Somatosensory and motor cortical activity in patients and carriers of Unverricht-Lundborg type progressive myoclonus epilepsy

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Brain Research Unit
Low Temperature Laboratory
Helsinki University of Technology

Helsinki 2003
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ACADEMIC DISSERTATION

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Helsinki 2003
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<tr>
<td>AP</td>
<td>Action potential</td>
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<tr>
<td>CNS</td>
<td>Central nervous system</td>
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<td>CSTB</td>
<td>Cystatin B</td>
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<td>ECD</td>
<td>Equivalent current dipole</td>
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<tr>
<td>EEG</td>
<td>Electroencephalography</td>
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<td>EMG</td>
<td>Electromyography</td>
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<tr>
<td>GABA</td>
<td>Gamma-aminobutyric acid</td>
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<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<tr>
<td>IPSP</td>
<td>Inhibitory postsynaptic potential</td>
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<tr>
<td>ISI</td>
<td>Interstimulus interval</td>
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<tr>
<td>MCE</td>
<td>Minimum current estimate</td>
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<tr>
<td>MEG</td>
<td>Magnetoencephalography</td>
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<td>MI</td>
<td>Primary motor cortex</td>
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<tr>
<td>MN</td>
<td>Median nerve</td>
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<tr>
<td>nAm</td>
<td>nanoampere•meter</td>
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<tr>
<td>PMC</td>
<td>Premotor cortex</td>
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<td>PME</td>
<td>Progressive myoclonus epilepsy</td>
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<tr>
<td>PPC</td>
<td>Posterior parietal cortex</td>
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<tr>
<td>SI</td>
<td>Primary somatosensory cortex</td>
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<tr>
<td>SII</td>
<td>Secondary somatosensory cortex</td>
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<td>SEF</td>
<td>Somatosensory evoked field</td>
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<tr>
<td>SEP</td>
<td>Somatosensory evoked potential</td>
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<td>SMA</td>
<td>Supplementary motor cortex</td>
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<tr>
<td>SQUID</td>
<td>Superconducting quantum interference device</td>
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<td>ULD</td>
<td>Unverricht-Lundborg disease</td>
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LIST OF PUBLICATIONS

This thesis is based on five publications, which will be referred to in the text by the Roman numerals I-V


In addition, some unpublished data are presented.
1. ABSTRACT

Unverricht-Lundborg disease (ULD) (OMIM 254800) is a subtype of progressive myoclonus epilepsy (PME). It is an autosomal recessive disorder caused by mutations in the cystatin B gene, clinically characterized by typical age of onset, generalized tonic-clonic seizures and stimulus-sensitive and action-related myoclonic jerks. To characterize somatosensory and motor cortical processing in ULD, we measured somatosensory evoked fields (SEFs) and monitored the reactivity of the 20-Hz motor-cortex rhythm to electric median nerve stimulus with a whole-head MEG in 7 ULD patients and compared the findings with those of healthy control subjects. We also measured cortical drive to isometrically contracting muscle in 8 ULD patients and 8 healthy control subjects by calculation of coherence between MEG and surface electromyography (EMG) signals during isometric contraction. In addition, we measured SEFs and cortex–muscle coherence in 2 genetically verified ULD patients who suffered from myoclonic jerks but no generalized tonic-clonic seizures, and the results were compared with those of ULD patients with generalized tonic-clonic seizures and healthy control subjects.

ULD patients showed greatly enhanced P30m responses to electric median nerve stimuli in the contralateral SI cortex. In addition, activation of the ipsilateral parietal cortex at 50–55 ms was observed in 5/7 patients. These findings suggest hyperreactivity of the contralateral SI cortex and decreased transcallosal inhibition in ULD patients.

The reactivity of the ~20-Hz motor cortex rhythm to electric median nerve stimulation was altered in ULD patients; the baseline mean amplitude of the ~20-Hz activity was 2 times higher than in the control subjects, and no enhancement of the ~20-Hz rhythm was observed within 0.8 s after the stimuli, in contrast to the controls. This lack of the ~20-Hz rebound indicates prolonged excitation of the motor cortex after somatosensory stimuli.

Coherence between oscillatory cortical and muscle signals recorded during isometric contraction of the first dorsal interosseus muscle was 2–4 times stronger in ULD patients compared with control subjects. Enhanced cortex–muscle coherence may be due to enhanced synchrony of firing of the motor cortex neurons.

In the atypical ULD patients without generalized tonic-clonic seizures, contralateral SI responses were within normal variation or only slightly increased, and no early ipsilateral SI responses were observed. This suggest that in ULD patients who have never experienced
generalized tonic-clonic seizures, the primary somatosensory cortex is not hyperexcitable and the transcallosal conduction is not disturbed, which in turn may lower their susceptibility to generalized seizures. In contrast, cortex–muscle coherence, which reflects cortical drive to isometrically contracting muscle, was remarkably enhanced suggesting that the function of the motor cortex is selectively altered.

To study the effect of 1 vs 2 deficient cystatin B (CSTB) alleles on clinical symptoms and neurophysiological findings, we measured SEFs, reactivity of the 20-Hz motor-cortex rhythm, and coherence between cortical and EMG signals in 6 ULD carriers with myoclonic symptoms, and in 6 totally symptom-free ULD carriers, and compared the findings with those of ULD patients and of healthy control subjects. Somatosensory cortical processing was normal both in symptomatic and asymptomatic ULD carriers. In both ULD carrier groups the strength of the cortex–muscle coherence was not enhanced and the reactivity of the ~20-Hz motor-cortex rhythm was normal, indicating that motor cortical activation is within normal variation in heterozygous ULD carriers. These findings also suggest that the generation of myoclonic jerks, observed in some ULD carriers, is not associated with continuously altered cortical sensorimotor functions.
2. INTRODUCTION

Progressive myoclonus epilepsies (PMEs) are a heterogeneous group of inherited disorders, which differ in clinical features, etiology and pathogenesis. One of the members of this group is Unverricht-Lundborg disease (ULD), which is clustered in Finland, and characterized by myoclonic jerks, generalized tonic clonic seizures, ataxia and progression of the disease (Koskiemi et al. 1974a; Norio and Koskiemi 1979). Recent molecular genetic studies have shown that a homozygous expansion mutation in the cystatin B (CSTB) gene accounts for the majority of ULD patients, and the diagnosis can be made by direct detection of this gene defect (Lafreri et al. 1997; Laloi et al. 1997; Virtaneva et al. 1997; Lehesjoki and Koskiemi 1998). Improved diagnostic methods have revealed variability in the clinical picture of ULD and in addition, carriers of ULD who suffer from myoclonic jerks have been identified.

Since the time when electroencephalographic (EEG) spikes were observed in association with myoclonic jerks (Grinker et al. 1938), and when the electric stimulation of peripheral nerves was noticed to elicit exaggerated cortical responses in patients with myoclonus epilepsy (Dawson 1946), PMEs have been of special interest in neurophysiological studies. Several EEG studies have shown enhanced early cortical somatosensory evoked responses to electric median nerve stimuli thereby suggesting hyperexcitability of the primary somatosensory (SI) cortex in PME patients (Shibasaki and Kuroiwa 1975; Chadwick et al. 1977; Brown et al. 1991; Reuters et al. 1993) but interpretation of the results has been difficult because of etiological variability of the patients.

This thesis focuses on sensorimotor functions in genetically verified ULD patients, studied by means of magnetoencephalography (MEG) that allows non-invasive investigation of cerebral currents with excellent temporal and relatively good spatial resolution. The etiologically homogeneous patient group enabled rather straightforward interpretations of the results and revealed pathophysiological mechanisms that may be related to the symptoms of the disease. As a clear advantage to an earlier MEG study of ULD patients (Karhu et al. 1994), we used a whole-scalp covering multichannel MEG device, which enables observation of simultaneous activation of multiple cortical areas. To deepen the perspective, we also studied sensorimotor functions in variant phenotypes of ULD and in symptomatic and asymptomatic ULD carriers to find out how one vs. two defective CSTB alleles would alter sensorimotor processing and whether these alterations would be reflected in the clinical symptoms.
3. REVIEW OF LITERATURE

3.1. Unverricht-Lundborg type progressive myoclonus epilepsy

3.1.1. Clinical symptoms

ULD begins typically between 6 to 15 years with myoclonic jerks or generalized tonic-clonic epileptic seizures (Koskineni et al. 1974a). Myoclonic jerks are essential for the diagnosis and they are the first symptom approximately in half of the patients (Norio and Koskineni 1979; Koskineni 1986; Koskineni 1987). They occur spontaneously or in association with action or external stimuli, such as touch, light or noise (Berkovic et al. 1986; Marseilles Consensus Group 1990). Myoclonic jerks may be focal or multifocal and they may generalize to a shaking attack and unconsciousness. Other typical symptom in ULD is the generalized tonic-clonic seizures that occur infrequently at the early stages of the disease and may later on, with anticonvulsve medication, cease entirely (Koskineni et al. 1974a; Norio and Koskineni 1979; Koskineni 1986). Some rare patients do not experience any generalized tonic-clonic seizures and some may have absence or even focal onset seizures (Koskineni et al. 1974a; Lehesjoki and Koskineni 1999).

Intelligence of ULD patients is considered normal and no progressive dementia has been reported. Previous studies have shown a decrease of about 10 point in the IQ in 10 years (Koskineni et al. 1974a; Koskineni 1986). Depression and emotional lability are often observed in ULD patients (Lehesjoki and Koskineni 1999).

In the initial phase of ULD, the clinical findings of the patients are normal but gradually intentional tremor, dysarthria and ataxia develop. Previously myoclonic jerks and ataxia progressed and resulted in severe motor disability and death on average at 24 years of age (Koskineni et al. 1974a). Nowadays, with advanced anticonvulsve medication, myoclonic jerks and ataxia do not necessary progress or the progression may be slow (Koskineni 1986; Lehesjoki and Koskineni 1998).

