical connections. The sensory input is reflected by an *evoked response* at stimulus frequency whereas underlying *cortical oscillations* perform task-related processed potentially unrelated to the stimulus characteristics. The mu-beta rhythm monitors the ongoing stimuli and the first stimulus is maintained in memory (as suggested in Romo and Salinas, 2003) although the cortical location is unknown , and is used to compare subsequent stimuli.

In a frequency discrimination task, two different stimuli are reflected in different patterns of neuronal activity. Whether these two stimuli can be distinguished is dependent on the amount of noise in the neuronal representation. Individuals with more GABA in the primary somatosensory cortex have less noise in the neuronal representation of different frequencies and are therefore better at discrimination.

In an adaptation task, the system is repeatedly driven at the frequency of the adapting stimulus, which is reflected in the evoked response of S1. The induced response integrates prior and ongoing network activity. Because the network is continuously driven at a certain frequency, the network dynamically changes its network properties to the adapting stimulus frequency under influence of GABA. This focuses the network to a particular frequency and enhances temporal acuity, which might underlie the improvement in discrimination ability. When the following stimulus is always 25 Hz, the number of active neurons is reduced to reduce the amount of 'redundant' activity as well as to sharpen the network response to that particular frequency, possibly under influence of GABAergic inhibition. This is reflected by a smaller mu-beta ERD/ERS.

9.5 Experimental conclusions and limitations

In this thesis, three different techniques were applied to the study of somatosensory processing. The combination of these techniques is a novel approach to understanding sensory processing in humans *in vivo*.

9.5.1 Behaviour

We have adapted the paradigm used by Goble & Hollins to induce adaptation to 25 Hz. Our simplified paradigm was successful in inducing adaptation, but it was too complex to elicit measureable changes in neuromagnetic activity with MEG. Repetitive stimulation lead to measurable MEG activity, but we were not able to measure task-related changes due to adaptation for the test-stimuli. For future studies it is important to realise the importance of simple behavioural tasks for MEG recordings. Whereas behavioural measures obtained outside of scanners (as performed in Chapter 6) can be useful to the understanding of the nature of these tasks, within a scan-session the experiment is limited by time constraints. Our psychophysical studies are not as thorough as earlier psychophysical studies and we did not perform the repeats of measurements of thresholds as have been previously used. Conversely, we used more participants than many psychophysical studies, and used participants naive to the experimental stimuli. The fact that we find a correlation between GABA concentration and behaviour suggest that we are measuring individual differences as they are expressed in the general population. This result may not hold in highly trained subjects as additional factors such as training might provide enough top-down influence to overcome any advantage conferred by differences in GABAergic processing across participants.

9.5.2 MEG

We have shown that we can measure task-related effects with MEG. The time-course of tactile processing can be measured with MEG and MEG is sensitive enough to detect small changes in power in functionally distinct frequency bands as

a result of single-digit stimulation. However, throughout the course of this thesis, we ran into several experimental limitations with MEG. The adaptation task by Goble and Hollins is complex in nature and we did not acquire a sufficient number of trials to obtain a good SNR to analyse the standard and comparison stimuli; the adapting stimuli were both in the flutter range and may not have shown a measurable effect on the neuromagnetic activity, and due to a large number of different stimulus occurrences in the frequency discrimination task, the power for different stimuli was insufficient to result in measurable and meaningful neuromagnetic activity. Therefore this task was simplified for Chapter 8, different stimulus frequencies were used and the possible amount of different stimuli was reduced.

9.5.3 MRS

GABA-MRS is a relatively novel technique and has been shown to be useful for understanding the role of baseline GABA concentration (Gusnard and Raichle, 2001). We have investigated and improved the methods for edited GABA-MRS of sensorimotor cortex. We explored the possibility of acquiring data from a smaller voxel region, but did not feel this optimised our scan-time. Due to experimental and anatomical limitations it was not possible to acquire GABA-MRS data from a specific somatosensory region. MRS is a useful tool in determining the role of GABA in neuronal processing. MRS is applicable to a range of disciplines and might be used to investigate individual differences, but also to detect changes in GABA concentration as a result of disease, stimulation or pharmacological manipulation. It is important that this technique goes toward general methods of analysis and that

the limitations of the technique are understood (see Puts and Edden, 2011, for a review).

