**Neural control of cooperative hand movements**

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# Contents

Summary 1

Zusammenfassung 4

General Introduction 7

Aims 10

Paper I 11

  Abstract 12

  Introduction 13

  Material and Methods 15

  Results 24

  Discussion 35

  Conclusion 40

Paper II 41

  Abstract 42

  Introduction 43

  Materials and Methods 45

  Results 47

  Discussion 49

Paper III 51

  Abstract 52

  Introduction 53
Consequences for therapy and robotic devices 108

General Discussion 111

Neural coupling of bimanual cooperative movements 111

Defective neural coupling after stroke 113

From bench to bedside 114

Limitations 115

Conclusion 116

Outlook 117

Physiology of neural coupling controlling cooperative hand movements 117

Pathology of neural coupling controlling cooperative hand movements 118

Implementation of cooperative training into neuro-rehabilitation 119

References 121

Glossary 134

Curriculum Vitae 136

Publications 139

Acknowledgements 140
Summary

Neuro-rehabilitation after injury of the central nervous system is focused on the improvement of functional movements of the affected body parts. This is usually achieved by training approaches based on neural mechanisms underlying these movements and according to the requirements of activities of daily living (ADL). In spite of advancements in rehabilitation research, stroke patients usually suffer from persisting deficits of arm and hand function and thus an impaired quality of life. Therefore, a better understanding of motor control of upper limb movements and, consequently of therapy strategies based on this knowledge are warranted.

Most studies concerning neural control of arm and hand movements have focused on unimanual or separate in- and out-phase bimanual tasks. Object-orientated, coupled movements such as opening a bottle or slicing bread have so far been neglected and neural mechanisms controlling such cooperative tasks are not yet well understood. The aim of this thesis was to investigate normal and impaired neural control of cooperative hand movements and the consequences for rehabilitation of hand function after a stroke.

In the first two studies (combined in paper I) of this thesis we analyzed cooperative upper limb movements by means of electrophysiology and functional magnetic resonance (fMRI) in healthy volunteers. The studies revealed a neural coupling mechanism that is involved in the control of cooperative hand movements. In the electrophysiological study we could show that only during cooperative hand movements bilateral electromyographic (EMG) reflex responses appeared following unilateral electrical nerve stimulation. During control conditions, reflex responses appeared only ipsilaterally to the stimulated side. The fMRI study revealed a task-specific activation of the secondary somatosensory (S2) cortical areas. The studies indicated that afferent input from each hand is task-specifically integrated in bilateral cortical S2 areas during cooperative upper limb movements. After processing of afferent signals, a joint release of efferent execution from supraspinal centers occurs to both arms.

The next study (paper II) was based on the observations made in paper I with the hypothesis that ipsilateral pathways play a major role in the neural coupling mechanism. Therefore, this study focused on the modulation of somatosensory information during cooperative movements. We analyzed the behavior of somatosensory evoked potentials (SSEPs) in healthy volunteers. The ratio of ipsilateral amplitude to contralateral amplitude was higher during cooperative movements.
Summary

when compared to non-cooperative bimanual tasks and resting. This result confirmed our assumption of a task-specific bilateral processing of afferent input as part of the neural coupling mechanism.

The question as to what extent the neural coupling is defective in stroke subjects was addressed in paper III. EMG reflex responses in the forearm muscles were analyzed following unilateral ulnar nerve stimulation in chronic post-stroke patients. The results revealed an intact neural coupling following stimulation of the unaffected arm, i.e. bilateral EMG responses appeared as in healthy subjects. In contrast, following stimulation of the affected arm polysynaptic EMG responses appeared neither in the stimulated nor in the contralateral unaffected arm muscles. This indicated an impairment of the neural coupling due to a defective processing of afferent input from the affected arm. A correlation between the severity of neurological deficit of the upper limb and impairment of the neural coupling mechanism could be shown. Additionally, an enhanced recruitment of ipsilateral efferent fibers for the compensation of the neurological deficit in severely affected patients was suggested.

The consequent next step was to analyze SSEPs evoked by ulnar nerve stimulation in stroke patients during cooperative movements (paper IV). An enhanced recruitment of ipsilateral afferent fibers from the affected arm to the unaffected hemisphere could be shown. However, this was only the case in moderately and mildly affected patients. More severely affected stroke-patients did not show any SSEPs from the affected arm. The results also indicated that ipsilateral ascending input is only one of the components of the neural coupling mechanism that is changed after a stroke. Another component seems to be a defective task-specific supraspinal processing of this information. This is in line with our previous fMRI findings concerning the role of S2 cortical areas in neural coupling.

The aim of the thesis was lastly to translate physiological and pathophysiological investigations to the application in neuro-rehabilitation. Thus, in paper V, training of cooperative hand movements as a rehabilitation strategy was investigated. Two chronic post-stroke patients participated in this single-subject ABAB design training study. Cooperative training was compared to conventional occupation therapy. Both approaches were applied for two blocks lasting four weeks each. The results revealed superior improvements related to cooperative hand movement training. This might indicate that training of cooperative hand movements required during ADL seems to be a valuable addition to current rehabilitation strategies.
Summary

The novel observation of a neural coupling mechanism controlling cooperative upper limb movements opens new ways into neuro-rehabilitation approaches as cooperative movements are frequently required in ADL. The understanding of the neural coupling mechanism and the correlation between integrity of this mechanism and the clinical impairment in post-stroke patients represents a scientific basis for research based rehabilitation approaches. Further studies analyzing the effect of cooperative training in different patient groups in an early stage after a stroke are needed. Nevertheless, with this thesis the gap between bench and bedside in therapy of impaired hand function becomes smaller.
Zusammenfassung

Trainingsstrategien in der Neurorehabilitation nach Verletzung des Zentralnervensystems sind am erfolgversprechendsten wenn sie auf Alltagsaktivitäten (ADL) ausgerichtet sind und auf neuralen Kontrollmechanismen von Bewegungen basieren. Trotz grosser Fortschritte in den Rehabilitationswissenschaften bleibt die Funktion der betroffenen oberen Extremität bei vielen Schlaganfallpatienten jedoch eingeschränkt. Es ist somit nach wie vor nötig, das Wissen über die motorische Kontrolle funktioneller Bewegungsabläufe zu vertiefen, um darauf basierend neue Trainingsstrategien zu entwickeln.


Zusammenfassung

In der anschliessenden Studie (Paper II) haben wir die Integration somatosensorischer Information bei kooperativen Handbewegungen mittels somatosensorisch evozierten Potentialen (Ulnaris-SSEPs) analysiert. Das Amplitudenverhältnis zwischen ipsilateralen und kontralateralen Potentialen war bei kooperativen Bewegungen grösser als bei nicht-kooperativen Kontrollbewegungen oder in Ruhe. Dies bestätigt die Annahme, dass afferente Information eines Armes bei kooperativen Bewegungen aufgabenspezifisch bilateral integriert wird und dies ein wichtiger Bestandteil der neuralen Kopplung darstellt.


SSEP-Analysen (Paper IV) bei Schlaganfallpatienten wiesen auf einen läsionsabhängigen kompensatorischen Beitrag der ipsilateralen afferenten Fasern vom betroffenen Arm zur nicht-betroffenen Hemisphere hin. Dies zeigte sich in erhöhtem Amplituden-Verhältnis bei Stimulation des betroffenen Arms. Allerdings war dies nur bei moderat oder schwach betroffenen Patienten der Fall, während schwerer beeinträchtigte Patienten keine SSEPs nach Stimulation des betroffenen Arms zeigten. Die Resultate dieser Studie wiesen auch darauf hin, dass die ipsilateral Afferenz nicht der einzige defizitäre Anteil der neuralen Kopplung ist in Schlaganfallpatienten.

Zusammenfassung

Funktionsgewinn bei kooperativem Training. Diese Ergebnisse weisen darauf hin, dass kooperatives Training eine wertvolle Ergänzung zu aktuellen Trainingsstrategien in der Neurorehabilitation sein kann.

General Introduction

From the time on that evolution led to an upright stand in primates, the arms became free for tool use. Differentiation of hand movements during evolution represents a basic requirement for human cultural development (Herder 1785). Humans have acquired a huge variability of specialized and skilled upper limb movements and motor control of the arms and hands has gained in complexity.

Manipulating objects usually requires the coordination of both hands. Bimanual movements and the pathways and networks controlling these tasks have been studied extensively (Kermadi et al. 2000; Wiesendanger and Serrien 2004; Carson 2005; Johansson et al. 2006; Janssen et al. 2010; Gooijers and Swinnen 2014; Shetty et al. 2014). Different neural networks including cortical areas, inter- and intra-hemispheric connections, cortical-spinal connections as well as spinal interneuron networks are involved in the control of upper limb movements. Studies using functional magnetic resonance imaging (fMRI) have shown activation of the supplementary motor area (SMA), the primary motor cortex (M1) and the premotor cortex (PMC) during bimanual tasks (Grefkes et al. 2008; Goble et al. 2010). Especially the SMA seems to be responsible for both ipsilateral and contralateral upper limb movements (Wiesendanger et al. 1996). Neural activity within the SMA has been shown to increase during bimanual tasks when compared to unilateral hand movements (Tanji et al. 1988). However, there are also studies indicating that these neural networks also control unilateral upper limb tasks and are thus not task-specifically activated during bimanual movements (Koenke et al. 2004). It was suggested that rather the complexity of a motor task than the number of limbs involved is responsible for recruiting specific brain areas.

Nevertheless, a task dependence of functional connectivity between M1, SMA and PMC could be shown (Grefkes et al. 2008). When a unilateral right hand movement is performed, all inter- and intrahemispheric connections between the above mentioned areas are inhibitory with the exception of the intra-hemispheric connections on the left hemisphere which are excitatory. In contrast, during a bimanual in-phase task all inter- and intra-hemispheric connections become facilitating. The importance of inter-hemispheric connections in bimanual tasks was also shown by the means of transcranial magnetic stimulation (TMS). Modulation of transcallosal connectivity between M1 and PMC was demonstrated to influence the performance of uni- and bimanual in- and out-phase movements (Liuzzi et al. 2011). Electroencephalography (EEG) studies have shown that the functional connectivity between the hemispheres during bimanual tasks changes over
different stages of motor learning (Gerloff and Andres 2002). These results suggest that rather a dynamic change of inter- and intra-hemispheric connections than an additional activation of specific brain areas is important for the motor control of bimanual coordination.

Most of the studies on motor control of the upper limb directed at better understanding neural mechanisms controlling bimanual movements have analysed separate bimanual or cycling-like movements. However, many activities of daily living (ADL) comprise cooperative hand movements such as opening a bottle or cutting bread. Surprisingly little is known about the neural control of such functionally important movements. This lack of knowledge might be due to the complexity of object oriented hand movements and the associated difficulties of analyzing these tasks (Obhi 2004).

Based on the knowledge that a neural coupling between arm and leg muscles exists during walking (Dietz 2002; Michel et al. 2008) we hypothesize that also the upper limbs might neurally be coupled when performing cooperative hand movements. Thus, the first part of this thesis addresses the question whether such a neural coupling of both arms/hands exists and what physiological mechanisms are involved in this coupling. The task-specific coupling during walking becomes manifested by electromyographic (EMG) reflex responses that appear in both arms and both legs following electrical stimulation of a peripheral nerve on one leg. In contrast, only bilateral EMG responses in the legs (without reflex responses in the arms) are observed during standing and only unilateral EMG responses appear in muscles of the stimulated leg during sitting. Therefore, a first approach to study a possible neural coupling of upper limbs is the analysis of EMG reflex responses in both arms following unilateral nerve stimulation during cooperative hand movements and then use further neurophysiological and imaging techniques to better understand the mechanism.

The inter-limb coupling mechanism during walking has been shown to be defective in post-stroke patients and relevance for neuro-rehabilitation has been suggested (Kloster et al. 2011). Consequently, in the second part of this thesis we investigated the neural coupling mechanism during cooperative hand movements in post-stroke patients.

A common consequence of an ischemic or hemorrhagic stroke is a spastic paresis of the contra-lesional body side. This hemiplegia due to stroke is one of the most frequent causes of disability (Adamson et al. 2004). Especially impairment of upper limb function has a great impact on self-independence and, consequently, quality of life. And even though training of the affected arm and hand is a main focus in current rehabilitation approaches, their effects on upper limb function is mostly incomplete, i.e. severe deficits in the affected arm usually remain (Hendricks et al. 2002; Kwakkel et
Even by increasing the amount of rehabilitative training the functional outcome of the patients is limited (Teasell et al. 2003). Today, a great variability of therapies is available and they all have been shown to improve functional outcome to some degree. Most commonly applied are constraint induced movement therapy (Taub et al. 1993) which aims to prevent or reverse the learned-nonuse phenomenon (Taub et al. 1994), bilateral training (Mudie and Matyas 2000) and robot assisted training (Lum et al. 2002). No distinct evidence exists favoring one of these approaches (Coupar et al. 2010) and only a few observations suggest, that patients’ characteristics might influence the success of a certain training approach (van der Lee et al. 1999). Both spontaneous recovery and motor training following stroke are based on central nervous system (CNS) plasticity. Regarding brain activity, plastic changes have been shown to either shift it towards normal function or towards compensatory patterns diverging from brain activity observed in healthy volunteers (Schaechter 2004). Another observed form of plasticity after stroke is the increased recruitment of ipsilateral connection between the undamaged hemisphere and the affected arm (Baker 2011).

It can be concluded that even though many rehabilitation training approaches exist, there is still a need to further understand the neural mechanisms behind the trainings and consequently optimize the training strategies and stratify the patients assigned to a certain approach on his or her characteristics.
Aims

The first aim of this thesis was to demonstrate that a neural coupling mechanism exists for the control of cooperative hand movements. The goal was to understand the nature of the neural coupling in healthy volunteers as good as possible and therefore analyzing it with different methods. The second aim was to elucidate the impaired function of this mechanism in post-stroke patients. Especially the contribution of ipsilateral pathways to the neural coupling after CNS lesion was analyzed. Consequently, the third aim was to introduce a new rehabilitation training based on the neural coupling mechanism and to demonstrate its effectiveness. It is of crucial importance to understand motor control of arm and hand movement in both physiology as well as pathology in order to optimize rehabilitation strategies.
Neural coupling of cooperative hand movements: A reflex and fMRI study

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1.1 Abstract

The neural control of ‘cooperative’ hand movements reflecting ‘opening a bottle’ was explored in human subjects by electromyographic (EMG) and functional magnetic resonance imaging (fMRI) recordings. EMG responses to unilateral non-noxious ulnar nerve stimulation were analysed in the forearm muscles of both sides during dynamic movements against a torque applied by the right hand to a device which was compensated for by the left hand. For control, stimuli were applied whilst task was performed in a static/isometric mode and during bilateral synchronous pro-/supination movements. During the dynamic cooperative task EMG responses to stimulations appeared in the right extensor and left flexor muscles, regardless of which side was stimulated. Under the control conditions responses appeared only on the stimulated side. fMRI recordings showed a bilateral extra-activation and interaction of the secondary somatosensory cortex (S2) during the dynamic cooperative but not during the control tasks. This activation might reflect processing of shared cutaneous input during the cooperative task. Correspondingly, it is assumed that stimulation-induced unilateral volleys are processed in S2, leading to a release of EMG responses to both forearms. This indicates a task-specific neural coupling during cooperative hand movements which has consequences for the rehabilitation of hand function in post-stroke patients.
1.2 Introduction

In contrast to the lower limbs a great variety of uni- and bimanual functional hand/arm movements exists which requires a specific neural control. In monkeys, it was suggested that the supplementary motor area (SMA) of one hemisphere influences the motor outflow of both hemispheres (Jenny 1979; Rouiller et al. 1994). Furthermore, the primary (Donchin et al. 1998; Kermadi et al. 1998), and non-primary motor cortex (Tanji et al. 1987) and the prefrontal cortex (Theorin and Johansson 2010) are assumed to play an essential role in the execution of bimanual tasks. Previous research has indicated that distributed neural networks coordinate interlimb coordination including cortical and subcortical areas (Kazennikov et al. 1999; Stephan et al. 1999; Kermadi et al. 2000; Debaere et al. 2001; Swinnen 2002), and their involvement might be task-specific (Ohki and Johansson 1999; Bracewell et al. 2003; Wiesendanger and Serrien 2004; White et al. 2008; Alberts and Wolf 2009; Heitger et al. 2012).

Most human studies on bimanual tasks involve the performance of bilateral separate, symmetrical or reciprocal movements (Kelso et al. 1979; Marteniuk et al. 1984; Fowler et al. 1991; Donchin et al. 1998; Swinnen 2002; McCombe Waller and Whitall 2008; Liuzzi et al. 2011), or object manipulation tasks (Johansson et al. 2006; Theorin and Johansson 2007), whilst cooperative hand movements are rarely studied (for review cf Obhi 2004). So far it has been demonstrated by fMRI (Puttemans et al. 2005; Johansson et al. 2006; Theorin and Johansson 2007; Grefkes et al. 2008; Goble et al. 2010) and magnetoencephalography (Disbrow et al. 2001) the brain activation pattern during bimanual hand movements is task- and condition-dependent. This includes the activation of the supplementary area (SMA), the primary motor cortex (M1), the premotor cortex (PMC), and the secondary somatosensory cortex (S2). However, the role of these cortical areas has not yet been investigated during cooperative hand movements.

The task investigated here, i.e., a ‘bottle opening’ movement against a defined resistance, requires bimanual cooperative movements, in which one hand supports the action of the other one in order to complete the task. Although many daily tasks involve cooperative hand movements, little is known about the underlying neural mechanisms.

The distribution of electromyographic (EMG) responses in arm muscles evoked by nerve stimulation allows analysis of the connectivity of neural circuits involved in a specific task (Michel et al. 2008; Kloter et al. 2011). In cases of uni- or bilateral separate arm movements this approach is known to evoke EMG-responses in the forearm muscles ipsilateral to the stimulation site (Zehr and Kido 2001). It is expected that during cooperative hand movements a neural coupling is reflected in reflex EMG responses to unilateral nerve stimulation in forearm muscles of both sides.
Additionally we used functional magnetic resonance imaging (fMRI) in order to identify brain areas involved in the cooperative hand movement task as complementary information. This includes the assumption that the supraspinal pathways and centres, mediating the bilateral reflex EMG responses, can be detected by the imaging study. The aim was to identify the cortical areas involved in the cooperative hand movements and whether they differ from those activated in the control conditions. We hypothesize that the neural coupling during cooperative hand movements is achieved by an exchange of information from each hand to both hemispheres at a brainstem level, followed by a processing of the afferent input in specific cortical sensory-motor areas (e.g. SMA, PMC, S2), which leads to an appropriate control of cooperative hand movements. Since S2 is suggested to be involved in the integration of information from the two sides of the body (Lin and Forss 2002) and is modulated by task effort (Heuninckx et al. 2005; Goble et al. 2010), we hypothesize that neural processing of cooperative hand movements can be achieved by an involvement of S2. Subsequently, we performed psycho-physiological interactions (PPI) and functional connectivity (FC) analyses to estimate the functional coupling between brain regions of interest with relation to task performance.
1.3 Material and Methods

General procedures and experimental conditions

This study was approved by the Ethics Committee of the Canton of Zurich and conformed to the standards set by the Declaration of Helsinki. All subjects were informed about the experiment and gave written consent for their participation. The electrophysiological recordings were performed in 12 right-handed (Oldfield 1971) healthy volunteers (mean age 27.3 years, range 25-32 years; 4 male). Twenty right handed healthy volunteers (mean age: 33 ± 9.8 years; 13 male) participated in the fMRI study. Four of the volunteers participated in both studies. Only right handed subjects were chosen in order to avoid an additional variable which could influence the results.

Electrophysiological study

A device which allows the performance of cooperative movement tasks of the upper limbs was constructed together with the Swiss Federal Institute of Technology Zurich (ETHZ) for the electrophysiological experiments (patent registration number E 11167554). The device comprises two handles representing a bottle which is placed horizontally in front of the subject (Figure 1.1A). The diameters of the two handles corresponded approximately to the two ends of a normal bottle (right 5 cm, left 7 cm). Torque and angular rotation sensors allowed the recording of torsional moments and the position of the handles on both sides. For the cooperative movement task, the two ends of the “bottle” were mechanically connected, i.e., when a torque was applied on one side it became transmitted to the other side. Therefore, the resistance was constant throughout a movement cycle on both sides. This cooperative mode was either used dynamically (the handles could be turned in opposite directions against a predefined resistance) or statically (the handles were blocked for reciprocal rotation).
Figure 1.1: **Experimental set-up and experimental conditions.** (A) Technical construction of the device. There are two torque sensors (typ: burster 8645) marked as (5). These two sensors separately measure the torque signals of the two sides. The main shaft (2) is unlocked resulting in one shaft connecting the two sides of the device for torque transmission. The two position sensors (4) were located on each handle separately. The device shown allows the performance of dynamic bilateral cooperative and static bimanual hand movements against a resistance. During the dynamic and static tasks, the torque exerted by the right hand was transmitted to the left hand for compensation and counteraction. The torques exerted on each side, were indicated on a screen in front of the subject. (B) Experimental conditions. Schematic drawings of all experimental conditions included in the study. The main condition was the performance of dynamic cooperative hand movements (a, dyn-coop). The controls were: bilateral pro-and supination movements (b, pro-sup); static (isometric) cooperative hand movements (c, stat-coop); synchronous up and down wrist movements (with approximately the same movement amplitude and velocity as in a) holding the device (d, non-coop-1) and, reciprocal left-right up and down wrist movements without device (e, non-coop-2). The conditions a and b were applied in both the electrophysiological and fMRI study. The condition c was only applied in the electrophysiological study and conditions d and e only in the fMRI study.
The maximum torque (MaxT) of each subject was determined in the static mode of the device. The highest value of three attempts was taken. For the two experimental conditions the resistance of the device was set to 20% MaxT. In the dynamic task (dyn-coop) subjects performed rhythmic ‘opening’ movements, i.e. extension of the right wrist and flexion of the left wrist, (frequency of 45/min, indicated by a metronome with auditory cues) with the right hand counteracted by the left hand, i.e. both hands were free to rotate during the dynamic task. A visual feedback about the torques applied to the device by both hands was provided through a potentiometer. In one control task (stat-coop) subjects exerted a bimanual reciprocal isometric/static torque of 20% MaxT on the handles (indicated again on a screen placed in front of the subject) with the right forearm extensors counteracted by the left flexor muscles thus mimicking the ‘opening’ task. An additional control task consisted in a frequently investigated upper limb task (Swinnen 2002), i.e. bilateral synchronous pro-/supination movements (pro-sup; frequency of 40-50/min) with freely held dumb-bells (1 or 2 kg, depending on the EMG background activity which had to match the EMG activity during the dynamic task as good as possible). This task was chosen for control as we expected a similar dynamic proprioceptive input (i.e., same forearm extensors and flexors as prime movers as in the coop task) becomes generated as during the dyn-coop task. Each of the 3 motor tasks was performed approximately 80 times. Figure 1.1B gives an overview over the setups for the different experimental conditions.

