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CORTICAL PLASTICITY IN RESPONSE TO MEDIAN NERVE TRAUMA

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Cortical plasticity in response to median nerve trauma

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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ABSTRACT

Median nerve injuries in adults, repaired with nerve suture, lead to incomplete functional recovery despite improved surgical technique. This results in a reduction in quality of life, poorer working ability and a considerable expense for society. Misrouting of axons at the suture site connects regenerating axons to the wrong distal end organs. When distorted signals are conveyed to the dorsal root ganglia, spinal cord, thalamus and the somatosensory cortex, somatotopic maps at all levels become reorganised in a disorderly fashion. Children often regain full sensory function after median nerve injury and repair despite impaired conduction across the injured segment. There is growing evidence that cortical plasticity is the main mechanism behind the superior recovery seen in young patients, but the exact pattern of reorganisation and its impact on functional recovery are not fully understood.

The general aim of this thesis was to investigate various aspects of cortical plasticity, in particular the response to median nerve injury. To this end we used two non-invasive brain imaging techniques, functional magnetic resonance imaging (fMRI) and magnetoencephalography (MEG). In Paper I we investigated the concept of audio-tactile interaction in a healthy population. We found an increased overlap between cortical activation areas (fMRI) in patients trained with coupled tactile and auditory stimuli indicating modulation of cortical plasticity induced by cross-modal training. In Paper II we studied age- and time-dependent effects on cortical activity patterns in patients with median nerve injury by correlating age at the time of injury and time passed since injury to sensory function, and cortical activation. We found a time-dependent decline in the size of the cortical activation area during stimulation of both the median and the ulnar nerve (fMRI). Furthermore, there was greater ipsilateral activation in the patient group than in a control group from a previous study. However, the results were not conclusive on this point because the stimulation paradigms differed between the two studies (event-related in the present and block paradigm in the previous study). Paper III was performed using MEG in order to further study cortical plasticity in patients with median nerve injury. We found decreased N1 and P1 amplitudes during stimulation of the injured median nerve, and an *increase* in these amplitudes during ulnar nerve stimulation. Paper IV was designed to reveal any possible differences in lateralisation of cortical activation after median nerve injury and to see if this was influenced by the stimulus paradigm used. By means of a laterality index (LI) the extent of contra- and ipsilateral activation was calculated. LI is decreased (more ipsilateral activation) in patients with a median nerve injury compared to controls. This means that median nerve injury causes a shift of activity from the contralateral to the ipsilateral SI. The type of stimulus paradigm (event-related or block) did not affect LI. Our findings add to the evolving knowledge of the cortical plasticity following median nerve injury.

LIST OF SCIENTIFIC PAPERS

I. Lundborg G., Björkman A., Hansson T., **Nylander L**., Nyman T., Rosén B. Artificial sensibility of the hand based on cortical audiotactile interaction: a study using functional magnetic resonance imaging. Scandinavian journal of plastic and reconstructive surgery and hand surgery. 2005; 39:370-372

II. **Fornander L**., Nyman T., Hansson T., Ragnehed M., Brismar T. Age- and time-dependent effects on functional outcome and cortical activation pattern in patients with median nerve injury: a functional magnetic resonance imaging study. Journal of Neurosurgery. 2010; 113, 122-128

III. **Fornander L**, Brismar T, Hansson T, Wikström H. Cortical plasticity in patients with median nerve lesions studied with MEG. Somatosensory and Motor Research. 2016; 20, 1-8

IV. **Fornander L**., Nyman T, Hansson T, Brismar T, Engström M. Interhemispheric plasticity in patients with median nerve injury. Neuroscience Letters. 2016; 15: 628:59-66

CONTENTS

LIST OF ABBREVIATIONS

fMRI	Functional Magnetic Resonance Imaging
BOLD	Blood Oxygenation Level Dependent
MEG	Magnetoencephalography
EEG	Electroencephalography
TMS	Transcrainal Magnetic Stimulation
DTI	Diffusion Tensor Imaging
2-pd	Two-point discrimination
LTM	Low Threshold Mechanoreceptors
LTP	Long Term Potentiation
LTD	Long Term Depression
SI	Primary somatosensory cortex
SII	Secondary somatosensory cortex
SEF	Somatosensory Evoked Field
ECD	Equivalent Current Dipole

1 INTRODUCTION

1.1 Nerve injuries of the hand

1.1.1 History

As early as the middle ages, surgeons attempted to suture injured nerves. However nerve repair to restore function was not generally accepted until the late 1800s [30]. The epineural suture was first described in 1873, but refined techniqes and better results came with the experiences gained from nerve injuries and repair during World Wars I and II. Techniques were further improved by the introduction of the operating microscope in the 1970s [31]. During the second half of the 20th Century research focused on the cells and growth factors involved in nerve regeneration, attempting to develop trophic factors that could enhance peripheral regeneration but without success. Despite extensive research on peripheral regeneration and improved techniques of adaptation the results of sensory recovery are depressing and the need for further research and development of rehabilitation strategies remains.

1.1.2 Epidemiology

Injuries of the hand and wrist account for 1/3 of the injuries admitted to emergency departments [2, 3]. Peripheral nerve injuries associated with hand trauma constitute a challenging problem because of the permanent effects on the patient's life due to disappointing recovery of motor and sensory function [32]. Median and ulnar nerves are the most commonly affected nerves [14] and the most frequent mechanism of injury is sharp cut nerve laceration with glass or a knife [14, 56, 68]. Most injuries occur during leisure activities with the dominant and non-dominant hands being equally affected. Peripheral nerve injury in the hand is especially prevalent in the male working age population [45, 68] causing substantial losses to society due to the inability of these patients to work.

1.1.3 Costs and employment

In Sweden the cost of a median nerve injury was calculated to be around 51238 Eur of which 87% sick leave (median 210 days) [68]. Bruyns and coworkers reported an average time off work of 24.2 weeks for median nerve injures and 30.7 weeks for ulnar nerve injuries. None of

the patients with combined median and ulnar nerve injuries had returned to work after one year [14]. Twenty-four percent were not able return to their former employment [45]. Patients with loss of grip strength, loss of pinch strength and poor sensory recovery seldom return to work within one year [14].

1.1.4 Hand function

Nerve injuries of the hand in adults result in residual loss of function and sometimes pain. Recovery after nerve injury continues for up to five years after nerve repair [52], but sensory function in particular seldom recovers fully [32]. In a study of 220 patients with median and/or ulnar nerve injury, good sensory and motor recovery was achieved in 21% and 49% respectively [45]. For those patients affected, a median nerve injury often leads to reduced sensibility, grip function, dexterity and other related sequelae associated with hand function. Many patients are also affected psychologically and suffer from depression and posttraumatic stress disorder [19].

Bailey and coworkers reported a 48% reduction in leisure activities in a group of patients with a nerve injury in the upper extremity and a 30% reduction in household activities. The rate of depression was 16%, this being correlated to loss of activity and pain [3]. A correlation with poor functional outcome, as measured with the SF-36 (Short form 36 health survey) and DASH (Disabilities of the Arm, Shoulder and Hand) and chronic neuropathic pain after nerve injury in the upper extremity, has also been reported [61].

1.1.5 Prognostic factors

In a prospective study on 61 patients with median and/or ulnar nerve injury in the forearm gender, age, level of education, number of injured structures, localisation of nerve injury, type of nerve and injury and posttraumatic stress at 1 and 3 months were presented as strong prognostic factors for functional outcome [43]. In a meta-analysis of predictors for motor and sensory recovery, the strongest predictors for motor function were age, site (proximal-distal), type of nerve and surgical delay. Age and delay predicted sensory outcome [75].

Many studies have shown excellent functional recovery in patients injured in childhood [18, 29]. There is, however, no correlation between functional recovery and the results of electroneurography testing [17]. Instead differences in clinical outcome following a median nerve injury may possibly be explained by cerebral plasticity [20].

1.1.6 Surgical techniques

Primary epineural suture is recommended for sharp laceration median nerve injuries explored within 5-7 days after injury. Proper alignment of fascicles can be aided by the localisation of surface vessels. However, group fascicular repair requiring intraneural dissection should not be performed. To avoid tension at the suture site, the wrist and fingers should be moderately flexed only. If this is not sufficient, another repair technique should be used. Gap bridging techniques include the use of nerve autografts, usually sural nerve grafts, collagen conduits and decellularised nerve allografts to provide support for the regenerating axons across the gap [64]. Although complications after direct nerve suture are unusual [84] nerve grafting may be associated with the formation of a neuroma because of failure of regenerating axons to enter the graft [55]. There are no differences between the results of primary microsurgical suture and collagen tubulisation for injuries with gaps less than 6 mm [11], nor between the use of collagen tubes or autografts in median nerve injuries with gaps less than 5 cm [91]. For larger gap injuries, however, gap-bridging techniques must be used. The well-established method of allografting has the disadvantage of donor site morbidity and this has driven the development of processed allografts. There is yet insufficient data concerning mixed nerve injuries regarding the choice of gap-bridging technique. Brooks and coworkers have shown 75% meaningful recovery following repair of median nerve injuries with an average gap of 30 mm using allografts from processed donated human nerves [12].

1.2 Anatomy and physiology

1.2.1 Peripheral nerve conduction

When touching an object, low threshold mechanoreceptors (LTM) of the skin are activated. There are four different types of receptors in the glabrous skin: Meissner, Merkel, Pacini and Ruffini corpuscles.

They are divided into fast or slowly adapting receptors, and are all specialised in different aspects of perception and recognition of tactile stimuli. Merkel cells are located in the basal layer of the epidermis. They have small receptive fields and are classified as slowly adapting receptors, *i.e.* they continue to fire throughout a mechanical stimulus. The Meissner corpuscles are located on the dermal-epidermal border and are fast adapting, *i.e.* they respond to initiation and termination of mechanical stimuli. Pacini (fast adapting) and Ruffini (slowly adapting) receptors are situated deeper in the skin and have large fields of reception.

Deformation of LTMs changes the ionic permeability of the receptor membrane, which in turn depolarises nerve endings and generates a receptor potential.

An action potential is triggered and propagated along the axons in the median, ulnar or radial nerve, to the cell body of the first-order neuron, located in the dorsal root ganglia of that specific spinal nerve. The central axons enter the spinal cord via the dorsal roots and ascend ipsilaterally in the dorsal column to couple with neurons of the cuneate nuclei in the lower medulla. These second-order neurons send axonal projections across the midline to form the medial lemniscus tract. Axons then project contralaterally to reach to the ventro-posterior lateral (VPL) nucleus of the thalamus. Axons of the third-order neurons located in the VPL convey signals from the thalamus to the cortical neurons of the primary somatosensory cortex (SI).