9.6 Future directions

9.6.1 Tactile processing

9.6.1.1 Advanced experimental and analysis methods for the current study

As discussed in detail, our implementation of the adaptation task by Goble and Hollins was not optimal for a relatively short-duration MEG scan and the amount of trials for the test stimuli was not sufficient for further analysis. However, in an optimal situation with enough trials, one approach to investigate discrimination with MEG would be to compare differences in neuromagnetic activity between correct and incorrect trials. Because the comparison stimulus is at threshold, it is possible that the standard and comparison stimulus are reflected by the same activity in incorrect trials, and by differences in beta-band activity in correct trials as shown by Haegens et al., (2011). Studies have shown that neuronal dynamics can change on a millisecond scale (Moore et al., 2010), but we measure the effect of adaptation by averaging over a 20 minute exposure. If the number of trials were not limited (by subject comfort) another approach is to compare the first *n* trials against the last *n* trials, to explore the time-course of adaptation with MEG and to investigate whether the effect of adaptation, as studied with MEG, is visible only after a certain duration or whether it presents itself after a relatively short duration.

Our studies have focused on the dynamics of the primary somatosensory cortex, but somatosensory processing occurs in a multitude of different regions. Not only

does S1 project to S2, posterior association areas and frontal regions, it is also affected by ipsilateral S1 and an effect of ipsilateral activity on discrimination performance has been shown previously (Haegens et al., 2010). S2 processing has been investigated with both invasive and noninvasive methods. Several studies have shown a differential response for S1 and S2 with respect to attention (Johansen-Berg and Lloyd, 2000; Bauer et al., 2006b; Dockstader et al., 2010) and analysis of S2 has shown that it has a role in integration of tactile stimuli (Villringer et al., 2009). Further analysis of S2 would explore the role of S2 in discrimination and adaptation, as well as exploring how S1 and S2 are related in our tasks.

9.6.1.2 The neural coding of Flutter-Vibration

As discussed in detail in section 9.2.1, it remains unclear how flutter is encoded by S1, it is apparent that population dynamics are involved in behaviour, although a number of studies have shown that single-neuron recordings can explain differences in discrimination ability (Houweling and Brecht, 2008). From a number of perspectives it is clear that the integration of multiple sources is a complex task involving many different processes. Reed et al. (2008) showed that a response to a single-digit stimulus results in a pattern of activity representing the entire hand-area. Among others, Favorov and Kelly (1994b) have suggested that discrimination is performed on the basis of spatiotemporal activity (also; Dykes et al., 1984). Furthermore, Moore et al., (2010) among others, have suggested that multiple processes involving different aspects of GABAergic inhibition, influence different aspects of sensory processing and integration. Understanding of different aspects

on a population level (such as MEG and LFP recordings) may provide an insight as to how different mechanisms are involved (see 9.3.2).

The effect of 180 Hz adapting stimulus on subsequent flutter or vibration probes was not investigated for this thesis. As described in Sections 1.3 and 8.1, vibration is differentially processed from flutter. Repetitive stimulation to vibration leads to a depression of neuronal activity as well as a loss of perception of the stimulus. In addition, the role of S1 in vibration processing is not clear. Our results have shown that the S1 location is the same for flutter and vibration and that the induced response does not differ for the two different types of cutaneous stimuli. In addition, Tommerdahl et al., (2005b) have shown effects of adaptation in both the vibration and flutter range. By investigating this task using MEG, it is possible to determine the role of S1 in vibration processing from the level of population dynamics. Several theories suggest that whereas S1 processes vibration and vibration has an effect on the processing of flutter, the primary locus for vibration processing lies elsewhere. Adaptation to vibration may result in a shift in peak location or the effect may not be visible in S1 but for instance in S2.