**Recording of biomechanical and electromyographic signals**

The angular position and torque signals were measured separately for both sides. EMG recordings were made using surface electrodes placed over the forearm flexor (M. flexor carpi ulnaris) and extensor (M. extensor carpi radialis) muscles and over the M. deltoideus pars clavicularis muscles of both arms. All signals were sampled at 1000 Hz and recorded using Soleasy v. 4.1 (ALEA Solutions GmbH Software & Instrumentation, Switzerland). EMG signals were amplified (10’000 fold) and band-pass filtered (30–300 Hz). Afterwards the signals were transferred together with biomechanical signals to a personal computer via an analog-to-digital converter. Further processing of the data was done using Soleasy v. 4.1. EMG signals were offset corrected, rectified and band-stop filtered (45-55 Hz) before RMS values, averages and grand averages were calculated.
Ulnar nerve stimulation

The distal part of the ulnar nerve was randomly stimulated 15 times per side at the onset of movement cycles at the right or left forearm for the release of reflex responses in the arm muscles. The stimulation electrodes (2.63cm$^2$ in size; Neuroline 700, Ambu, Ballerup, Denmark) were placed over the ulnar nerve at both wrists with an inter-electrode space of 2cm.

Electrical pulses were administered by an Electro Stimulator (AS 100, Alea Solutions, Zurich, Switzerland). Each electrical stimulus consisted of a train of four biphasic pulses with 2ms duration per pulse and a frequency of 200 Hz resulting in total stimulus duration of 17ms. The intensity was set on 1.5 times motor threshold (MT). MT was defined as the lowest intensity leading to a visible twitch of the M. abductor digiti minimi. It was determined by stepwise increasing the intensity. This non-noxious stimulation intensity (1.5 MT) is known to evoke cutaneous reflexes (Yang and Stein 1990). A similar stimulus paradigm has been previously used to investigate neural limb coupling during locomotion in healthy subjects (Michel et al. 2008; Dietz and Michel 2009) and post-stroke patients (Kloter et al. 2011).

In a pilot study we compared the effects of median, radial and ulnar nerve stimulation. The bilateral responses to ulnar nerve stimulation were more reproducible, stronger, and the stimuli were less painful than median nerve stimulation. Superficial radial nerve stimulation was more difficult to perform (perception threshold is a quite subjective measure). Therefore we used ulnar nerve stimulation in our study. The stimulation electrodes were attached with surgical tape after determining the optimal stimulation site. The MT of each person was checked to confirm that it was constant during and after the experiment.

The stimuli were randomly released every third to sixth cyclic movement triggered by a change in torque of 0.035 Nm, i.e. about 100 ms after the start of the ‘opening’ (dyn-coop) movement of the right hand and every 5 to 9 seconds during the control tasks. A dummy signal was released at the same time point without stimulation and was used to record the non-stimulated EMG, i.e. background activity.
Reflex data analysis

Reflex responses were analysed by calculating the root mean square (RMS) of EMG signals including all samples within a time window of 50 to 200 ms after start of stimulation. The window was chosen according to the appearance of the main components of the reflex responses. The responses were compared to the RMS of background EMG activity within the same time window following ‘dummy’ stimulations using a multivariate General Linear Model (GLM) with post hoc paired t-Test with Bonferroni correction. The factors for the GLM were condition, stimulation side and recording site.

The responses were mostly suppressive. The amplitude of the reflex response (maximal negativity/suppression; maximal positivity/facilitation) was compared to that of background activity in the non-stimulated recordings. The onsets of reflex responses were determined manually. The differences in latency were also compared by a GLM with post hoc paired t-Test with Bonferroni correction. Pearson correlations were applied in order to evaluate the similarities between the shape of the EMG response patterns recorded from the right forearm extensors and left forearm flexor muscles during the dyn-coop opening task following unilateral nerve stimulation on the ipsilateral and contralateral side, respectively.

fMRI study

For the fMRI study a hand-held MR-compatible device was used corresponding to the ‘bottle’ device used in the EMG experiment, constructed by the ETHZ. In total, twenty volunteers performed the dyn-coop condition with the left hand resisting the ‘opening’ movements guided by the right hand (ca. 20% MaxT) with a frequency of 45/min, so that the right extensors and left flexors were activated.

For control, the same participants performed a bilateral synchronous pronation/supination (pro-sup) movement task of both arms with a frequency of 45/min while holding fMRI compatible dumb-bells of 500g. Additionally, 13 (out of 20) randomly selected volunteers performed two additional ‘non-coop’ tasks. During the ‘non-coop-1’ task, they held the device and executed symmetrical wrist extension and flexion movements (Figure 1.1B, non-coop-1). The rationale for this choice of task was to have a control task in which subjects performed synchronized but non-cooperative wrist movements. This control was included to see whether activation of a brain
region is exclusively influenced by the factor ‘cooperative movement’ or by the factor wrist movement during extension and flexion movements in the dyn-coop task. During the ‘non-coop-2’ task, subjects performed the same reciprocal hand wrist movements as during the dyn-coop condition but without holding the device (Figure 1.1B, non-coop-2). Thus, the factor ‘bimanual hand movement’ was kept constant but the factor ‘hand cooperation’ was minimized.

For all tasks, metronome pacing was achieved by visual cues, which were shown via a mirror system, indicating the start of the requested movement (movement onset was further controlled by the experimenter). The synchronization between the fMRI clock and the temporal onset of the visual cues was controlled by Presentation (www.neurobs.com/presentation). Each task was performed separately (6min duration) and was arranged in a block design: during each block, seven opening or pro-sup movements were followed by rest periods of 8-10s. For each experimental condition, participants performed ten trials. Task presentation was randomized across subjects to avoid order effects. To prevent head movements during the fMRI scan a neck-pad was used to fixate the head. In addition, subjects were only included if overall head motion was smaller than 1.5 mm in translation and 1.5 degrees in rotation. We therefore believe that any task-related activity was not influenced by head motion.

fMRI acquisition

For all tasks, fMRI was performed at the University Hospital of Zurich on a Philips Ingenia 3-T whole-body MRI system (Philips Medical Systems, Best, The Netherlands) and an eight-channel head coil. Functional data was obtained in 180 scans per run using 30 transverse slices covering the whole brain in oblique orientation. Slices were acquired in interleaved order, using a sensitivity encoded (SENSE, factor 1.8), single-shot echo planar imaging (EPI) technique [echo time = 35 ms, repetition time = 2000 ms, field of view = 220 × 220 mm, voxel size: 2.75 × 2.75 × 4 mm, re-sliced: 1.72 × 1.72 × 4 mm, flip angle = 78°]. SENSE imaging was applied to shorten readout trains in single-shot in EPI, to reduce susceptibility artefacts and to improve spatial resolution (Boujraf et al. 2009). Four dummy scans were acquired at the beginning of each run and discarded in order to establish a steady state in T1 relaxation for all functional scans.
Preprocessing

Data was analysed using MATLAB 7.9 (Mathworks Inc., Natick, MA, USA) and SPM8 (Wellcome Department of Cognitive Neurology, London, UK). For each subject functional images were realigned, normalized to the EPI-template provided by the Montreal Neurological Institute (MNI brain), re-sliced to $2 \times 2 \times 2$ mm voxel size, and smoothed using 8-mm full width-at-half-maximum Gaussian kernel. An autoregressive model of the first order was used to account for serial correlations. Highpass filtering with standard 128 s cutoff eliminated slow signal drifts. The Anatomical Automatic Labeling Toolbox for SPM8 was used to identify activated regions (Tzourio-Mazoyer et al. 2002).

Regions of interest (ROI) analysis

Before testing for fMRI signal changes related to cooperative hand movements (i.e., dyn-coop versus pro-sup), we first estimated the average activation strength (i.e., parameter estimates) across the dyn-coop and pro-sup tasks (F-contrast, $p < 0.05$, family-wise error (FWE) corrected for multiple comparisons) in ROIs known to be involved in bimanual hand movements (Puttemans et al. 2005; Grefkes et al. 2008). The spatial coordinates of the individuals ROIs were determined following the definition of Grefkes et al. [45]: The M1 region has to be located in the precentral gyrus and central sulcus near the hand knob (MNI coordinates in this study: left M1: -34 -26 56, right M1: 36 -26 56), the PMC in the lateral precentral sulcus (MNI coordinates: left PMC: -50 -2 44, right PMC: 52 -2 46), the SMA in the dorsal medial wall within the interhemispheric fissure (MNI coordinates: 0 -19 48), and the S2 region in the upper bank of the Sylvian fissure (MNI coordinates: left S2: -56 -16 14, right S2: 54 -14 18). Subsequent analysis was performed for ROIs, placed in S1 and in the insular cortex. For each subject, the time series of all ROIs were extracted in a sphere region (radius = 4 mm).

fMRI data analysis

A standard hemodynamic response function was used for convolution of the model regressors. First-level analyses were conducted using a voxelwise General Linear Model (GLM), which reflects a flexible generalization of an ordinary/simple linear regression (Friston et al. 1995). Each task was entered as regressor into four separate GLMs: dyn-coop and pro-sup ($n = 20$), and non-coop-1 and
non-coop-2 (n = 13). Session specific motion parameters were modelled as covariates of no interest. Second-level random effect analyses were conducted using a full-factorial design. The following two contrasts were estimated: ‘dyn-coop versus pro-sup’ (n=20) and ‘dyn-coop versus average (pro-sup and non-coop-1/2)’ (n=13). All fMRI results were shown on a cluster-corrected voxel threshold of p < 0.001 (spatial extent: k ≥ 42 voxels). The cluster size threshold for the selected p-values was estimated using Monte Carlo simulations (http://afni.nimh.nih.gov/pub/dist/doc/program_help/AlphaSim.html). The cluster threshold method was applied to control for the overall type I error. Only grey matter-related fMRI signal changes will be reported.

**Psycho-Physiological Interactions (PPI) and functional connectivity (FC)**

PPI is a brain imaging method of estimating the functional coupling between a brain region and the rest of the brain with relation to the performance of a particular cognitive task (Friston et al. 1997). In this study, the psychological vector was separately modelled for two types of bimanual hand movements: (1) dyn-coop and (2) pro-sup. For example, the PPI-specific General Linear Model (GLM) for the dyn-coop task contains three regressors: the interaction term (BOLD response x psychological vector), main effect of BOLD activity from a given ROI, and main effect of psychological vector (e.g. dyn-coop). To extend the concept of factorial designs to PPI’s the basic idea is to substitute (neural) activity from one cerebral region for one of the factors:

\[ Y = ROI*\beta_1 + (B2-B1)*\beta_2 + (ROI \times (B2-B1))*\beta_3 + G*\beta_4 + \epsilon \]

with ROI: functional region of interest (substitutes original factor A), \(\beta\): beta weights B1/B2: factor B (with two levels), and \(\epsilon\): error term. (ROI \times (B2-B1))*\beta_3 reflect the interaction term, ROI*\beta_1 and (B2-B1)*\beta_2 reflect the main effects. The following seeds were used for the PPI analysis: right and left, S2, right and left M1, and right and left PMC, and SMA. The analysis was computed for the contrast ‘dyn-coop task > pro-sup task’ and for the reversed contrast.

We used the SPM toolbox conn (v13i, http://www.nitrc.org/projects/conn/) to perform the FC analysis. White matter, cerebrospinal fluid, and the six motion parameters were used as covariates of no interest. Only the white matter and cerebrospinal fluid signals were removed to avoid any bias introduced by removing the global signal (i.e., grey matter) (Behzadi et al. 2007; Murphy et al. 2009). This approach should 'normalize' the distribution of voxel-to-voxel connectivity values as effectively as
including the global signal as a covariate of no interest, but without the potential problems of the latter method. Although we did not record respiration and cardiac responses, it has been demonstrated that non-neuronal physiological noise (e.g., cardiac and respiratory signal) can successfully be removed by the CompCor algorithm (Behzadi et al. 2007) as implemented in the conn toolbox. Bivariate correlations were calculated as a measure of strength of functional connectivity, to examine cross-correlations of BOLD signal time series between ROIs. For each individual, the fMRI time-series were extracted for each ROI using MarsBaR (Brett et al. 2002; http://marsbar.sourceforge.net/) after the fMRI time-series had been spatially smoothed, temporally filtered (0.01 – 0.1 Hz), normalized (to the MNI template) and motion corrected. The signal of a given ROI was then averaged for 4-mm-diameter spheres. The same ROIs as for the PPI analysis were used. Task-specific (dyn-coop and pro/sup) statistical results were represented at p ≤ 0.05 (FWE corrected for multiple comparisons).
1.4 Results

Electrophysiological recordings

An opening movement cycle took a mean of 485 ms. The movement onset of the left and right hands during the dyn-coop task was analysed using the changes in hand position (angular rotation sensors) at both handles. According to this analysis both hands started almost simultaneously with the dynamic ‘opening’ torque exerted by the right hand and the left hand compensatory torque (difference between right and left hand: 18±18ms).

**Figure 1.2**: Electromyographic and biomechanical signals. Individual example of the dyn-coop and the stat-coop tasks. Biomechanical signals and right forearm extensor muscle EMG (average of 15 trials) from one subject. (A) dynamic-task and, (B) static (bimanual) task. The stimulated (left and right ulnar nerve) and non-stimulated conditions are displayed. In addition the torque (solid lines) and position (dotted lines) signals of the right (black) and left (grey) side are shown. Negative sign stands for flexion while positive sign stands for extension torque. Note the two ordinate scales on the left side of every graph (EMG activity [µV] on the outside and position [°] on the inside) and one on the right side (torque [Nm]). Note also the different amplitude calibrations for the EMG signals in A and B.
During the different tasks reflex responses measured in proximal and distal arm muscles following unilateral ulnar nerve stimulation showed approximately the same behaviour, i.e. a unilateral (ipsilateral) EMG response during the control tasks and a bilateral response during the dyn-coop task. For further analysis, only the forearm flexors and extensor muscles were included. Figure 1.2 shows an example of the EMG signal recordings from the right extensor muscles and the biomechanical signals of torque and position of both sides from one volunteer during the performance of the dyn-coop (Figure 1.2A) and of the stat-coop (Figure 1.2B) task. During the dynamic task segmented EMG responses appeared in the right extensor muscles following right and left ulnar nerve stimulation whereas in the non-stimulated movements they did not. In the static task a response was only discerned in the ipsilateral muscle.

In the dyn-coop task the reflex EMG responses evoked by the stimulation consisted mainly of suppressive responses on both sides. These were further evaluated for their similarities in terms of latency, duration and amplitude (Figure 1.3a and b). In contrast, during the stat-coop task reflex responses appeared only ipsilateral to the same stimulation (Figure 1.3c). Figure 1.3 shows the overall averages of the reflex EMG responses in the right extensor and left flexor muscles to ipsilateral (Figure 1.3A) and contralateral (Figure 1.3B) ulnar nerve stimulation during dyn-coop movements (Figure 1.3a and b). The mean background EMG activity is shown as grey area. The RMS values of all reflex responses (time window 50-200 ms) in the dyn-coop task were significantly different from background EMG activity (stimulation ipsilateral p<0.01, contralateral p<0.05). The same difference was found after ipsilateral stimulation in the stat-coop task (Figure 1.3c; p<0.05) and in the pro-sup task (Figure 1.3d; p<0.005) but not following contralateral stimulation, where no response could be detected.
Figure 1.3: Cooperative hand movements: Electrophysiological recordings. Grand averages (n=12 subjects) of the EMG responses in the right forearm extensor (a) and the left flexor (b) during the dynamic cooperative ‘opening’ task to ipsilateral (A) and contralateral (B) ulnar nerve stimulations. (c) Grand averages (n=15 subjects) of the EMG responses in the right extensors during the static/isometric opening task and (d) during the pro-supin task to ipsilateral (A) and contralateral (B) ulnar nerve stimulation. Stimulations were randomly released either on the right or left side at the beginning (about 15%) of the movement cycle. The stimulation artefact last over the first 17ms. The grey area shows the level of background activity. Early responses (ER), first negativity (N1) and first positivity (P1) appeared only after ipsilateral stimulations (a). Peak negativity (N2) and positivity (P2) occurred in all four conditions displayed. Note the different amplitude calibrations in the extensor and flexor recordings.
In figure 1.3 the stimulation artefact (SA) lasted for the first 17ms on both sides. The responses in the right extensor muscles to right (ipsilateral) ulnar nerve stimulation (Figure 1.3Aa) appeared with an early response (ER) that had a latency of 25±3.1ms followed by a first suppressive response (N1) peaking at a latency of 43±6.0ms after onset of right side stimulation and a first facilitative wave (P1) with a peak at 59±7.2ms. The peak of the second suppressive wave (N2) was reached at 86±10.1ms and the following plateau-like activity (P2) was observed at 120±20.3ms. Following left (contralateral) ulnar nerve stimulation (Figure 1.3Ba) the EMG response onset in the right extensor was at 61±8.8ms, the peak of the suppressive wave (N2) occurred at a latency of 87±7.7ms, and the plateau like activity (P2) was reached again at 104±8.7ms. The grand averages of late right extensor responses to ipsi- and contralateral nerve stimulation were similar in their latencies, durations and amplitudes.

Figure 1.3 also shows the corresponding EMG responses in the left flexor muscles following left and right ulnar nerve stimulation (Figure 1.3b). Following left (ipsilateral) ulnar nerve stimulation (Figure 1.3Ab), an early response (ER) (onset 24±3.7ms) was followed by a suppressive peak (N1) with a latency at 48±5.8ms), a facilitative peak (P1) (69±10.8ms) and a second suppressive peak (N2) at 96±18.4ms. The plateau like activity (P2) was reached again at 121±22.9ms. Stimulation of the right (contralateral) ulnar nerve (Figure 1.3Bb) was followed by a suppressive EMG response in the left flexor muscle starting at 66±8.5ms with a peak suppression (N2) at 88±7.7ms, followed by a peak facilitation (P2) at 109±5.1ms.

The background activity and EMG response amplitudes were about 3 times larger in the extensor than in the flexor muscles. In addition, the response amplitudes in the extensor muscles (suppressive peaks) were smaller following contralateral compared to ipsilateral stimulations (not significant, p=0.057).

Figure 1.3c and d show the EMG responses in the right forearm extensors following ipsilateral (Figure 1.3Ac and d) and contralateral (Figure 1.3Bc and d) ulnar nerve stimulation during the control tasks: static (Figure 1.3c) and pro-sup (Figure 1.3d) task. In contrast to the responses in the right arm muscles during the dyn-coop task (Figure 1.3Ba and b), no distinct EMG responses could be detected in the right extensor muscle when the left (contralateral) ulnar nerve was stimulated (Figure 1.3Bc and d). In the static task (Figure 1.3c) the EMG responses in the right extensor muscle following ipsilateral nerve stimulation appeared with a suppressive peak (N1) at a latency of 47±5.6 ms, followed by a facilitative peak (P1) at a latency of 66±6.5 ms, a second suppressive
peak (N2) at 96±15.3 ms and a late facilitative peak (P2) at 141±21.7 ms. Similar responses appeared in the left flexor muscle following left ulnar nerve stimulation (not shown). Also in the pro-sup task (Figure 1.3d) EMG responses were only present in the muscles of the stimulated arm. The EMG responses in the right extensor muscle following ipsilateral nerve stimulation appeared with a suppressive peak (N1) at a latency of 52±4.8 ms, followed by a facilitative peak (P1) at a latency of 73±6.3 ms, a second suppressive peak (N2) at 106±12.7 ms and a late facilitative peak (P2) at 148±29.8 ms.

The response pattern (early and late reflex complex; ER to P2) to ipsilateral stimulation was similar in latency, duration and amplitude during the dyn-coop and the control tasks (Figure 1.3A). Only during the dyn-coop task a late reflex complex (N2, P2) appeared also at the contralateral, non-stimulated side (Figure 1.3B) which again was similar in latency, duration and amplitude to the late ipsilateral reflex complex.

The level of background activity in the extensor muscles was about three times larger (RMS values: 154 to 45 µV) in the dynamic compared to the static condition. When the amplitude of the EMG responses was compared to the amplitude of background EMG (same time interval in non-stimulated movements) it amounted to 65% in the dynamic and 60% in the static task. The difference in background activity is thought to be due to the fact that a dynamic movement against a torque of 20% MaxT produces a stronger muscle activation compared to the static/isometric muscle contraction with a 20% MaxT.