1.2.2 Cortical processing

The primary somatosensory cortex is divided into four discrete regions; Brodmann Areas 1, 2, 3a and 3b. Area 3b is thought to respond to simple tactile stimuli and Area 1 to tactile stimulation having a specific direction. Area 2 neurons process both tactile and proprioceptive information and specialise in shape recognition and finger coordination, whereas Area 3a responds mainly to stimulation of proprioception receptors. Each of these areas has a somatotopic map with complete representation of the body, where regions of the body with greater receptor density, *i.e.* the hand and face, are assigned a greater area of representation. Areas 1 and 3 contain maps of the contralateral side of the body whereas Area 2 seems to represent both the right and left side of the body [44].

The various SI regions are intrinsically interconnected, and there are projections from the SI to several other higher-order cortical areas. The secondary somatosensory area (SII) is situated in the upper bank of the lateral sulcus just below the SI. The SII receives input from the ipsilateral VPL and SI, and projects to the contralateral SI and SII. The function of the SII is not yet fully understood, but it is thought to be involved in sensorimotor integration, tactile learning and memory.

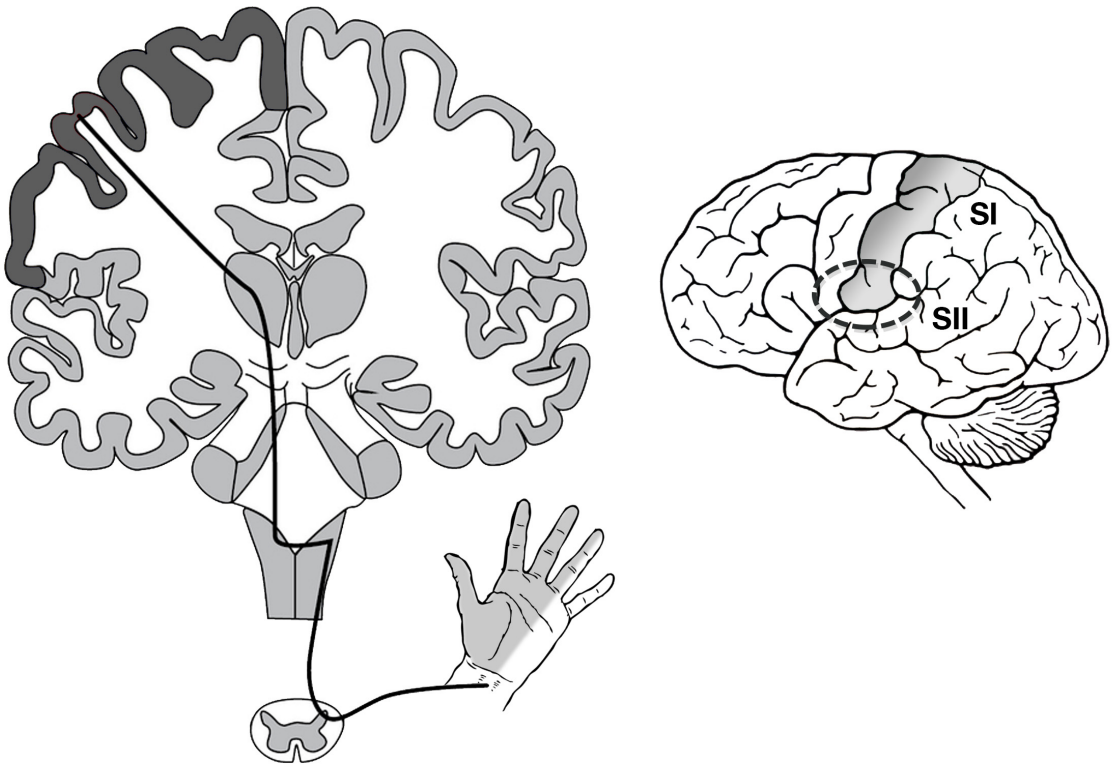

Figure 1. Left: the somatosensory pathway from the innervation area of the median nerve (shaded) to the median nerve representational area in the SI (dark grey). Right: location of the SI (shaded) at the postcentral gyrus and the SII at the upper bank of the lateral sulcus (striated).

1.3 Nerve regeneration

1.3.1 Peripheral regeneration

A peripheral nerve injury affects all levels of the peripheral and central nervous system. Wallerian degeneration develops within a week following transection of a nerve. This process breaks down myelin and axons in the distal nerve stump to facilitate ingrowth of regenerating axons. The breakdown of the axonal cytoskeleton begins with an inflow of extracellular ions, primarily Ca^{2+} and Na^{+}, which triggers a cascade of events. Macrophages migrate to the area, clearing the myelin debris [81] and Schwann cells de-differentate and proliferate to form tubes known as the bands of Bügner and produce molecules that promote axonal growth [21, 66].

Axonal transection also leads to axonal loss at the proximal nerve end caused by neuronal apoptosis, and there may be 20-50% loss of cells in the dorsal root ganglion [1]. The

axotomised neurons undergo morphological change from a transmitting state to a growth mode and axons begin to sprout [83]. At the distal tip of the sprouting axons a growth cone is formed that interacts with activated Schwann cells promoting regeneration by the release of neurotrophic factors [58]. There is always misdirection of axons at the site of section and repair with sensory axons growing into the Schwann cell tubes of motor nerves and vice versa [90].

1.3.2 Brain plasticity

Most early studies on plasticity were performed on non-human primates. They showed that at the representational area of the median nerve in the cortex, a peripheral median nerve injury leads to a silent "black hole" that is almost immediately invaded by adjacent areas. Within one week the cortical territory of the median nerve is completely occupied by the area of the radial and the ulnar nerve [59]. Similar changes have also been seen in the ventroposterior lateral nucleus (VPL) of the thalamus [33]. If the median nerve does not regenerate, this reorganisation remains. However, if the nerve is repaired, further remodeling will occur. Due to misdirection at the suture site the axons will now innervate skin areas that differ from the original ones and when the nerve regenerates, it will reactivate parts of its former territory but the somatotopic disorganisation remains [33]

Human brain mapping studies are in agreement with the studies on primates. Patients with median nerve compression, *i.e.* carpal tunnel syndrome, display a smaller interdigit distance between Digits III and IV in the cortex *i.e.* between median and ulnar nerve territories indicating a greater overlap between the areas [25, 54]. Short-range plasticity is also found in patients with median or ulnar nerve injury and repair. Larger areas are activated in the contralateral somatosensory cortex following tactile stimulation of the injured and sutured nerve, but the degree of activation falls with progressive regeneration [29, 36].

Furthermore, plasticity is not restricted to the contralateral, deprived cortex, but also affects the ipsilateral hemisphere. Interhemispheric transfer of information between left and right SII and SI exists under normal conditions, but deafferentation of one hemisphere seems to have an impact on the interhemispheric balance between excitation and inhibition [15, 23, 88]. Deafferentation of a hemisphere by forepaw nerve transection in rats causes upregulation of interneuron activity resulting in increased activity in the ipsilateral hemisphere [65]. Increase in ipsilateral activity is also seen following stimulation of the intact extremity. It is suggested that the upregulated callosal connections might act to protect the denervated hemisphere from take-over by adjacent areas [92]. The finding of interhemispheric plasticity following

stimulation of injured and healthy nerve has been demonstrated in brain imaging studies of patients with median nerve injury [20, 29]. Increased ipsilateral activation volume is seen in patients above the age of 14 but preadolescent patients display normal activation patterns [20].

The process of plasticity is present throughout life though it declines with age [63]. Age has proved to be the strongest predictor for outcome after peripheral nerve injury [29, 75] and it has been hypothesised that this is due to the superior ability to adapt to changes in neural input in younger persons. This hypothesis is well supported by the finding of incomplete peripheral nerve regeneration in patients with median nerve injury, regardless of age and functional outcome [20].

Plasticity does not respect the boundaries of the sensory modality affected. There are many examples of cross-modal reorganisation of the brain. After deprivation of input to the auditory cortex in deafness, there is invasion from the visual cortex and enhanced vision has been described in deaf persons [39, 77]. In blind readers of braille, activation of the visual cortex is seen when performing a tactile discrimination task [57], and transcranial magnetic stimulation (TMS) of the visual cortex produced tactile sensations in the fingertips correlating to the number of hours spent reading Braille each day [67].

1.3.3 Mechanisms of plasticity

The mechanisms involved in plasticity are complex. During development, the formation of functional networks is dependent on experience-dependent strengthening and weakening of synapses referred to as long-term potentiation (LTP) [10] and long-term depression (LTD) [4]. These mechanisms are mediated by NMDA receptor activation, responding to rate and degree of postsynaptic depolarisation [40]. These long-term-plasticity mechanisms are also present in adults, and the classic example of use-dependent plasticity in adults is the string instrument player where cortical representational areas of the fingers are enlarged [26, 38].

Peripheral nerve injury is thought to evoke three main mechanisms of plasticity occurring over different time periods.

1. Immediately after injury: loss of inhibition of excitatory neurons causes **unmasking of already existing synapses**. In human studies of transient deafferentation by ischaemic anaesthesia or nerve block, cortical changes are found only minutes after deafferentation [9, 73]. The representational areas of different fingers seem to be interconnected so that sensory input from one finger normally

inhibits input from adjacent fingers. Finger deafferentation by anaesthetic blockade thus leads to loss of that inhibition allowing adjacent areas to spread [86, 87]. The explanation of this short-term plasticity is probably loss of GABAergic inhibition, suggested by the finding of decreased levels of GABA in the SI after deafferentation [50], but rapid unmasking of connections can also be caused by increased neurotransmitter release and increased density of postsynaptic receptors [22].

2. During the first month after nerve injury: removal of excitation and inhibition due to deafferentation allows for weaker or previously latent synapses to strengthen through NMDA receptor activation. The NMDA receptor increases inflow of Ca^{2+} into the neuron which augments synaptic strength. This causes residually strengthened connections and new patterns of activation through the process of **LTP** [60].
3. Long-term plasticity: involves the **growth of new connections** through sprouting of axons [46], but to what extent this occurs is not fully understood. In studies on amputees, widespread cortical reorganisation is observed and considered to be cortico-cortical sprouting [28]. The extension of connections over long distances, however, is unlikely and the explanation is probably found in reorganisation of the thalamus where short-distance changes can lead to large projections at the cortical level.