9.6.2 Understanding the GABA signal

9.6.2.1 Combined MEG/MRS

The role of GABA in adaptation is not well understood (see Section 9.2.2) but the mu-beta rhythm has been linked to GABAergic inhibition. In the experiment described in Chapter 8, all but one subject showed a decrease in the mu-beta ERD/ERS for the 25 Hz probe. It is possible that the absolute difference in mu-beta ERD/ERS between probes reflects the ability of the system to ‘tune’ to a certain

frequency. This ability could be limited to baseline GABA concentration. Whereas a correlation between GABA and behavioural effect of adaptation was absent, it is possible that the effect of adaptation as reflected by mu-beta power correlates with GABA. This would be the first finding showing that tactile stimulus-induced changes in cortical activity are driven by GABAergic inhibition, without inducing a change in GABA concentration. A different way of examining whether GABA is *also* involved in determining the flexibility of a system (as briefly discussed in Section 7.4) is by inducing adaptation to one stimulus, followed by inducing adaptation to a second stimulus. This will force the network to shift its' focus and might reflect flexibility of the system to shift as a result of external stimuli.

9.6.2.2 Regional correlations

Current studies have not yet shown any regional correlations in GABA concentrations and the current findings suggest a local role of GABA rather than a global effect. However, it is well known that some regions, such as bilateral primary somatosensory cortex, are anatomically and functionally connected (e.g. McGlone et al., 2006). Findings that show bilateral activity have also suggested a role of interhemispheric inhibition between bilateral primary somatosensory cortex (Tommerdahl et al., 2006; Haegens et al., 2010b; Haegens et al., 2011a; Reed et al., 2011) through a distributed S1 network. The functional relation between bilateral S1 is a possible target for the investigation of regional correlations. In a different study, parallel to this thesis, a strong positive correlation between right and left primary somatosensory cortex was found. This is an interesting effect and needs further investigation. In addition, acquiring basic MEG data on the mu-rhythm

during left- and right digit stimulation might shed a light on the relation between GABA and cortical oscillations in a study combining MEG and MRS.

9.6.2.3 GABAergic function and sensory processing in neuropsychiatric disorders

Research into several neuropsychiatric disorders has suggested links between abnormal sensory processing and impaired GABAergic processing; for instance Autistic Spectrum Disorder (Tannan et al., 2008; Tommerdahl et al., 2008) and Schizophrenia (Chang and Lenzenweger, 2005). Rather than a deficit to a single neurotransmitter system, these disorders have been linked to a multitude of causes and ranges of factors. By investigating the link between GABA and behaviour it might be possible to target specific features of the disorder, including sensory processing, by specifically modulating GABA. It is possible that GABAergic interventions may represent a future goal for drug development.

Summary and final conclusion

In this thesis we have shown it is possible to use non-invasive neuroimaging techniques to investigate the dynamics of somatosensory processing. On a human-level it is possible to investigate mechanisms on a macro-scale such as behaviour, cortical oscillations and cortical-area scale baseline GABA concentration. These measures may reflect known processes that have been investigated using invasive measures. It may not be possible to combine the investigation of the macro- and micro-level, but by probing a mechanism that has been investigated on a micro-level and investigating it on a macro-level, it is possible to investigate ongoing dynamic mechanisms and a common understanding of a system might be formed.

For instance, the functional role of task-related cortical oscillations measured with MEG can be explained by understanding the neuromagnetic correlates of such a task. In addition, understanding of the role of cortical oscillations may provide an understanding as to how these neuron-to-neuron interactions act in a larger cortical network (e.g. the finger area of S1). By probing baseline GABA levels it is possible to see how GABA acts on a network-level and this might be understood by understanding how GABA acts on a neuron-to-neuron level.

In this thesis we have shown that in a single set of experiments, the network from molecules to differences in individual behaviour can be probed using a combination of different techniques. It may not provide direct evidence as to how neuron-to-neuron interactions lead to differences in behaviour but by investigating different aspects of a system, comparing it to other findings, the interactions between the systems can be understood.