**fMRI recordings**

Figure 1.4A shows robust mean fMRI signal changes across the dyn-coop and pro-sup tasks in the SMA, PMC, and M1 as well as in the cerebellum and higher visual areas (including V5). However, the parameter estimates (Figure 1.4 B) did not differ between dyn-coop and pro-sup in these regions.
Figure 1.4: Cooperative and pro-/sup hand movements: fMRI recordings. The two- and three-dimensional illustrations in (A) show the mean activity across the contrast ‘dyn-coop and pro-sup’ task (p < 0.001, FWE corrected, F-contrast). Robust activations were seen in the SMA, M1, and PMC regions as well as in the cerebellum and higher visual areas. The parameter estimates (with standard deviations) shown in (B) were not different between the dyn-coop and pro-sup task.

The contrast ‘dyn-coop versus pro-sup’ elicited exclusively bilateral activation patterns in the secondary somatosensory cortex (S2, Brodmann area (BA) 43) at a voxel threshold of p < 0.001 (cluster-corrected, k ≥ 42 voxels) as shown in figure 1.5 A and table 1.1 A. Parameter estimates were significantly higher for the dyn-coop than the pro-sup condition (Figure 1.5A, right S2: p < 0.001 and left S2: p < 0.001). As shown in figure 1.5B and table 1.1B, the contrast between ‘dyn-coop versus average pro-sup and non-coop’ still revealed bilateral S2 activation at a voxel threshold of p < 0.001 (cluster-corrected, k≥ 42 voxels), and a weaker activation of the left insular cortex (BA 13), cerebellum (lobe 3-5), right posterior thalamus, and bilateral S1 (BA 2/3). S1 activity was especially strong if the dyn-coop task was compared to the non-coop-2 task (p < 0.0001, data not shown), i.e. a task in which subjects did not hold any device but performed out-phase hand movements.
Figure 1.5: Cooperative versus non-cooperative hand movements: fMRI recordings. The three-dimensional illustration in (A) shows bilateral S2 activation for the contrast dyn-coop versus pro-sup (p < 0.05, cluster-corrected with k ≥ 42). Additionally, in both hemispheres parameter estimates for S2 were significantly higher for the dyn-coop than the non-coop tasks (** p < 0.001). Figure 1.5B shows the bilateral S2 activations for the contrast dyn-coop versus pro-sup (labeled in red) and for the contrast dyn-coop versus non-coop (labeled in green) on an axial slice. L: left hemisphere, R: right hemisphere.
Table 1.1

A) Areas of brain showing significantly greater BOLD signal changes for the contrast ‘dyn-coop task > pro-sup task’ (p < 0.001, cluster-corrected with k ≥ 42)

<table>
<thead>
<tr>
<th>Region</th>
<th>Brodmann area</th>
<th>MNI Coordinates</th>
<th>t-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>x</td>
<td>y</td>
</tr>
<tr>
<td>Left Postcentral Gyrus (S2)</td>
<td>43</td>
<td>-58</td>
<td>-16</td>
</tr>
<tr>
<td>Right Postcentral Gyrus (S2)</td>
<td>43</td>
<td>54</td>
<td>-16</td>
</tr>
</tbody>
</table>

B) Areas of brain showing significantly greater BOLD signal changes for the contrast ‘dyn-coop task > average pro-sup task and non-coop tasks’ (p < 0.001, cluster-corrected with k ≥ 42)

<table>
<thead>
<tr>
<th>Region</th>
<th>Brodmann area</th>
<th>MNI Coordinates</th>
<th>t-value</th>
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<tr>
<td></td>
<td></td>
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<tr>
<td>Left Postcentral Gyrus (S2)</td>
<td>43</td>
<td>-58</td>
<td>-16</td>
</tr>
<tr>
<td>Right Postcentral Gyrus (S2)</td>
<td>43</td>
<td>54</td>
<td>-16</td>
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<tr>
<td>Left insula</td>
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<td>-14</td>
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<tr>
<td>Cerebellum</td>
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<td>-22</td>
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</tr>
<tr>
<td>Right Postcentral Gyrus (S1)</td>
<td>2/3</td>
<td>50</td>
<td>-20</td>
</tr>
</tbody>
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At a more liberal threshold (p < 0.005, cluster corrected) also in the contrast ‘dyn-coop versus pro-sup’ additional activations to S2 were observed bilaterally in S1 (Figure 1.6). Only the left insular cortex (MNI: -38 -14 12, Table 1B, p = 0.01) but not its homologues counterpart (MNI: 38 -14 12) or literature-based insular cortex ROIs (Heuninckx et al., 2005; MNI: -36 24 -8 -36 -2 6) showed stronger activity for dyn-coop relative to all control conditions.
The age of the subjects was not correlated to the right S2 \( (r = -0.21, p = 0.37) \) or left S2 \( (r = 0.35, p = 0.13) \) activity for the dyn-coop task, as assessed by Pearson’s correlations.

The PPI analysis for the between-task comparison showed significant results only for the contrast ‘dyn-coop task versus pro-sup task’ \( (p < 0.001, \text{ cluster-corrected with } k \geq 42) \) but not vice versa (see table 1.2). A main finding was that a preselected seed was never “connected” to its homologus counterpart. In general, results were rather unspecific and did not show a unique pattern within intra-regional or inter-regional seeds. In contrast, the FC analysis revealed that the two S2 ROIs were functionally connected during the dyn-coop only \( (t=3.75) \). In addition, the left M1 and right M1 ROIs show a functional connection \( (t = 6.21) \). At this statistical threshold, no functional coupling between any of the ROIs was observed for the pro/sup task.
Table 1.2: Results of the PPI

Areas of brain showing significantly greater BOLD signal changes for the contrast ‘dyn-coop task > pro-sup task’ (p < 0.001, cluster-corrected with k ≥ 42) using different seed regions. For SMA no significant effects were observed.

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<tr>
<td>Right supramarginal gyrus</td>
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<tr>
<td>Right middle frontal gyrus</td>
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<tr>
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<tr>
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1.5 Discussion

The aim of this study was to explore the neural control of cooperative hand movements by electrophysiological and fMRI recordings. The main result was that only during the performance of a cooperative ‘opening’ movement EMG responses appeared in the forearm muscles of both arms, independent of which side was stimulated. Correspondingly, fMRI showed a bilateral extra-activation of the S2 during the dyn-coop task but not in the control tasks. It is assumed that in most bimanual tasks an integrated control structure is used. However, the present study suggests relevant task-specific differences in the neural control.

Task-specific neural hand coordination

In the present study we observed a task-specific neural coupling during dynamic cooperative hand movements. Previous EMG research in this field has focused mainly on the execution of unilateral or separate bimanual movements, (Donchin et al. 1998; Swinnen 2002; White et al. 2008). A task-dependent amplitude modulation of unilateral EMG responses in upper limb muscles to magnetic brain stimulation (Datta et al. 1989) and to cutaneous nerve stimulation (Zehr and Kido 2001) with larger amplitudes during a dynamic compared to a static muscle contraction (Zehr and Kido 2001) was described. Only ipsilateral EMG responses were also recorded in both control conditions investigated here.

This reflex behaviour differs profoundly from that found during cooperative hand movements and represents the novel result of this study. Exclusively during the dynamic cooperative hand movements a distinct contralateral EMG response pattern (N2-P2 complex) appeared in forearm muscles with approximately the same latency (80ms) as the late complex (N2-P2) of the ipsilateral, stimulated side. Therefore, different neural circuitries are suggested to be involved during cooperative and non-cooperative hand movements.

In the muscles ipsilateral to the nerve stimulation the response pattern was more complex, combining early (ER and N1-P1 complex), -similar to reflex responses elicited during control tasks-, and long-latency (N2-P2 complex) reflex components-present also on the contralateral, non-stimulated side only during the cooperative task. The distribution of reflex responses to a unilateral afferent volley reflects a task-specific, functionally meaningful, neural coupling of upper limbs, i.e. the processing of an artificial input by (coupled) neural circuits. This coupling obviously
depends on the performance of cooperative hand movements ('opening a bottle'), as the coupling did not occur when the task was mimicked in the static (stat-coop) condition or during the dynamic pro-sup task. The mostly suppressive action of the responses might reflect a transient blockage of processing the natural afferent input.

It might be argued that the high level of background EMG in the forearm extensor (compared to flexor) muscles (Figure 1.3a) contributes to the bilateral N2-P2 responses. However, this is rather unlikely as, 1. the extensor background EMG level was similar during the pro-sup control task (Figure 1.3d) but a response appeared only ipsilateral to the stimulation site and, 2. the same bilateral N2-P2 pattern was obtained in the left flexors (Figure 1.3b) during the dyn-coop task although the background EMG level was low compared to the extensor activity (Figure 1.3a).

**Task specific involvement of CNS areas**

We suggest that such a task-specific neural coupling represents an operational rule which occurs within the spinal cord, brainstem and supraspinal circuitries. There is spinal decussation and bilateral termination of cervical corticospinal projections in the cervical cord of monkeys (Rosenzweig et al. 2009) which could account for the bilateral long-latency response pattern seen in the dyn-coop condition. Also stimulation of reticulospinal cells can lead to bilateral response patterns (Drew et al. 1996; Brocard et al. 2010). Such a mechanism was assumed to mediate bilateral arm muscle responses to unilateral leg nerve stimulation during locomotion of stroke subjects (Kloter et al. 2011). Although locomotor function differs basically from cooperative hand movements, the underlying task-dependent neural coupling of limbs might be achieved in a similar way. Nevertheless, it is not possible to conclude from the present experiments whether either one or both mechanisms are involved.

One has to be aware that the electrophysiological and fMRI recordings represent connected studies which provide complementary information since both experimental parts examined cooperative hand movements. They can, however, not directly be linked with each other. Thus the fMRI study does not allow direct conclusions to be drawn in relation to the electrophysiological findings. Based on our fMRI findings, we suggest that the pathways and brain areas involved in the generation of the bilateral reflex responses become reflected in the bilateral activation of S2 during cooperative hand movements (i.e., ‘dyn-coop versus pro-sup’) although no direct
relationship between S2 activation and amplitude of the N2-P2 complex was found in a small subject sample. Using a different set-up this assumption is supported by observations in humans (Disbrow et al. 2001) and non-human primates (Whitsel et al. 1969) as S2 receives afferent inputs from receptor fields of both hands.

Earlier it was hypothesized that a single integrating centre is involved in the control of bimanual movements (Wiesendanger and Miles 1982; Peters 1985; Jagacinski et al. 1988) and that the connectivity between the homologous primary motor cortices is mediated by the corpus callosum (Liuzzi et al. 2011). The latter mechanism would be in line with the observation that patients who undergo callosotomy are unable to perform these tasks (Preilowski 1972).

However, our fMRI results do not provide evidence for a single integrating centre but for the existence of a brain region, i.e. S2 bilaterally, which is specifically involved in the coordination of cooperative hand movements but not in the control tasks. If the contrast dyn-coop versus pro-sup task was analysed, one could argue that we have not identified regions that are responsive to cooperative hand movements as the observed activation differences could simply be due to different proprioceptive input to S2. However, our results do not support this assumption, as bilateral S2 activation was still present after subtraction of the non-coop tasks, which involve both in-phase (‘non-coop-1’ task) and out-of-phase (‘non-coop-2’ task) bimanual hand movements. Thus, S2 activation appears to be task-dependent and, importantly, might be modulated by an ‘additional/stronger coordinative effort’ (Heuninckx et al. 2005; Wenderoth et al. 2005; Goble et al. 2010) during the dyn-coop task. Therefore, the ‘task-specific’ bilateral activation of the S2 areas does not exclude an S2 activation in other bimanual movement tasks not investigated here.

In addition, a S1 activation was found especially in the contrast ‘dyn-coop versus non-coop-2’, although it was weaker then S2 activation. This S1 activation has to be due to cooperative hand movements and not to outphase wrist movements. Hence, we would conclude that successful integration of active cooperative hand movements (integration sensory information of both body parts) is primarily achieved by S2 with an additional involvement of S1.

It has been hypothesized that S2 plays a role in the human mirror system (Avikainen et al. 2002) because an active suppression of S2 occurred during action observation. Yet, this finding is unlikely to explain our results as visual observation of the hand was minimized during scanning. Furthermore, S2 seems to play an important role in proprioception (Lin et al. 2000). Although proprioception from the wrist movements was similar for both the dyn-coop and the pro-sup task
the shared bimanual afferent input during the dyn-coop task might be meaningful for the S2 activation. It is known that S2 activity increases during attentive cutaneous tasks (e.g. Nelson et al. 2004; Jung et al. 2012[65,66]). It would thus seem reasonable that the dyn-coop condition included greater attention to the bimanual cutaneous information that signalled the activity of the opposite limb during rotation of the device.

S2 is also suggested to be involved in the exchange and integration of information from the two sides of the body (Lin and Forss 2002). After unilateral limb stimulation S2 cortices of both hemispheres are activated and thus, S2 is thought to have a role in combining somatosensory information from the two sides of the body to allow its interhemispheric unification (Hari et al. 1998), which is in line with our fMRI results. In addition, the spatial extent of fMRI activation in the S2 (and ventral parietal areas) in humans is larger for bilateral hand stimulation than it is for unilateral (Disbrow et al. 2001). This further supports our suggestion that S2 is engaged and required in the interhemispheric processing of afferent input during cooperative hand movements. The functional connectivity analysis revealed that the left and right S2 areas (in addition to M1) were functionally connected only for the dyn-coop condition. Thus a stronger connectivity between the right and the left S2 exists for the dyn-coop task relative to the pro-sup task. This finding supports the idea of an interaction and coupling between the two cortical areas involved in the execution of the cooperative task.

There was no specific dyn-coop related activation of the supplementary motor area (SMA), (right) insular cortex (Heuninckx et al. 2005), or any other cortical regions (e.g., M1 and PMC; Grefkes et al., 2008) [45] nor an age-related increase in S2 activity within our subject sample (Heuninckx et al. 2005; Goble et al. 2010), suggested to be characteristic for other bimanual hand movements, during the dyn-coop condition. The absence of an age-by-task correlation is not too surprising, as it has been shown that activations (including S2) for coordinated bimanual hand movements do only show an age-dependency for the elderly (subjects > 60 years; Heuninckx et al., 2005; Goble et al., 2010) but not for young adults.

However, the non-subtracted fMRI data show robust activation of the SMA, PMC, and M1 in all experimental hand movement tasks performed in this study, i.e., these cortical areas are obviously non-specifically involved in all bimanual movement tasks. The main difference in the neural organization of cooperative hand movements is the involvement of S2.
Consequences for hand rehabilitation

Task-specific training effects are well established on the basis of both animal experiments (Edgerton et al. 1997; de Leon et al. 1998a; de Leon et al. 1998b) and studies in humans (for reviews see Dietz 2002, Dietz 2008[71,72]). An established approach for hand rehabilitation after a stroke is “constraint-induced movement therapy” (Liepert et al. 1998; for reviews see Taub et al. 1999; Liepert 2010; Nijland et al. 2011) [73-76]. Using this approach the paretic limb is trained exclusively and compensation or involvement of the unaffected limb is avoided. According to observations made here the neural structure and inter-hemispheric information transfer mediating cooperative movements should additionally be trained in order to achieve an improved performance in everyday tasks that require cooperative hand movements.
1.6 Conclusion

On the basis of both reflex and fMRI analyses we conclude that combining and integrating somatosensory information from dynamic cooperative hand movements is task-specifically mediated by S2. Consequently, the afferent volley produced by unilateral nerve stimulation is thought to be processed by an interaction of the S2 areas through the corpus callosum. The similarity in latency of the N2-P2 complex of the reflex EMG responses indicates a joint release from supraspinal centres to the arm muscles of both sides. This together with the simultaneous start of bilateral hand movements to ‘open the bottle’ suggests a common release of executor signals to forearm extensors and flexors. The present data provide evidence that bilateral S2 areas are involved in such cooperative bimanual actions. It remains for further studies to determine the exact pathways involved in the generation of the bilateral reflexes described.
Task-specific role of ipsilateral pathways: Somatosensory evoked potentials during cooperative hand movements

Miriam Schrafl-Altermatt, Volker Dietz

Neuroreport (2014)
2.1 Abstract

Task-specific neural coupling during cooperative hand movements has been described in healthy volunteers, manifested by bilateral reflex EMG-responses in forearm muscles following unilateral ulnar nerve stimulation and by task-specific activation of secondary somatosensory cortical areas (S2) in fMRI. The aim of this study was to investigate the role of sensory input to the ipsi- and contralateral cortex during a cooperative task. Somatosensory evoked potentials (SSEP) from the ulnar nerve were recorded over the ipsi- and contralateral cortex during resting and during cooperative and non-cooperative hand movements. Ipsilateral potentials with smaller amplitude were present in all conditions in almost all subjects. In relation to the resting condition, the amplitudes of both the ipsi- and the contralateral potential were reduced during the cooperative and the non-cooperative tasks. Nevertheless, the reduction in amplitude was similar for the ipsi- and the contralateral potentials in the non-cooperative task but less on the ipsilateral compared to the contralateral side during the cooperative task. The ratio of ipsi-/contralateral SSEP amplitude was thus significantly larger during the cooperative task when compared to the control task and the resting condition. This indicates a functional role of ipsilateral pathways connecting the cervical spinal cord with the cortex during the cooperative task. These observations favor the idea of a task-specific mediation of sensory input from both hands to the ipsi- and contralateral hemispheres (most likely S2) as the basis of neuronal coupling.
2.2 Introduction

The neural control of bimanual movements in humans is less well understood than that of unilateral reaching and grasping movements. Cortical structures such as the supplementary motor area (Jenny 1979), the primary and non-primary motor cortex (Tanji et al. 1987; Donchin et al. 1998), the prefrontal cortex (Theorin and Johansson 2010) as well as subcortical structures such as the corticospinal tract (Davidoff 1990) and the reticulospinal tract (Nathan and Smith 1955) are assumed to play an essential role. These structures are thought to form distributed neural networks that task-specifically control interlimb coordination (Wiesendanger and Serrien 2004).

Only recently has research suggested that cooperative hand movements, such as opening a bottle, underlie a task-specific neural coupling. This neural coupling manifests itself as a bilateral electromyographic (EMG) reflex response in the forearm muscles following unilateral ulnar nerve stimulation (Dietz et al. 2015). The exact mechanism underlying such neural coupling is not yet clear. However, it is likely that ipsilateral fibers of the corticospinal tract, transcallosal connections as well as reticulospinal pathways are involved. In a recent functional magnetic imaging study (fMRI) (Dietz et al. 2015) specific activation of secondary somatosensory (S2) cortical areas was observed during cooperative hand movements and was assumed to represent a task-specific processing of shared afferent input from both hands during these cooperative movements. The S2 cortical areas are suggested to have similar intracerebral connections as the primary somatosensory cortical areas (S1) (Krubitzer and Kaas 1990). Both the S1 and S2 areas have ipsilateral connections with each other, as well as with several other cortical areas such as the parietal ventral area (PV), the rostrolateral parietal cortical area (PR), the primary motor cortex (M1), the supplementary motor area (SMA) and the limbic cortex (Krubitzer and Kaas 1990). Both S1 and S2 have callosal connections with S2 and PV. Additionally, S2 cortical areas are ipsilaterally connected to the frontal eye fields and the frontal part of the ventral stream (Krubitzer and Kaas 1990). The face and trunk areas of S1 cortical areas have additional callosal connections to the contralateral S1 cortical areas which are projected to both homotopic and non-homotopic areas (Pandya and Vignolo 1968). In humans, it is suggested, that by arm nerve stimulation S1 and S2 cortical areas become sequentially activated within one hemisphere contralaterally to the side of stimulation with latencies of 20ms (S1) and 80-100ms (S2). The ipsilateral S2 cortical area is assumed to be activated in parallel via direct thalamic connections with latencies of 80-100ms (Forss et al. 1999). Regarding ipsilateral fiber tracts, it remains unclear whether the afferent or the efferent signals project ipsi- and contralaterally and therefore lead to bilateral responses, or whether a combination of such projections exists. Until
recently, research has focused on motor pathways mediating bimanual interactions (Carson 2005). It has been shown that ipsilateral upper limb muscle responses to transcranial magnetic stimulation (TMS) can be evoked although this mainly occurs in axial and proximal limb muscles (Bawa et al. 2004). The aim of this study was to gain insight into the involvement of ipsilateral somatosensory fibers in neural coupling during cooperative hand movements by analyzing ipsi-and contralateral somatosensory evoked potentials (SSEPs) following ulnar nerve stimulation. It is hypothesized that ipsilateral afferent pathways are more strongly involved in the control of cooperative hand movements than they are in bimanual control tasks.
2.3 Materials and Methods

This study was approved by the Ethics Committee of the Canton of Zurich and conformed to the standards set by the Declaration of Helsinki. All subjects were informed about the experiment and gave written consent for their participation. The recordings were performed in 13 right-handed healthy volunteers (3 male) with a mean age of 26.4 ± 3.7 years and a mean height of 1.73 ± 0.09 meters.

SSEPs were evoked during three experimental conditions, 1. resting (rest), 2. performing non-cooperative bimanual in-phase movements in the form of a bilateral pronation-supination task with dumbbells (pro-sup) and, 3. dynamic cooperative movements (dyn-coop) performed with a device previously used for the fMRI analysis of cortical activation during cooperative movements (Dietz et al. 2015). The order of tasks and stimulation side was varied randomly. The volunteers were in a supine position with their eyes closed and lips open and they were asked not to speak or swallow during the recordings to avoid artefacts. The elbows were placed on the bench by the side of the body during all conditions, so that only forearm movements occurred. For ulnar nerve stimulation, self-adhesive electrodes (5.96mm$^2$, CareFusion, Middleton, Wi, U.S.) were placed over the ulnar nerve at both wrists with an interelectrode distance of 2cm. SSEPs were recorded by needle-electrodes (12mm, Spes Medica S.r.l., Battipaglia, Italy) placed over Fz, C3, C4 and Pz. C3 was referenced to Fz to record the contralateral potential during stimulation of the right wrist and ipsilateral potential during stimulation of the left wrist. C4 was referenced to Fz for measurement of left contralateral and right ipsilateral potential. Pz was referenced to Fz as a control to exclude cephalic signal irradiation which might not be fully subtracted by using the cephalic Fz as reference. Stimulation intensity was set at 0.5mA stronger than motor threshold, i.e. first visible twitching of the M. abductor digiti minimi. Stimulation frequency was set at 3.1 Hz and 2 traces at 200 stimulations were applied per side and condition.