3.1.2. Genetics

ULD is inherited in an autosomal recessive mode. The gene defect has been located on chromosome 21 (Lehesjoki et al. 1991; Lehesjoki et al. 1993a; Lehesjoki et al. 1993b). Molecular genetic studies have shown that a homozygous minisatellite expansion mutation in the
CSTB gene accounts for the majority of ULD patients in the world (Lafranchi et al. 1997; Lalioti et al. 1997; Virtanen et al. 1997). In Finland, the expansion mutation accounts for 99% of disease alleles (A-E Lehesjoki, personal communication). Direct detection of this mutation is used to confirm the clinical ULD diagnosis (Lehesjoki and Koskiniemi 1998).

3.1.3. Epidemiology

Progressive myoclonus epilepsy (PME) syndrome was first described by Heinrich Unverricht in Estonia (Unverricht 1891) and by Herman Lundborg in Sweden (Lundborg 1903). Definition of the clinical and pathologic features of the Finnish PME patients showed that the PME syndrome, described by Unverricht and Lundborg, is one single clinical entity (Haltia et al. 1969; Koskiniemi et al. 1974a; Norio and Koskiniemi 1979). Unverricht-Lundborg disease (ULD) is most prevalent around the Baltic Sea, especially in Finland. Mediterranean myoclonus, which is present in southern Europe and in North Africa, is both phenotypically and genotypically similar to ULD (Marseilles Consensus Group 1990; Malafosse et al. 1992; Lehesjoki et al. 1994). ULD is also found sporadically worldwide in caucasians, blacks, and in Japanese (Eldridge et al. 1983; Marseilles Consensus Group 1990). During the last decades, prevalence of ULD seems to have been increasing partly because of better treatment, which has resulted in more benign course of the disease (Koskiniemi 1986; Koskiniemi 1987), but also due to advanced diagnostic methods (Lehesjoki and Koskiniemi 1998). However, there are no recent epidemiological studies that would demonstrate the prognosis of ULD in Finland or in other countries. The incidence of ULD in Finland is 1 in 20,000 births (Norio and Koskiniemi 1979; Eldridge et al. 1983). At present about 160 ULD patients live in Finland (Lehesjoki and Koskiniemi 1999).

3.1.4. Treatment

Since 1970’s valproic acid has been the drug of choice in the treatment of ULD, whereas the previously used phenytoin is considered toxic to these patients (Eldridge et al. 1983). In general, carbamazepine, phenytoin, tiagabine, vigabatrin, and gabapentin may precipitate myoclonic jerks, and their use in ULD is not recommended (Perucca 2001). Favourable results with valproic acid treatment have been obtained although double-blind randomised studies have not been performed (Iivanainen and Himberg 1982; Somerville and Olanow 1982; Koskiniemi 1986). Clonazepam and piracetam serve as effective add-on therapy in ULD (Iivanainen and Himberg 1982; Somerville and Olanow 1982; Brown et al. 1993; Koskiniemi et al. 1998).
From the new antiepileptic drugs, zonisamide has been demonstrated to reduce the number of myoclonic jerks and generalized tonic-clonic seizures in studies with small number of ULD patients (Mather and Shah 2002). Accordingly, levetiracetam and topiramate have been shown to suppress myoclonus in some preliminary studies (Uldall and Buchholt 1999; Genton and Gelisse 2000; Frucht et al. 2001; Schauer et al. 2002; Kasteleijn-Nolst Trenite and Hirsch 2003; Kinriors et al. 2003). Lamotrigine has been demonstrated to be a useful alternative in the treatment of juvenile myoclonus epilepsy (Buchanan 1996). Recently reduced seizures and improved cerebellar dysfunction were reported in one ULD patient by vagus nerve stimulation (Smith et al. 2000). However, no randomized, controlled studies with ULD patients have yet been conducted. With all the above-mentioned new treatments.

3.1.5. Neuropathological findings

There are only few studies describing the neuropathological changes in ULD patients. 3 patients with typical ULD symptoms showed a severe loss of cerebellar Purkinje cells, and neuronal loss and degeneration in the medial part of thalamus (Haltia et al. 1969). Similar degeneration of the cerebellum was demonstrated in another study of 6 ULD patients (Koskiemi et al. 1974a). However, it is not clear whether the degeneration of Purkinje cells was related to the disease itself, and especially to the tonic-clonic seizures, or to the antiepileptic medication.

3.1.6. Neuropathological findings of ULD

Electroencephalogram (EEG) of ULD patients displays characteristic spike-and-slow wave paroxysms, which increase after photic stimulation (Koskiemi et al. 1974b; Koskiemi 1986). Progression of the disease results in diminution of beta and alpha activity and accentuation of theta and delta activity in ULD patients treated with conventional anticonvulsive medication (Mervaala et al. 1986). Although myoclonic jerks are provoked by light and noise, the strength of visual evoked potentials (Mervaala et al. 1986) and brainstem auditory evoked responses (Hari et al. 1983) have been normal. In contrast, several previous neuropathological studies of PME patients have shown remarkably enhanced early cortical somatosensory evoked responses to electric median nerve stimuli suggesting hyperexcitability of the primary somatosensory (SI) cortex (Shibasaki and Kuroiwa 1975; Chadwick et al. 1977; Brown et al. 1991; Reuntes et al. 1993; Karhu et al. 1994). Activation of the ipsilateral SI cortex after unilateral median nerve stimuli has been observed in some cortical myoclonus patients with generalized seizures (Brown
et al. 1991). Such interhemispheric spread of excitation was suggested to represent an additional and separate patophysiological deficit, which may contribute to the generalization of seizures.

3.1.7. Differential diagnosis

Diagnosis of ULD may be difficult at the beginning of the disease as the clinical and electroencephalographic (EEG) features may resemble some other epileptic syndromes (Berkovic et al. 1993).

Juvenile myoclonus epilepsy (JME) is an inherited autosomal dominant disorder, which appears around puberty. JME is characterized by bilateral, single or repetitive, irregular myoclonic jerks, occurring preferentially shortly after awakening, and by generalized tonic-clonic seizures or absence seizures (Delgado-Escueta and Enrile-Bacsal 1984; Commission on Classification and Terminology of the International League Against Epilepsy 1989). EEG of JME patients shows 4/6 Hz spike wave variants or polyspike and waves and photosensitivity (Janz 1969; Delgado-Escueta and Enrile-Bacsal 1984).

Lafora disease is a PME syndrome in which abnormal deposit material accumulates in tissues: neurons, heart, skeletal muscle, liver, and sweat-gland duct cells (Lafora 1911; Carpenter and Karpati 1981; Berkovic et al. 1986). Lafora disease is common in southern Europe. It is an autosomal recessive disorder, which begins between 10 to 18 years with myoclonus, tonic-clonic seizures, and mental retardation (Van Heycop ten Ham and de Jager 1963).

Myoclonus epilepsy and ragged-red fibers syndrome (MERFF) is one of the most common causes of PME (Fukuhara et al. 1980; Berkovic et al. 1989; Marseilles Consensus Group 1990). The clinical spectrum of MERFF is broad, including myoclonus, tonic-clonic seizures, dementia, ataxia, myopathy, neuropathy, deafness, and optic atrophy. Symptoms can begin at any age and there may be marked intrafamily variation in the age of onset and in the clinical severity (Rosing et al. 1985; Berkovic et al. 1989).

Neuronal ceroid lipofuscinosis (NCL) is a group of diseases characterized by accumulation of abnormal amounts of lipopigment in lysosomes. In contrast to ULD, patients suffering from NCL syndromes show visual, cognitive and extrapyramidal features whereas tonic-clonic and myoclonic seizures are a relative minor manifestation of the disease.

3.2. Somatosensory afferent pathways and cortices

3.2.1. Afferent pathways

Somatosensory sensations are divided into 4 modalities: touch, pain, position, and temperature, each with distinct receptors. In the following, only the receptors and fibers concerning touch are considered. Touch is mediated via slowly and rapidly adapting mechanoreceptors in the skin.

Sensory information is transferred to the spinal cord by afferent fibers, which form together with efferent fibers for the same body part, a peripheral nerve. Almost all sensory information enters the spinal cord through the dorsal roots of the spinal nerves.

From the spinal cord to the brain the sensory signals are carried mainly through the dorsal column–lemniscal system. The nerve fibers of this system ascend to the medulla, where they synapse in the cuneate and gracile nuclei. The second-order neurons decussate immediately to the opposite side, and then continue upward to the thalamus through the medial lemnisci. In the thalamus, the medial lemniscal fibers terminate in the ventral posterior nucleus. From the thalamus, third-order nerve fibers project to the primary somatosensory cortex (SI), to the secondary somatosensory cortex (SII), and to the posterior parietal cortex (PPC).

Some fibers mediating somatosensory impulses enter the anterolateral system, which decussate to the opposite side already in the spinal cord. The anterolateral system mediates mainly thermal and pain impulses but also some tactile information, which allows only crude localization of the stimulus on the surface of the body.

3.2.2. Primary somatosensory cortex (SI)

The somatosensory fibers project from the thalamus to SI, which is located in the posterior bank of the central fissure and in the postcentral gyrus. SI is divided into four cytoarchitectonic regions (Broadmann areas 3a, 3b, 1, and 2; Figure 1), which are extensively interconnected so that both serial and parallel processing is involved in sensory information. Most thalamic fibers terminate in areas 3a and 3b, which project their axons between each other and to areas 1 and 2. Thalamic neurons also send some direct projections to areas 1 and 2. These four regions of the SI differ functionally. Area 3a receives information mainly from the muscle receptors, 3b and 1 from mechanoreceptors on the skin, and area 2 from proprioceptors.

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In addition to the projections within the SI area, neurons send their axons to primary motor cortex (MI), to SII and to PPC.