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Appendix A - List of Figures

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Chapter 3

Appendix B – List of Abbreviations

2AFC	Two alternative forced choice
ASA	Accelerated stochastic approximation
BOLD	Blood oxygenation level dependent
Cho	Choline
CNS	Central Nervous System
Cr	Creatine
CSF	Cerebrospinal fluid
CSI	Chemical shift imaging
CUBRIC	Cardiff University Brain Research Imaging Centre
EEG	Electroencephalography
EPSP	Excitatory post-synaptic potential
ERD	Event-related desynchronisation
ERF	Event-related field
ERP	Event-related potential
ERS	Event-related synchronisation
FSE	Fast-spin echo
GABA	γ-amino butyric acid
Glx	Glutamate/Glutamine/Glutathione complex
fMRI	Functional MRI
IPSP	Inhibitory post-synaptic potential
ISI	Inter-stimulus interval
ITI	Inter-trial interval
LFP	Local field potential
MEG	Magnetoencephalography

MEGA-PRESS	Mescher-Garber PRESS
MM	Macromolecule
MRI	Magnetic resonance imaging
MRS	Magnetic resonance Spectroscopy
MUA	Multi unit activity
NAA	N-acetyl aspartate
NMDA	N-methyl d-aspartate
NMR	Nuclear magnetic resonance
Occ	Occipital
PET	Positron emission tomography
PEST	Paired estimation by sequential testing
PPC	Posterior-parietal cortex
ppm	Parts per million
PRESS	Point Resolved Spectroscopy
PVA	Parietal ventral area
RAI	Rapidly adapting units type 1 (Meissner's corpuscles)
RAII	Rapidly adapting units type 2 (Pacinian corpuscles)
RF	Radiofrequency
S1	Primary somatosensory cortex
S2	Secondary somatosensory cortex
SAI	Slowly adapting units type 1
SAII	Slowly adapting units type 2
SAM	Synthetic aperture magnetometry
SM	Sensorimotor
SNR	Signal-to-noise
SQUID	Superconducting quantum interference device

SSR	Steady-state response
STEAM	Stimulated echo acquisition mode
T (e.g.3T/7T)	Tesla
TE	Echo time
TR	Repetition time
V1	Primary visual cortex
VPI	ventroposterior inferior nucleus of the thalamus
VPL	ventroposterior lateral nucleus of the thalamus
VPM	ventroposterior medial nucleus of the thalamus
VPS	ventroposterior superior nucleus of the thalamus

Appendix C - My Amazing Brain

I have a brain inside my head;

It tells me when to go to bed.

It tells me when it's time to eat;

It tells my heart how fast to beat.

I need my brain to act and think;

It tells me when I need a drink

I need my brain to hear and talk.

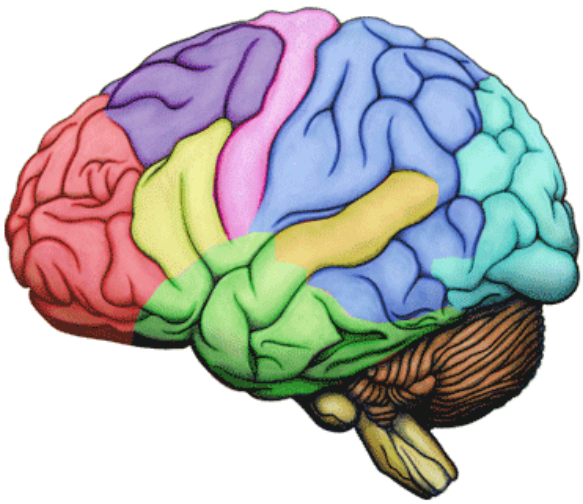
I need my brain to take a walk.

My brain never takes a rest;

It always has to be its best.

Even when it sleeps, it dreams.

I owe my brain a lot, it seems!



FOR MY PUBLIC ENGAGEMENT ACTIVITIES SEE http://tinyurl.com/7f95epl