SSEPs were elicited and recorded by KeyPoint XP (Medtronic A/S, Skovlunde, Denmark). Signals were transferred to a personal computer for further analysis including filtering, averaging, latency, amplitude and ratio of amplitude calculation, all of which was performed using Soleasy (ALEA Solutions GmbH Software & Instrumentation, Zurich, Switzerland). A Butterworth band stop filter was used between 45Hz and 55Hz to exclude 50Hz noise from the recorded signal. Latencies were calculated as minima in the time window of 16.5ms to 21.5ms (N20) and maxima in the window of 20ms to 30ms (P25). Amplitudes were calculated as the difference between P25 and N20. The ratio of amplitudes between the two sides was calculated as ipsilateral (stimulation side).
amplitude divided by contralateral amplitude. Time normalization was then calculated by setting the N20 peak of each trace of every subject to 0 and then calculating the average of all traces between 5ms before the peak and 25ms after the peak for illustration purposes. Statistics were calculated with IBM SPSS Statistics 19 (Armonk, New York, U.S.). Differences in latencies and amplitudes were calculated with Friedman’s and Wilcoxon signed-rank tests with Bonferroni corrections.
2.4 Results

Volunteers were able to perform the three requested movement tasks while holding the rest of the body and the head still. Only minimal artefacts therefore occurred during the movement tasks compared to the resting condition. The nerve stimulations were perceived but remained below the pain threshold in all cases. No differences were found regarding stimulation of the dominant or the non-dominant arm and thus no separation of stimulation site was made for further analysis. No potentials could be recorded at the Pz location. All subjects showed contralateral potentials in all three conditions while only 11 out of the 13 volunteers showed ipsilateral potentials. These 11 volunteers showed ipsilateral potentials in all conditions, while the remaining 2 subjects only showed contralateral potentials.

Figure 2.1: (A-C) Time normalized EEG traces. Average of all subjects, N20 at 0ms. The N20-P25 potentials were evoked during (A) the dyn-coop and, (B) the pro-sup tasks. (C) resting condition. Grey traces: contralateral potential; black traces: ipsilateral potentials. Note different calibration of ordinate scales. (D) amplitude ratio: amplitude of ipsilateral potential divided by amplitude of contralateral potential. Significant differences between dyn-coop and pro-sup as well as dyn-coop and rest. * = ps0.05.
Figure 2.1 shows the time normalized EEG traces for all three conditions (Figure 2.1A-C) and the differences in amplitude ratios (Figure 2.1D). In the dyn-coop task (Figure 2.1A), the N20-P25 amplitude of the ipsilateral side was 0.59 ± 0.33µV whereas the contralateral potential had an amplitude of 1.23 ± 0.85µV. Hence, the ratio for the dyn-coop task was 0.70 ± 0.22. In this task the ipsilateral N20 latency was 17.84 ± 0.43ms and the contralateral latency was 18.78 ± 1.56ms (n.s.).

In the pro-sup task (Figure 2.1B), the amplitude ratio was 0.41 ± 0.10 with an ipsilateral amplitude of 0.93 ± 0.31µV and contralateral amplitude of 2.34 ± 0.77µV. The N20 latency was 19.26 ± 1.35ms on the ipsilateral and 19.24 ± 1.25ms on the contralateral side. A similarly low ratio was found in the rest task (Figure 2.1C). The ratio was 0.40 ± 0.09 with an ipsilateral amplitude of 1.47 ± 0.42µV and a contralateral amplitude of 3.85 ± 1.14µV. The latency was 18.87 ± 1.43ms for the ipsilateral N20 and 18.98 ± 1.26ms for the contralateral N20.

The ratio of amplitudes was significantly larger (p<0.05) during the dyn-coop task compared to both the pro-sup and the rest tasks (Figure 2.1D). There was no significant difference between the latter two conditions. The absolute amplitudes of ipsi-and contralateral SSEP were largest during the rest task with significant differences (p<0.05) to the other two conditions for both the ipsilateral and the contralateral potentials. The SSEP amplitudes did not differ significantly between the dyn-coop and the pro-sup task, but there was a trend for larger amplitudes during the pro-sup task compared to the dyn-coop task (p<0.08). The contralateral amplitudes differed significantly from the ipsilateral amplitudes in all conditions. There were no significant differences in SSEP latency between all three conditions.
2.5 Discussion

We show, for the first time, that task-specifically modulated, robust ipsilateral SSEPs evoked by electrical stimulation of the ulnar nerve occurred in most volunteers. So far, ipsilateral potentials have only been shown in 10% of patients with epilepsy (Noachtar et al. 1997). These potentials were evoked by electrical stimulation of the median nerve and had a longer latency (up to 18ms) than the contralateral potentials.

In this study, the ipsilateral potentials were task-specifically modulated and moderately increased in amplitude in the dynamic cooperative upper limb task only. This finding suggests that the afferent volley induced by nerve stimulation becomes task-specifically gated to the ipsi- and contralateral cortex possibly leading to the neural coupling underlying cooperative hand movements (Dietz et al. 2015). This coupling is reflected in the bilateral reflexes elicited following unilateral nerve stimulation and a bilateral activation of S2 cortical areas in fMRI recordings. The fact that no difference existed in the latencies of the ipsilateral and contralateral SSEPs suggests an involvement of the ipsilateral S1 cortical area as potentials in the S2 cortical area to nerve stimulation have longer latencies (80-100ms, Forss et al. 1999). It further suggests that the ipsilateral potentials are due to ipsilateral projections from the cervical spinal cord to the cortex and are unlikely due to mediation through transcallosal fibers as such callosal connections would be expected to cause a time difference between the responses in both hemispheres due to callosal delay (Caminiti et al. 2013).

The N20 latencies amounted to an average of about 18ms. This might indicate that this represents the N18 peak generated in the brainstem rather than the N20 peak originating in the cortex. However, the fact that an N18 can only be recorded if a non-cephalic reference is used (Lee and Seyal 1998) rules this possibility out, as our reference was on the cephalic Fz point. Thus the absence of potentials at the Pz location makes it unlikely that the ipsilateral potentials reflect an irradiation phenomenon instead of a genuine potential.

The general attenuation of ipsi- and contralateral SSEP during the motor tasks compared to resting might be due to the fact that SSEPs are superimposed on a high background noise due to signals arising during the bilateral movements. Consequently the impact of the synchronized volley evoked by electrical stimulation becomes smaller during a movement task in which the afferent pathways are more involved than during rest.
The fact that the ipsilateral potential is less diminished than the contralateral potential during the dyn-coop task, i.e. that the SSEP become similar in amplitude, is thought to reflect the task-specific bilateral S2 activation during the dyn-coop task in the fMRI recordings (Dietz et al. 2015). We suggest that neural coupling during the dyn-coop task is based on the processing of shared input from both hands to the ipsi- and contralateral S2 cortical areas, respectively and represented in the bilateral appearance of SSEPs.

The similar latencies of both the ipsilateral and the contralateral potential are in line with the earlier findings of EMG reflex responses in both forearms arising with the same latencies (Dietz et al. 2015). This suggests a task-specific involvement of ipsi- and contralateral sensory and motor pathways in the control of cooperative upper limb movements. The present study provides evidence towards the mechanisms underlying task-dependent neural coupling. A better understanding of this mechanism will inform rehabilitation of hand function in post-stroke subjects.
Cooperative hand movements in post-stroke subjects: Neural reorganization

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Clinical Neurophysiology (2015)

- I would like to thank Alexandra Schättin who assisted me during the conduction of this study –
3.1 Abstract

Recent research indicates a task-specific neural coupling controlling cooperative hand movements reflected in bilateral electromyographic reflex responses in arm muscles following unilateral nerve stimulation. Reorganization of this mechanism was explored in post-stroke patients in this study. Electromyographic reflex responses in forearm muscles to unilateral electrical ulnar nerve stimulation were examined during cooperative and non-cooperative hand movements. Stimulation of the unaffected arm during cooperative hand movements led to electromyographic responses in bilateral forearm muscles, similar to those seen in healthy subjects, while stimulation of the affected side was followed only by ipsilateral responses. No contralateral reflex responses could be evoked in severely affected patients. The presence of contralateral responses correlated with the clinical motor impairment as assessed by the Fugl-Meyer test. The observations suggest that after stroke an impaired processing of afferent input from the affected side leads to a defective neural coupling and is associated with a greater involvement of fiber tracts from the unaffected hemisphere during cooperative hand movements. The mechanism of neural coupling underlying cooperative hand movements is shown to be defective in post-stroke patients. The neural re-organizations observed have consequences for the rehabilitation of hand function.
3.2 Introduction

Bimanual tasks are assumed to require a specific form of interlimb coordination controlled by distributed neural networks, involving cortical and subcortical areas (Donchin et al. 1998; Kazennikov et al. 1999; Kermadi et al. 2000; Debaere et al. 2001; Swinnen 2002). Alongside these general control mechanisms, task-specificity of neural control seems to exist for different bimanual movements (Ohki and Johansson 1999; Bracewell et al. 2003; Wiesendanger and Serrien 2004; White et al. 2008; Alberts and Wolf 2009). In particular, a task-specific, meaningful coordination of bimanual (Dimitriou et al. 2012) (Diedrichsen et al. 2010; Omrani et al. 2013) or postural (Marsden et al. 1981) motor responses to single limb perturbations was shown to occur.

Cooperative hand movements represent a special type of bimanual task. They differ from other bimanual movements in that not only both hands are acting in synchrony but that, in order to accomplish the task, the action of one hand is supported by an appropriate counteraction of the other one, e.g. in opening a bottle. The neural control of a cooperative task has recently been studied in healthy subjects by investigating reflex responses following unilateral arm nerve stimulation (Dietz et al. 2015). Unilateral non-noxious electrical stimulations of the ulnar nerve were followed by bilateral reflex electromyographic (EMG) responses in the forearm muscles of both sides during the cooperative movement task, indicating a task-specific neural coupling. In contrast, only ipsilateral reflex responses were generated during non-cooperative bimanual control tasks. In addition, ipsilateral somatosensory potentials were larger in amplitude during cooperative hand movements compared to bimanual control tasks indicating a task-specific involvement of ipsilateral pathways in this neural coupling (Schrafl-Altermatt and Dietz 2014).

A similar task-dependent neural coupling of arm and leg movements underlies interlimb coordination during walking (Dietz et al. 2001; Michel et al. 2008), a mechanism which is defective in stroke patients (Kloter et al. 2011).

The aim of this study was to investigate the reorganization of neural coupling underlying cooperative hand movements in post-stroke patients. The goal was to evaluate in how far this mechanism is defective and to what extent this is related to the clinical impairment of hand functions required during activities of daily living (ADL).
3.3 Materials and Methods

This study was approved by the local ethics committee (Kantonale Ethikkommission Zürich) and all participants gave their written informed consent.

The study was performed on 15 post-stroke patients (4 females) with a mean age of 56.2 ± 10.5 years (Table 3.1). All subjects had a mild to moderate hemiparesis (FM score of the affected upper limb: 51.1 ± 6.7; (Woodbury et al. 2013)) and slightly impaired perception of light touch on the affected side resulting from either an ischaemic or haemorrhagic stroke occurring at least 6 months before enrolment (time since stroke: 64.4 ± 51.0 months). Post-stroke subjects were selected according to their clinical impairments, i.e. hemiparesis resulting in the Fugl-Meyer (FM) score of the upper limb between 35 and 60 and without or only mild affection of sensory perception. Radiology reports were only available from the acute phase and did little relate to the clinical impairments. Patients with clinically apparent cognitive deficits preventing from a full understanding task instructions and patients with diseases other than stroke impairing arm or hand function were excluded. 12 age (55.3 ± 10.4 years) and gender (4 females) matched healthy volunteers served as a control group.

General procedures and experimental conditions

The study protocol comprised of two different movement conditions, a cooperative movement task ('coop') using a device previously described (Dietz et al. 2015) and a bimanual synchronous but non-cooperative pro-/supination task ('pro-sup') with dumb-bells.

In the coop condition, patients with a right hemiparesis and right handed volunteers performed rhythmic opening movements (extension of the right wrist and flexion of the left wrist) in a continuous manner. Patients with a left hemiplegia and left handed volunteers performed corresponding closing movements. Although, handedness has been ruled out as influencing factor in previous experiments (not published) the set set-up for this study was chosen in a way that all patients performed the wrist extension movement of the coop task with their paretic hand, while healthy volunteers performed this extension movement with their dominant hand.

The resistance to the movements was set at 20% maximal voluntary force which was tested at the beginning of the experiment. For the pro-sup task, subjects held a dumb-bell in each hand. The
weight was adjusted depending on the EMG background activity which was set to approximately match the EMG background activity during the coop condition. As in the coop condition, the movements were performed continuously and rhythmically. The frequency was set at 45/min for both tasks which was indicated by a metronome. Therefore, an entire movement cycle lasted for about 1.33 seconds and the subsequent cycle started immediately after completion of the previous one. Every subject completed a total of about 120 movement cycles per condition.

**EMG recordings**

For the EMG recordings, dual surface electrodes with an interelectrode distance of 2 cm were placed over the wrist flexor (flexor carpi ulnaris) and extensor (extensor carpi radialis) muscles of both arms. According to pilot EMG recordings, these muscles were most involved in the performance of the movement tasks investigated. EMG signals were sampled at 1500 Hz and recorded with a wireless EMG system (Noraxon, Scottsdale, AZ, USA). The signals were filtered with a band-pass filter (10-10’000 Hz), amplified (500-fold) and transferred to a personal computer. Further processing was accomplished using Soleasy (ALEA Solutions GmbH Software & Instrumentation, Switzerland). Before determining root mean squares (RMS) and averages, the EMG signals were offset corrected, rectified and band-stop filtered (45-55 Hz).

**Ulnar nerve stimulation for reflex release**

Each ulnar nerve was stimulated 15 times with the AS100 stimulator (ALEA Solutions GmbH Software & Instrumentation, Switzerland) at non-noxious intensity during both conditions in order to evoke reflex responses. The intensity was set at 150% of the motor threshold, previously determined as the lowest intensity leading to a visible twitch of the abductor digiti minimi muscle. The stimulation electrodes (2.63 cm2) were placed over the ulnar nerve at both wrists, with an interspace of approximately 1 cm. Each stimulus consisted of a 200Hz train of four biphasic pulses of 2ms duration. This resulted in total stimulus duration of 17ms. The non-noxious stimulation intensity is known to evoke cutaneous reflexes (Yang and Stein 1990). A similar stimulus paradigm has previously been used to investigate neural coupling during cooperative hand movements (Dietz et al. 2015) and during locomotion (Dietz and Michel 2009) in healthy subjects. Electrical stimuli to trigger the EMG reflex responses as well as dummy stimuli for calculation of background activity were randomly
released 200ms after onset of a movement cycle, i.e. during the initial phase of muscle contraction of the target muscles.

*Reflex data analysis*

Reflex responses and background EMG were analyzed by calculating the RMS of EMG signals, including all samples for each condition in each subject within a time window of 50 to 200ms following stimulation. The rationale for this time period was that it was already taken for analysis in a preceding study on healthy volunteers (Dietz et al. 2015) as it became obvious that this window also covers the N2/P2 reflex complex (cf. Figures 3.1 and 3.2). Latencies were calculated as maxima or minima within the same time frame. Determination of peak latencies instead of deviation onsets was chosen for two reasons: (1) this method is more exact and was previously used to investigate the neural coupling in healthy individuals (Dietz et al. 2015). Application of the same method allows a better comparison between healthy and post-stroke patients. (2) Peak latencies can be set automatically whereas deviation onsets would require manual latency placement. In addition, the number of contralateral reflex responses elicited by stimulation of the affected and unaffected arm in the coop task was calculated. For this, the gradient between the most negative point between 75 and 125ms and the most positive point between 100 and 200ms after stimulation was calculated and then compared with the corresponding gradients in the background activity over the same time windows. To be confirmed as a reflex response, the gradient of the response had to be at least 20% higher than the gradient in the background activity. A maximum of two reflex responses was possible, if stimulation of both the affected and the unaffected arm was followed by contralateral EMG responses.

Differences in RMS and latencies were calculated with Wilcoxon signed rank tests with Bonferroni corrections. The correlation between N2 latencies and FM score was calculated as Spearman correlation. Differences between FM score and presence of contralateral reflex responses were calculated using a Friedman test.
3.4 Results

All experimental procedures were well tolerated by both patients and healthy volunteers. Electrical stimulations at 150% of MT were perceived as non-noxious in all cases. In healthy volunteers MTs were achieved at 4.5±1.2mA and 4.25±1.2mA at the dominant and the non-dominant hand, respectively. In stroke patients MTs were achieved at 4.8±2.8mA at the affected arm and 4.0±1.2mA at the unaffected arm. No significant differences between the thresholds were found either in intra- or in inter-group comparisons. All subjects were able to complete the movement tasks with the requested frequency. There was no difference in timing of the movement cycles between healthy volunteers and stroke subject. Also, there was no difference in task execution between post-stroke patients with lower FM compared to those with higher FM. The resistance to movement performance (set according to maximal voluntary force) was lower in patients than in control subjects. Consequently, EMG background activity was smaller in post-stroke patients than in healthy volunteers. During the performance of both, the cooperative and the control task the EMG activity was generally stronger in the forearm extensor than in the flexor muscles (cf. Figure 3.3A, no stimulation). EMG responses to unilateral ulnar nerve stimulation were usually most prominent in the extensor muscles of the affected (patients) or dominant (healthy volunteers) arm and flexor muscles of the contralateral arm. Hence, we only included these two forearm muscle groups for further analysis.

The focus of this study was the difference in the task-specific neural coupling, reflected in the appearance of EMG responses in forearm muscles of both sides to unilateral arm nerve stimulation during cooperative hand movements, between healthy age-matched and post-stroke subjects. Disparities between motor conditions (cooperative versus non-cooperative tasks) were therefore only briefly described and discussed. A preceding study in healthy volunteers (Dietz et al. 2015) addressed this subject extensively.

Healthy volunteers: Characteristics of reflex responses

The reflex responses to unilateral ulnar nerve stimulation in the aged-matched healthy volunteers showed approximately the same characteristics during the coop and the pro-sup task as observed in our previous study in a cohort of young healthy volunteers (Dietz et al. 2015). Even though latencies in the grand average seem to be longer in the present study compared to those in the previous
one done in young healthy subjects (Dietz et al. 2015), there was no statistically significant difference. Figure 3.1A and B shows the grand averages of the EMG responses in healthy subjects following unilateral ulnar nerve stimulation during the coop task. Figure 3.1A shows the ipsilateral reflex responses composed of an ER with a mean latency of 36.1 ± 7.0ms, a first response complex (N1: 52.0 ± 4.6ms and P1: 70.3 ± 9.6ms) and a second response complex (N2: 115.3 ± 16.8ms and P2: 160.5 ± 22.7). Contralateral stimulation only consisted of the second response complex with N2 at 100.9 ± 16.3ms and P2 at 159.3 ± 28.6ms (Figure 3.1B).

During the pro-sup task (not shown), no contralateral reflex response was elicited. The ipsilateral response consisted of an ER at 33.3 ± 8.0ms and a first response complex with N1 at 51.9 ± 3.5ms and P1 at 72.3 ± 9.7ms.

**Stroke patients: Characteristics of reflex responses**

In post-stroke patients stimulation of the unaffected arm led to ipsilateral (Figure 3.1C) and contralateral (Figure 3.1D; Table 3.1) reflex responses which were similar to those observed in healthy controls. Ipsilaterally, an ER was followed by a first (N1-P1) and second (N2-P2) response complex. Contralateral stimulation was followed by the second response complex in the forearm muscles of the affected side. Latencies were similar to those seen in healthy volunteers in both the unaffected (ER: 36.7 ± 6.9ms, N1: 49.7 ± 5.2ms, P1: 75.7 ± 11.6, N2: 109.0 ± 13.7ms, P2: 152.3 ± 29.1ms) and affected arm (N2: 100.6 ± 18.7ms, P2: 145.1 ± 23.8ms).

In contrast, the reflex characteristics during the coop task differed from those observed in healthy volunteers when the nerve of the affected arm was stimulated. Stimulation of the affected arm (Figure 3.1E and F) elicited ipsilateral to the stimulation site (Figure 3.1E) only an early response (ER) with a mean latency of 33.78 ± 6.70ms, followed by three to four small peaks. At the contralateral, unaffected side (Figure 3.1F) no EMG response was recorded.