Different parts of the body are represented in SI in a distinct spatial orientation, a somatotopic map. The head is represented in the most lateral portion of SI, whereas the lower limb is represented medially (Bostroem et al. 1936). The size of the SI representation area is determined by the innervation density of the body part; areas with high number of sensory receptors in the skin, such as lips and fingertips, have disproportionately large representations in SI.

3.2.3. Secondary somatosensory cortex (SII)

The secondary somatosensory cortex (SII) is located on the superior bank of the Sylvian fissure. It also shows signs of somatotopic organization although to a lesser extent than SI; the face is presented anteriorly, the arms centrally, and the legs posteriorly (Penfield and Jasper 1954; Haight 1972; Hari et al. 1993; Ruben et al. 2001). Unilateral tactile stimulation activates SII.
bilaterally but typically the contralateral activation is slightly earlier and stronger (Whitsel et al. 1969; Hari 1983; Hari et al. 1984; Allison et al. 1989). In addition to the serial input of information from SI, SII receives parallel information directly from the thalamus (Burton and Robinson 1987; Forss et al. 1999).

The functional significance of SII in humans is still unclear. Direct electric stimulation of human SII during neurosurgery produced sensation of tingling and an “urge to move” (Penfield and Jasper 1954). In monkeys, both bilateral and unilateral lesions of SII impair tactile learning and retention, as well as intermanual transfer of the learned skill (Ridley and Ettlinger 1976; Garcha and Ettlinger 1978). Lesions of the SII area in humans do not usually produce severe impairment of tactile skills. However, SII may be involved in object recognition; SII lesions may impair texture and shape discriminations (Murray and Mishkin 1984), and a large lesion in the ventrolateral somatosensory association cortex, including SII, lead to tactile agnosia (Caselli 1993).

Recent MEG studies have shown that SII, with left-hemisphere dominance, has a role in integration of information from bilateral body parts (Simões and Hari 1999; Simões et al. 2001; Alary et al. 2002), and it may be related to the maintenance of body scheme (Hari et al. 1998b).

Electric median nerve stimulation evokes enhanced SII responses during simultaneous movements of the fingers and during isometric contraction of hand muscles, suggesting that SII integrates somatosensory and motor information (Huttunen et al. 1996; Forss and Joumsäki 1998). The modulating effect of motor activity on SII activation depends on the topographical proximity of the stimulated and contracted body parts; SII responses are enhanced during the thenar and deltoid muscle contraction but decreased during the masseter and tibialis anterior contraction (Lin et al. 2000). In addition to sensorimotor integration, animal studies have suggested that SII play a part in tactile learning and memory (Ridley and Ettlinger 1976).

3.2.4. Other somatosensory cortices

Posterior parietal cortex (PPC), also known as parietal association area, includes the parietal cortex posterior to area 2 (Brodmann areas 5 and 7). In humans, it is not rigidly delineated. It is considered to participate in higher processing of somatosensory information, such as integration of tactile and proprioceptive information with visual properties of the objects touched (Mountcastle et al. 1975; Arezzo et al. 1981; Hyvärinen 1982). PPC is also important for the spatial organization of movement during visually guided movements (Sakata and Taira 1994). In
addition, mesial cortex is associated in the somatosensory processing and is activated when the subjects are actively observing the stimuli (Penfield and Jasper 1954; Forss et al. 1996).

3.3. Motor cortices and motor pathways
Motor commands are regulated by serial and parallel activation of the motor cortical areas, the descending systems of the brain stem and the spinal cord. Whereas the spinal cord is able to generate muscle activation in the form of reflexes and rhythmic motor patterns such as walking, the cortical motor areas are needed for execution of skilled voluntary movements and for coordinating and planning of complex movements. These areas, consisting of premotor and primary motor cortices, are located anterior to the central sulcus, and occupy approximately the posterior third of the frontal lobes (Figure 1).

3.3.1. Primary motor cortex
The primary motor cortex (MI, Brodmann’s area 4) lies anterior to the central sulcus, beginning laterally in the sylvian fissure and extending superiorly to the uppermost portion of the brain folding over into the longitudinal fissure between the hemispheres. Topographical organization of MI resembles the somatotopical organization of SI: the face and mouth regions are located most laterally, the arm and hand area in the middle, the trunk near the apex and the leg and foot areas in the part that dips into the longitudinal fissure. Body parts with dense motor innervations have large representations in MI; more than half of the entire MI is concerned with controlling the hands and the muscles involved in speech production. Motor skill learning may alter cortical representations of MI (Karni et al. 1995; Nudo and Milliken 1996).

The main output neurons of MI are pyramidial cells, which lie in the fifth cortical layer. Motor corticospinal neurons make direct connections to spinal motoneurons of several muscles. Synergistic muscles are usually simultaneously excited, whereas antagonistic muscles are inhibited. In addition, corticospinal neurons terminate on interneurons in the spinal cord, which is important for coordinating larger groups of muscles in behaviours such as reaching and walking. For instance one target muscle of hand may be influenced by corticospinal neurons that are dispersed throughout the hand presentation area. The cells activated will depend on the task in which the muscle is used (Maier et al. 1993). The changes in neural activity in MI begin about 100 ms or more before the onset of movement and a small number of MI cells may be activated during ipsilateral movements (Evarts 1966; Matsunami and Hamada 1981; Tanji et al. 1988).
Corticospinal neurons signal the direction and amplitude of muscle force required to produce a movement (Evarts 1968). Movement in a particular direction is considered to be determined by the net action of a large population of neurons (Georgopoulos et al. 1982). MI neurons receive somatosensory information from SI and directly from the thalamus, and indirectly from the posterior parietal cortex via premotor areas. In addition, MI receives input from the cerebellum and basal ganglia. Thus the activation of MI is significantly modulated by a continuous stream of tactile, visual, and proprioceptive input, which is needed to make voluntary movement both accurate and properly sequenced.

3.3.2. Premotor cortices

Premotor areas comprise Brodmann’s area 6 anterior to MI. The supplementary motor area (SMA), which involves the dorsomedial part of area 6, projects to MI and to the reticular formation. The premotor cortex (PMC) comprises the ventrolateral part of area 6. The neurons of PMC project directly to MI, the red nucleus, the reticular formation, and basal ganglia. They are also indirectly connected to the cerebellum. In addition, premotor neurons involving activation of proximal and axial muscles project directly to the spinal motoneurons. These direct monosynaptic connections suggest that the premotor neurons can also control movements independently of the primary motor cortex.

PMC receives major input from prefrontal association areas and from the posterior parietal cortex, and it is thus important for the integration of sensory inputs from the environment during the planning and execution of movements. SMA has been suggested to have a role in coordinating bilateral movements of the body, and in preparing movement sequences from memory. Activity of premotor areas increases with enhanced complexity of a movement, indicating that the premotor areas are likely to be important in planning and organizing of complex movements (Roland et al. 1980; Rao et al. 1993). Further, mental rehearsal of a movement activates the same patterns of activity in the premotor and posterior parietal cortical areas as those that occur during performance of the movement (Jeannerod 1995; Stephan et al. 1995).

3.3.3. Efferent pathways

The human corticospinal tract consists of about one million axons, of which a third originate from MI, another third from the premotor areas and the remaining third from SI. These axons
descend through the subcortical white matter, the internal capsule and the cerebral peduncle. As the fibers of the corticospinal tract descend they form the medullary pyramids, prominent protuberances on the ventral surface of the medulla, and thus the entire projection is also called the pyramidal tract. Most of the corticospinal fibers cross the midline in the medulla at the pyramidal decussation and descend in the dorsal part of the lateral columns of spinal cord forming lateral corticospinal tracts. About 10% of the fibers do not cross in the medulla. They descend in the ventral columns of spinal cord as ventral corticospinal tracts, and project bilaterally in the spinal cord having reached the level at which they will terminate. Both corticospinal tracts make monosynaptic connections with spinal motor neurons and with interneurons in the intermediate zone of spinal cord.

Corticospinal neurons that synapse with the spinal motoneurons control mainly distal muscles of extremities, especially the hand muscles. In monkeys, sectioning of the medullary corticospinal tracts, which interrupts the projection of corticospinal axons from MI and premotor areas, produces contralateral weakness, which recovers after periods of months but the movements remain clumsy and the ability to move fingers independently is permanently lost (Lawrence and Kuypers 1968). The partial recovery is possible because cortical commands have indirect access to spinal motoneurons through the descending pathways of the brain stem; two pathways of brain stem neurons, the medial and lateral, receive input from the cerebral cortex and subcortical nuclei and project to the spinal cord, thereby contributing to the control of posture and reflexes, and to the control of limb muscles.

3.3.4. Motor units

The cell bodies of motor neurons lie in the anterior horn of the spinal cord. The axon of each motor neuron exits the spinal cord through the ventral root and transverses progressively smaller branches of peripheral nerves until it enters the muscle it controls. Each motoneuron that leaves the spinal cord innervates many different muscle fibers, but each muscle fiber is normally innervated by only one motor neuron. A motor unit is formed by a single motor neuron and all muscle fibers that it innervates; the motor unit is activated in an all-or-none fashion. The number of muscle fibers in a single motor unit varies in different parts of the body. In muscles that react rapidly and whose control must be exact, like extraocular muscles, the motor units are small, consisting of about 10 muscle fibers. Motor units of small muscles of hand consist of about 100 muscle fibers. Large muscles, such as gasterognemius, do not require fine control and therefore
have several hundred muscle fibers in one motor unit. Individual muscles contain varying amounts of the individual motor unit types depending on the functional demands of the muscles. The muscle fibers of each motor unit are spread out in the muscle and lie among muscle fibers from other motor units.

The muscle fibers of small motor units can produce only low force but they are able to contract for a long time. Large motor units consist of fibers, which can produce high force, but they fatigue rapidly. The threshold for activation is lower for small than large motor neurons. During muscle contraction, the small motor units are activated first and when the synaptic input increases i.e. higher contraction force is needed, larger motor units are recruited. This minimizes the development of fatigue in muscles and provides skilled control of muscle force needed for the task.