2 AIMS OF THE THESIS

The general aim of this thesis was to study changes in cortical activation patterns of patients with median nerve injury and repair.

Specific aims were:

- to inverstigate the phenomenon of audiotactile interaction and specifically explore the possibility of substituting tactile sensibility with hearing (Paper I)
- to explore how age at the time of injury and time passed since injury influences changes in cortical activity patterns and functional outcome (Paper II)
- to study how the MEG response in the SI correlates with the size of the BOLD signal, peripheral neurography readings, and with clinical outcome (Paper III)
- Paper II showed greater activation of the ipsilateral hemisphere in patients with median nerve injury compared to the healthy controls of an earlier study performed with a different stimulation paradigm. The aim of Paper IV was to investigate if median nerve injury influences the degree of ipsilateral activation, and if the type of fMRI paradigm (event-related or blocked) affects the degree of ipsilateral activation.

3 MATERIALS AND METHODS

3.1 Study outlines

3.1.1 Paper I

The phenomenon of audiotactile cortical interaction was investigated using fMRI in six healthy volunteers. The subject's right index finger was equipped with a miniature microphone that picked up the friction sound elicited by tactile stimulation of the finger pulp (sensor glove system [53]). Tactile stimuli were delivered to the volar aspect of the index finger by an air-driven brush. The auditory stimulus generated by the tactile stimulation was transposed to an earphone in the right ear, making it possible for the subject to ‘‘listen to the touch’’. Three subjects were trained with the equipment 15 minutes a day for one week and three subjects were not trained. The subjects were given either tactile stimulation, auditory stimulation or simultaneous tactile and auditory stimulation delivered according to a block-design paradigm with 30s of activity and 30 s at rest. fMRI was used to map activation in the somatosensory and auditory cortices in response to the three different sets of stimuli.

3.1.2 Paper II

Eleven patients with unilateral median nerve injury at the wrist were investigated with clinical sensory examination (2-pd and monofilaments) and fMRI. Tactile stimuli were delivered with an air-driven brush to the volar aspects of Digits II-III and IV-V of the injured and healthy hand, respectively. To perform the statistical analysis, we included eight subjects from a previous study [36] even though it had been performed with a slightly different stimulation paradigm. Activation ratios between the injured and healthy hands were calculated and correlated to sensory recovery, age at the time of injury and time passed since injury.

3.1.3 Paper III

MEG was used to examine cortical plasticity following median nerve injury. Nine patients were examined with MEG at the Biomag Laboratory, Helsinki University Hospital, Helsinki, Finland. The evoked electromagnetic cortical response was recorded during electrical stimulation of the median nerve at the wrist. Peripheral nerve regeneration was evaluated with electroneurography. Amplitudes, latencies and coordinates of the somatosensory evoked magnetic fields were described.

3.1.4 Paper IV

Paper IV was designed to further investigate activation in the ipsilateral hemisphere in patients with median nerve injury. Four patients who had had unilateral median nerve injury at the wrist repaired with an epineural suture were included. A group of ten healthy control subjects were examined for comparison. Tactile stimulation was delivered by an air-driven brush to Digits II-III and Digit V of both hands and the event-related fMRI signals studied. The group of healthy controls was studied both with block and event-related stimulation in order to evaluate if the stimulation paradigm affected the degree of ipsilateral activation. A laterality index (LI) was calculated to quantify the hemispheric distribution of activation.

3.2 Clinical sensory examination

3.2.1 Static Two-Point Discrimination Test

The static 2-point discrimination (2-pd) test measures multiple overlapping peripheral receptive fields and innervation density. 2-pd is measured using calipers with two blunt pins applied until blanching occurs, in a longitudinal direction perpendicular to the skin (Disk-Criminator, Baltimore, Maryland). The test instrument is randomly applied in two radial and two ulnar positions on the volar aspect of Digits II–V distal to the distal interphalangeal joint. Testing began with a gap of 5-mm between the pins of the caliper and the gap then increased or decreased according to the subject's response; normal (0–5 mm), fair (6–10 mm), poor (11–15 mm), protective (only one point perceived), and anaesthetic (no points perceived) according to the norm scale of the American Society for Surgery of the Hand. The threshold is defined as the minimum caliper gap the subject experiences as being two stimuli.

3.2.2 Touch and Pressure Threshold Test

Semmes-Weinstein nylon filaments were used for testing light touch and deep pressure thresholds. Each monofilament (1.65–6.65; Semmes-Weinstein Pressure Aes-thesiometer Kit, North Coast Medical Inc.) was applied perpendicular to the skin for approximately 1.5 seconds. The radial and ulnar side of the distal phalanx of each digit were tested with the following thresholds: normal (monofilament 1.65–2.83, 0.008–0.08 g); diminished light touch (monofilament 3.22–3.61, 0.172–0.217 g); diminished protective sensation (monofilament 3.84–4.31, 0.445–2.35 g); loss of protective sensation (monofilament 4.65–6.65, 4.19–279.4 g); and loss of deep pressure sensation (mono- filament > 6.65, > 279.4 g).

3.3 Neurophysiology

Electroneurography

Electroneurography is used to measure the signal amplitude and conduction velocity of a nerve. A percutaneous depolarising current is delivered via surface electrodes and the action potential is measured at different distances from the stimulus. The conduction velocity (m/s) is calculated from the measured latencies (ms) and distances (mm). The amplitude of the response is correlated to the number of transmitting axons and the latency is affected by the properties of the myelin sheath.

In Paper III sensory and motor nerve conduction velocity and action potential amplitudes were determined bilaterally in the median and ulnar nerves using standard techniques. All patients were examined in a warm room (21-23 °C) and warmed for at least 10 minutes prior to the examination with heating pads and blankets. Sensory conduction velocity (CV) and amplitude were recorded with surface electrodes placed at the wrist proximal to the scar. The median nerve was stimulated with ring electrodes on Digit III and the ulnar nerve on Digit V.

3.4 Brain imaging

3.4.1 fMRI

fMRI is a non-invasive brain imaging technique that measures the haemodynamic response to brain activity. The most common way of performing an fMRI experiment is to deliver a stimulus and thereafter map the subsequent cortical response. Increased firing of cortical neurons in a specific area increases the blood flow to that area. Oxygenated blood is delivered in excess of the metabolic requirement and there is a net increase in oxygenated haemoglobin. Haemoglobin is diamagnetic when oxygenated and paramagnetic when deoxygenated, and the presence of paramagnetic deoxyhaemoglobin produces magnetic field distortions that are detected by the MR-scanner. This is called the Blood Oxygenated Level-Dependent effect (BOLD effect) [62] [48].

In the early days of fMRI the BOLD effect was considered a measurement of neuronal activity. However, nowadays it is agreed that it is more a measurement of the haemodynamic response triggered by neuronal activity. The BOLD response is not only affected by neuronal activity itself but also by neurovascular coupling, *i.e.* the relationship between neural signaling and blood flow [35]. The complex signaling mechanisms of neurovascular coupling are not completely understood, but recent studies propose a model of interplay between astrocytes, pericytes, interneurons and blood vessels [41].

There is a physiological delay of several seconds from the stimulus to the peak haemodynamic response and return to baseline, which limits temporal resolution. However, the haemodynamic response has a tight spatial coupling to neuronal activity and the limits of spatial resolution are generally the performance of the MRI-equipment itself.

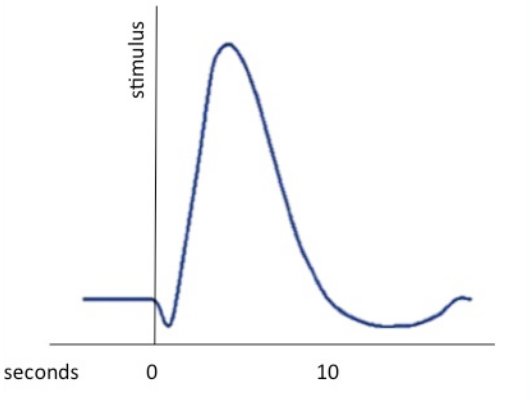

Figure 2. Time scale of the BOLD response curve

In Papers I, II and IV tactile stimulation of the median and ulnar nerves was applied by means of a pneumatic brush. Stimuli were applied to the glabrous skin over Digits II–III, IV–V (Paper II) and V (Paper IV), respectively. Brush strokes were delivered according to an event-related paradigm (Paper II and IV) or block paradigm (Paper I and IV). In the event-related paradigm the stimuli are delivered as single brushstrokes. The event-related paradigm consisted of a single brushstroke every 20 seconds (Paper II) or in a series with pre-randomised inter-stimuli intervals of 14, 16, 18 or 20 seconds (Paper IV). The block paradigm comprises blocks of repeated stimulations (30 seconds of 1Hz brush strokes) alternating with blocks at rest (30 seconds).

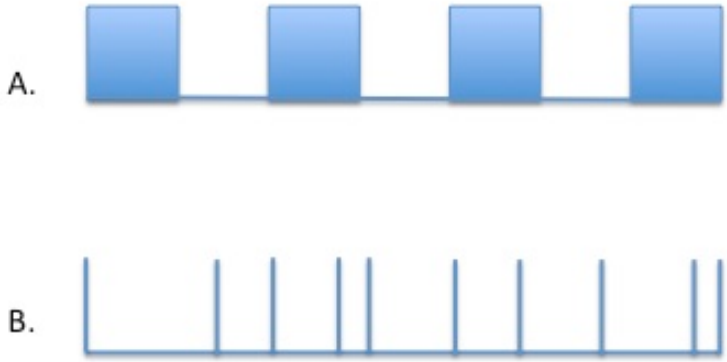

Figure 3. Stimulation paradigms

A: block paradigm with 30 seconds of activity (1Hz brush strokes) and 30 seconds of rest.

B: event-related paradigm with single brushstrokes delivered in a prerandomised sequence with varying inter-stimuli intervals.