During the pro-sup control task (not shown), no contralateral reflex responses appeared. The ipsilateral reflex response pattern following stimulation of the unaffected arm consisted of an ER (34.5 ± 7.9ms), N1 (48.6 ± 4.5ms) and P1 (78.1 ± 14.5ms, i.e. the response was similar to control subjects. The ipsilateral response following stimulation of the affected arm consisted of an early response (ER) at 36.6 ± 8.0ms frequently followed by three to four small peaks.
Figure 3.1: Reflex responses during the cooperative task in healthy volunteers and stroke subjects. Grand averages of healthy volunteers (A, B; n=12; all healthy subjects) and post-stroke patients (C-F; n=15; all post-stroke subjects) of the EMG responses in flexor (A, C, F) and extensor (B, D, E) muscles to unilateral ulnar nerve stimulation. In healthy subjects, reflex responses were seen in both ipsilateral (A) and contralateral (B) forearm muscles. In stroke subjects, stimulation of the unaffected arm led to bilateral responses. Stimulation of the affected arm resulted in an ipsilateral early response followed by three to four small, segmented responses on the ipsilateral side. Shaded area represents the level of background EMG. Vertical arrows in A and C indicate the onset of electrical stimulation. ER = early response, N1 = negativity of the first complex, P1 = positivity of the first complex, N2 = negativity of the second complex, P2 = positivity of the second complex. Note different calibrations in B. Left: schematic drawings of stimulation site. Affected part of the body is marked in grey.
### Table 3.1: Characteristics of stroke subjects included in the study

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Left: Clinical characteristics of the post-stroke subjects included in the study with Fugl-Meyer (FM) score and the cause of spastic paresis (isch=ischemia, haem=hemorrhage). Right: Characteristics of the contralateral responses to nerve stimulation of the affected (aff) and unaffected (unaff) arm respectively. Contralateral N2 response ‘aff arm’: responses in affected forearm muscles following nerve stimulation of the unaffected arm and vice versa for the response ‘unaff arm’. The “reflex group” refers to the number of contralateral reflex responses and the “latency group” refers to a short latency (1) and a long latency (2) of the contralateral reflex response in the affected arm to stimulation of the unaffected arm.
**Quantification of reflex responses**

Figure 3.2 shows the quantified RMS values of the EMG responses between 50 and 200ms following arm nerve stimulation in healthy (Figure 3.2A) and post-stroke (Figure 3.2B) subjects during the coop condition. Nerve stimulation in healthy subjects (Figure 3.2A) and of the unaffected arm of stroke subjects (Figure 3.2B) differed significantly ($p < 0.05$) from the background activity in both arms. No significant difference was calculated for the EMG responses following stimulation of the affected arm of stroke subjects. Only in 4 less affected patients (high FM score, cf. Table 3.1, reflex group 2) did stimulation of the affected arm lead to contralateral responses.

RMS values of EMG reflex responses during the non-coop condition (not shown) were significantly higher than background activity only ipsilateral to the stimulated side in healthy volunteers and of the unaffected arm in post-stroke patients.

**Figure 3.2: Quantified reflex responses.** Root mean square (RMS) values of EMG responses within a 50 - 200ms time window after unilateral ulnar nerve stimulation in all healthy volunteers (A) and all post-stroke subjects (B). Grey bars represent the responses in the affected arm in patients and the dominant arm in healthy volunteers; white bars represent the responses in the unaffected arm in patients and the non-dominant arm in volunteers. 'No stimulation': background EMG. Ipsilateral reflex responses are represented by hatched bars; contralateral responses and background activity are represented by non-hatched bars. Laterality of stimulation is coded as: A = affected arm, U = unaffected arm, D = dominant arm, N = non-dominant arm, * = $p < 0.05$. Error bars: standard error.
**Latencies of contralateral N2**

During the coop task, the latencies of the N2 responses in the affected arm to stimulation of the unaffected arm varied between 74 and 131ms (Table 3.1) and were inversely related to clinical impairment (Figure 3.3). Figure 3.3A shows the relationship between the latencies of the contralateral N2 responses to nerve stimulation of the unaffected arm in stroke subjects and the FM score. Five out of the fifteen patients could not be included in this part of the analysis as contralateral responses were elicited neither to stimulation of the unaffected nor of the affected arm (Table 3.1; reflex group 0). Two groups of the remaining ten patients (reflex group 1 and 2) could be defined, one with shorter latencies (Figure 3.4C; $83.7 \pm 5.2$ms) and lower FM scores (48.8...
± 4.1 latency group 1; n=5) and another one with longer latencies (Figure 3.4B 117.5 ± 9.9ms) and higher FM scores (57.0 ± 2.8; latency group 2; n=5). The only exception of this grouping was one patient (S15; table 3.1) who was in group 1 because only after stimulation of the unaffected side a contralateral reflex response appeared but this response had a long latency (124ms) associated with a high FM score (56). Accordingly this patient was attributed to the group with longer response latencies. Both latencies and FM scores differed significantly (p < 0.05) in an intergroup comparison. Additionally, latencies of both groups differed significantly from the values obtained in healthy volunteers (100.1 ± 12.9ms). Contralateral N2 latencies following stimulation of the affected arm which were obtained in four post-stroke patients (97.3 ± 5.7ms) did not differ from latencies in healthy subjects.

**Stroke patients: Relationship between contralateral reflexes and FM score**

Figure 3.4 displays the relationship between the number of contralateral reflex responses to stimulation of either the unaffected or affected arm during the coop task and the FM score in all fifteen post-stroke subjects. The number of contralateral reflex responses are represented by integers 0 to 2, with 0 indicating that no contralateral reflex response could be evoked during the coop task, 1 indicating a contralateral reflex response in the affected arm following stimulation of the unaffected arm and 2 indicating that stimulation of both the affected and the unaffected arm resulted in contralateral reflex responses. The distribution of FM scores differed significantly over the three groups according to the Kruskal-Wallis test. However, post-hoc Wilcoxon signed rank test only showed significant difference between group 0 and 2. The absence of statistical significance between other pairings is most likely due to the small number of 4 to 6 patients included in each group, respectively. However, it indicates that the higher the FM, the more contralateral reflex responses were present.
Figure 3.4: Fugl-Meyer score and contralateral reflex responses. Box plots of the three patterns of contralateral reflex responses are shown: Four patients showed contralateral reflex responses to stimulation of both the affected and unaffected arm (response 2). Six patients showed only contralateral reflex responses to stimulation of the unaffected arm but not following stimulation of the affected arm (response 1), and five patients showed contralateral reflex responses to nerve stimulation of neither the affected nor the unaffected arm (response 0).
3.5 Discussion

The aim of this study was to investigate the neural control of cooperative hand movements in post-stroke patients by means of electrophysiologic recordings. Electrical nerve stimulation of the unaffected arm led in the majority of patients to bilateral forearm EMG responses with profiles similar to those obtained in the healthy control group, despite the difference in background activity. In contrast, nerve stimulation of the affected arm usually elicited only early ipsilateral EMG responses. These ERs showed some abnormalities on the affected side, i.e. they were stronger compared to the non-affected side. This most probably reflects the known reflex behavior in spastic paresis with exaggerated tendon reflexes but a loss of long-latency responses (Dietz and Sinkjaer 2007). It has to large part to remain open what pathways might be involved in mediation the different reflex responses Therefore, the discussion of this study will be focused on the behavior of the contralateral reflex components to stimulation of both the unaffected and affected arm of post-stroke patients, respectively, that reflects the mechanism of neural coupling (Dietz et al. 2015).

Cooperative hand movements: defective neural coupling

Impaired hand function in chronic post-stroke subjects is associated with a defective neural coupling of upper limbs. This was reflected in the relationship between FM score and the appearance of contralateral reflex responses. In mildly impaired patients bilateral responses appeared following stimulation of the affected as well as the unaffected arm which coincides with the results observed in healthy subjects (Dietz et al. 2015). In patients, with moderate impairment, bilateral responses could only be evoked by stimulation of the unaffected arm. The more severely affected patients did not show any contralateral reflex responses regardless of the stimulation side. Task-specific neural coupling was previously described in locomotion (Dietz et al. 2001; Dietz 2002). In this condition, unilateral tibial nerve stimulation during stepping was followed by bilateral EMG responses in proximal arm muscles. It was assumed that this coupling is mediated at a midbrain level. In post-stroke patients, this neural coupling during locomotion was shown to be impaired. Stimulation of the tibial nerve of the unaffected leg produced normal EMG responses in proximal arm muscles bilaterally, but stimulation of the paretic leg elicited neither ipsilateral nor contralateral reflex responses in the arms (Kloter et al. 2011). This was interpreted as being due to an impaired processing of afferent input by the damaged corticospinal tract.
Although the cooperative hand movement task studied here differs fundamentally from automatized stepping movements and seems to be mediated at a cortical level, we suggest that a similar mechanism might underlie this for the first time described observed defective neural coupling during cooperative hand movements in post-stroke subjects.

**The role of ispi- and contralateral cortical-spinal connections**

In our post-stroke patients, a discrepancy between the lack of contralateral responses following stimulation of the affected arm but preserved contralateral responses on the paretic side following stimulation of the unaffected arm was observed. This indicates an impaired processing of afferent input from the affected limb. In contrast, the efferent output to the paretic arm muscles, i.e. the amplitude of the N2/P2 reflex response complex to nerve stimulation of the unaffected limb, was largely preserved even in patients with a distinct paresis. It favors the idea that the control of afferent input by the corticospinal tract (Lemon 2008) is more affected after stroke than it might be expected from the clinical assessment, i.e. light touch perception was only slightly impaired on the affected side.

The largely preserved efferent output on the paretic side may be explained by transmission of the efferent EMG volley to the contralateral, paretic limb by a task-dependent increase in excitability of descending ipsilateral pathways, such as the ipsilateral, non-crossing corticospinal or the cortico-reticulo-propriospinal tract of the undamaged hemisphere (Bradnam et al. 2013). In healthy subjects, ipsilateral tract fibers can be activated under specific task conditions (Bradnam et al. 2010; Howatson et al. 2011). Accordingly, recordings of ipsilateral SSEP potentials were higher in amplitude during cooperative compared to bimanual control conditions suggesting stronger activation of afferent ipsilateral projections (Schrafl-Altermatt and Dietz 2014). Ipsilateral pathways are suggested to provide a substrate for the recovery of hand function after stroke (Baker 2011; Bradnam et al. 2013). Ipsilateral afferent/efferent fibers may, in fact, play an important role in stroke recovery and patients with more severe lesions may recruit this pathway more frequently (Teasell et al. 2005).

The observation that patients with low FM scores showed shorter N2 latencies of the contralateral responses in the affected arm than healthy volunteers would support the hypothesis that afferent and/or efferent ipsilateral pathways are recruited to compensate for the defective
interhemispheric interactions. This might not be restricted to cooperative but also in other meaningful bimanual movements (Dimitriou et al. 2012).

In patients with a moderate FM score contralateral responses occurred with an unusual long latency. These longer N2 latencies of patients with higher FM score might reflect a compensatory inclusion of alternative non-affected pathways leading to longer delays for impulse transmission.

**Therapeutic consequences**

In our view, the observations made in this study are of significance for therapeutic interventions as they give evidence for the reorganization of the defective task-specific neural coupling during cooperative hand movements. The results might serve as a basis for new approaches to restore hand function in post-stroke subjects. The observations made in chronic patients could lead to specific treatments of motor deficits in the more acute stage at least in moderately affected post stroke patients when interventions are usually provided. Compensatory neural adaptations following a stroke could be supported by a specific training approach based on the present experiments. The observation that the unaffected hemisphere influences the activity of the paretic forearm muscles (i.e. nerve stimulation of the unaffected arm leads to a response in the affected arm muscles during cooperative hand movements) might be used for the rehabilitation of hand function. From the (established) training of only paretic arm muscles (e.g. reach and grasp) a limited exploitation of neuroplasticity of the damaged hemisphere can be expected. However, by a combination of training approaches, including cooperative tasks, an involvement of the undamaged hemisphere in the performance of the paretic hand movement occurs which might lead to a better outcome of hand function.

Based on experiments in rodents, current approaches exploiting neuroplasticity after stroke are mainly directed at training of specific motor tasks which are required during ADL. Improvement of function thus depends on the specific task and its underlying neural control to be trained (Edgerton et al. 2004; Buma et al. 2013). Many ADL require cooperative hand movements. We would therefore propose that current therapeutic approaches with an emphasis on training the affected limb should be supplemented by training of bimanual and, specifically cooperative hand movement tasks at an early stage after stroke. This field nevertheless remains in need of further experimental and clinical studies (Sleimen-Malkoun et al. 2010).
3.6 Conclusion

Neural coupling of cooperative upper limb movements is defective in stroke patients. The training of such movements should therefore be integrated in rehabilitation approaches of hand function after stroke.
Neural coupling during cooperative hand movements after stroke: Role of ipsilateral afference

Miriam Schrafl-Altermatt, Volker Dietz

Neurology (submitted)

- I would like to thank Nadja Enz for her help in data acquisition -
4.1 Abstract

The objective of this study was to investigate alterations of ipsilateral afference to the brain as part of the neural coupling mechanism underlying cooperative hand movements of stroke subjects. Therefore, ipsi- and contralateral somatosensory evoked potentials (SSEPs) were recorded following ulnar nerve stimulation of both the affected and the unaffected arm of twelve post-stroke subjects and of eight age-matched healthy volunteers. Recordings were performed during cooperative hand movements as well as during bimanual control conditions. The amplitude ratio, i.e. ipsilateral amplitude divided by contralateral amplitude, was calculated as main outcome measure and was compared between the conditions. In post-stroke subjects, the amplitude ratio was significantly higher during cooperative compared to control hand movements when the affected arm was stimulated. The increase in amplitude ratio during the cooperative movement condition was also greater in the patients compared to healthy volunteers. The presence of ipsilateral SSEPs from the paretic arm to the unaffected hemisphere of was closely correlated with the functional state of patients’ hand function. The observation of an enhanced afferent input from the affected arm to the unaffected hemisphere in post-stroke subjects is assumed to be part of the neural coupling mechanism which was shown to be preserved from the unaffected to the affected limb. This suggests an involvement of the unaffected hemisphere in the control of paretic hand/arm movements. This might have consequences for neuro-rehabilitation.
4.2 Introduction

Cooperative hand movements are required in activities of daily living (ADL). They are defined as counteractive movements of both hands with a force transfer between the hands via a kinematic chain, i.e. one hand supports the action goal of the other one (e.g. opening a bottle). These object oriented movements are controlled by a task-specific neural coupling of both hemispheres (Schrafl-Altermatt and Dietz 2014; Dietz et al. 2015; Schrafl-Altermatt and Dietz 2016a). The mechanism of neural coupling differs from those of distributed neural networks underlying the control of interlimb coordination in non-cooperative bimanual tasks (Kermadi et al. 2000; Wiesendanger and Serrien 2004; Carson 2005; Johansson et al. 2006; Grefkes et al. 2008; Janssen et al. 2010; Gooijers and Swinnen 2014; Shetty et al. 2014). The neural coupling is reflected in the appearance of electromyographic (EMG) reflex responses in activated forearm muscles of both sides following unilateral stimulation of the ulnar nerve (Dietz et al. 2015). In contrast, only ipsilateral reflex responses appear during non-cooperative bimanual hand movements. The neural coupling mechanism is also reflected in a task-specific activation of bilateral secondary somatosensory (S2) cortical areas during cooperative hand movements in fMRI recordings (Dietz et al. 2015).

These observations indicate that each hemisphere is task-specifically involved in the performance of cooperative hand movements. The neural coupling might be achieved by an involvement of ipsilateral pathways. In fact, ipsilateral somatosensory potentials evoked by ulnar nerve stimulation (SSEP) were larger during cooperative compared to bimanual control movements. This indicates an upregulation of afference to the ipsilateral hemisphere during cooperative movements, i.e. it is part of the neural coupling mechanism (Schrafl-Altermatt and Dietz 2014).

In post-stroke subjects, the neural coupling mechanism was shown to be preserved from the unaffected side but defective from the affected arm/hand due to an impaired processing of afferent input (Schrafl-Altermatt and Dietz 2016a). This suggests an involvement of the unaffected hemisphere in the control of paretic hand movements (Schrafl-Altermatt and Dietz 2016a; Schrafl-Altermatt and Dietz 2016b). Ipsilateral pathways are known to play a role in the recovery after stroke (Teasell et al. 2005; Baker 2011). Therefore the question arises about the strength of ipsilateral afferent input to the unaffected hemisphere from the affected limb and, consequently, its influence on the recovery of paretic hand function after a stroke. An analysis of ipsilateral and contralateral SSEP from the unaffected and affected side in post-stroke patients can contribute to answer this question. It is hypothesized that in post-stroke patients the ratio of ipsi- to contralateral ulnar SSEP is higher.
following stimulation of the affected arm during cooperative hand movements, i.e. a stronger afference to the unaffected hemisphere, reflecting an involvement in movement control of the paretic arm/hand.
4.3 Materials and Methods

This study conformed to the Declaration of Helsinki and was approved by the local ethics committee (Ethics Committee of the Canton of Zurich). All subjects were previously informed about the study and gave written informed consent before enrolment.

Twelve chronic, i.e. insult more than 6 months before study onset, post-stroke subjects (61.7±8.4 years; 3 females) participated in this study. Characteristics of the patients are shown in table 4.1. All patients had participated in a previously published study on the neural coupling mechanism analyzing bilateral EMG reflex responses to unilateral arm nerve stimulations during cooperative hand movements (Schrafl-Altermatt and Dietz 2016a). Fugl-Meyer (FM) scores were newly rated at the beginning of the experiment and differed slightly from the FM scores in the previous study (Schrafl-Altermatt and Dietz 2016a). Eight age-matched (61.0±8.0 years; 2 females) healthy volunteers (Table 4.1) served as control and underwent the same study protocol.

Table 4.1: Characteristics of patients and healthy volunteers

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<td>C05</td>
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<td>C06</td>
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<td>4.0</td>
<td>56</td>
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</table>

Left: Clinical characteristics of the post-stroke subjects included in the study including Fugl-Meyer (FM) score. Right: Characteristics of healthy volunteers.
SSEPs were evoked in a supine position with head fixation. The participants were instructed to close their eyes and keep their mouth slightly open. They were further instructed to avoid any head movements or extensive swallowing during the recordings in order to minimize artifacts. Stimulation of the ulnar nerve was applied by KeyPoint XP (Medtronic A/S, Skovlunde, Denmark) through self-adhesive surface electrodes (5.96 mm², CareFusion, Middleton, Wi, U.S.) which were placed over both wrists with an inter-electrode distance of 2cm. Stimulation intensity of the ulnar nerve was set at 0.5 mA above motor threshold (MT), i.e. lowest intensity resulting in visible twitching of the M. abductor digiti minimi. Stimulation frequency was set at 3.1Hz to minimize interference of 50Hz noise and the pulse width was set at 0.2ms.

Cortical potentials were bilaterally recorded by KeyPoint XP (Medtronic A/S, Skovlunde, Denmark) through needle-electrodes (12mm, Spes Medica S.r.l., Battipaglia, Italy) placed over Fz (as reference), C3 and C4. Signals were recorded with a frequency of 12000Hz and band-pass filtered between 500Hz and 1Hz.

Patients had to perform three different tasks during the recording: (1) resting without any movement (rest), (2) bimanual non-cooperative pro- and supination movements with dumbbells (pro-sup) and (3) cooperative hand movements (dyn-coop) using a device previously described (Schrafl-Altermatt and Dietz 2014; Dietz et al. 2015). Electrical ulnar nerve stimulation was consecutively applied at both wrists. The order of stimulated side and task performed during the measurement was randomly varied to avoid ordering effect. Each side and condition was recorded in four traces of 100 stimuli each.

Signals were transferred to a personal computer for further analysis with Soleasy (ALEA Solutions GmbH Software & Instrumentation, Zurich, Switzerland). A Butterworth band-stop filter set between 45Hz and 55Hz was applied to exclude any remaining 50Hz noise from the signal. All four traces per side and condition were averaged for every subject before calculation of latencies and amplitudes. Latencies were automatically set at minima between 19ms and 27ms (N20) and maxima between 23ms and 32ms (P25). Latencies were also visually verified and the time window for automatic latency calculation adjusted if needed. Amplitudes were calculated as differences between N20 and P25. Division of ipsilateral amplitude by contralateral amplitude resulted in the amplitude ratio. Time normalisation for illustrating purposes was achieved by setting the individual N20 peak of every trace to zero. Background EEG was quantified and potentials were defined as EEG responses with amplitudes that exceed the mean of background EEG by at least
one standard deviation. Statistics were calculated with IBM SPSS Statistics 19 (Armonk, New York, U.S.). Differences in thresholds, latencies, amplitude ratios and FM scores were calculated using Wilcoxon signed-rank tests.
4.4 Results

All participants tolerated the experiments well and complied with all instructions. In patients, both sensory thresholds (ST) and motor thresholds (MT) were slightly higher in the affected (ST: 5.4±1.4mA; MT: 9.7±1.9mA) than in the non-affected arm (ST: 4.5±0.8; MT: 9.1±3.1). However, the differences were statistically not significant. The control group showed lower values (ST: 3.7±0.6mA; MT: 7.7±1.7mA). While differences in the ST were significant for both the affected and the unaffected arm of stroke patients, differences in MTs were not significant. Stimulation intensity of 0.5mA above MT intensity was perceived as non-noxious in all cases.