Contraction force of a muscle can be increased also by enhancing the firing rate of motor neurons. Each action potential in the motor neuron releases transmitter, acetylcholine to depolarize the postsynaptic membrane of the muscle fiber. All muscle fibers of a healthy motor unit respond synchronously to each action potential of the motor neuron. For isometric muscle contraction, the lowest firing rate of motor units is about 8 Hz, and it increases up to 25 Hz with increasing force of contraction (Monster and Chan 1977) and the motor units of the same muscle tend to fire in synchrony (Sears and Stagg 1976; Datta and Stephens 1990; Farmer et al. 1993). Various motor units are driven asynchronously by the spinal cord, which enables the various contractions produced by all active motor units to blend together in a smooth contraction.

3.4. Myoclonus

The term “myoclonus” is used to describe sudden, brief, involuntary muscle jerks of extremities, face, or trunk (Fahn et al. 1986). Myoclonic jerks, which are caused by muscle contraction, are called positive myoclonus, whereas jerks caused by sudden loss of muscle contraction are called negative myoclonus (Artieda et al. 1992; Shibasaki 1995b). Myoclonus can be cortical, subcortical or spinal (Shibasaki 1995a). Cortical myoclonus may be spontaneous or elicited by stimuli, such as touch, light, or sound, and it may manifest as positive or negative myoclonus. Cortical myoclonus is most commonly seen in PMEs with various ethiology and in JME but also in post-anoxic encephalopathy, Alzheimer’s disease, Creutzfeldt-Jakob disease, metabolic encephalopathy, olivopontocerebellar atrophy, and cortical-basal ganglionic degeneration (Hallet
et al. 1979; Obeso et al. 1983; Rothwell et al. 1983; Kakigi and Shibasaki 1987). In cortical myoclonus, large pre-myoclonus spikes can be observed in polygraphic EEG or MEG recordings, whereas smaller spikes can be detected by jerk-lock back averaging method (Shibasaki and Kuroiwa 1975; Shibasaki et al. 1978; Shibasaki et al. 1985; Shibasaki et al. 1986; Uesaka et al. 1996; Mima et al. 1998a).

According to (Shibasaki 2000) subcortical myoclonus includes e.g. essential myoclonus, which is a nonprogressive disorder with periodic myoclonus and palatal tremor but without any neurologic deficits. Subcortical myoclonus occurs also at rest, is not stimulus-sensitive, and the jerks are not shock-like. Spinal myoclonus tends to involve a group of muscles innervated by a certain spinal segment. The jerks can be stimulus-sensitive but no cortical spikes preceding the muscle discharge can be observed in electrophysiological polygraphic recordings.

3.5. Magnetoencephalography

The following review of MEG is mainly based on review by Hämäläinen et al 1993. The transport of ions through the nerve cell membrane and the development of electrical potentials across it is the basis of nerve cell function. Magnetoencephalography (MEG) allows non-invasive investigation of magnetic fields generated by activation of the cerebral cortical neurons. The development of multichannel whole-head covering MEG devices has enabled investigation of simultaneously activated cortical areas.

3.5.1. Neural origin of magnetic fields

Electric currents in apical dendrites of cortical pyramidal cells are assumed to be the primary generators of magnetic field measured outside the head. These dendrites are orthogonal to the cortical surface and parallel to each other, which permits summation of magnetic fields from thousands of cells with minimal cancellation.

Post-synaptic currents associated with excitatory or inhibitory potentials (PSPs) are the probable source of extracranially measured magnetic fields for the following reasons: neuronal currents associated with action potentials (APs) are quadrupolar and the associated magnetic field diminishes as 1/r^3 with the distance r. Instead, the magnetic field of a PSP current is dipolar and it decreases considerably more slowly as 1/r^2. Further, the duration of an AP is only one
millisecond, whereas the duration of a PSP is at least 5–10 milliseconds, which allows summation of several simultaneous PSPs (Lopes da Silva and Van Rotterdam 1992; Hari 1993).

Since the estimated cortical activation area for typical evoked potential is about 100–250 mm² (Hari 1990) simultaneous activation of tens of thousands of pyramidal cells is needed in the generation of an evoked magnetic response.

3.5.2. Instrumentation

Since the magnetic fields produced by the brain are very weak (5–50 x 10^-14 T) compared for example with the Earth’s magnetic field (5 x 10^-5 T), MEG measurements are performed in a magnetically shielded room to attenuate external magnetic and electric noise.

Registration of weak cerebral magnetic fields is possible by using SQUID (Superconducting Quantum Interference Device) sensors, which are kept immersed in liquid helium at the temperature of 4 K. Magnetic field is first recorded by a set of pickup coils, which convert the magnetic flux into electric current. The pickup coils form closed loops with the input coils, which are coupled to the SQUIDs.

The sensitivity of the device is improved by proper design of the pickup coil system (Hämäläinen et al. 1993). Magnetometers, that have only one loop in the pickup coil, easily record signals from the heart and environmental noise. First-order gradiometers consist of a pickup coil and a compensation coil, which are wound in opposite direction. These gradiometers measure the difference between the field strength recorded by the pickup and the compensation coils, and they are effective in measuring the inhomogeneous magnetic fields produced by nearby sources.

In axial gradiometers, the pickup coils and the compensation coils are connected in series. When the coil distance is a few centimeters, axial gradiometers provide sufficient rejection of the background field without severe attenuation of the signal. In a planar first-order gradiometer, the two coils are coupled in a figure-of-eight structure, and the device measures the tangential derivative of the magnetic field. Planar gradiometers are more compact in size than axial gradiometers and can be fabricated easily (Hämäläinen et al. 1993). They collect the strongest signals right above a local source area, thereby offering a relatively good spatial resolution even in quite noisy environments.

Two helmet-shaped whole-head covering neuromagnetometers were used in the present work: a 122-channel planar first-order SQUID neuromagnetometer (Neuromag 122™) and a
306-channel neuromagnetometer (Vectorview™) comprising 204 planar first-order SQUID sensors and 102 magnetometers (Figure 2).

Fig. 2. During the measurements 306-channel Vectorview™ neuromagnetometer is brought close to the subject’s head. Registration of cerebral magnetic fields is possible by using SQUID sensors, which are kept immersed in liquid helium inside the device. First-order gradiometers consist of a pickup coil and a compensation coil, which are wound in opposite direction. Adapted from Vectorview Users Guide.

3.5.3. Source analysis

A conductor model for the head is needed for computing the magnetic field produced by a cerebral source. A realistic head model would be the most accurate but as it is time consuming to compute, a simple spherical conductor model is normally used. The head geometry around sensorimotor cortex, which was the area of main interest in this study, is reasonably well approximated with a sphere (Hännäläinen and Sarvas 1989). Since in a spherically symmetric structure only currents tangential to the surface produce magnetic fields outside, MEG measures mainly activity of the fissural cortex. However, currents in convexal cortex with some deviation from the radial orientation can also contribute to the measured signals (Hari 1993).

The most likely source area of an evoked response or of a spontaneous signal can be roughly estimated from the distribution of the magnetic signals measured with planar...
gradiometers. The sites, strengths and orientations of the source currents are computed to obtain more information about the source area. The fact that many current configurations could produce the same magnetic field distribution is called the inverse problem. In MEG studies, currents in the brain may be approximated with equivalent current dipoles (ECDs), which can be characterized by five parameters: three spatial coordinates, orientation, and strength. The ECD which best explains the measured field distribution is usually determined by a least-squares search, and the adequacy of the model may be quantified by the goodness-of-fit value, which indicates how much of the measured field variance is accounted for by the dipole model.

If several brain areas are simultaneously active, multidipole models are needed to interpret the complex field patterns. In multidipole models, the strengths of the equivalent current dipoles, best describing local source currents at the peak of the response, are allowed to change as a function of time whereas their locations and orientations are kept fixed.

3.6. Somatosensory evoked responses

3.6.1. Stimulation

Physiology and functional organization of somatosensory cortex and pathways can be investigated by measuring somatosensory evoked potentials (SEPs) and somatosensory evoked fields (SEFs). Single cortical evoked responses are averaged time-locked to the stimuli to distinguish responses from noise produced by brain activity itself or by the environment.

Sufficient number of signals has to be averaged to obtain a satisfactory signal-to-noise ratio. Electric stimuli are widely used to study the somatosensory system, because they are easy to apply and produce clear and reproducible responses in most subjects. However, they are less physiological than mechanical stimuli, such as tapping, vibration, and airpuffs. Electric stimuli produce highly synchronized activation but they may activate both deep and superficial receptors and their fibers with different conduction velocities. The electric stimuli are typically applied on peripheral nerves, usually on median and tibial nerves, at intensities just above the motor threshold. The amplitudes of all deflections of median nerve SEPs increase significantly as stimulus intensity is raised from sensory threshold to motor threshold, and at higher intensities no further notable changes occur (Joumáni and Forss 1998). Optimal detection of long latency responses to median nerve stimulation requires interstimulus intervals (ISIs) of 3–4 s to each nerve (Pratt et al. 1980; Hari 1983; Allison et al. 1992; Huttunen et al. 1992).
3.6.2. Somatosensory evoked potentials

Somatosensory evoked potentials (SEPs) may be recorded directly from the cortex during surgery or noninvasively from the scalp. The skull and other extracerebral tissues have different electric conductivities, which alter the electric potentials measured on the scalp and therefore weaken the spatial resolution of EEG. Nevertheless, scalp SEP measurements are widely used because they are easy to perform and some current sources, like very deep and radial sources, are most reliably detected by EEG. To electric median nerve stimulation, a surface negative deflection (N20) over the parietal cortex is observed at about 20 ms after the stimulus, followed by a positive deflection at about 30 ms (P30). A waveform of similar latency but of opposite polarity is recorded from the frontal scalp (Allison et al. 1991). Several scalp and intracranial SEP studies suggest that these waveforms are generated by one tangential source in area 3b and one radial source in area 1 of SI (Wood et al. 1985; Allison et al. 1989; Allison et al. 1991; Baumgartner et al. 1991). However, some studies have suggested generation of P30 and P25 in the motor cortex (Desmedt and Cheron 1981; Mauguire et al. 1983) or generation of radial dipole in the motor cortex (Deiber et al. 1986; Desmedt et al. 1987).