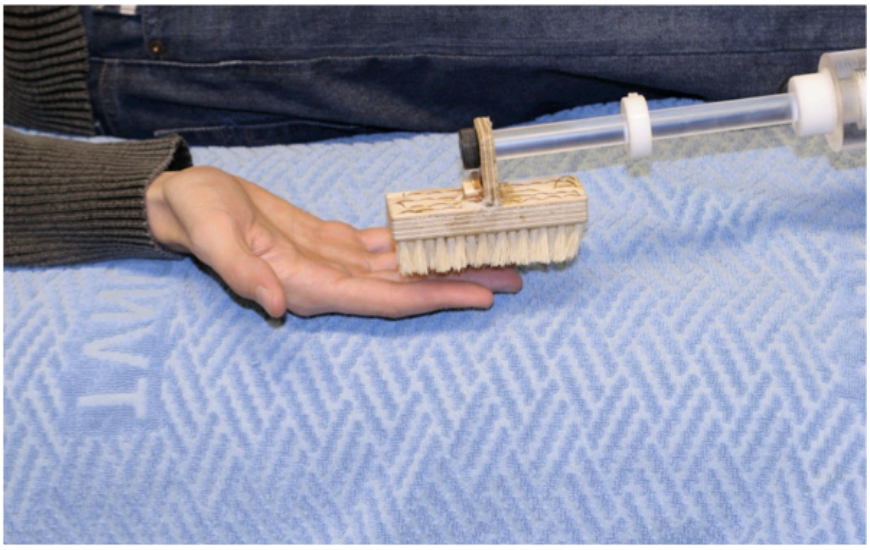

Figure 4. The pneumatic brush used for tactile stimulation in the fMRI experiments.

3.4.2 MEG

Electric current flowing through a conductor produces a magnetic field. MEG measures the somatosensory-evoked fields (SEFs) produced by postsynaptic currents in the cortex and projected on the surface of the scalp. Determination of the location of the source of the electric activity is called the inverse problem. Because there is an infinite number of primary currents capable of producing similar externally measured field distributions, a source model must be used to interpret the MEG data. The current dipole is a commonly used source model that is suitable for situations where neuronal activation is supposed to occur in a small

cortical area, for example the hand representational area of the SI. It is assumed that the centre of gravity of the activated brain area can be modelled by a point-like electric dipole and that the brain is modelled as a sphere. The least-squares method is applied to estimate the magnitude, direction and position of the equivalent current dipole (ECD) that best agrees with the data.

The MEG experiments (Paper III) were performed in a magnetically shielded room with a helmet-shaped sensor array consisting of 306 detectors: 204 planar gradiometers and 102 magnetometers (Elekta, Neuromag®, Elekta Oy, Helsinki, Finland). Before the measurement session electrodes for the detection of electro-oculograms (EOGs) were placed above and below the right eye and on the right and left temples. In addition, position indication coils were placed behind both ears. The positions of the coils were determined according to anatomical landmarks on the skull using a three-dimensional digitizer (Polhemus) to construct an individual Cartesian coordinate system. In this system, the pre-auricular points determined the x-axis, to which the y-axis was perpendicular pointing towards the nasion, and the z-axis was perpendicular to these pointing upwards. Before each measurement session a current was passed through the coils to determine their positions within the detector helmet.

Stimulation with non-painful electrical pulses (duration 0.2 ms) was delivered at the wrist with an inter-stimuli interval of 3 s. The position and the strength of the stimulus were adjusted to produce a non-painful twitch in Digit I (median nerve) or Digit V (ulnar nerve), respectively. The SEFs were averaged online using a band pass of 0.03-260 Hz and responses containing electro-oculography (EOG) artifacts exceeding 150 μV were rejected online. At least 100 responses were gathered at each recording session.

3.4.2.1 SEF-waveforms

A typical SEF waveform over the contralateral SI in response to electrical stimulation of the median nerve is composed of an initial activation peak 20 ms after stimulation (N20) followed by a peak of opposite polarity at 30-40 ms (P35), a 45 ms-peak (N45) and a peak at 60 ms (P60).

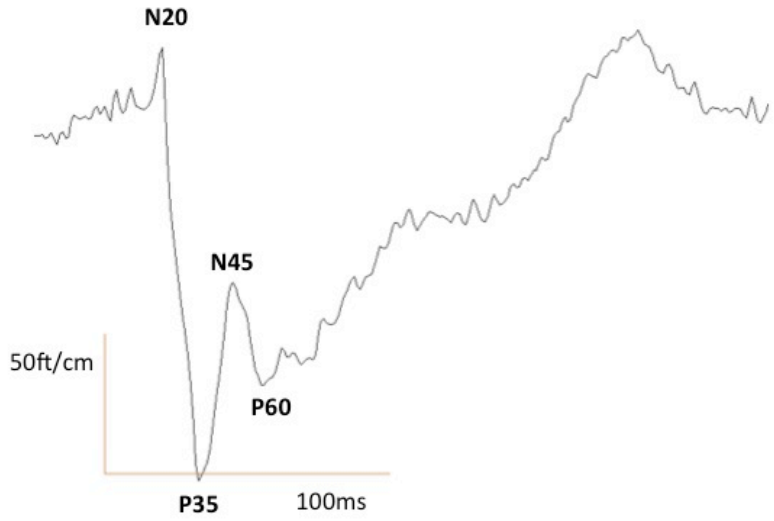

Figure 5. Typical SEF waveform over the contralateral SI in response to electrical stimulation of the median nerve.

4 SUMMARY OF RESULTS

For more details the reader is referred to the Results sections of Papers I-IV. The following is a summary of the results.

4.1 Paper I

The impact of training on audiotactile interaction was explored. Tactile stimulation activated the contralateral somatosensory cortex in all subjects. Simultaneous tactile - auditory stimulation activated the contralateral somatosensory and auditory cortex in all subjects. Auditory stimulation alone activated only the auditory cortex in the untrained subjects, whereas in all trained subjects it activated areas in both the auditory and the somatosensory cortices.

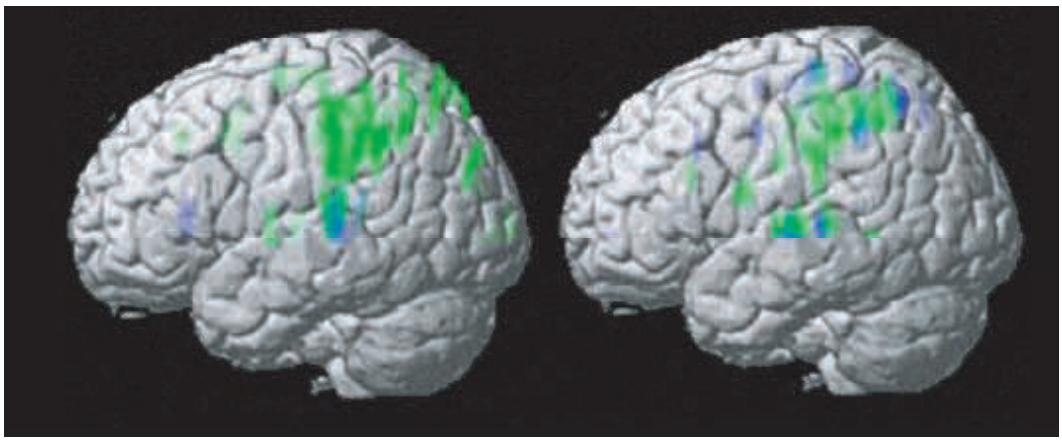

Figure 6. Activity in response to tactile stimulation of Digit II (green) and to auditory stimulation (blue). Left: untrained subject. Right: trained subject with overlap in cortical activation.

4.2 Paper II

Eleven patients with a median nerve injury at the wrist were examined with fMRI. Eight patients from a previous study [36] were included in the statistical analysis. Two-point discrimination was decreased in all patients. The strongest predictor of sensory outcome was age at the time of injury ($p < 0.0048$). Activation ratios were calculated from the quota of activated voxels in the contralateral hemisphere in response to stimulation of the injured and the healthy hand. The activation ratio for stimulation of Digits II-III was positively correlated to time passed since injury ($p < 0.041$). The activation ratio for stimulation of

Digits IV-V correlated to both age at the time of injury ($p < 0.048$) and time passed since injury ($p < 0.033$). The ratio of contalateral and ipsilateral activation to stimulation of Digits II-III was 0.55 for the injured hand and 0.66 for the healthy hand.

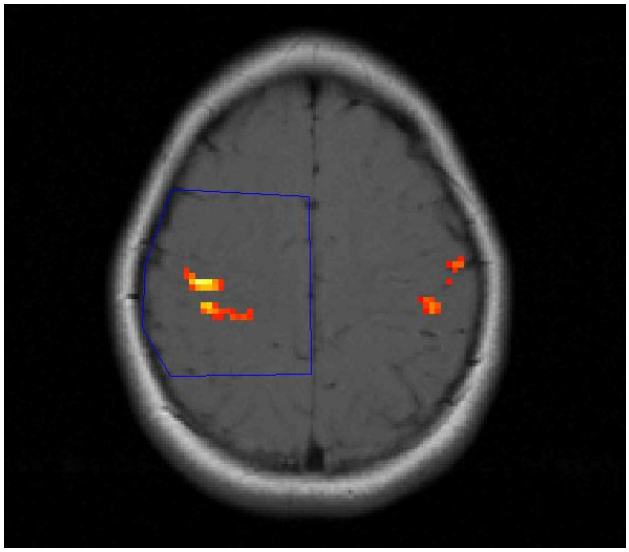

Figure 7. Cortical activity in response to median nerve stimulation of the right hand in one of the patients and the contralateral ROI used for analysis (blue).

4.3 Paper III

MEG was used to map cortical activity in response to electrical stimulation of the median and ulnar nerves of nine patients with median nerve injury at the wrist. The N1 and P1 responses to stimulation of the injured median nerve at the wrist were lower in amplitude compared to the healthy side ($p<0.04$). Stimulation of the ulnar nerve of the injured hand produced a response with a *larger* N1 amplitude compared to stimulation of the ulnar nerve of the healthy hand ($p<0.04$). There was no correlation between N1 amplitude and degree of cortical activation in fMRI.

4.4 Paper IV

Four patients with median nerve injury at the wrist and ten healthy volunteers were examined with tactile stimulation and fMRI. A laterality index (LI) was calculated to further investigate the distribution of activity between the contra- and ipsilateral hemispheres. Stimulation of Digits II-III resulted in less contralateral activation in patients compared to controls. The patients had a lower LI (0.21 ± 0.15) compared to the healthy

controls (0.60 ± 0.26) indicating augmented activation of the ipsilateral primary somatosensory cortex.

The possible effect of the stimulation paradigm used on the degree of ipsilateral activation recorded was tested in a separate set of experiments on the healthy controls. We found no difference in LI obtained from recordings using the event-related paradigm and those using the block stimulus paradigm.