Table 4.2: SSEP latencies and amplitude ratios

<table>
<thead>
<tr>
<th></th>
<th>rest</th>
<th>pro-sup</th>
<th>dyn-coop</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unaffected arm</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contralateral latency</td>
<td>22.03±1.43</td>
<td>21.41±1.13</td>
<td>21.93±2.63</td>
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<tr>
<td>Ipsilateral latency</td>
<td>24.09±2.76</td>
<td>21.57±1.72</td>
<td>22.84±2.24</td>
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<td>Amplitude ratio</td>
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<td>0.53±0.16</td>
<td>0.61±0.22</td>
</tr>
<tr>
<td><strong>Affected arm</strong></td>
<td></td>
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<tr>
<td>Contralateral latency</td>
<td>23.34±1.56</td>
<td>22.94±2.27</td>
<td>23.33±2.69</td>
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<tr>
<td>Ipsilateral latency</td>
<td>22.54±2.21</td>
<td>24.08±2.57</td>
<td>20.98±1.84</td>
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<tr>
<td>Amplitude ratio</td>
<td>0.62±0.33</td>
<td>0.62±0.29</td>
<td><strong>1.23±0.28</strong></td>
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<tr>
<td><strong>Healthy volunteers</strong></td>
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<tr>
<td>Contralateral latency</td>
<td>23.49±2.18</td>
<td>22.56±2.05</td>
<td>23.53±2.34</td>
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<tr>
<td>Ipsilateral latency</td>
<td>23.27±2.49</td>
<td>23.53±2.34</td>
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<tr>
<td>Amplitude ratio</td>
<td>0.66±0.27</td>
<td>0.62±0.21</td>
<td><strong>0.99±0.44</strong></td>
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</tbody>
</table>

Mean values and standard deviations of latencies and amplitude ratios (ipsi- to contralateral amplitude) of somatosensory evoked potentials (SSEPs). The highlighted values are significantly higher than the other amplitude ratios.
Figure 4.1: Time-normalized EEG traces of post-stroke patients. Grand averages (N=12) of the somatosensory evoked potentials (SSEPs) are shown. They were evoked during three different conditions, i.e. resting (rest, A and D), non-cooperative bimanual movements (pro-sup, B and E) and cooperative hand movements (dyn-coop; C and F). Electrical stimulation of the ulnar nerve was applied at the affected (A-C) and the unaffected (D-F) arm of the stroke patients. Grey traces: contralateral potential; black traces: ipsilateral potentials.
Figure 4.1 shows the grand averages of time-normalized EEG traces of post-stroke patients. Ipsilateral potentials (black traces; rest affected arm: 0.45±0.19µV; rest unaffected arm: 0.49±0.21µV; pro-sup affected arm: 0.46±0.19µV; pro-sup unaffected arm: 0.39±0.18µV; dyn-coop unaffected arm: 0.40±0.26µV) were usually smaller than contralateral potentials (grey traces; rest affected arm: 0.74±0.47µV; rest unaffected arm: 1.05±0.55µV; pro-sup affected arm: 0.66±0.33µV; pro-sup unaffected arm: 0.62±0.26µV; dyn-coop unaffected arm: 0.54±0.24µV). However, when stimulating the affected arm of post-stroke patients during dyn-coop the ipsilateral potential had a higher amplitude (0.57±0.26µV) than the contralateral (0.46±0.21µV) one (Figure 4.1C). Therefore, the amplitude ratio (Table 4.2) was above one in this condition and differed significantly from the other conditions (Figure 4.2A). No other condition showed a statistically significant difference in amplitude ratio, i.e. the ratio was always around 0.7. In healthy volunteers, the amplitude ratio during dyn-coop was significantly higher compared to the other conditions (Figure 4.2B). The amplitudes of both the ipsilateral (rest: 1.14±0.70µV; pro-sup: 0.78±0.53µV; dyn-coop: 1.03±0.53µV) and the contralateral (rest: 1.84±0.93µV; pro-sup: 1.26±0.69µV; dyn-coop: 1.16±0.58µV) potentials were higher in healthy volunteers compared to those in stroke patients.
Figure 4.2: Amplitude ratios. (A): Amplitude ratio, i.e. ipsilateral amplitude divided by contralateral amplitude, in post-stroke patients during cooperative hand movements (dyn-coop), non-cooperative bimanual movements (pro-sup) and resting (rest) elicited by stimulation of the ulnar nerve of the affected (a; grey bars) and unaffected (u; white bars) arm. (B): Amplitude ratio, i.e. ipsilateral amplitude divided by contralateral amplitude, in healthy volunteers during cooperative hand movements (dyn-coop), non-cooperative bimanual movements (pro-sup) and resting (rest). * = p < 0.05.
Amplitude of background EEG was similar in all participants during all conditions (0.31±0.05 µV). Therefore, minimal amplitude for potentials was set at 0.35µV. During the dyn-coop condition, all healthy volunteers showed an ipsilateral potential. In post-stroke patients, however, there were two groups: one without an ipsilateral potential when stimulating the affected arm during dyn-coop and with lower FM scores and one group showing ipsilateral potentials in the unaffected hemisphere during dyn-coop and higher FM scores. The FM scores of the upper extremity of these two groups differed significantly (Figure 4.3).

![Figure 4.3: Relationship between ipsilateral potentials and hand function.](image)

Relationship between Fugl-Meyer scores of upper extremities and the presence of ipsilateral potentials elicited by stimulation of the affected arm during cooperative hand movements in post stroke patients. * = p < 0.05. Ipsilateral potentials not present n=5; present n=7.

Latencies (Table 4.2) were similar for all conditions for both the affected and the unaffected arm and also showed no difference when comparing ipsi- and contralateral potentials. Furthermore, no difference was found between post-stroke patients and healthy volunteers.
4.5 Discussion

Recent research observations suggest a task-specific neural coupling of upper limbs during cooperative hand movements (Dietz et al. 2015). The involvement of both hemispheres in the control of each hand in such tasks is reflected, first in the appearance of EMG responses in the activated forearm muscles to unilateral nerve stimulation and, second by prominent ipsilateral SSEPs during the performance of cooperative in comparison to separate hand movements (Schrafl-Altermatt and Dietz 2014). Thus the enhanced ipsilateral ascending input to the brain seems to be part of the specific neural coupling during such movements. In post stroke subjects the neural coupling mechanism was shown to be defective (Schrafl-Altermatt and Dietz 2016a). In most patients the coupling was preserved from the unaffected to the affected side but not from the affected to the unaffected limb.

The aim of this study was to explore the involvement of the ipsilateral ascending input in the defective neural coupling mechanism in post-stroke patients. Up to now, only ipsilateral efferent cortical-spinal connections have been studied in patients with CNS lesions (Turton et al. 1996; Netz et al. 1997; Liepert et al. 1998; Teasell et al. 2005). Furthermore, the importance of afferent input in motor learning (Mechsner et al. 2001; Carson et al. 2002) and its consequences for neuro-rehabilitation (Staines et al. 1997; Jackson et al. 2000; Carson and Swinnen 2002) has been demonstrated. Nevertheless, the role of ipsilateral afference in the reorganization of the neural coupling mechanism after a stroke is not yet known. Here we show a task-specific modulation and dominance of ipsilateral afferent input from the paretic arm to the unaffected hemisphere during cooperative hand movements of moderately affected post-stroke patients. The results suggest an involvement of the unaffected hemisphere in the control of the paretic hand during cooperative movements. This might have consequences for the rehabilitation of hand function after a stroke.

Modulation of ascending input from unaffected and affected arms

As in young healthy volunteers (Schrafl-Altermatt and Dietz 2014) contra- and frequently also ipsilateral SSEPs could be recorded following ulnar nerve stimulation in both stroke patients and age-matched healthy volunteers in all three tasks. The older cohort of healthy volunteers in the present study showed higher amplitude ratios (ipsilateral amplitude divided by contralateral amplitude) compared to the younger cohort. As in young subjects the ratio was significantly higher
during the dyn-coop task than in the other two conditions indicating a task-specific involvement of ipsilateral pathways.

In stroke patients the amplitude ratio was not modulated by the task, i.e. it stayed at the same level in all three conditions when the unaffected arm was stimulated. However, stimulation of the affected arm lead to a significantly higher amplitude ratio and in most patients the amplitude of the ipsilateral SSEP even exceeded the amplitude of the contralateral potential. Thus the afference of the affected arm to the unaffected hemisphere becomes strengthened during cooperative hand movements. The enhanced ipsilateral afference observed in the moderately affected patients might converge in S2 cortical area. In healthy subjects a task-specific activation of the S2 cortical areas in the fMRI during cooperative movements was found (Dietz et al. 2015). These areas are the appropriate candidates for the convergence and processing of shared afferent input from both hands, as each area is known to receive afferent input from both hands (Hari et al. 1998; Lin and Forss 2002).

The observation of an ipsilateral enhanced afference suggests that the unaffected hemisphere is involved in the movement control of paretic hand/arm as a part of the preserved neural coupling mechanism from the unaffected hemisphere to the paretic arm/hand.

*Input of ipsilateral ascending to the neural coupling mechanism in post-stroke patients*

All patients included in this study have previously participated in experiments analysing reflex responses during cooperative hand movements (Schrafl-Altermatt and Dietz 2016a). In most patients, a contralateral reflex response could be elicited in the affected arm by stimulation of the unaffected arm. Only severely affected patients showed neither ipsi-nor contralateral responses to stimulation of the affected and non-affected arm. All these patients did also not show an ipsilateral SSEP following stimulation of the affected arm.

These findings support the assumption that ipsilateral ascending input to the unaffected hemisphere from both the affected and the unaffected arm plays a major role in the neural coupling mechanism of cooperative hand movements in post-stroke subjects.
Impact of ipsilateral afference to the unaffected hemisphere and outcome of hand function

The fact that only patients with a moderate or high FM score showed ipsilateral SSEPs from the affected wrist to the unaffected hemisphere indicates an association of ipsilateral afference with the outcome of hand function after stroke.

In moderately affected patients neural coupling is preserved from the unaffected to the affected side, but not vice versa (Schrafl-Altermatt and Dietz 2016a), i.e. in these patients the unaffected hemisphere mediates the EMG responses to nerve stimulation of the unaffected arm to both, the unaffected and affected forearm muscles during cooperative hand movements. This suggests an involvement of the unaffected hemisphere in movement performance of the paretic hand.

In the group of severely affected patients no such responses could be evoked in the earlier study (Schrafl-Altermatt and Dietz 2016a) and, correspondingly, no ipsilateral SSEP responses could be elicited in the present study. This patient group can obviously not profit from an involvement of the unaffected hemisphere in the performance of cooperative movements by the paretic hand. For the severely affected patients the neural coupling mechanism might not be accessible due to the extent of brain damage or, the other way around, these patients have difficulties to use this mechanism for the support of paretic hand function. Additional studies are required to identify the structures required to allow a neural coupling.

The present observations might be seen at variance to the finding that ipsilateral motor tracts are involved in the neural reorganization of severely affected patients (Netz et al. 1997; Teasell et al. 2005). However, it is debated whether the involvement of ipsilateral motor pathways represents a functional improvement during rehabilitation or is merely due to a transcallosal shift following the loss of contralateral fibres (Carson and Swinnen 2002).

From our results it can hardly be decided whether a favourable outcome of hand function is due to a strengthening of ipsilateral pathways from the affected limb leading to a preserved coupling from the unaffected hemisphere to the paretic limb. Alternatively, these patients might have been primarily less affected compared to the patients without a preserved neural coupling.

Longitudinal studies are required to answer these questions. However, it can be speculated that the involvement of the unaffected hemisphere in the control of movements of the paretic hand requires some preserved involvement of the affected hemisphere and interactions between the hemispheres. As a consequence we would expect that it is decided quite early after a stroke
whether a beneficial effect on the outcome of hand function can be expected from a strengthening of the afference from the affected hand to the unaffected hemisphere.

Conclusion

Our observations indicate an involvement of the unaffected hemisphere in the control of the paretic arm during cooperative hand movements in post-stroke patients. This is reflected in an enhanced ipsilateral afference from the paretic arm to the unaffected hemisphere as the afferent link of the preserved part of neural coupling from the unaffected to the affected side. The functioning of this mechanism is associated with a favourable outcome of paretic hand function. A next step will be to explore which factors (e.g., extent of brain damage) determine the functioning of this part of neural coupling and, consequently, the outcome of hand function. With this knowledge patients could be stratified at an early stage into two groups, i.e. patients who might not and patients who might profit from a training of cooperative movements, required during ADL, for the recovery of their hand function (Schrafl-Altermatt and Dietz 2016b).
Effect of cooperative hand movement training in post-stroke subjects: a single-subject designed study

Miriam Schrafl-Altermatt, Volker Dietz

British Journal of Occupational Therapy (submitted)

- I would like to thank Fanny Leimgruber and the occupational therapists from Klinik Lengg for conducting the trainings and Werner Popp who assisted during the assessments –
5.1 Abstract

Cooperative hand movements have been shown to be controlled by a specific ‘neural coupling’ mechanism which is defective in stroke patients. This study in ABAB single-subject design with two chronic post-stroke patients (S01 and S02) aimed at analysing the effect of cooperative training compared to conventional occupational therapy on the outcome of hand function in stroke patients. Cooperative arm training (COOP) and conventional occupational therapy (OT), respectively were applied in two blocks each (twice four weeks) over a total of 16 weeks of therapy with four training session lasting over 45 minutes every week. All following measures were assessed four times at baseline (BL) and then every two weeks during the intervention: Fugl-Meyer upper limb score, Chedoke arm and hand activity inventory, Box and Block test assessed by a blinded examiner. Additionally, electromyographic reflex responses reflecting neural coupling were recorded. Improvement in the Box and Block test of the impaired hand was greater in the COOP compared to OT (S01: 10.75 BL, 16.0 COOP, 16.5 OT; S02: 36.25 BL, 42.0 COOP, 36.75 OT). In the Chedoke test an improvement was obtained during both therapies only in patient S01 (S01: 35.5 BL, 42.25 COOP, 42.1 OT). The Fugl-Meyer score did not change over the course of training. A modulation of the ‘neural coupling’ mechanism occurred over the course of therapy in S02. It was concluded that cooperative training seems to enhance outcome of some aspects of hand function in single post-stroke subjects.
5.2 Introduction

Stroke is one of the most common causes of disability (Adamson et al. 2004) mainly due to the deficit of arm and hand function (Kwakkel et al. 2003). Impairment of upper limb function has a great impact on self-independence and, thus quality of life. Therefore, arm and hand training are a main focus in the rehabilitation of post-stroke patients. Despite various rehabilitation procedures usually only a poor outcome of hand function can be achieved. Therefore, a search for new treatment strategies is warranted. These should be based on insights from basic research studies.

Recently, a neural coupling mechanism has been shown to be involved in the control of cooperative upper limb movements (Schrafl-Altermatt and Dietz 2014; Dietz et al. 2015). In healthy volunteers, this neural coupling during cooperative arm movements leads to bilateral electromyographic (EMG) reflex responses in forearm muscles following unilateral distal nerve stimulation (Dietz et al. 2015), task-specific activation of bilateral secondary somatosensory (S2) cortical areas (Dietz et al. 2015) and enlarged ipsilateral somatosensory evoked potentials (SSEPs) (Schrafl-Altermatt and Dietz 2014) compared to non-cooperative unimanual and bimanual control tasks. This neural coupling mechanism was shown to be defective in stroke patients (Schrafl-Altermatt and Dietz 2016a). The extent of this defect was related to the patient’s clinical impairment: Severely affected patients showed no contralateral reflex EMG responses following stimulation of both the affected and the unaffected arm. In moderately affected patients a contralateral reflex response appeared in the affected arm elicited by stimulation of the unaffected arm while no contralateral reflex response was evoked by stimulation of the affected arm. The fact that during cooperative hand movements the unaffected hemisphere contributes to movement performance of the affected arm/hand in post-stroke patients should have in impact on the improvement of upper limb function after a stroke. Furthermore, cooperative hand movements are required in activities of daily living (ADL) but are so far usually not included in arm/hand rehabilitation (Coupar et al. 2010). This aspect of hand rehabilitation has not been studied so far (Obhi 2004). Today it seems to be essential to train task-specifically in order to optimize improvement of limb function (Bayona et al. 2005; Hubbard et al. 2009) even though limited transfer to related motor tasks is possible (Schaefer et al. 2013). For these reasons we studied the effect of a cooperative arm/hand training compared to conventional occupational hand therapy in two chronic post-stroke subjects.
5.3 Materials and Methods

This study was approved by the Ethics Committee of the Canton of Zurich and conformed to the Declaration of Helsinki. The subjects were informed about the experiment and gave written informed consent before study onset.

Participants

Two male post-stroke patients (ages 64 and 48) with a chronic ischemic infarction in the right hemisphere participated in this single-case training study. Figure 5.1 provides information about lesion area and demographic data of the two patients. Both patients were right handed and had a moderate Fugl-Meyer (FM) score of the affected upper extremity at enrolment (44 and 46 out of 66). Both patients had no therapy for at least 6 months before study onset.

![Figure 5.1: Characteristics of study participants.](image)

Figure 5.1: Characteristics of study participants. Magnetic resonance imaging scans with plotted lesions in black are shown for patient S01 (A) and S02 (B). Demographic and clinical characteristics are given for both patients. FM: Fugl-Meyer.
**Training protocol**

This study was designed as a single-case training study, meaning that each patient served as own control. The study protocol included 16 weeks of therapy. Patient S01 started with four weeks of cooperative training (COOP), followed by four weeks of conventional occupational therapy (OT) and then repeated both training blocks. Patient S02 started with four weeks of OT and continued accordingly with COOP. Four therapy sessions were conducted every week, each of them lasting for 45 minutes. Thus, every training block consisted of 16 sessions. The COOP was conducted at Balgrist University Hospital (Zürich) by two human movement scientists. It consisted of training using a previously described device (Dietz et al. 2015; Schrafl-Altermatt and Dietz 2016a) which allows cooperative upper limb movements interacting with virtual realities (VRs). The VRs consisted of ADLs and games. The cooperative movements consisted of reciprocal rotation of the handles which were coupled by a clutch. OT was conducted at the Clinic Lengg (Zürich) by two occupational therapists. The therapists were instructed to conduct the therapy at their own appraisal with the sole limitation of not including cooperative hand movements. Besides mobilization, the main focus of OT was the performance of unilateral reach and grasp movements trained conventionally and with the support of robotic devices, e.g. Amadeo (Sale et al. 2012) and Armeo (Klamroth-Marganska et al. 2014).

**Assessments**

Assessments were conducted every two weeks during the training phase starting with a baseline of four assessments and ending with a follow-up assessment three months after the end of training. Hence, each patient completed a total of 13 assessments.

Three clinical outcome measures were included in the study: the FM score for the upper limb (Fugl-Meyer et al. 1975), Chedoke arm and hand activity inventory (CAHAI, version: CAHAI-8, (Gustafsson et al. 2010)) and Box and Block test (BBT, (Mathiowetz et al. 1985)). The clinical outcome measures were conducted by a blinded examiner to avoid biased outcome. The last part of the assessments comprised recordings of EMG reflex responses in forearm muscles of both sides elicited by unilateral ulnar nerve stimulation during cooperative hand movements as described before (Dietz et al. 2015; Schrafl-Altermatt and Dietz 2016a).
Data analysis

All data but the EMG reflex responses were analyzed as follows. Absolute values of the outcome measures were averaged separately for assessments conducted during baseline and after two weeks of COOP and of OT, respectively, in each patient and the standard deviation was calculated. Changes between the assessments were calculated as differences between two consecutive assessments. Averages and standard deviations of changes were calculated separately for changes during baseline, during COOP and during OT, respectively, in each patient. Statistically significant improvements were calculated using c-statistics (Tryon 1982). Z-values were accepted as significant if they exceeded 1.64 (p<0.05) or 2.17 (p<0.01). Time series analyses were conducted for all outcome measures by calculating Pearson correlations between assessment number and dimension of change in both patients.

EMG data was preprocessed as described before (Schrafl-Altermatt and Dietz 2016a). Root mean squares (RMS) in a time window of 50 to 200ms after stimulation onset were calculated for each trial. All trials of an assessment were averaged according to the stimulation site, i.e. nerve stimulation of the affected arm or of the unaffected arm for reflex responses or dummy stimulation for background activity calculation. Gradients between global minima and maxima between 50 and 200ms were calculated in the averaged curves to determine whether a reflex response was present, i.e. the gradient had to be at least 20% higher than the gradient in the background activity, or not, i.e. the gradient was smaller or less than 20% higher as the gradient in the background activity. Correlations between RMS and assessment number were calculated as Spearman correlations.
5.4 Results

Both patients fulfilled the 16 weeks of training and completed all the assessments. COOP resulted in minor blisters in both subjects at the beginning of the training sessions which were further prevented by the use of cycling gloves. No other adverse effects were reported.

Clinical outcome measures

Table 5.1 gives an overview about all outcome measures in both patients but the EMG reflex responses. Average values show the mean performance values in all assessments during baseline and following two weeks of either COOP or OT. The Δ values represent changes due to either COOP or OT training calculated as performance after two weeks of training minus performance of the previous training session. Table 5.2 shows the Z-values of the c-statistics for changes due to training in general (COOP and OT), due to COOP and due to OT, respectively. A Value above 2.17 indicates a significant effect (p<0.01) due to the training phase included in the calculation.

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<tr>
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<tr>
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<tr>
<td>S02</td>
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<td>46±1.87</td>
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</tbody>
</table>

Average: Average of absolute values ± standard deviation; Δ values: Average of inter-assessment changes ± standard deviation, highlighted values indicate greater change compared to the other intervention; BL: Baseline, COOP: cooperative training, OT: occupational therapy; FM: Fugl-Meyer score; CAHAI: Chedoke arm and hand activity inventory; BBT: Box and Block test.
Table 5.2: C-Statistics: Z values

<table>
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</tr>
</thead>
<tbody>
<tr>
<td>S01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COOP + OT</td>
<td>3.31*</td>
<td>-0.36</td>
<td>2.69*</td>
</tr>
<tr>
<td>COOP</td>
<td>2.68*</td>
<td>1.20</td>
<td>2.37*</td>
</tr>
<tr>
<td>OT</td>
<td>2.43*</td>
<td>-2.1</td>
<td>2.70*</td>
</tr>
<tr>
<td>S02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COOP + OT</td>
<td>0.91</td>
<td>0.17</td>
<td>0.50</td>
</tr>
<tr>
<td>COOP</td>
<td>2.51*</td>
<td>1.64</td>
<td>0.43</td>
</tr>
<tr>
<td>OT</td>
<td>-1.35</td>
<td>-0.15</td>
<td>0.68</td>
</tr>
</tbody>
</table>

COOP + OT: all interventions (cooperative and occupational therapy); COOP: cooperative training, OT: occupational therapy; FM: Fugl-Meyer score; CAHAI: Chedoke arm and hand activity inventory; BBT: Box and Block test. * P < 0.01 if Z-value > 2.17.