3.6.3. Somatosensory evoked fields

Although somatosensory evoked fields (SEFs) reflect mainly currents tangential to the skull surface, the same primary currents in the brain generate both the electric and magnetic signals. Therefore the temporal behaviour of SEFs is similar to SEPs. The earliest evoked magnetic response to median nerve stimulation peaks at about 20 ms (N20m). The corresponding ECD points anteriorly and agrees with direction of the intracellular current from deep towards superficial layers of area 3b of the SI cortex. The next deflection has opposite polarity and peaks at 30–35 ms after MN stimulation with the source area located in SI (Tiitinen et al. 1989). MEG studies have shown that the somatotopic distribution of the generator areas of the somatosensory evoked fields in SI agrees with the homuncular organization of SI cortex (Brenner et al. 1978; Hari et al. 1984) in line with findings in cortical stimulation (Penfield and Jasper 1954) and intracranial recordings of somatosensory evoked potentials (SEPs) (McCarthy et al. 1993).

Activation of the human secondary somatosensory cortices was first demonstrated noninvasively in MEG studies (Hari 1983; Teszner et al. 1983; Hari et al. 1984). The

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Activation of the human secondary somatosensory cortices was first demonstrated noninvasively in MEG studies (Hari 1983; Teszner et al. 1983; Hari et al. 1984). The
somatosensory evoked responses from the SII cortex are bilateral to unilateral stimuli but typically slightly earlier and stronger to contralateral than ipsilateral stimuli (Hari 1983; Hari et al. 1984). The responses peak around 100 ms to upper-limb stimuli and 10–30 ms later to lower-limb stimuli. Although demonstration of the somatotopical organization of the SII cortex with MEG is difficult because of the small size of the SII area, signs of somatotopical arrangement have been observed (Hari et al. 1993).

MEG recordings have also revealed activation of the posterior parietal cortex (PPC) in the walls of the postcentral sulcus (Forss et al. 1994) at 70–110 ms. Activation of the mesial cortex in the paracentral lobule is found when the subjects are actively observing the stimuli (Forss et al. 1996; Mauguire 1997).

3.7. Sensorimotor cortical electromagnetic rhythms

According to EEG and MEG recordings, neurons of cortical sensory projection areas oscillate with their own intrinsic rhythms. The neurones in the thalamus exhibit intrinsic oscillations, which play essential role in the development of cortical rhythms (Steriade and Llinas 1988), and certain cortical cells contain intrinsic membrane properties, which give rise to oscillatory behaviour (Llinas 1988; Gray and McCormick 1996). The intensity of the brain oscillations is determined mainly by the proportion of neurones that fire synchronously, not by the total level of electrical activity in the brain.

Although analysis of cortical rhythms forms an essential part of clinical EEG evaluation, the functional significance of cortical rhythms has remained largely unknown. Cortical rhythms have been suggested to be by-products, epiphenomena, without any functional significance, or an idling rhythm, which would allow the system to start more rapidly (Kuhlman 1978; Bouyer et al. 1983).

3.7.1. Mu rhythm

The cortical rhythm over the sensorimotor cortex, the mu rhythm, was first demonstrated in intraoperative cortical recordings (Jasper and Penfield 1949; Gastaut 1952). It consists of occasionally phase-locked 10-Hz and 20-Hz components, which form typical “comb-like” shape of the rhythm. With MEG, and with appropriately analysed EEG recordings (Pfurtscheller and Aranibar 1979), mu rhythm can be found in most subjects. Electrocoorticographic recordings have localized 25-Hz activity strictly in the motor cortex and demonstrated predominance of the 10-
Hz activity in the postcentral sulcus (Jasper and Penfield 1949; Papakostopoulos 1980). In line, MEG studies have shown that sources of 20-Hz component of the mu rhythm cluster anterior to the sources of the 10-Hz component (Salmelin and Hari 1994). Therefore, the 10-Hz component of the mu rhythm is suggested to generate predominantly in the somatosensory cortex close to the hand SI area, and the 20-Hz component in the anterior wall of the central sulcus, in the primary motor cortex. However, it has also been debated whether the ~20-Hz oscillations measured with different electrophysiological methods are reflections of the same phenomena or whether there is more than one source of ~20-Hz oscillations in the motor cortex (Pfurtscheller et al. 1997).

3.7.2. Reactivity of the mu rhythm

Already electrocortical recordings have shown that voluntary and reflex movements e.g. grip of the hand, and to a lesser extent somatosensory stimulation, block the mu rhythm (Jasper and Penfield 1949). The mu rhythm begins to decrease 1–2 s before the voluntary finger movements and increases substantially 1–2 s after the movements (Pfurtscheller 1992; Salmelin and Hari 1994; Toro et al. 1994). The rhythm is strongest during rest or a steady contraction (Pfurtscheller et al. 1996; Baker et al. 1997). The movement-related changes occur in both components of the mu rhythm, but they are faster and stronger for the 20-Hz rhythm than for the 10-Hz rhythm (Pfurtscheller 1992; Salmelin and Hari 1994; Salmelin et al. 1995; Salenius et al. 1997c). Unilateral movements/stimuli tend to block the mu rhythm bilaterally but the reactivity of the rhythm is stronger in the contralateral hemisphere, especially for the 20-Hz component.

Electric median nerve stimulation or touch of the hand, cause at first slight decrease of the mu rhythm level, which is then followed by a strong enhancement, “rebound”, within 1 s (Chatrian et al. 1959; Salmelin and Hari 1994; Salenius et al. 1997c). The rebounds are seen in both hemispheres but again, are stronger in the contralateral hemisphere. The enhancement of 20-Hz mu rhythm has been suggested to reflect inhibition and its suppression excitation or disinhibition of the motor cortex (Salmelin and Hari 1994). In line, the 20-Hz rebound of mu rhythm following median nerve stimulation is suppressed by simultaneous finger movements (Salenius et al. 1997c; Schnitzler et al. 1997), motor imagery (Schnitzler et al. 1997) and even by viewing another person making finger movements (Hari et al. 1998a). Accordingly, a transcranial magnetic study showed decreased motor cortex excitability from 200 ms to 1000 ms
after median nerve stimulation, a time course corresponding well to the rebound of the 20-Hz rhythm (Chen et al. 1999).

3.8. Cortex–muscle coherence
The mu rhythm is suppressed in association with movements but during isometric contractions the rhythm reappears (Jasper and Penfield 1949; Chatriaen et al. 1959); simultaneously the motor units of the contracting muscle fire in synchrony (Sears and Stagg 1976; Datta and Stephens 1990). During voluntary movements, the most prominent peaks in EMG occur around 10, 20, and 40 Hz and have been suggested to reflect rhythmicity in the central motor commands (McAuley et al. 1997). Motor cortical oscillations display coherence with EMG signals during isometric contraction (Conway et al. 1995; Salenius et al. 1997b; Halliday et al. 1998; Mima and Hallett 1999). The frequency of cortex–muscle coherence depends on the contraction force; during weak or moderate contraction coherence is seen around 20 Hz whereas during strong contraction it is shifted to around 40 Hz (Brown et al. 1998; Mima et al. 1999). The peak frequency of cortex–muscle coherence varies across individuals but remains fairly stable across different measurements and muscles in the same individual (Hari and Salenius 1999). Strength of the cortex–muscle coherence covaries with 20-30 Hz motor-cortex rhythm, thereby suggesting a relationship between these two oscillatory activities (Salenius et al. 1997a). The localization of maximal coherent activity shows homuncular organization for the coherent activity corresponding to upper and lower limb muscle contractions (Salenius et al. 1997b). Patients with cerebellar cortical stroke and parkinsonian patients withdrawn from levodopa show reduction in the cortex–muscle coherence (Pohja et al. 2000; Salenius et al. 2002) suggesting that the basal ganglia and cerebello-thalamo-cortical pathways have effect on synchronization between cortex and muscle during sustained contraction.

Timelags between the cortical and muscle signals suggest that the coherence between cortex and muscle is probably mediated by fast corticospinal axons and their monosynaptic cortico-motoneuronal connections (Brown et al. 1998).

Several studies have shown task dependence of cortex-muscle coherence, which may reflect its importance in recalibration of the motor system after a movement and a role in the sensorimotor integration (Kilner et al. 1999; Feige et al. 2000; Kilner et al. 2000; Kilner et al. 2003). Coherence increases when attention is directed to the motor task and when the task needs after median nerve stimulation, a time course corresponding well to the rebound of the 20-Hz rhythm (Chen et al. 1999).

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high precision; thus the cortical oscillations at ~20 Hz have been proposed to be related to
attention directed towards the motor task (Kristeva-Feige et al. 2002).
4. AIMS OF THE STUDY

The aims of this study were to evaluate functional properties of the somatosensory and motor cortices in patients suffering from Unverricht-Lundborg type progressive myoclonus epilepsy and in carriers of the disease.

The specific aims were:

1) To compare somatosensory cortical processing in ULD patients and in healthy control subjects by measuring somatosensory evoked responses to median nerve stimulation.

2) To probe the functional state of the motor cortex after external somatosensory stimulus by investigating the reactivity of the ~20-Hz motor-cortex rhythm after median nerve stimulation in ULD patients.

3) To investigate the oscillatory cortical control to contracting muscle in ULD patients and in healthy subjects.