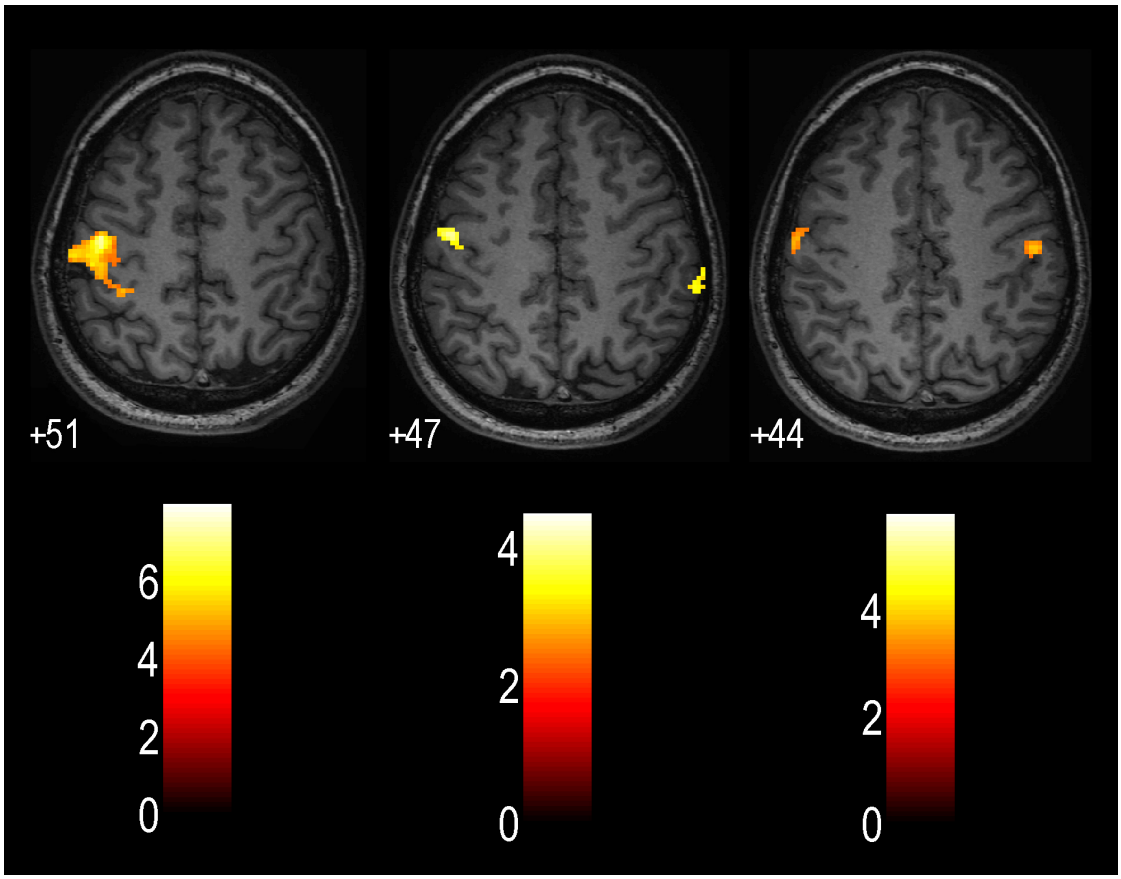

Figure 8. Activation caused by stimulation of Digits II-III. Left panel: group data from stimulation of the right hand of healthy controls. Middle panel: stimulation of Digits II-III of the injured (right) hand of Patient 4. Right panel: stimulation of the healthy (left) hand of Patient 4. Threshold $p<0.001$ uncorrected. The colour bar indicates T-statistics. Images are displayed according to neurological convention i.e. left hemisphere displayed on the left.

5 DISCUSSION

The poor recovery of hand function after nerve injury has been a challenge to surgeons and researchers for decades. Great efforts have been made to improve surgical repair technique at the suture site but results have been disappointing. Prognostic factors for recovery have not been so much the surgical technique used but rather static factors such as age and gender, that are insensitive to surgical performance [45]. We concluded in Paper II that age at the time of injury is an important factor for good sensory recovery but found no statistically significant correlation between the results of sensory testing and fMRI. In a more recent study with longer follow-up and a larger patient cohort this finding was confirmed and furthermore a correlation with the cortical activation pattern was found [20]. This finding finally confirms the assumption that recovery after peripheral nerve injury is mainly dependent on central processes, and that efforts now must be put into revising rehabilitation strategies with training that manipulates plasticity in the central nervous system.

5.1 Cross-modal plasticity

Under normal conditions interaction between the different senses provides us with a reliable and enhanced perception of the environment. The concept of visuotactile and audiovisual interaction is well known but audiotactile interaction has not been studied to the same extent. The sound of friction from fingers exploring a surface contributes to the tactile perception of that object, and posterior areas of the auditory cortex are activated by tactile stimulation of the fingertips indicating sharing of cortical areas in the processing of tactile and auditory inputs [76]. The interaction between these two sensory modalities was studied by delivering simultaneous tactile and auditory stimuli to healthy subjects. The response to simultaneous stimulation was affected by the respective salience of the two stimuli. When tactile stimulation was salient there was inhibition of the auditory cortex [34] and conversely salient auditory stimuli suppressed the SII. We investigated the reorganisation of the audiotactile interaction in response to training and found an increased overlap of cortical activation to tactile and auditory stimulation in trained subjects. There is now growing evidence for a multisensory area at the superior temporal sulcus that may serve as a non-specific modality network for integration of sensory inputs [5, 6]. Multisensory processing can be beneficial in cases of deprivation of a sense since the intact senses continue to respond to stimuli, thereby preventing total stimulus deprivation of that area [49]. This is the principle behind early sensory re-education programmes using mirror visual feedback [72].

5.2 Inter-hemispheric plasticity

Haemodynamic responses in the brain are dichotomously controlled by excitation and inhibition. A simple interpretation would be that an increase in cerebral blood flow (CBF) *i.e.* a positive BOLD signal is induced by excitation and that neuronal deactivation, which causes a decrease in CBF, is the source of a negative BOLD signal. However the excitation of inhibitory interneurons can also produce a positive BOLD signal and the exact mechanisms of interplay between excitation and inhibition and its role in plasticity mechanisms are not fully understood.

In healthy individuals tactile stimulation of one hand typically produces a positive BOLD signal in the contralteral SI and in the SII bilaterally. A negative BOLD signal is found in the ipsilateral SI. The negative BOLD signal is shown to correlate to functional inhibition as unilateral stimulation of the right hand in healthy subjects correlated to increased perception thresholds in the contralateral unstimulated hand [47]. In patients with median nerve injury we found a positive BOLD signal in the ipsilateral hemisphere following stimulation of both the injured and the healthy hand (Paper IV). Stimulation of an injured hand supposedly leads to an increase in ipsilateral SI-activity by loss of transcallosal inhibition. Similar results were seen in a study on lower limb amputees where increased ipsilateral activity was seen on fMRI following stimulation of the stump. Furthermore, structural changes of the corpus callosum were found with diffusion tensor imaging (DTI) [78]. Increased ipsilateral motor activation is also seen in children with cerebral palsy, and it has been suggested that this correlates with poor function and mirror movement, *i.e.* involuntary movement of the opposite arm when muscles of the paretic arm are activated [42, 85].

On the other hand, increased ipsilateral SI activity in response to healthy hand stimulation, i.e. increased activity in the deprived SI, is thought to arise from an increase in activity of inhibitory interneurons. In a study on rats combining fMRI, electrophysiology and immunostaining, stimulation of the injured paw caused increased ipsilateral fMRI-activity associated with increased local field potentials (LFP) and an increase in responding single units. However, neurons in the *deprived* cortex responding to *healthy* paw stimulation were identified as interneurons, and the increase in fMRI-activity was not accompanied by an increase in LFP [65].

Increased inhibitory interneuron activity in the deprived cortex may impair rehabilitation after peripheral nerve injury, and efforts have been made to guide the plasticity process, by manipulating transcallosal pathways. In an animal study using optogenetic technology, a light

signal of a specific wavelength was used to trigger the hyperpolarisation of subsets of neurons, thereby decreasing their firing rate. This technique was used to decrease transcallosal signalling from the intact hemisphere to the deprived cortex in rats with a denervated paw. The result was increased excitatory and decreased inhibitory activity in the deprived cortex that was reflected in the fMRI response as a positive BOLD signal [51]. This may possibly provide us with a new rehabilitation strategy for patients with a nerve injury, where modification of interhemispheric plasticity is achieved by means of transcranial magnetic brain stimulation (TMS). However, there is also evidence, that upregulated transcallosal signalling has a protective role. In a study on rats with a denervated paw, upregulation resulted in protection of the denervated area from take-over by adjacent areas [92]. It has been proposed that activation of the ipsilateral hemisphere is a compensatory mechanism to improve performance when function of the affected hemisphere is lost or impaired. Nevertheless, patients with poor recovery display activation patterns which extend ipsilaterally. It is uncertain what significance the increase in ipsilateral activation has for the the functional outcome of the patient.

The involvement of both hemispheres in the plasticity process, displaying changes in the brain activation pattern to stimulation of both the healthy and the injured hand is problematic when the healthy hand of the patient is used as a control. When the experiments in Paper II were planned and performed, the process of interhemispheric plasticity was not fully understood and we therefore used the patient's healthy hand as a control. The large areas of ipsilateral activation in response to stimulation of both the healthy and the injured hand was an incidental finding of Paper II that encouraged us to perform the experiments in Paper IV in which a group of ten healthy controls was used for comparison.

The ipsilateral activity found in the patients in Paper II was increased compared to healthy controls in a previous study [37], however the methodology differed slightly. In the fMRI experiments of Paper II, tactile stimulation was given with single brush strokes according to an event-related paradigm, whereas in the previous study tactile stimulation was delivered in blocks of 30 seconds of alternating brush strokes and rest. It has been suggested that the rate and pattern of stimulation can have an impact on cortical activation. We were therefore unsure as to whether the increase in ipsilateral activity was an effect of the median nerve injury or of the stimulation paradigm employed. By performing the experiments in Paper IV we could clearly exclude the effect of different stimulation paradigms on hemispheric plasticity, as there was no difference in the LI response.