Figure 5.2 shows the Box and Block test. S01 (Figure 5.2A) improved significantly (p<0.01) during both OT and COOP. However, the improvements were larger during COOP (Table 5.1). S02 (Figure 5.2B) showed a significant (p<0.01) improvement in the BBT test during the COOP training but no improvement during OT. No significant change was observed in the FM due to the COOP or OT training approach (not shown). In the CAHAI test (Figure 5.3) patient S01 showed significant improvement during COOP while patient S02 did not improve at all. Time series analysis (not shown) revealed no correlation between training time point and improvement in function for any assessment.
Mean baseline = 10.75  
SD = 0.43

Mean baseline = 36.25  
SD = 1.48

+2SD = 11.62

-2SD = 9.88

Follow-up

Patient S01

Patient S02

Figure 5.2: Box and block test.
Figure 5.3: Chedoke arm and hand activity inventory.
**Functional EMG reflex responses**

Subject S01 initially showed only an ipsilateral EMG reflex responses following nerve stimulation of the unaffected arm. This response did not change over the course of training. Patient S02 showed bilateral EMG reflex responses following stimulation of the unaffected arm but no contralateral responses following stimulation of the affected arm. Also this pattern did not change over time. However, the EMG reflex response in the affected arm to contralateral stimulation increased significantly over time (Figure 5.4).

![Graph](image-url)

**Figure 5.4: Contralateral electromyographic reflex response.** The root mean square of the reflex response recorded in the left (impaired) wrist extensor following right (unimpaired) electrical ulnar nerve stimulation increased significantly over time in subject 2. The gradual increase can hardly be assigned to a specific training, but it is likely due to the enhanced use of the neural coupling mechanism. Note that assessment 5 is missing due to technical problems.
5.5 Discussion

The aim of this study was to investigate the effect of a cooperative upper limb training compared to a conventional occupational therapy on the outcome of hand function in two chronic post-stroke patients. The overall changes were rather small with only a few significant improvements of hand function, but all favoring more the COOP training approach. The limited effects might be due to the chronicity of both patients. Although it is known that functional recovery can still occur in a chronic state, it declines over time (for review see (Simpson and Eng 2013)). Patient S01 who showed beneficial effects from both COOP and OT had lower assessment values at the beginning of training and started with COOP, while Patient S02 who had higher baseline values and started with OT showed beneficial effects only during COOP training. Therefore, the difference in the therapeutic effect between the patients might be attributed either to an order effect and/or to a higher efficacy of the therapy in patients with lower baseline function. However, as time series analysis showed no significant correlation between time and amount of change, it is rather unlikely that the difference between the patients is due to an ordering effect.

Outcome of clinical measures

The most important result is the improvement in the Box and Block test, a unilateral reach and grasp test. Both patients improved in this test during COOP and, although one improved also during OT, changes were greater during COOP. This finding would be in line with other studies showing a beneficial effect of bilateral training on unilateral tasks (Mudie and Matyas 2000; Cauraugh and Kim 2002; Cunningham et al. 2002). However, a systematic review (Coupar et al. 2010) did not favor bilateral compared to unilateral arm training. As bimanual movements have also been trained in OT, the result might support the assumption of a positive effect in the Box and Block test due to the training of cooperative compared to other bimanual tasks, i.e. a superiority of COOP over other bimanual therapies.

FM score showed no changes either after COOP or after OT training sessions. Even though the FM score is routinely used as outcome measure (Sivan et al. 2011) it might not be sensitive enough to detect small changes in chronic post-stroke subjects. It is suggested that the variability in the FM score can rather be attributed to factors such as variability in spastic muscle tone and daily fitness than to training.
The CAHAI was specifically chosen as outcome measure in this study as it includes both bimanual and cooperative ADL. The lacking change in this test in one patient could be due to the gross evaluation scale which might not allow detection of small changes. Another explanation might be that this patient reported that he did not transfer the trained movements into daily living activities. Patient S01 improved both during COOP and OT in the CAHAI test compared to baseline. Nevertheless improvements were higher during COOP training sessions.

*Functional reflex behavior/Neural coupling mechanism*

The observation that patient S01 did not show a contralateral EMG reflex response in the affected arm following nerve stimulation of the unaffected arm was unchanged, i.e. the neural coupling mechanism did not recover following both therapies. There is some evidence that ipsilateral fibers from the undamaged hemisphere can be recruited after stroke to compensate for the impaired control by the damaged hemisphere (Baker 2011; Bradnam et al. 2012; Bradnam et al. 2013) and that these fibers become stronger activated during cooperative tasks (Schrafl-Altermatt and Dietz 2014; Dietz et al. 2015; Schrafl-Altermatt and Dietz 2016a). However, this might be the case only in an early post-stroke rehabilitation stage.

Patient S02 showed contralateral EMG reflex response in the affected arm at study onset. This response increased in RMS over the time of both COOP and OT training. This observation indicates that the neural coupling mechanism can be modulated by a training of hand function leading to an increased activation of paretic arm muscles following stimulation of the unaffected arm.

*Limitations*

This pilot study was conducted in two patients. Therefore it can only be interpreted as an indicator for what might be expected in a study performed in a larger population. The two chronic post-stroke patients were selected based on FM score and lesion side with appropriate handedness. Even though the FM score was similar, reach and grasp movements measured as BBT and ADL measured in the CAHAI test differed between the two patients. Therefore, the data of each patient has to be viewed on its own. The number of assessments allowed for the use of c-statistics which might not be sensitive enough to detect small differences between training effects. Hence, also differences in absolute values have to be considered and might be important.
5.6 Conclusion

This study for the first time evaluates training effects of cooperative arm/hand movements, which are based on a specific neural control, on paretic hand function. The study performed in two single patients indicates that such training can improve hand function, especially reach and grasp movements of the affected hand/arm. However, the results are not fully conclusive and thus have to be verified in a larger study including patients early after a stroke before such a training can be integrated in a neuro-rehabilitation program.
Appendix

**Normal and Impaired Cooperative Hand Movements: Role of Neural Coupling**

Miriam Schrafl-Altermatt, Volker Dietz

Appendix

A.1 Abstract

Recent research indicates that a task-specific, interhemispheric neural coupling is involved in the control of cooperative hand movements required for activities of daily living. This neural coupling is manifested in bilateral electromyographic reflex responses in the arm muscles to unilateral arm nerve stimulation. In addition, fMRI recordings show a bilateral task-specific activation and functional coupling of the secondary somatosensory cortical areas (S2) during the cooperative, but not during bimanual control tasks. This activation is suggested to reflect processing of shared cutaneous input during the cooperative task in both cortical areas. In chronic post-stroke patients, arm nerve stimulation of the unaffected arm also leads to bilateral electromyographic responses, similar to those seen in healthy subjects in the cooperative task. However, stimulation of the affected side is frequently followed only by ipsilateral responses. The presence/absence of contralateral electromyographic responses correlates with the clinical motor impairment measured by the Fugl-Meyer score. The observations suggest impaired processing of afferent input from the affected side leading to defective neural coupling during cooperative hand movements after stroke. In severely affected patients, movement execution seems to rely on the involvement of the ipsilateral cortico-spinal tract arising in the non-damaged hemisphere. According to these results, hand rehabilitation of stroke patients, currently focused on reach and grasp movements of the affected side, should be supplemented with the training of cooperative hand movements required during activities of daily living.
A.2 Introduction

In contrast to lower limb movements, a great variety of uni- and bi-manual functional hand/arm movements exist that requires a specific neural control. In monkeys, it was suggested that the supplementary motor area (SMA) of one hemisphere influences the motor outflow of both hemispheres (Jenny 1979; Rouiller et al. 1994). Furthermore, the primary (Donchin et al. 1998; Kermadi et al. 1998) and non-primary motor cortex (Tanji et al. 1987) as well as the prefrontal cortex (Theorin and Johansson 2010) are assumed to play an essential role in the execution of bimanual tasks. Previous research has indicated that distributed neural networks coordinate interlimb coordination of bimanual tasks, including cortical and subcortical areas (Donchin et al. 1998; Kazennikov et al. 1999; Stephan et al. 1999; Kermadi et al. 2000; Debaere et al. 2001; Swinnen 2002).

Alongside these general control mechanisms, task-specificity of neural control seems to exist for various bimanual movements (for review cf. (Wiesendanger and Serrien 2004)). It has been shown, that interhemispheric connections between the primary motor cortices are involved in the control of uni- and bilateral in-phase movements while connections between the premotor cortex and the contralateral primary motor cortex regulate bimanual anti-phase movements (Liuzzi et al. 2011). In bimanual reactive restrain tasks, digits are coupled by a neural control that facilitates reaction of one digit when another digit is preloaded and when digit-specific afferent input is present (Ohki and Johansson 1999). Furthermore, rapid grip force adjustments are modulated by sensory input from the contralateral hand, whereas arm reaction movements in the same task are independent from the contralateral arm (White et al. 2008). During a two hand grasp, bimanual reflex responses occurred following a unilateral mechanical hand perturbation with short latency (30ms) and a delay in the non-perturbed side of about 15ms (Lum et al. 1993).

Cooperative hand movements represent a special type of bimanual tasks. They differ from other bimanual movements in that not only both hands are acting in synchrony but that, in order to accomplish the task, the action of one hand is counteracted by the other, e.g. in opening a bottle. Although many daily tasks involve cooperative hand movements, little is known about the underlying neural mechanism. This chapter gives an overview of recent research on the neural mechanisms underlying cooperative hand movements. A task-specific neural coupling will be demonstrated as the main mechanism controlling cooperative hand movements and its function and dysfunction in post-stroke subjects will be discussed. These novel aspects of normal and impaired hand movement control have consequences for rehabilitation of hand function in post-
stroke subjects. In a first part of this chapter we will describe specific aspects of neural control of cooperative hand movements. In the second part we will discuss the neural adaptations of the impaired task-specific control in post-stroke subjects and in the last part we will establish the consequences of the first two parts for the rehabilitation of hand function and implementation of new technology.
A.3 Task-specific neural control of hand function: Neural coupling

Many daily life activities require cooperative hand movements. Therefore it is surprising to see that little is known about their neural control (Obhi 2004). Recently, a task-specific neural coupling during cooperative hand movements has been described (Dietz et al. 2015). Exclusively during dynamic cooperative hand movements (e.g. opening a bottle), a distinct contralateral EMG response pattern (N2–P2 complex) appears in forearm muscles with approximately the same latency (80 ms) as the late reflex complex (N2–P2) in the forearm muscles of the ipsilateral, stimulated side (Figure A.1). In accordance with previous electrophysiological research on hand function that has focused mainly on the execution of unilateral or separate bimanual movements, a task-dependent amplitude modulation of unilateral EMG responses in upper limb muscles with larger amplitudes during a dynamic compared with a static muscle contraction was described (Datta et al. 1989; Zehr and Kido 2001; Dietz et al. 2015). Also, only ipsilateral EMG responses to arm nerve stimulations were recorded in synchronously performed pro-/supination movements of both hands (Swinnen 2002; Dietz et al. 2015). This reflex behavior differs profoundly from that found during cooperative hand movements, the latter being a novel observation. Therefore, different neural circuitries are suggested to be involved during cooperative and non-cooperative bimanual hand movements.

The bilateral distribution of reflex responses elicited by a unilateral afferent volley reflects a task-specific, functionally meaningful, neural coupling of upper limbs. That is, the processing of an artificial afferent input produced by nerve stimulations is processed by coupled neural circuits within both hemispheres. This coupling seems to depend on the execution of cooperative hand movements, as the neural coupling does not occur when the task is mimicked in a static condition or during a bimanual pro-/supination task. A corresponding observation of a task-specific neural coupling, i.e. the appearance of bilateral arm muscle responses to unilateral leg nerve stimulation during locomotion in healthy subjects (Michel et al. 2008) has recently been described. Although locomotor function differs basically from cooperative hand movements, the underlying mechanism of a task-dependent neural coupling of limbs might be achieved in a similar way (Dietz et al. 2001).

Based on fMRI findings (Dietz et al. 2015), it is suggested that the pathways and brain areas involved in the control of cooperative hand movements and in the generation of the bilateral reflex responses to unilateral nerve stimulation are partly reflected in the task-specific bilateral activation of secondary somatosensory (S2) cortical areas. Using a different set-up, this assumption is
supported by observations in humans (Disbrow et al. 2001) and nonhuman primates (Whitsel et al. 1969) where it could be shown that S2 cortical areas of both hemispheres receive afferent inputs from receptor fields of both hands.

Figure A.1: Reflex responses during a cooperative and a non-cooperative control task in healthy volunteers. Grand averages (n=24) of the EMG recordings in forearm muscles of the non-dominant (A-B) and the dominant (C-D) arm following electrical unilateral ulnar nerve stimulation on the non-dominant arm. Ipsilateral responses (A-B) are similar for both task and consist of an early reflex response (ER) followed by a first component composed of a first negativity (N1) and a first positivity (P1) followed by a late component (N2 and P2). On the contralateral side, a reflex response is only observed during the cooperative task. This response only consists of the late components, i.e. N2 and P2. The stimulation artefact lasting for the first 20ms is seen in both tasks in the ipsilateral muscles. Upper part: schematic drawings of the movement tasks performed and stimulation site. Shaded areas represent the level of background EMG. Vertical arrows in A and B indicate the onset of electrical stimulation. Note the different calibrations.
S2 is suggested to be involved in the exchange and integration of information from both sides of the body (Lin and Forss 2002). After unilateral limb stimulation, S2 cortices of both hemispheres are activated and thus, S2 is thought to have a role in combining somatosensory information from the two sides of the body to allow its interhemispheric unification (Hari et al. 1998). This assumption is in line with the fMRI results obtained during cooperative hand movements (Dietz et al. 2015). In addition, the spatial extent of fMRI activation in the S2 (and ventral parietal) cortical areas in humans is larger for bilateral than for unilateral hand stimulations (Disbrow et al. 2001). This further supports the suggestion that the S2 areas are engaged and required in the inter-hemispheric processing of afferent input during cooperative hand movements. In addition, a functional connectivity analysis shows that the left and right S2 areas (in addition to M1) are functionally connected only during the cooperative task (Dietz et al. 2015). Thus, a stronger connectivity between the right and the left S2 exists for a cooperative hand movement task in comparison to a bimanual pro-/supination task. This finding supports the idea of an interaction and coupling between the two cortical areas involved in the execution of the cooperative task.

Non-subtracted fMRI data show robust non task-specific activation of the SMA, PMC, and M1 in many bimanual and also cooperative, hand movements. Consequently, these cortical areas are obviously nonspecifically involved in bimanual movement tasks. The main difference in the neural organization of cooperative hand movements is the stronger involvement of the S2 cortical areas compared to other bimanual in- and out-phase movement tasks.

The role of sensory input to both the ipsi- and contralateral cortex during a cooperative hand movement task becomes apparent when somatosensory evoked potentials (SSEP) from the ulnar nerve are recorded over the ipsi- and contralateral cortex during cooperative and non-cooperative hand movements (Schrafl-Altermatt and Dietz 2014). In relation to the resting condition, the amplitudes of both the ipsi- and the contralateral potential are reduced during cooperative and non-cooperative tasks. The reduction in amplitude is similar for the ipsi- and the contralaterally recorded potentials in the non-cooperative task. However, during the cooperative task the ipsilateral potential is less reduced compared to the contralateral side. Consequently, the ratio of ipsi-/contralateral SSEP amplitude is significantly larger during the cooperative task when compared to the control task. This indicates a major functional role of ipsilateral pathways connecting the cervical spinal cord with the cortex during the cooperative hand movement task. These observations favor the idea of a task-specific mediation of sensory input from both hands to the ipsi- and contralateral hemispheres, respectively, as the basis of neural coupling.
A.4 Neural control of cooperative hand movements in post-stroke subjects

In post-stroke patients, it is known that both anticipatory postural adjustments (Massion et al. 1999) as well as bimanual coordination, due to somatosensory limitations, (Torre et al. 2013) are reduced. Also the task-specific neural coupling previously discussed in this chapter is defective after a stroke (Schrafl-Altermatt and Dietz 2016a). The extent of the impairment is related to the clinical impairment of motor function. Electrical nerve stimulation of the unaffected arm leads to bilateral forearm EMG responses with characteristics similar to those obtained in healthy subjects. In contrast, nerve stimulation of the affected arm usually elicits only ipsilateral EMG responses (Schrafl-Altermatt and Dietz 2016a).

This striking discrepancy in post-stroke patients between the lack of contralateral responses in unaffected arm muscles following stimulation of the affected arm and the frequently preserved contralateral responses on the paretic side following stimulation of the unaffected arm indicates an impaired processing of afferent input from the paretic side, but a largely preserved efferent reflex transmission to affected arm muscles. This suggests that the processing of afferent input by the corticospinal tract (Lemon 2008) is disproportionately affected after stroke with regard to the generation of efferent output. This is the case although in the clinical testing of these patients the paresis dominates while light touch perception is only slightly impaired on the affected side. The findings are in line with the alteration of a task-specific neural coupling during locomotion in stroke subjects (Kloter et al. 2011). Stimulation of the tibial nerve of the unaffected leg produces normal EMG responses in bilateral proximal arm muscles, but stimulation of the paretic leg elicits neither ipsilateral nor contralateral reflex responses in the arms. This was also assumed to be due to an impaired processing of afferent input by the damaged corticospinal tract.

In severely affected patients, both contralateral reflex responses, i.e. in the paretic as well as in the unaffected arm, are absent while moderately affected patients show the above described preservation of the contralateral reflex in the affected arm. Mildly affected patients do not differ from healthy individuals. Such an observation is important as, after CNS damage, improvement in function depends on the training of both motor tasks required in daily life activities (ADL) and those based on specific neural control (Edgerton et al. 2004). Consequently, cooperative hand movements should be included in training approaches following a stroke. However, rehabilitation of hand function is currently mainly focused on unimanual reach and grasp function of the affected arm and hand.
Appendix

Figure A.2: Contralateral reflex responses in stroke subjects during cooperative movement task. Averages of EMG recordings from the affected side to 15 nerve stimulations of the unaffected side of an exemplary patient with a high Fugl-Meyer score and longer (compared to healthy subjects) N2 latency (A) and a low Fugl-Meyer score and shortened N2 latency (B). The N2 latency seen in A is somewhat longer than that observed in healthy volunteers, indicating a slower processing of afferent input. The latency seen in B is shorter than those observed in healthy volunteers, indicating an involvement of ipsilateral efferent, non-crossing pathways. Shaded areas represent the level of background EMG. Note the different calibrations.

The largely preserved efferent output on the paretic side may be explained by transmission of the efferent EMG volley to the contralateral, paretic limb via an alternative pathway. A suggestion for this pathway is the ipsilateral, non-crossing corticospinal tract or the cortico-reticulo-proprioospinal pathway of the undamaged hemisphere. These fibers can in healthy subjects be activated under specific conditions (Bradnam et al. 2010; Howatson et al. 2011). It is also assumed, that ipsilateral tract fibers become involved in movement performance after stroke for a partial compensation of the deficit on the paretic side (Baker 2011). The observation that severely affected patients with low FM scores show shorter N2 latencies of the contralateral responses in the affected arm than healthy volunteers (Figure A.2) would support such an assumption. It would imply the recruitment of ipsilateral pathways replacing or compensating for the defective inter-hemispheric interactions (Edwards et al. 2013). Ipsilateral fibers of the corticospinal tract may, in fact, play an important role in stroke recovery especially in patients with more severe lesions (for a review see (Teasell et al. 2005)). However, one has to be aware that in such cases motor deficits and functional impairments can concern both ipsi- and contralesional arms (Schaefer et al. 2007). The pathways suggested to be involved in bimanual separate and cooperative movements in healthy subjects as well as in post-stroke subjects are displayed in figure A.3.
Figure A.3: Schematic illustration of the pathways involved in bimanual movement control. This illustration shows the pathways suggested being involved in the neural control of bimanual separate (e.g. pro-/supination movements; A) and in cooperative movements of healthy (B) and post-stroke (C) subjects. During cooperative movements in addition to the pathways involved controlling bimanual separate hand movements, ipsilateral, non-crossing ascending and descending pathways as well as S2 cortical areas become involved and play a role in the compensation of sensorimotor deficits after stroke.
A.5 Consequences for therapy and robotic devices

After stroke, impairment of the affected limb is usually compensated by utilizing the unaffected limb, leading to the non-use phenomenon (Taub et al. 2006). To avoid this, constraint induced movement therapy (CIMT; Taub et al. 1999) is well established. This approach demands exclusive training of the affected arm/hand. No clear evidence of superiority has been demonstrated when CIMT became compared with bimanual training (Stoykov et al. 2009; Lin et al. 2010; van Delden et al. 2013). However, bimanual training of reaching and grasping tasks in stroke patients has been shown to be more effective in improving unilateral execution of these tasks with the affected arm than unilateral training alone (Mudie and Matyas 2000). And there is also some evidence in post-stroke patients that ipsilateral pathways from the unaffected hemisphere support movement performance of the affected limb during bimanual movements. For example, the observation that stroke patients perform a simple tapping task faster when they use both arms/hands compared to execution of the task only with the paretic arm/hand (Jung and Dietz 1975) is in line with the idea of an involvement of the unaffected hemisphere in task performance. Furthermore, children with cerebral palsy can use mirrored movements to accomplish a task with the more affected arm (Carr et al. 1993; Mayston et al. 1999).

Based on experiments in rodents, improvement of function appears to depend on the specific task and its underlying neural control to be trained (for a review (Edgerton et al. 2004; Buma et al. 2013)). Therefore, current approaches to exploit neuroplasticity after stroke are directed at training specific motor tasks required during ADL. The neural coupling mechanism underlying cooperative bimanual movements should therefore also be included in the rehabilitation of hand function after a stroke as many ADL require bimanual cooperation. A single-subject pilot study comparing cooperative training to conventional occupational therapy has in fact indicated an enhanced improvement in affected hand function due to cooperative training (Schrafl-Alttermatt and Dietz 2016b) This field nevertheless remains in need of further experimental and clinical studies (Sleimen-Malkoun et al 2010).