4) To further characterize the whole spectrum of neurophysiological findings in ULD by measuring activation of the somatosensory cortex and cortical drive to contracting muscle in two atypical ULD patients without generalized tonic-clonic seizures to evaluate which neurophysiological findings are related to different phenotypes of ULD.

5) To compare clinical symptoms, activation of the somatosensory cortex, and cortical drive to contracting muscle in ULD carriers with myoclonic jerks with asymptomatic ULD carriers, ULD patients and healthy control subjects to explore how different genotypes of cystatin B gene are reflected in phenotype.
5. MATERIALS AND METHODS

5.1. Subjects
Some of the subjects participated in many studies, and altogether 10 ULD patients, 12 cystatin B gene mutation carriers and 15 healthy control subjects were studied. We investigated the somatosensory responses and reactivity of the ~20-Hz rhythm in seven ULD patients (age 18–35 yrs; 4 males, 3 females) and seven age- and gender-matched control subjects (age 18–35 yrs; 4 males, 3 females). Cortex–muscle coherence was investigated in eight ULD patients (age 18–35 yrs; 5 males, 3 females) and eight age- and gender-matched control subjects (age 18–35 yrs; 5 males, 3 females). In addition we studied, 2 sisters who suffered from ULD but did not have generalized tonic-clonic seizures, and six carriers of ULD who had myoclonic jerks, as well as six asymptomatic ULD carriers. Table 1 summarizes the clinical symptoms and neurophysiological findings in all patients investigated in this study.

The patients were selected in co-operation with the Rehabilitation Division of the Department of Clinical Neurosciences of the Helsinki University Central Hospital. The ULD carriers with myoclonic jerks and asymptomatic ULD carriers were selected in co-operation with the Department of Genetics of the Helsinki University Hospital. DNA analysis showed in all patients a homozygous unstable minisatellite expansion mutation in the CSTB gene and in all carriers an unstable minisatellite expansion mutation in one allele.

An informed consent was obtained from all individuals participating in these studies, and the experimental protocols were accepted by the Ethical Committee of the Department of Neurology of Helsinki University Hospital and by the Ethical Committee of the Department of Genetics of Helsinki University Hospital.

5.2. Stimulations
The left and right median nerves were stimulated electrically with 0.3-ms transcutaneous current pulses. The intensity of the stimulus was adjusted just above the motor threshold. The stimuli were delivered alternately at the wrists with an 1.5-s interval, resulting in a 3-s interstimulus interval to each median nerve. One patient received left and right median nerve stimuli alternatingly also at 3- and 6-s interstimulus intervals.
For cortex-muscle coherence calculations, the subjects upheld for 4 min isometric contractions of the left and right first dorsal intersosseus muscles in consecutive sessions. The contraction force was submaximal and optimized for each individual to reveal synchronized motor unit potentials in the surface electromyogram (EMG). MEG and EMG signals were recorded and stored on magneto-optic disks for off-line analysis.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age/ onset</th>
<th>Gen. seizures/ year</th>
<th>Myoclonic jerks</th>
<th>Ataxia</th>
<th>Medication/ day</th>
<th>Walking</th>
<th>Disability</th>
<th>Strength of P30m nAm RH LH</th>
<th>Ipsi SI</th>
<th>SII</th>
<th>Cx-muscle Coherence RH LH</th>
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<tr>
<td>M</td>
<td>33/9</td>
<td>1</td>
<td>+++</td>
<td>+++</td>
<td>Valproate 1.5 g</td>
<td>Short distances, takes support</td>
<td>Severe</td>
<td>293.5 207.8</td>
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<td>++</td>
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<td>Without aid, clumsy</td>
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<td>112.8 322.3</td>
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<td>+++</td>
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<td>No uses a wheelchair</td>
<td>Severe</td>
<td>344.6 163.8</td>
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<td>0.19</td>
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</tr>
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<td>No more</td>
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<td>+</td>
<td>Valproate 1.5 g</td>
<td>Almost normal</td>
<td>Slight</td>
<td>144.3 149.4</td>
<td>+ -</td>
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<tr>
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<td>++</td>
<td>++</td>
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<td>Short distances, clumsy</td>
<td>Moderate</td>
<td>168.9 142.5</td>
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<td>0.19</td>
</tr>
<tr>
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<td>No more</td>
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<td>++</td>
<td>Valproate 1.3 g</td>
<td>Without aid, clumsy</td>
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<td>109.4 211.6</td>
<td>- +</td>
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<td>+</td>
<td>+</td>
<td>Valproate</td>
<td>Almost normal</td>
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<td>- -</td>
<td>0.46</td>
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<td>+</td>
<td>Valproate</td>
<td>Almost normal</td>
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<td>76.7 83.3</td>
<td>- -</td>
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</tr>
<tr>
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<td>Mean + 2 SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>85.5 64.7</td>
<td>- -</td>
<td>0.16</td>
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Table 1. Clinical and neurophysiological data of ULD patients. Score for myoclonic jerks: + = occasional, ++ = frequent, +++ = very frequent myoclonic jerks. Score for ataxia: + = slight, ++ = moderate, +++ = severe. Score for disability: slight = barely detectable, moderate = can walk without help, clumsiness, severe = needs support in walking or uses a wheelchair. RH = right hemisphere, LH = left hemisphere. SI = primary somatosensory cortex, SII = Secondary somatosensory cortex, Cx-muscle = cortex-muscle.
5.3. Recordings

Cortical magnetic signals were recorded in a magnetically shielded room made of three layers of aluminium and mu metal to attenuate external magnetic and electric noise by up to 90–110 dB (Kelhälä et al. 1982). The recordings were made with a whole-head 122-channel Neuromag-122™ neuromagnetometer housing 122 planar first-order SQUID gradiometers placed in pairs at 61 recording sites, and a 306-channel Vectorview™ neuromagnetometer housing 204 planar gradiometers and 102 magnetometers placed at 102 recording sites. The planar gradiometer pairs measure two orthogonal tangential derivatives of the magnetic field component at the sensor unit locations.

During the measurement, the subject sat relaxed with the eyes open and the head supported against the helmet-shaped bottom of the device. Head position with respect to the sensor array was determined by measuring magnetic signals from four indicator coils placed on the scalp. The coil locations with respect to anatomical landmarks on the head were identified with a 3-D digitizer to align the MEG and MRI coordinate systems. The MRI images of three ULD patients were acquired with a 1-T Siemens Magnetom™ system.

5.4. Data analysis

5.4.1. Somatosensory evoked fields

Somatosensory evoked fields (SEFs) were obtained by averaging 100–150 responses to electric MN stimuli. The signals were bandpass filtered through 0.03–320 Hz, digitized at 1 kHz or through 0.03–200, digitized at 1 kHz. The 600-ms analysis period consisted of 100–200 ms prestimulus baseline. Responses during which the amplitude of the simultaneously recorded vertical electro-oculogram (EOG) exceeded 150 µV were automatically rejected from the analysis.

5.4.2. Current dipole modeling

Sources of the measured evoked fields (SEFs) were first identified visually in 2-ms steps to create the initial estimate of the number of active sources within that time period and to estimate the stability of the magnetic field pattern measured by the gradiometers. Then the equivalent current dipole (ECD) that best described a local source current at the peak of the response was...
found by a least-squares search using a subset of channels (usually 16–20) over the response area. These calculations resulted in a three-dimensional location, orientation and strength of the ECD in a spherical conductor. Goodness-of-fit ($g$) of the model was calculated to ascertain what percentage of the measured signal variance was accounted for by the dipole; only ECDs with $g \geq 85\%$ at selected periods of time in the subset of channels were used for the further analysis. Then the analysis period was extended to all channels and to the entire measurement epoch in computing a time-varying multidipole module. The validity of the multidipole model was evaluated by comparing the measured signals with responses predicted by the model. The details of this approach have been presented by Hämäläinen et al. 1993.

5.4.3. Minimum current estimates

We also used minimum current estimates (MCE) to study the generators of the SEFs. MCE enables calculation of current distribution where the total current is smallest possible (Uutela et al. 1999). MCE does not require initial estimates and thereby allows independent evaluation of brain activation. The estimated source distribution is visualized by projecting the current to the surface of the three-dimensional brain model.

5.4.4. Temporal spectral evolution

The reactivity of the ~20-Hz motor cortex with respect to MN stimuli was quantified from the spontaneous MEG activity with temporal spectral evolution method (TSE; Salmelin and Hari 1994). The ~20-Hz rhythm level was quantified from the MEG channel over the contralateral sensorimotor cortex that showed the strongest stimulus-related changes. The signals were first filtered through a passband centered around 20 Hz, as suggested by individual spectral analysis. Then the signals were rectified and finally averaged over about 55–90 trials time-locked to the stimulus. The baseline was determined from 300 ms preceding the stimulus and the rebound was quantified as the mean level 400–800 ms after the stimulus or 200 ms before and after the peak of the rebound. The analysis period of 5 s started 2.5 s before the stimulus.

To ascertain that the TSE frequency bands were optimally chosen for each subject, time-frequency representations were calculated from 5 to 35 Hz over the whole analysis period and then averaged time-locked to the stimuli. This approach provides estimates for the energy of the signal as a function of time and frequency.
5.4.5. Cortex–muscle coherence

Coherence spectra were calculated between MEG and the rectified EMG with a frequency resolution of 1 Hz. The spectra were calculated from at least 500 epochs (overlap half of the epoch) and averaged over the 4-min contraction. To evaluate the statistical significance of the observed peaks in the coherence spectra, the noise level was estimated by calculating the coherence with EMG signals shifted by 2 s, thereby abolishing any true coherence (Salenius et al. 1997b; Brown et al. 1998; Gross et al. 2000). For comparison, we also calculated the noise levels for 3 ULD patients with the method of Rosenberg et al. (1989) and the results were within 7% the same as with our method.