5.3 Mechanisms and levels of plasticity

We studied cortical plasticity in patients with a median nerve injury and found an increased cortical response to median nerve stimulation that declined with time, (Paper II). We also confirmed the results of others, describing expansion of the ulnar nerve cortical area, when using fMRI (Paper II) and MEG (Paper III). Furthermore, we saw a time-dependent decrease in activation after stimulation of the ulnar nerve (Paper II). These findings are probably only partially explained by strict corticocortical plasticity processes, more likely it is a combined effect of plasticity at the periphery, dorsal root ganglia (DRG), spinal cord, thalamus and cortex levels. Misrouting at the section site causes regenerating axons to re-innervate tissue with poor specificity. When target organs become re-innervated by nerve fibers with deviant function, these axons are eliminated [13] giving adjacent neurons the opportunity to capture the disconnected skin areas by distal sprouting. Intact sensory neurons (Aβ) show enlarged projections at the level of the DRG when the input of adjacent afferents is decreased. Furthermore, the increased synaptic strength of undamaged fibres can activate second-order neurons originally responding to the injured nerve afferents [60]. This increase in size of the receptive field of the intact adjacent neurons could contribute to the increased size of the ulnar nerve response area that we found in the SI.

Following axotomy there is a change in the ion channel expression of the axons of the injured neurons as well as in the neurons in the DRG, contributing to hyperexcitability in those cells. The apoptotic cell death of neurons in the DRG in response to axotomy, which preferentially affects inhibitory interneurons, adds to the increased excitation of ascending sensory pathways [60]. This may partially explain the increase in the BOLD signal in response to injured median nerve stimulation.

Changes in the spinal cord and at the subcortical level following nerve injury were not studied in this thesis, but must be taken into account when interpreting the results and drawing conclusions from the present findings.

5.4 Methodological considerations

5.4.1 Patients

A general limitation of the studies in this thesis is the small number of patients included. In Sweden, median nerve injuries are fortunately relatively rare, and only a few of the patients found in our diagnosis register replied to our invitation to take part in the study. Many of the patients we were unable to contact had a history of abuse and self-destructive behaviour and

their injury was the consequence of aggressive behaviour. fMRI is a demanding investigation for patients as they must lie completely still in the scanner, and is thus not suitable for patients with claustrophobia, tremor or mental distress. MEG involves sitting still for long periods of time and furthermore we had to fly the patients to Helsinki to perform the experiments since at that time MEG was not available in Sweden.

5.4.2 Clinical sensory testing

There are several methods that may be used for the evaluation of outcome after nerve repair. We used 2-pd and Semmes-Weinstein monofilaments for measurement of sensory recovery.

The static 2-pd test is one of the most commonly used methods for evaluation of nerve function. It is, however, highly dependent on the examiner as the force applied varies both between examiners and between repeated applications of a single examiner. The patient may be able to solve the task by discriminating between a light or heavy force instead of perceiving one or two points. The test also proves not to be static as vibrations from the examiner are propagated through the test instrument onto the examined digit [7]. The 2-pd test thus has a low repeatability.

Semmes-Weinstein monofilaments provide more reproducible results as the diameter of the filament corresponds to a known force applied before buckling. The direction of application and the examiners grip of the equipment may, however, affect the results.

The Rosen score [70] is said to provide more reliable results of sensory recovery since it embraces various aspects of sensory and motor hand function including the static 2-pd test, Semmes-Weinstein monofilaments, the standardised Sollerman hand function dexterity test [79] and the STI-test for identification of shapes and textures [71].. It also includes Jamar grip strength measurements for evaluation of motor function, estimation of pain and discomfort, estimation of impact on ADL, and classification according to the Medical Research Council Scale of Sensory Recovery (S0-S4).

In Paper II, we could not demonstrate a significant correlation between the amount of cortical activation and the level of sensory recovery, as measured by the 2-pd test and Semmes-Weinstein monofilaments. This was possibly due to the small number of patients and to the inexact and non-specific measurements of sensory performance. In another fMRI study on patients with median nerve injury, using the Rosen Score as a measurement of recovery, significant correlations were found between age at the time of injury, cortical activity pattern and clinical sensory outcome [20].

5.4.3 fMRI and MEG

fMRI measures the BOLD signal that reflects increases or decreases in CBF, which in turn is controlled by the neurochemical processes triggered by neuronal activity. Thus, local changes in cerebral blood flow not only reflect neuronal activity, but the neurovascular coupling also influences the BOLD response. Physiological noise such as cardiac and respiratory activity, are known to generate BOLD artifacts [8] and the stimulus itself can sometimes provoke responses that co-vary with the expected cortical response. Motor stimulation such as finger grasping can provoke movement artifacts that cannot be filtered without neglecting the response measured. This is less probable with passive tactile stimulation and when using an event-related paradigm with randomised inter-stimuli intervals, the effect of both physiological noise and anticipation is diminished [24].

MEG offers a more concise way of examining the cortical response as it measures the magnetic field produced by the neuronal current itself. The source of the deflection on the scalp is not the potential of a single neuron, but rather the sum of the postsynaptic potentials of a population of pyramidal cells situated perpendicular to the cortex. Source modelling in Paper III was performed using the current dipole model, which is suitable for situations where the dipole is thought to originate from a limited area such as the SI, but the spatial resolution cannot be expected to exceed 5-8 mm [80]. In a previous MEG study on patients with median nerve compression, *i.e*. carpal tunnel syndrome, changes in the cortical somatotopy of hand representation were found [82]. In Paper III we hypothesised that the dipole coordinates would change in response to median nerve injury but found no such difference. Considering the low spatial resolution, the interindividual and interhemispheric variability [89] and the small changes in position to be expected, this study was underpowered to reliably reject that hypothesis. Although we were not able to detect displacement of the coordinates, a significant decrease in the median nerve amplitude and a concurrent increase in ulnar nerve amplitude were seen.

6 CONCLUSIONS

- There are training-induced effects on audio-tactile interaction.
- Age at the time of injury is an important predictor of functional outcome after median nerve injury.
- The initial increase in cortical activation following median nerve injury declines with time passed since injury. This was also shown to be the case for the ulnar nerve in patients with median nerve injury.
- The MEG response to stimulation of the injured hand of patients with a median nerve injury shows decreased median nerve amplitude and increased ulnar nerve amplitude compared to stimulation of the healthy hand. There is no correlation between MEG amplitude and cortical activation size as measured by fMRI.
- There is hemispheric redistribution of SI activity in patients with median nerve injury. Laterality index calculations showed that stimulation of both the injured and the healthy hand of patients resulted in increased levels of ipsilateral activity compared to controls.
- There is no difference in measured ipsilateral activation in response to stimulation in healthy subjects, regardless of whether a block or an event-related paradigm is used.

7 CLINICAL IMPLICATIONS AND FUTURE PERSPECTIVES

Most studies on patients with a peripheral nerve injury include small heterogeneous groups. Factors such as age, age at the time of injury and time passed since injury are confounding when investigating changes in brain activation with fMRI (Paper II). The need for larger studies with more homogeneous groups is great, and multicenter studies may well be the only solution.

Traditionally, no active rehabilitation is carried out immediately after nerve injury and repair and prior to regeneration of the nerve. More recently, however, research has shown that cross-modal interaction in this early phase maintains input to the deafferented SI . In Paper I we describe the ability to substitute tactile sensibility with hearing. Sustained improvement in tactile gnosis was thereafter found in patients with median nerve injury after audiotactile stimulation training [69]. Early sensory re-education programmes, using cross-modal interaction are already in clinical practice and a randomised controlled trial of patients with median and ulnar nerve injuries has shown improvement in discriminative touch following mirror training with observation of touch [72].

The increase in ipsilateral activation (decrease in LI) following nerve injury was the main finding in Paper IV. There is still some uncertainty regarding the impact of ipsilateral hemispheric activation on functional outcome. In children with a median nerve injury and good functional outcome, contralateral hemispheric dominance is restored [20]. In patients with cerebral palsy, ipsilateral projections seem to correlate with poor performance in motor tasks [42]. Thus ipsilateral activation of the deafferented hemisphere by stimulation of the healthy hand may disturb the recovery process, and restriction of the use of the healthy hand may be advantageous. Constraint-induced movement therapy (CIMT) is a rehabilitation programme led by an occupational therapist in which intensive training is performed while the unaffected arm is immobilised in a splint or cast. It has been used in adults with stroke and children with cerebral palsy [27] with promising results. A recent study showed improved results in patients with median or ulnar nerve injury using a modified CIMT programme [74]. Measurement of changes in LI with fMRI may provide insight into the mechanisms behind the effects of this form of therapy.

Recovery after peripheral nerve injury involves regenerative changes at all levels from the periphery to the cortex. In Paper III we used MEG to capture more directly the changes in brain activation due to plasticity after peripheral nerve injury. Changes in the function of the somatosensory pathways were indicated by the delayed cortical MEG response associated with direct electrical nerve stimulation. This would have been easier to detect using EEG recordings. The reason we used MEG was its superior spatial resolution compared to the EEG. However, in patients such those as in the present study group, the evoked signal was often small and its source difficult to localise. The present studies have focused on sensory mechanisms but in other studies, corresponding changes in plasticity in the motor cortex after nerve injury have been described [16]. The use of navigated TMS, which has recently become available, will enable the mapping of motor cortex excitability in future studies.

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7 REFERENCES

[1] H. Aldskogius, J. Arvidsson, Nerve cell degeneration and death in the trigeminal ganglion of the adult rat following peripheral nerve transection, Journal of neurocytology 7 (1978) 229-250.

[2] P. Angermann, M. Lohmann, Injuries to the hand and wrist. A study of 50,272 injuries, Journal of hand surgery (Edinburgh, Scotland) 18 (1993) 642-644.

[3] R. Bailey, V. Kaskutas, I. Fox, C.M. Baum, S.E. Mackinnon, Effect of upper extremity nerve damage on activity participation, pain, depression, and quality of life, The Journal of hand surgery 34 (2009) 1682-1688.

[4] M.F. Bear, W.C. Abraham, Long-term depression in hippocampus, Annual review of neuroscience 19 (1996) 437-462.

[5] M.S. Beauchamp, See me, hear me, touch me: multisensory integration in lateral occipital-temporal cortex, Current opinion in neurobiology 15 (2005) 145-153.

[6] M.S. Beauchamp, N.E. Yasar, R.E. Frye, T. Ro, Touch, sound and vision in human superior temporal sulcus, NeuroImage 41 (2008) 1011-1020.

[7] J.A. Bell-Krotoski, W.L. Buford, Jr., The force/time relationship of clinically used sensory testing instruments, Journal of hand therapy : official journal of the American Society of Hand Therapists 10 (1997) 297-309.

[8] R.M. Birn, J.B. Diamond, M.A. Smith, P.A. Bandettini, Separating respiratory-variation-related fluctuations from neuronal-activity-related fluctuations in fMRI, NeuroImage 31 (2006) 1536-1548.