A wide variety of rehabilitation technology for upper limb training after stroke is available today (for review cf. (Laffont et al. 2014)). Robot assisted training has shown superior effects on functional improvements in post-stroke patients when compared to conventional therapy (Lum et al. 2002; Klamroth-Marganska et al. 2014). Currently available robotic devices provide training for the affected hand, e.g. Amadeo (Sale et al. 2012), or arm, e.g. Armeo (Guidali et al. 2011), MIT Manus (Ang et al. 2015).
for bimanual training, e.g. Bi-Manu Track (Formaggio et al. 2013), or mirror movement training, e.g. MIME (Lum et al. 2004) (for review cf. (Basteris et al. 2014)). However, none of these devices enables support for the training of cooperative hand movements and, thereby, the neural coupling mechanism. We suggest that robot-assisted therapy should be complemented by a technology that allows training of cooperative hand and arm movements covering a great range of upper limb tasks needed in ADL.
General Discussion

The objective of this thesis was to demonstrate and discuss a newly discovered neural coupling mechanism which is involved in the motor control of cooperative upper limb movements. The goal was to illustrate both its neurophysiological characteristics in healthy volunteers and its pathology in post-stroke patients.

Neural coupling of bimanual cooperative movements

We were able to show that cooperative hand movements are task-specifically controlled by a neural coupling mechanism. Even though it might not be exclusive, we limited the term “cooperative hand movements” to tasks, where both hands act reciprocally and are coupled by a kinematic chain transferring the force from one hand to the other, i.e. one hand supports the action goal of the other hand. Examples for such movements from ADL are opening a bottle or jar, cutting bread, pumping a bike tire or peeling carrots.

In the first study (paper I) we were able to demonstrate that bilateral EMG reflex responses following unilateral ulnar nerve stimulation are task-specifically elicited during cooperative hand movements, while only ipsilateral EMG reflex responses are observed during bimanual control tasks. Thus, we could demonstrate for the first time a neural coupling mechanism that functionally couples both arms and controls cooperative hand movements. The similarity to the neural coupling of legs and arms during walking (Dietz 2002) suggests a similar neural mechanism, i.e. unilateral afferent input leads to an efferent output to both body sides. However, based on latencies we concluded that in contrast to the coupling during walking which appears to be an operational rule at a brain stem level the neural coupling during cooperative hand movements seems to include cortical areas.

In the second study (paper I) we further analyzed this neural coupling mechanism by the means of fMRI. We were able to show a task-specific activation of bilateral S2 areas during cooperative movements. In addition, previously described cortical areas involved in the control of bimanual tasks such as SMA, M1, PMC and cerebellum (Grefkes et al. 2008) were also activated. However, in contrast to other studies showing no specifically activated cortical areas when comparing unimanual and bimanual tasks (Koenke et al. 2004), we found an integration of S2 areas of both
hemispheres into the tasks-specific control of cooperative hand movements. Additionally, we could demonstrate a functional connectivity between S2 areas of both hemispheres only during the cooperative task. S2 has previously been suggested to play a role in integrating sensory information from both body sides (Lin and Forss 2002) by interhemispheric unification of afferent input (Hari et al. 1998). These findings support our assumption that each S2 cortical area being involved in the neural coupling during cooperative hand movements by a joint processing of afferent input from both hands. Such an organisation would explain our observation that a unilateral afferent volley leads to muscle responses on both sides.

In the next study (paper II) we analyzed the involvement of ipsilateral afferent pathways in the neural coupling mechanism. We could show ipsilateral somatosensory evoked potentials (SSEPs) in all conditions, i.e. resting, non-cooperative bimanual tasks and cooperative hand movements. However, the amplitude ratio, i.e. amplitude of the ipsilateral potential divided by the amplitude of the contralateral potential, was significantly higher during cooperative movements. The fact that there was no latency difference between ipsi- and contralateral potentials suggest that direct spinal-cortical pathways are rather involved than connections via the corpus callosum or via other supraspinal connections (Caminiti et al. 2013). Such task-specific up-regulation of involvement of ipsilateral pathways in the mediation of afferent volleys is suggested to play an important role in the neural coupling mechanism.

Consequently, in a next step (paper in progress, not included in this thesis) we aimed to analyze the possible involvement of ipsilateral efferent pathways in the neural coupling by the use of TMS. We hypothesized a stronger activation of ipsilateral fibers leading to enlarged ipsilateral motor evoked potentials (MEPs). However, we were only able to show a slight increase in root mean square (RMS) of ipsilateral MEPs due to longer duration during cooperative movements when compared to a non-cooperative control task while the number of elicited ipsilateral MEPs was the same for both conditions. The fact that the increase in RMS was not due to enhanced peak-to-peak amplitude but to longer duration of the ipsilateral MEP might suggest an involvement of additional pathways such as cortico-reticulo-propriospinal pathways (Nakajima et al. 2000). Nevertheless, the findings concerning the role of ipsilateral motor pathways in the neural coupling of cooperative hand movements were rather small and inconclusive.
Defective neural coupling after stroke

In paper III we showed that the neural coupling controlling cooperative hand movements is defective in stroke subjects. We analyzed EMG reflex responses in forearm muscles following unilateral stimulation of the ulnar nerve on both the affected and the unaffected arm. Stimulation of the unaffected arm usually led to a normal EMG reflex response pattern, i.e. bilateral responses during cooperative movements and only ipsilateral responses during control tasks. In contrast, stimulation of the affected arm usually failed to elicit a contralateral response during cooperative hand movements. We suggested that this lack of coupling is due to an impaired processing of afferent input from the affected arm. This is in line with the often neglected role of the corticospinal tract in the processing of afferent input (Lemon 2008). The preserved efferent output to the affected arm following stimulation of the unaffected arm indicates an intact coupling, i.e. an involvement of the unaffected hemisphere in movement control of the paretic hand. We found a correlation between the extent of deficit in the neural coupling and the clinical sensorimotor deficits of the post-stroke patients measured by the Fugl-Meyer (FM) score. Additionally, a stronger involvement of ipsilateral motor pathways took place during cooperative movements in more severely affected patients compared to less affected patients. This is in line with previous findings indicating an involvement of ipsilateral tracts in the recovery of function after stroke (Teasell et al. 2005; Baker 2011; Bradnam et al. 2013) and might indicate a compensatory mechanism for lost contralateral connections. The involvement of these fibers in the neural coupling mechanism might therefore be a pathological compensation mechanism for lost contralateral connections rather than a physiological part of the neural coupling of cooperative hand movements.

Consequently, in the next study (paper IV) we analyzed the processing of afferent input during cooperative hand movements in post-stroke patients. In analogy with the healthy volunteers (paper II) we investigated ipsi- and contralateral SSEPs during resting and during cooperative and non-cooperative hand movements. In the grand average, ipsilateral potentials could be elicited from both the affected as well as the unaffected arm during all conditions. However, the amplitude ratio was task-specifically modulated only when the affected arm was stimulated. In contrast to healthy volunteers where the ipsilateral potential were always smaller than the contralateral SSEP, in most post-stroke patients during cooperative hand movements the ipsilateral potential was larger following stimulation of the affected arm when compared to the contralateral potential. Comparison between individual ipsilateral SSEPS during cooperative hand movements and clinical deficit revealed that only less affected patients, i.e. patients with a high
General Discussion

FM score, were able to recruit ipsilateral afferent pathways and thus showed large ipsilateral SSEPs. Consequently, the amplitude of the ipsilateral potential elicited by stimulation of the affected arm correlated with the FM score. This confirms the assumption, that processing of afferent input to the unaffected hemisphere might play a major compensatory role in the neural coupling mechanism of cooperative hand movements in post-stroke patients. It further indicates that strengthening of ipsilateral pathways between the affected arm and the unaffected hemisphere by training of cooperative hand movements might have a beneficial effect for hand function in post-stroke patients.

From bench to bedside

The above findings led to a pilot training study (paper V) using a single-subject ABAB design with two chronic post-stroke patients. The hypothesis was that cooperative training might lead to an enhanced improvement of upper limb function when compared to conventional occupational therapy (OT). Both patients showed greater improvements in the box and block test (BBT) during cooperative training than during OT. This was of interest as the BBT is a test that only evaluates unilateral reach and grasp function which was trained in OT but not by the performance of cooperative hand movements. We suggest a facilitating influence from the unaffected hemisphere to the affected hand by the neural coupling during cooperative hand movements. This might provide the basis for an improved unilateral hand and arm function. It has previously been shown, that bimanual training can lead to better unilateral function than unimanual training (Cauraugh et al. 2010). However, the OT in our study was not restricted to unilateral training but also included bimanual tasks. We therefore suggest that the cooperative training specifically led to functional improvements due to the neural coupling mechanism rather than due to the bimanual approach.

In the training study we could also show an increase in amplitude of the contralateral reflex response following stimulation of the unaffected arm in one of the patients. Even though this increase could be attributed to neither OT nor cooperative training, it indicates a modulation and up-regulation of the pathways involved in the neural coupling mechanism. This might provide an explanation for the functional improvement.

Today, many robotic devices are used in neuro-rehabilitation (Laffont et al. 2014) and superiority for robot assisted training over conventional training has also been shown (Lum et al. 2002). However,
none of the existing devices enables training of cooperative hand movements and, thereby, the neural coupling mechanism. Therefore, we are currently on the way to further develop our device used to investigate the neural coupling mechanism together with an industrial partner to a commercial version.

**Limitations**

In the studies on post-stroke patients we had the drawback of the heterogeneity of population due to lesion location, lesion causes, time since stroke and impairment severity. The variation in impairment severity turned out in so far to be an advantage due to the fact that we could correlate neurological deficit to the observed reflex behavior. The other aspects of the phenotypic variance in our patients made it more difficult to draw conclusions about affected structures. It is still impossible to conclude on a stratification of patients who might benefit from cooperative training.

The training study was only conducted in two chronic post-stroke patients. Even though they showed some beneficial effect from cooperative training, we cannot yet generalize the effects for larger patient populations. We also don’t know yet, in how far patients might have a greater benefit from a cooperative training in an early state when the potential for neuro-plasticity is enhanced (Traversa et al. 1997).
Conclusion

Cooperative hand movements are task-specifically controlled by a newly discovered neural coupling mechanism. This functional coupling of both arms/hands depends on an interplay of various cortical areas of both hemispheres. Ipsilateral ascending and descending pathways and the activation and connectivity of S2 areas between the two hemispheres seem to play a major role in this neural coupling. Post-stroke patients show a deficit in the neural coupling which is related to the clinical impairment. This deficit is associated with an impaired processing of afferent input from the affected side, while neural coupling is preserved from the unaffected to the paretic side. Consequently, cooperative training as part of neuro-rehabilitation might enhance the functional outcome of the paretic arm in post-stroke patients based on the influence from the undamaged hemisphere to the affected side.
Outlook

Physiology of neural coupling controlling cooperative hand movements

There are still some unanswered questions regarding the neural coupling mechanism of cooperative hand movements in healthy volunteers. Involvement of both ipsilateral pathways and transcallosal connections in the neural coupling are not yet fully understood. Also possible task-specific changes of intra-hemispheric connections during cooperative movements have to be further studied. Different research approaches may be applied to address these questions:

Ipsilateral efferent pathways

There are essentially five possible ipsilateral connections between M1 and target muscles in the forearm: (1) Ipsilateral corticospinal fibers, i.e. fibers that fail to cross at the medulla oblongata (Kuypers 1978), (2) corticospinal fibers that re-cross on spinal level (Rosenzweig et al. 2009), (3) connections via the bilaterally organized reticulospinal tract (Jankowska et al. 2003), (4) impulses that travel over C3/C4 propriospinal neurons (Sasaki et al. 2004) or (5) via transcallosal connections (Witelson 1989) to the target muscle. Investigation of ipsilateral efferent pathways is usually done by the use of TMS and analysis of iMEPs. It has been shown that it is possible to elicit iMEPs in healthy volunteers, mainly under strong background activity and high stimulation intensities (Wassermann et al. 1991; Ziemann et al. 1999). We were not yet able to show meaningful modulation of iMEPs during cooperative compared to non-cooperative hand movements in a corresponding study. Nevertheless there might still be a possibility for the involvement of one or more of the above mentioned pathways in the neural coupling mechanism that could not be activated by our approach. The reason for the only slight increase in RMS of iMEPs might be the chosen set-up. A change of stimulation site and/or of level of background EMG might reveal more about the role ipsilateral motor pathways during cooperative hand movements.
Outlook

**Inter- and intrahemispheric connections**

Transcallosal connections play an important role in bimanual coordination (Gooijers and Swinnen 2014). This interhemispheric communication can be both inhibitory and excitatory depending on the task at hand (Takeuchi et al. 2012). The task-specific role and organization of transcallosal connections during cooperative hand movements is not yet fully understood. Even though different methods allow for studying such hemispherical interplay, i.e. EEG, magnetoencephalography (MEG) or TMS, the method of choice here would be diffusion tensor imaging (DTI) based on fMRI with dynamic casual modelling (DCM) and fractional anisotropy (FA) as main outcome measures. The effective connectivity, i.e. inhibitory and excitatory connections and their strengths, between the cortical areas that have been shown to be activated during the conduction of cooperative hand movements (Dietz et al. 2015), i.e. SMA, M1, PMC, cerebellum and S2 might be elucidated. Not only transcallosal but also intrahemispheric connectivity could be measured using this method. FA would be especially interesting in a long-term study when looking at changes of the water diffusion directionality after intensive training of cooperative hand movements.

**Pathology of neural coupling controlling cooperative hand movements**

More studies are needed to better understand the specific deficits in the neural coupling mechanism controlling cooperative hand movements after CNS injuries. This knowledge would serve as basis for stratification of patients that could benefit from a cooperative training in rehabilitation.

**Post-stroke patients**

We already have gained insight into the impairment of neural coupling in post-stroke patients. Nevertheless, it would be interesting to complement the conducted studies by analyses of MEPs and DTI. Previous studies have shown that ipsilateral cortical-spinal connections may play a role in reorganization following stroke (Baker 2011) and that the extent of involvement of such fibers might depend on the severity of functional impairment (Teasell et al. 2005). Our findings in the study analyzing EMG reflex responses in post-stroke patients (Schräfl-Altermatt and Dietz 2016a) are in line with these suggestions. A detailed analysis of the motor part of the neural coupling by the use of TMS
might be insightful. DTI might be interesting to be evaluated in a longitudinal study. Furthermore, analysis of DCM and FA before and after training of cooperative hand movements in acute and chronic post-stroke patients might reveal both spontaneous and training induced reorganization of the neural structures involved in the control of cooperative hand training. ROIs would be the cortical areas shown to be activated in healthy volunteers during cooperative hand movements (Dietz et al. 2015) as well as the corpus callosum and the corticospinal and reticulospinal tract.

**Patients with cerebral palsy**

Patients with cerebral palsy, especially those with unilateral spastic cerebral palsy (USCP), show similarities to post-stroke patients in upper limb function. The main difference is the age at which the CNS lesion is acquired. While stroke usually happen in older patients, USCP occurs either already during pregnancy or during or shortly after birth and thus in a premature state of the CNS. The premature CNS has more reorganizational possibilities and hence phenomena such as mirror movements (Farmer et al. 1991; Carr et al. 1993) which are suggested to be due to an over-taking of the ipsilateral cortical-spinal projections from the contralesional hemisphere as compensation for the lost contralateral control from the ipsilesional hemisphere are more frequently observed in USCP children than in post-stroke subjects (Adler et al. 2015). As a consequence, the enhanced significance of ipsilateral pathways might also have an impact on the neural coupling of cooperative hand movements.

**Implementation of cooperative training into neuro-rehabilitation**

The final goal of this project is to implement the cooperative training approach into neuro-rehabilitation. Even though preliminary observations indicated promising training effects (Schrafl-Altermatt and Dietz 2016b) training studies in larger cohorts are necessary. Motor recovery is most effective in an early state after CNS damage (Hendricks et al. 2002) due to brain plasticity than can be exploited during in the same timeframe (Traversa et al. 1997). This suggests inclusion of both chronic (> six months after lesion) as well as acute (one to six months after lesion) post-stroke patients. Regarding the involvement of S2 area in neural coupling during cooperative movements it might be interesting to analyze whether a difference exists between patients with and without a damage
Outlook

of S2 area. For clinical outcome measures the FM score, the CAHAI and the action research arm test (ARAT) should be applied. In addition, assessments such as fMRI with DTI, EMG reflex responses, SSEPs and MEPs should be included in the training study in order to differentially analyze the benefit from the training and to consequentially stratify patient groups that might be most responsive and therefore benefit the most from cooperative training.
References


References


References


References


References


References


References


<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>ADL</td>
<td>activities of daily living</td>
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<td>ARAT</td>
<td>action research arm test</td>
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<td>BA</td>
<td>brodmann area</td>
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<td>box and block test</td>
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<td>CAHAI</td>
<td>chedoke arm and hand activity inventory</td>
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<td>CIMT</td>
<td>constraint induced movement therapy</td>
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<td>CNS</td>
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<tr>
<td>FA</td>
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<td>fugl-meyer</td>
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<td>M1</td>
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<td>magnetoencephalography</td>
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<td>MEP</td>
<td>motor evoked potential</td>
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<td>MNI</td>
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<td>MT</td>
<td>motor threshold</td>
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<td>N1</td>
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<td>non-coop</td>
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<tr>
<td>rest</td>
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<td>root mean square</td>
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<td>region of interest</td>
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<td>secondary somatosensory area</td>
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<td>stimulation artefact</td>
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<td>sensitivity encoded</td>
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<td>supplementary motor area</td>
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<td>somatosensory evoked potential</td>
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<tr>
<td>TMS</td>
<td>transcranial magnetic stimulation</td>
</tr>
<tr>
<td>USCP</td>
<td>unilateral spastic cerebral palsy</td>
</tr>
</tbody>
</table>
Curriculum Vitae

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Children: November 28\textsuperscript{th} 2013

Education

2012-2015 \textbf{PhD at ETH Zurich}, Switzerland
Research conducted at the Spinal Cord Injury Center, Balgrist University Hospital, Zurich, Switzerland

2012-2015 \textbf{International PhD Program in Neuroscience}
Neuroscience Center Zurich (ZNZ), University of Zurich and ETH Zurich

2010-2015 \textbf{MAS SHE at ETH Zurich}, Switzerland
Master of Advanced Science for Secondary and Higher Education in Biology and Physical Education

2009-2011 \textbf{MSc HMS at ETH Zurich}, Switzerland
Master of Science in Human Movement Science

2006-2010 \textbf{BSc HMS at ETH Zurich}, Switzerland
Bachelor of Science in Human Movement Science
Work Experience

2012 – 2015  **Balgrist University Hospital, University of Zurich**, Zurich (ongoing)
Research in recovery of upper limb function after stroke and cervical spinal cord injury. Working with electrical stimulations, EMG, EEG, TMS, Lokomat and a new upper limb training device (bottle simulator).

2011 – 2015  **Prodorso AG**, Zurich (ongoing)
Assistance during neurology consults. Electrophysiological measurements including SSEPs (somatosensory evoked potentials), NCVs (nerve conduction velocities) and EMGs (electromyograms).

2011 – 2013  **Neurophysiology, Balgrist University Hospital**, Zurich
Electrophysiological measurements including SSEPs (somatosensory evoked potentials), MEPs (motor evoked potentials), CHEPs (contact heat evoked potentials), NCVs (nerve conduction velocities) and EMGs (electromyograms) in in-house patients and patients of the out-patient clinic. Intraoperative neurological monitoring during spinal surgeries.

2010 – 2011  **Balgrist University Hospital, University of Zurich**, Zurich

2010  **Children’s Rehabilitation Center**, Affoltern a. A.
Sport therapy including nordic walking, biking, strength and endurance training, climbing, gait training and different ball games; mainly with children after traumatic brain injury or with cerebral palsy.

Teaching Experience

2012 – 2015  Supervisions of Master's Theses in Human Movement Science, ETH Zurich.

2012 – 2015  "Functional Assessment of Human Spinal Cord Injury": Annual conduct of the block course attended by undergraduate students in biology of the University of Zurich and ETH Zurich.

Curriculum Vitae

Attended Conferences

**ZNZ Symposium**, Zurich, 2015
Poster: Neural coupling of cooperative hand movements in stroke patients

**International CRPP Symposium**, Ittingen, 2015
Poster: Neural coupling of cooperative hand movements in stroke patients

**Hand, Brain and Technology**, Monte Verità, 2014
Talk (invited speaker) and Poster: Neural coupling of cooperative hand movements

**EFNS-ENS Joint Congress of European Neurology**, Istanbul, 2014
Talk: Neural coupling of cooperative hand movements in stroke patients

**World Congress of Neurology (WCN)**, Vienna, 2013
Poster: Neural coupling of cooperative hand movements in stroke patients

**International Neurorehabilitation Symposium (INRS)**, Zurich, 2103
Poster: Neural coupling of cooperative hand movements in stroke patients

**ZNZ Symposium**, Zurich, 2013
Poster: Neural coupling of cooperative hand movements in stroke patients
Publications

Original Articles


Reviews


Book chapters

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I would like to thank Huub van Hedel for sharing his enthusiasm with me and for being an example of how family and career can actually be compatible. His critical but also positive input to the project was always very helpful and taught me to sometimes think outside the box.

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Il faut avoir déjà beaucoup appris de chose pour savoir demander ce qu’on ne sait pas.

Jean-Jaque Rousseau

*Julie, ou la Nouvelle Héloïse (1761)*