To locate the cortical sites of maximum coherence, cross-correlograms between MEG and EMG signals were calculated by applying an inverse Fourier transform to the normalized MEG–EMG cross-spectrum. Sources of MEG signals corresponding to the maximum cortex–muscle coherence were modeled in time domain as equivalent current dipoles that were found by a least-squares search of the spatial distribution of the cross-correlogram peaks. The source analysis was restricted to a subset of 20–40 channels over the sensorimotor cortex in each hemisphere. The locations, orientations, and strengths of the ECDs were determined at the strongest cross-correlogram peak corresponding to postero-anterior current direction; only sources that accounted for more than 85% of the field variance were accepted.

Strengths of the somatosensory evoked responses, amplitudes of the TSE curves, and coherence strengths were compared between patients and controls with Student's two-tailed t-test. Before the statistical tests, the square roots of the coherences were normalized with Fisher's z-transform.

5.5. DNA analysis

DNA analysis was performed at the Department of Medical Genetics of Helsinki University. DNA was extracted either from peripheral blood or lymphoblastoid cells using standard procedures. The minisatellite expansion mutation was analyzed as previously described (Virtaneva et al. 1997) from PstI digested DNA using Southern blotting and the CSTB cDNA as a probe. The three exons and their immediate exon-intron boundaries in the CSTB gene were sequenced from PCR-amplified genomic DNA fragments using an ABI377 sequencer.

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6. EXPERIMENTS

6.1. Somatosensory cortical processing is modified in ULD patients

Cortical responses to electric MN stimuli were investigated in seven patients suffering from Unverricht-Lundborg type progressive myoclonus epilepsy, verified by DNA analysis, and in seven healthy control subjects.

6.1.1. Results

Figure 6.1.1 shows whole-scalp somatosensory evoked fields and magnetic field patterns during the peaks of the responses to left median nerve stimuli for one patient. The earliest cortical response (N20m) peaks at 21 ms over the right parietal cortex (insert A). N20m is followed by a strong deflection of opposite polarity at 30 ms (P30m). Another response is observed ipsilateral to the stimulus, over the left parietal cortex (insert B). This response peaks at 54 ms, and it is well explained by a single dipole pointing in the anterior direction.

Figure 6.1.2 shows the source locations and the source waveforms of one patient and one control subject. The sources of N20m and P30m were located in the posterior wall of the central fissure, agreeing with the activation of the contralateral SI cortex (SI_1). The strength of P30m response is giant in the patient compared with the control subject. In contrast to the control, the patient is showing an ipsilateral response, presumably in the SI cortex (SI_1). This source showed triphasic activity, with the largest response at about 55 ms. To ascertain the absence of the ipsilateral SI activity in the control subject, an ipsilateral source (white circle) was inserted to the model according to the location of the P30m to right median nerve stimuli, but no significant ipsilateral SI activity was observed during the analysis period. The control subject displayed bilateral long-latency responses in the upper lips of the sylvian fissures in the sites of SII cortices with strongest activation at about 90 ms. In contrast, no SII activation was observed in the patient. Source areas estimated according to locations of the SII cortices of the control subject did not display any transient activity in the patient.
In all patients and control subjects, N20m and P30m were easily identified. In patients, P30m was four- to five-fold in patients compared with control subjects (p < 0.02 to left median nerve and p < 0.001 to right median nerve stimuli), whereas the N20m amplitudes did not differ statistically significantly between the groups.

In five out of seven patients, P50m(i) response was observed both to left and right median nerve stimuli in the ipsilateral parietal cortex at 48–61 ms. On average, the source of P50m(i) was 8 mm inferior, 5 mm medial, and 6 mm posterior to the N20m of the same hemisphere but the source locations did not differ significantly. These five patients with ipsilateral SI responses showed no activity in SII regions.
In agreement with the results of dipole modeling, the minimum current estimates of Fig. 6.1.3 visualize the clearly different distributions of active areas in a control subject and in a patient. The contralateral SI is strongly activated at 27 to 29 ms in the patient and at 30 ms to 32 ms in the control subject. The ipsilateral SI region is activated at about 60 ms in this patient (arrow), and activation remains in both SI regions at least up to 110 ms. In the control subject, contralateral SII activity starts to appear at about 80 ms and the ipsilateral SII activity is strongest at 95 to 110 ms (arrows). The patient shows no signals attributable to SII activation.
6.1.2. Discussion

In line with previous studies of cortical myoclonus patients (Shibasaki and Kuroiwa 1975; Chadwick et al. 1977; Brown et al. 1991; Reutens et al. 1993; Karhu et al. 1994), our ULD
patients showed enhanced P30m responses to electric median nerve stimuli over the contralateral SI cortex, suggesting hyperreactivity of the SI cortex. In our study, the N20m amplitudes were not enhanced suggesting that the thalamocortical projection system functions relatively normally in these patients.

In five out of seven patients, median nerve stimuli elicited responses also over the ipsilateral parietal cortex. These responses were generated at or slightly posterior to the generation site of N20m response, indicating their origin in the SI cortex. Further, MEG–MRI coregistration in two patients indicated the generation site of ipsilateral responses in the SI cortex. The ipsilateral responses peaked at 50–55 ms, about 20 ms later than the giant P30m. This temporal behavior of ipsilateral cortical activation agrees with previous studies showing ipsilateral responses in etiologically heterogeneous cortical myoclonus patients (Brown et al. 1991), and suggest spread of activation through corpus callosum.

The five patients with ipsilateral SI responses showed no SII activation. These five patients had more severe motor symptoms and more generalized tonic-clonic seizures than the other two patients. As previous studies have suggested participation of SII in sensorimotor integration (Huttunen et al. 1996; Forss and Jousmäki 1998; Lin et al. 2000) lack of SII in ULD patients may reflect disturbed sensorimotor integration contributing to impaired movement coordination.

6.2. Reactivity of the ~20-Hz motor cortex rhythm is abnormal in ULD patients

Our aim in this study was to monitor the reactivity of the ~20-Hz motor-cortex rhythm after left and right median nerve stimulation in genetically homogeneous ULD patients to reveal the functional state of their motor cortex. The baseline was determined from 300 ms preceding the stimulus and the rebound was quantified as the mean level 400–800 ms after the stimulus.

6.2.1. Results

Determined from the MEG amplitude spectra during rest, the ~20-Hz rhythm peaked at lower frequencies in patients than in controls (15.9 ± 0.8 Hz vs 20.5 ± 1.2 Hz over the left and 16.3 ± 1.0 Hz vs 21.6 ± 1.5 Hz over the right hemisphere; p < 0.01 and p < 0.05, respectively). The strength of the ~20 Hz spectral peak was ~2 fold in patients compared with controls (21.3 ± 2.9
fT/cm vs 8.7 ± 1.0 fT/cm over the left hemisphere and 25.6 ± 3.3 fT/cm vs 10.6 ± 1.0 fT/cm over the right hemisphere; p < 0.001 for both differences).

The mean (± SEM) source locations of the rhythmic signals of one patient, superimposed on her MR images, agreed with the location of the hand region of the central sulcus. The mean location was 4 mm more anterior (p < 0.05) for the ~20-Hz than for the ~10-Hz oscillations, in agreement with previously suggested generation of the higher mu rhythm component in the precentral motor cortex.

Figure 6.2.1 shows reactivity of the ~20-Hz level over the right sensorimotor cortex in two control subjects and in two ULD patients; the left median nerve was stimulated at time 0. In all traces, the transient increase immediately after the stimulus reflects the evoked response. In the control subjects, the ~20-Hz rhythm is first suppressed and then strongly enhanced; this “rebound” reaches its peak amplitude at 500–700 ms. In the two patients, the behavior of the 20-Hz rhythm differs in many respects; the baseline level is much stronger than in the controls, the suppression is stronger and prolonged, and there is no rebound.

In the patient group, the baseline of the ~20-Hz activity was about double compared with that of the controls: the mean (± SEM) amplitudes were 44.2 ± 4 fT/cm vs 19.2 ± 2 fT/cm (p < 0.005) in the left and 50.0 ± 4 fT/cm vs 22.0 ± 1 fT/cm (p < 0.001) in the right hemisphere for patients and controls, respectively.
Fig. 6.2.1 Stimulus-related changes in the ~20-Hz mu rhythm level of two control subjects and two patients. Light gray shadowed areas show the suppression and dark gray shadowed areas the rebounds 400–800 ms after the stimulus (at 0 ms).

Figure 6.2.2. shows the time-frequency representation of the 5–35 Hz energy after L MN stimulation for one control subject and one patient. In the control subject, the energy is strongly enhanced, representing the rebound of the 15–20 Hz band 500–700 ms after stimulus, whereas in the patient the energy decreases for about 1 s after the stimulus.

Figure 6.2.3. illustrates the mean (+ SEM) strengths of the rebounds in both subject groups. In controls, the rebounds were statistically significant on both left (p < 0.05) and right (p < 0.01) hemispheres. In patients, the mean amplitudes were negative with respect to the baselevel and did not differ statistically significantly from zero in either hemisphere.
Fig. 6.2.2. Time-frequency representation of the energy at 5–35 Hz from 500 ms before to 1500 ms after LMN stimulation of one control subject and one patient. The scale of the energy is logarithmic.