[9] A. Bjorkman, A. Weibull, B. Rosen, J. Svensson, G. Lundborg, Rapid cortical reorganisation and improved sensitivity of the hand following cutaneous anaesthesia of the forearm, The European journal of neuroscience 29 (2009) 837-844.

[10] T.V. Bliss, A.R. Gardner-Medwin, Long-lasting potentiation of synaptic transmission in the dentate area of the unanaestetized rabbit following stimulation of the perforant path, The Journal of physiology 232 (1973) 357-374.

[11] M.E. Boeckstyns, A.I. Sorensen, J.F. Vineta, B. Rosen, X. Navarro, S.J. Archibald, J. Valss-Sole, M. Moldovan, C. Krarup, Collagen conduit versus microsurgical neurorrhaphy: 2-year follow-up of a prospective, blinded clinical and electrophysiological multicenter randomized, controlled trial, The Journal of hand surgery 38 (2013) 2405-2411.

[12] D.N. Brooks, R.V. Weber, J.D. Chao, B.D. Rinker, J. Zoldos, M.R. Robichaux, S.B. Ruggeri, K.A. Anderson, E.E. Bonatz, S.M. Wisotsky, M.S. Cho, C. Wilson, E.O. Cooper, J.V. Ingari, B. Safa, B.M. Parrett, G.M. Buncke, Processed nerve allografts for peripheral nerve reconstruction: a multicenter study of utilization and outcomes in sensory, mixed, and motor nerve reconstructions, Microsurgery 32 (2012) 1-14.

[13] T.M. Brushart, J. Gerber, P. Kessens, Y.G. Chen, R.M. Royall, Contributions of pathway and neuron to preferential motor reinnervation, The Journal of neuroscience : the official journal of the Society for Neuroscience 18 (1998) 8674-8681.

[14] C.N. Bruyns, J.B. Jaquet, T.A. Schreuders, S. Kalmijn, P.D. Kuypers, S.E. Hovius, Predictors for return to work in patients with median and ulnar nerve injuries, The Journal of hand surgery 28 (2003) 28-34.

[15] M.B. Calford, R. Tweedale, Interhemispheric transfer of plasticity in the cerebral cortex, Science (New York, N.Y.) 249 (1990) 805-807.

[16] T. Carlstedt, T. Hultgren, T. Nyman, T. Hansson, Cortical activity and hand function restoration in a patient after spinal cord surgery, Nature reviews. Neurology 5 (2009) 571-574.

[17] A. Chemnitz, G. Andersson, B. Rosen, L.B. Dahlin, A. Bjorkman, Poor electroneurography but excellent hand function 31 years after nerve repair in childhood, Neuroreport 24 (2013) 6-9.

[18] A. Chemnitz, A. Bjorkman, L.B. Dahlin, B. Rosen, Functional outcome thirty years after median and ulnar nerve repair in childhood and adolescence, The Journal of bone and joint surgery. American volume 95 (2013) 329-337.

[19] A. Chemnitz, L.B. Dahlin, I.K. Carlsson, Consequences and adaptation in daily life - patients' experiences three decades after a nerve injury sustained in adolescence, BMC musculoskeletal disorders 14 (2013) 252.

[20] A. Chemnitz, A. Weibull, B. Rosen, G. Andersson, L.B. Dahlin, A. Bjorkman, Normalized activation in the somatosensory cortex 30 years following nerve repair in children: an fMRI study, The European journal of neuroscience 42 (2015) 2022-2027.

[21] P. Chen, X. Piao, P. Bonaldo, Role of macrophages in Wallerian degeneration and axonal regeneration after peripheral nerve injury, Acta neuropathologica 130 (2015) 605-618.

[22] R. Chen, L.G. Cohen, M. Hallett, Nervous system reorganization following injury, Neuroscience 111 (2002) 761-773.

[23] J.C. Clarey, R. Tweedale, M.B. Calford, Interhemispheric modulation of somatosensory receptive fields: evidence for plasticity in primary somatosensory cortex, Cerebral cortex (New York, N.Y. : 1991) 6 (1996) 196-206.

[24] A.M. Dale, Optimal experimental design for event-related fMRI, Human brain mapping 8 (1999) 109-114.

[25] K. Druschky, M. Kaltenhauser, C. Hummel, A. Druschky, W.J. Huk, H. Stefan, B. Neundorfer, Alteration of the somatosensory cortical map in peripheral mononeuropathy due to carpal tunnel syndrome, Neuroreport 11 (2000) 3925-3930.

[26] T. Elbert, C. Pantev, C. Wienbruch, B. Rockstroh, E. Taub, Increased cortical representation of the fingers of the left hand in string players, Science (New York, N.Y.) 270 (1995) 305-307.

[27] A.C. Eliasson, B. Bonnier, L. Krumlinde-Sundholm, 'Clinical experience of constraint induced movement therapy in adolescents with hemiplegic cerebral palsy--a day camp model', Developmental medicine and child neurology 45 (2003) 357-359.

[28] S.L. Florence, H.B. Taub, J.H. Kaas, Large-scale sprouting of cortical connections after peripheral injury in adult macaque monkeys, Science (New York, N.Y.) 282 (1998) 1117-1121.

[29] L. Fornander, T. Nyman, T. Hansson, M. Ragnehed, T. Brismar, Age- and time-dependent effects on functional outcome and cortical activation pattern in patients with median nerve injury: a functional magnetic resonance imaging study, Journal of neurosurgery 113 (2010) 122-128.

[30] A.H. Friedman, W.J. Elias, R. Midha, Introduction: peripheral nerve surgery--biology, entrapment, and injuries, Neurosurgical focus 26 (2009) E1.

[31] K. Fugleholm, The surgery of peripheral nerves (including tumors), Handbook of clinical neurology 115 (2013) 781-802.

[32] S.P. Galanakos, A.B. Zoubos, I. Ignatiadis, I. Papakostas, N.E. Gerostathopoulos, P.N. Soucacos, Repair of complete nerve lacerations at the forearm: an outcome study using Rosen-Lundborg protocol, Microsurgery 31 (2011) 253-262.

[33] P.E. Garraghty, J.H. Kaas, Functional reorganization in adult monkey thalamus after peripheral nerve injury, Neuroreport 2 (1991) 747-750.

[34] R. Gobbele, M. Schurmann, N. Forss, K. Juottonen, H. Buchner, R. Hari, Activation of the human posterior parietal and temporoparietal cortices during audiotactile interaction, NeuroImage 20 (2003) 503-511.

[35] E.L. Hall, S.E. Robson, P.G. Morris, M.J. Brookes, The relationship between MEG and fMRI, NeuroImage 102 Pt 1 (2014) 80-91.

[36] T. Hansson, T. Brismar, Loss of sensory discrimination after median nerve injury and activation in the primary somatosensory cortex on functional magnetic resonance imaging, Journal of neurosurgery 99 (2003) 100-105.

[37] T. Hansson, T. Brismar, Tactile stimulation of the hand causes bilateral cortical activation: a functional magnetic resonance study in humans, Neuroscience letters 271 (1999) 29-32.

[38] I. Hashimoto, A. Suzuki, T. Kimura, Y. Iguchi, M. Tanosaki, R. Takino, Y. Haruta, M. Taira, Is there training-dependent reorganization of digit representations in area 3b of string players?, Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology 115 (2004) 435-447.

[39] N. Hauthal, P. Sandmann, S. Debener, J.D. Thorne, Visual movement perception in deaf and hearing individuals, Advances in cognitive psychology / University of Finance and Management in Warsaw 9 (2013) 53-61.

[40] G. Hess, K.M. Jacobs, J.P. Donoghue, N-methyl-D-aspartate receptor mediated component of field potentials evoked in horizontal pathways of rat motor cortex, Neuroscience 61 (1994) 225-235.

[41] E.M. Hillman, Coupling mechanism and significance of the BOLD signal: a status report, Annual review of neuroscience 37 (2014) 161-181.

[42] L. Holmstrom, B. Vollmer, K. Tedroff, M. Islam, J.K. Persson, A. Kits, H. Forssberg, A.C. Eliasson, Hand function in relation to brain lesions and corticomotor-projection pattern in children with unilateral cerebral palsy, Developmental medicine and child neurology 52 (2010) 145-152.

[43] C.A. Hundepool, J. Ultee, T.H. Nijhuis, P. Houpt, S.E. Hovius, Prognostic factors for outcome after median, ulnar, and combined median-ulnar nerve injuries: a prospective study, Journal of plastic, reconstructive & aesthetic surgery : JPRAS 68 (2015) 1-8.

[44] Y. Iwamura, Bilateral receptive field neurons and callosal connections in the somatosensory cortex, Philosophical transactions of the Royal Society of London. Series B, Biological sciences 355 (2000) 267-273.

[45] J.B. Jaquet, A.J. Luijsterburg, S. Kalmijn, P.D. Kuypers, A. Hofman, S.E. Hovius, Median, ulnar, and combined median-ulnar nerve injuries: functional outcome and return to productivity, The Journal of trauma 51 (2001) 687-692.

[46] J.H. Kaas, Plasticity of sensory and motor maps in adult mammals, Annual review of neuroscience 14 (1991) 137-167.

[47] A. Kastrup, J. Baudewig, S. Schnaudigel, R. Huonker, L. Becker, J.M. Sohns, P. Dechent, C. Klingner, O.W. Witte, Behavioral correlates of negative BOLD signal changes in the primary somatosensory cortex, NeuroImage 41 (2008) 1364-1371.

[48] K. Kwong, J. Belliveau, D. Chesler, Dynamic magnetic resonance imaging of human brain activity during primary sensory stimulation, Proc Natl Acad Sci U S A 89 (1992) 5675-5679.

[49] E. Ladavas, Multisensory-based approach to the recovery of unisensory deficit, Annals of the New York Academy of Sciences 1124 (2008) 98-110.

[50] L.M. Levy, U. Ziemann, R. Chen, L.G. Cohen, Rapid modulation of GABA in sensorimotor cortex induced by acute deafferentation, Annals of neurology 52 (2002) 755-761.

[51] N. Li, J.E. Downey, A. Bar-Shir, A.A. Gilad, P. Walczak, H. Kim, S.E. Joel, J.J. Pekar, N.V. Thakor, G. Pelled, Optogenetic-guided cortical plasticity after nerve injury, Proc Natl Acad Sci U S A 108 (2011) 8838-8843.

[52] G. Lundborg, B. Rosen, L. Dahlin, J. Holmberg, I. Rosen, Tubular repair of the median or ulnar nerve in the human forearm: a 5-year follow-up, Journal of hand surgery (Edinburgh, Scotland) 29 (2004) 100-107.

[53] G. Lundborg, B. Rosen, S. Lindberg, Hearing as substitution for sensation: a new principle for artificial sensibility, The Journal of hand surgery 24 (1999) 219-224.

[54] Y. Maeda, N. Kettner, J. Holden, J. Lee, J. Kim, S. Cina, C. Malatesta, J. Gerber, C. McManus, J. Im, A. Libby, P. Mezzacappa, L.R. Morse, K. Park, J. Audette, M. Tommerdahl, V. Napadow, Functional deficits in carpal tunnel syndrome reflect reorganization of primary somatosensory cortex, Brain : a journal of neurology 137 (2014) 1741-1752.

[55] M.F. Meek, J.H. Coert, P.H. Robinson, Poor results after nerve grafting in the upper extremity: Quo vadis?, Microsurgery 25 (2005) 396-402.

[56] P.M. Meiners, J.H. Coert, P.H. Robinson, M.F. Meek, Impairment and employment issues after nerve repair in the hand and forearm, Disability and rehabilitation 27 (2005) 617-623.

[57] P. Melzer, V.L. Morgan, D.R. Pickens, R.R. Price, R.S. Wall, F.F. Ebner, Cortical activation during Braille reading is influenced by early visual experience in subjects with severe visual disability: a correlational fMRI study, Human brain mapping 14 (2001) 186-195.

[58] R.M. Menorca, T.S. Fussell, J.C. Elfar, Nerve physiology: mechanisms of injury and recovery, Hand clinics 29 (2013) 317-330.

[59] M.M. Merzenich, J.H. Kaas, J.T. Wall, M. Sur, R.J. Nelson, D.J. Felleman, Progression of change following median nerve section in the cortical representation of the hand in areas 3b and 1 in adult owl and squirrel monkeys, Neuroscience 10 (1983) 639-665.

[60] X. Navarro, M. Vivo, A. Valero-Cabre, Neural plasticity after peripheral nerve injury and regeneration, Progress in neurobiology 82 (2007) 163-201.

[61] C.B. Novak, D.J. Anastakis, D.E. Beaton, J. Katz, Patient-reported outcome after peripheral nerve injury, The Journal of hand surgery 34 (2009) 281-287.

[62] S. Ogawa, T.M. Lee, A.R. Kay, D.W. Tank, Brain magnetic resonance imaging with contrast dependent on blood oxygenation, Proc Natl Acad Sci U S A 87 (1990) 9868-9872.

[63] A. Pascual-Leone, C. Freitas, L. Oberman, J.C. Horvath, M. Halko, M. Eldaief, S. Bashir, M. Vernet, M. Shafi, B. Westover, A.M. Vahabzadeh-Hagh, A. Rotenberg, Characterizing brain cortical plasticity and network dynamics across the age-span in health and disease with TMS-EEG and TMS-fMRI, Brain topography 24 (2011) 302-315.

[64] W.C. Pederson, Median nerve injury and repair, The Journal of hand surgery 39 (2014) 1216-1222.

[65] G. Pelled, D.A. Bergstrom, P.L. Tierney, R.S. Conroy, K.H. Chuang, D. Yu, D.A. Leopold, J.R. Walters, A.P. Koretsky, Ipsilateral cortical fMRI responses after peripheral nerve damage in rats reflect increased interneuron activity, Proc Natl Acad Sci U S A 106 (2009) 14114-14119.

[66] M.J. Politis, K. Ederle, P.S. Spencer, Tropism in nerve regeneration in vivo. Attraction of regenerating axons by diffusible factors derived from cells in distal nerve stumps of transected peripheral nerves, Brain research 253 (1982) 1-12.

[67] M. Ptito, A. Fumal, A.M. de Noordhout, J. Schoenen, A. Gjedde, R. Kupers, TMS of the occipital cortex induces tactile sensations in the fingers of blind Braille readers, Experimental brain research 184 (2008) 193-200.

[68] H.E. Rosberg, K.S. Carlsson, S. Hojgard, B. Lindgren, G. Lundborg, L.B. Dahlin, Injury to the human median and ulnar nerves in the forearm--analysis of costs for treatment and rehabilitation of 69 patients in southern Sweden, Journal of hand surgery (Edinburgh, Scotland) 30 (2005) 35-39.

[69] B. Rosen, G. Lundborg, Enhanced sensory recovery after median nerve repair using cortical audio-tactile interaction. A randomised multicentre study, The Journal of hand surgery, European volume 32 (2007) 31-37.

[70] B. Rosen, G. Lundborg, A model instrument for the documentation of outcome after nerve repair, The Journal of hand surgery 25 (2000) 535-543.

[71] B. Rosen, G. Lundborg, A new tactile gnosis instrument in sensibility testing, Journal of hand therapy : official journal of the American Society of Hand Therapists 11 (1998) 251-257.

[72] B. Rosen, P. Vikstrom, S. Turner, D.A. McGrouther, R.W. Selles, T.A. Schreuders, A. Bjorkman, Enhanced early sensory outcome after nerve repair as a result of

immediate post-operative re-learning: a randomized controlled trial, The Journal of hand surgery, European volume 40 (2015) 598-606.

[73] P.M. Rossini, G. Martino, L. Narici, A. Pasquarelli, M. Peresson, V. Pizzella, F. Tecchio, G. Torrioli, G.L. Romani, Short-term brain 'plasticity' in humans: transient finger representation changes in sensory cortex somatotopy following ischemic anesthesia, Brain research 642 (1994) 169-177.

[74] H.R. Rostami, M. Akbarfahimi, A. Hassani Mehraban, A.R. Akbarinia, S. Samani, Occupation-based intervention versus rote exercise in modified constraint-induced movement therapy for patients with median and ulnar nerve injuries: A randomized controlled trial, Clinical rehabilitation (2016).

[75] A.C. Ruijs, J.B. Jaquet, S. Kalmijn, H. Giele, S.E. Hovius, Median and ulnar nerve injuries: a meta-analysis of predictors of motor and sensory recovery after modern microsurgical nerve repair, Plastic and reconstructive surgery 116 (2005) 484-494; discussion 495-486.

[76] M. Schurmann, G. Caetano, Y. Hlushchuk, V. Jousmaki, R. Hari, Touch activates human auditory cortex, NeuroImage 30 (2006) 1325-1331.

[77] A. Sharma, H. Glick, Cross-Modal Re-Organization in Clinical Populations with Hearing Loss, Brain sciences 6 (2016).

[78] E.L. Simoes, I. Bramati, E. Rodrigues, A. Franzoi, J. Moll, R. Lent, F. Tovar-Moll, Functional expansion of sensorimotor representation and structural reorganization of callosal connections in lower limb amputees, The Journal of neuroscience : the official journal of the Society for Neuroscience 32 (2012) 3211-3220.

[79] C. Sollerman, A. Ejeskar, Sollerman hand function test. A standardised method and its use in tetraplegic patients, Scandinavian journal of plastic and reconstructive surgery and hand surgery / Nordisk plastikkirurgisk forening [and] Nordisk klubb for handkirurgi 29 (1995) 167-176.

[80] J. Solomon, S. Boe, T. Bardouille, Reliability for non-invasive somatosensory cortex localization: Implications for pre-surgical mapping, Clinical neurology and neurosurgery 139 (2015) 224-229.

[81] G. Stoll, J.W. Griffin, C.Y. Li, B.D. Trapp, Wallerian degeneration in the peripheral nervous system: participation of both Schwann cells and macrophages in myelin degradation, Journal of neurocytology 18 (1989) 671-683.

[82] F. Tecchio, L. Padua, I. Aprile, P.M. Rossini, Carpal tunnel syndrome modifies sensory hand cortical somatotopy: a MEG study, Human brain mapping 17 (2002) 28-36.

[83] W. Tetzlaff, M.A. Bisby, G.W. Kreutzberg, Changes in cytoskeletal proteins in the rat facial nucleus following axotomy, The Journal of neuroscience : the official journal of the Society for Neuroscience 8 (1988) 3181-3189.

[84] L. Thomsen, C. Schlur, [Incidence of painful neuroma after end-to-end nerve suture wrapped into a collagen conduit. A prospective study of 185 cases], Chirurgie de la main 32 (2013) 335-340.

[85] Y. Vandermeeren, G. Sebire, C.B. Grandin, J.L. Thonnard, X. Schlogel, A.G. De Volder, Functional reorganization of brain in children affected with congenital hemiplegia: fMRI study, NeuroImage 20 (2003) 289-301.

[86] J.T. Wall, C.G. Cusick, Cutaneous responsiveness in primary somatosensory (S-I) hindpaw cortex before and after partial hindpaw deafferentation in adult rats, The Journal of neuroscience : the official journal of the Society for Neuroscience 4 (1984) 1499-1515.

[87] J.T. Wall, J. Xu, X. Wang, Human brain plasticity: an emerging view of the multiple substrates and mechanisms that cause cortical changes and related sensory dysfunctions after injuries of sensory inputs from the body, Brain research. Brain research reviews 39 (2002) 181-215.

[88] K.J. Werhahn, J. Mortensen, R.W. Van Boven, K.E. Zeuner, L.G. Cohen, Enhanced tactile spatial acuity and cortical processing during acute hand deafferentation, Nature neuroscience 5 (2002) 936-938.

[89] H. Wikstrom, R.O. Roine, O. Salonen, H.J. Aronen, J. Virtanen, R.J. Ilmoniemi, J. Huttunen, Somatosensory evoked magnetic fields to median nerve stimulation: interhemispheric differences in a normal population, Electroencephalography and clinical neurophysiology 104 (1997) 480-487.

[90] C. Witzel, C. Rohde, T.M. Brushart, Pathway sampling by regenerating peripheral axons, The Journal of comparative neurology 485 (2005) 183-190.

[91] M. Yang, J.L. Rawson, E.W. Zhang, P.B. Arnold, W. Lineaweaver, F. Zhang, Comparisons of outcomes from repair of median nerve and ulnar nerve defect with nerve graft and tubulization: a meta-analysis, Journal of reconstructive microsurgery 27 (2011) 451-460.

[92] X. Yu, A.P. Koretsky, Interhemispheric plasticity protects the deafferented somatosensory cortex from functional takeover after nerve injury, Brain connectivity 4 (2014) 709-717.