Fig. 6.2.3. Mean (± SEM) amplitude of the ~20-Hz motor cortex rebound in patients and in control subjects.
6.2.2. Discussion

The present results revealed clear abnormalities in the reactivity of the ~20-Hz motor-cortex rhythm in patients with ULD: (1) the peak frequency was about 5 Hz lower in patients than in controls, (2) the baseline mean amplitude of the ~20-Hz activity was double compared with that of the controls, and (3) there was no rebound following median nerve stimuli. Source analysis of the signals in one patient confirmed that the higher frequency oscillations of the mu rhythm were mainly generated in the motor cortex, in line with previous studies in healthy subjects (Jasper and Penfield 1949; Salmelin and Hari 1994). Thus the on average ~8 Hz and ~16 Hz mu rhythm components of our ULD patients seem to correspond to the on average ~11 Hz and ~21 Hz oscillations of healthy subjects. The slowing of the mu rhythm in patients could be due to degenerative changes in the brain or due to anticonvulsive polytherapy. The time-frequency representation confirmed that the frequency bands for the TSE analysis were chosen correctly and did not miss rebounds at any other frequencies. Since the rebound of mu rhythm is supposed to reflect inhibition of the motor cortex (Salmelin et al. 1995; Chen et al. 1999), the observed lack of the 20-Hz rebound indicates prolonged excitation/disinhibition of the motor cortex after somatosensory stimuli, in line with previous trancranial magnetic stimulation studies, which showed hyper excitability of the motor cortex in patients with cortical myoclonus (Brown and Marsden 1996; Valzania et al. 1999).

6.3. Oscillatory cortical drive to isometrically contracting muscle is enhanced in ULD patients

In this study eight ULD patients and eight healthy control subjects upheld for 4 min isometric contraction of the first dorsal interosseus muscle. The MEG and surface EMG signals were recorded and coherence between the signals was calculated. In addition, sources of the coherent activity were located.
Fig. 6.3.1. 4-s periods of MEG over the left sensorimotor cortex and of EMG from the right interosseus muscle at rest and during isometric contraction. The signals were filtered through 3-30 Hz. On the right the corresponding MEG and rectified EMG spectra from the whole 4-min measurement period are shown.

6.3.1. Results

Figure 6.3.1 shows the MEG–EMG coherence spectrum from one channel and the corresponding EMG spectrum for one patient during isometric left interosseus contraction. The coherence was strongest (0.21) on this channel over the right sensorimotor area, with a peak frequency at 15 Hz; the simultaneously recorded EMG showed the strongest peak at the same frequency.
Fig. 6.3.2 shows for all subjects the MEG–EMG coherence spectra from one MEG channel over the contralateral sensorimotor cortex during left- and right-sided contractions. The spectra peaked in the control subjects at 19 ± 2 Hz (mean ± SEM) and 20 ± 3 Hz for left and right-sided contractions, respectively. The corresponding values for the patients were 17 ± 1 Hz (range 15–24 Hz) and 17 ± 1 Hz (15–21 Hz); the differences between the patients and controls were not statistically significant. Six patients and six controls displayed another, weaker peak with a highly variable frequency (5–44 Hz). The coherence was significantly stronger in patients than in controls (0.22 ± 0.03 vs. 0.05 ± 0.01 and 0.15 ± 0.03 vs. 0.07 ± 0.02 during left- and right-sided contractions, p < 0.001 and p < 0.01, respectively). In addition, during contraction, the 17–20 Hz motor cortex rhythm was significantly stronger in patients than in controls (23.2 ± 4.3 ft/cm vs. 7.9 ± 1.3 ft/cm, p < 0.05 for left hand and 16.9 ± 1.6 ft/cm vs. 7.1 ± 0.6 ft/cm, p < 0.001 for right hand).

In control subjects, significant cross-correlogram peaks were observed only over the contralateral primary motor cortex, in agreement with previous findings (Salenius et al. 1997b; Brown et al. 1998). In contrast, five ULD patients showed additional cross-correlogram peaks over the ipsilateral sensorimotor cortex. Figure 6.3.3 shows for one patient the contra- and ipsilateral magnetic field patterns at the peaks of the MEG–EMG cross-correlograms during left hand contraction and the corresponding source areas superimposed on her MR image. The
sources of the most coherent activity are located in the contralateral and in the ipsilateral central sulci.

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**6.3.2. Discussion**

The main finding in this study was the remarkably enhanced coherence between contralateral motor cortex and muscle in ULD patients compared with control subjects. This finding may be explained by a higher tendency of the motor cortex neurons to fire synchronously. During contraction this synchronisation involves the corticospinal projections, which may lead to a higher EMG activity at the same frequency, and higher coherence between MEG and EMG signals.

Similarly as in healthy subjects (Gross et al. 2000), also in the ULD patients the time lags determined from the phase spectra showed that the cortex was driving the muscle signal. The 15–30 ms time lag agrees with cortex–muscle conduction delays observed in previous EEG–EMG coherence and TMS studies of patients with cortical myoclonus (Brown et al. 1999; Valzania et al. 1999).

In contrast to the strictly contralateral cortex–muscle coherence in the control group, five out of eight patients showed additional coherent activity between the ipsilateral motor cortex and muscle. This finding may suggest altered corticospinal conduction but should be regarded as preliminary due to the relative weakness of the ipsilateral coherence.
6.4. The primary somatosensory cortex is not hyperreactive but the activation of the motor cortex is altered in Unverricht-Lundborg disease patients without generalized seizures

In this study we investigated cortical functions in two Unverricht-Lundborg disease patients who suffered from myoclonic jerks but did not have generalized tonic-clonic seizures. Somatosensory cortical responses were recorded to median nerve stimuli and coherence was calculated between cortical and muscle signals during isometric contraction of hand muscle.

6.4.1. Results

In the two ULD patients without generalized tonic-clonic seizures (Patients 1 and 2), the strongest SEFs to left and right median nerve stimulation were observed over the contralateral SI. No responses occurred in the homologue area of the ipsilateral hemisphere in Patient 1 but in Patient 2, weak ipsilateral SI activity was observed at about 100 ms. Significant activation was not observed outside SI region in either patient; responses from the SII, typically observed at about 90–110 ms in the Sylvian fissures bilaterally in healthy subjects, were lacking in both patients.

Figure 6.4.1 compares the strengths of the P30m responses of these two patients with corresponding values of ULD patients with generalized tonic-clonic seizures and with healthy control subjects. In Patients 1 and 2, the P30m amplitudes were within normal variation in the right hemisphere (51 nAm and 77 nAm, respectively, vs 86 nAm, mean ± 2 SD in controls), and only slightly enhanced in the left hemisphere (75 nAm and 83 nAm, respectively, vs 65 nAm, mean ± 2 SD in controls). P30m amplitudes of the two patients were clearly smaller than in eight ULD patients with generalized seizures, in whom the mean strength of P30m was 4–6 fold compared with the healthy control subjects (range 93–345 nAm in the right hemisphere, 143–322 nAm in the left hemisphere).
Fig. 6.4.1. Strengths of P30m of two ULD patients without generalized tonic-clonic seizures (black circles), ULD patients with generalized tonic-clonic seizures (GTCS) (white circles), and the mean strength of P30m + 1 SD and 2 SD of control subjects (dashed lines).

Figure 6.4.2 shows the coherence spectra of Patients 1 and 2 over the right and left motor cortices during contralateral interosseous muscle contraction, and the corresponding mean (± 2 SD) values of ULD patients with generalized tonic-clonic seizures and of the healthy control subjects. In Patient 1, coherence is clearly stronger than in controls (0.46 in the right hemisphere and 0.32 in the left hemisphere). In Patient 2, coherence is stronger than in controls in the right hemisphere and within normal variation in the left hemisphere (0.35 and 0.15 over right and left hemisphere).
hemispheres, respectively). In both patients, significant coherent activity was observed also over the ipsilateral sensorimotor cortex, in contrast to the strictly unilateral coherent activity in the control subjects.

![Graph](image)

**Fig. 6.4.2.** Coherence spectra for one channel over the right and left hemispheres during contralateral interosseus muscle contraction of two ULD patients without generalized tonic-clonic seizures (solid and dashed lines). Dashed horizontal lines show the mean + 2 SD strengths of coherence of ULD patients with generalized tonic-clonic seizures (GTCS) and of healthy control subjects.

In line with ULD patients with generalized tonic-clonic seizures, the ~20-Hz rebounds to electric median nerve stimulation were lacking in ULD patients without generalized tonic-clonic seizures (unpublished data).

### 6.4.2. Discussion

As a clear difference to previous studies of PME patients (Shibasaki and Kuroiwa 1975; Chadwick et al. 1977; Brown et al. 1991; Reutens et al. 1993; Karhu et al. 1994), contralateral SI responses in our ULD patients without generalized tonic-clonic seizures were within normal variation or only slightly increased, and like in healthy controls, no early ipsilateral SI responses were observed.

Cortex–muscle coherence, which reflects cortical oscillatory drive to contracting muscle (Salenius et al. 1997b), was remarkably enhanced in our ULD patients without generalized seizures, in line with our previous findings of ULD.

![Graph](image)

**Fig. 6.4.2.** Coherence spectra for one channel over the right and left hemispheres during contralateral interosseus muscle contraction of two ULD patients without generalized tonic-clonic seizures (solid and dashed lines). Dashed horizontal lines show the mean + 2 SD strengths of coherence of ULD patients with generalized tonic-clonic seizures (GTCS) and of healthy control subjects.

In line with ULD patients with generalized tonic-clonic seizures, the ~20-Hz rebounds to electric median nerve stimulation were lacking in ULD patients without generalized tonic-clonic seizures (unpublished data).

### 6.4.2. Discussion

As a clear difference to previous studies of PME patients (Shibasaki and Kuroiwa 1975; Chadwick et al. 1977; Brown et al. 1991; Reutens et al. 1993; Karhu et al. 1994), contralateral SI responses in our ULD patients without generalized tonic-clonic seizures were within normal variation or only slightly increased, and like in healthy controls, no early ipsilateral SI responses were observed.

Cortex–muscle coherence, which reflects cortical oscillatory drive to contracting muscle (Salenius et al. 1997b), was remarkably enhanced in our ULD patients without generalized seizures, in line with our previous findings of ULD.
The present results suggest that in ULD patients who have myoclonic jerks but have never experienced generalized tonic-clonic seizures, the primary somatosensory cortex is not hyperreactive in contrast to clearly altered activation of the motor cortex.

6.5. Unverricht-Lundborg type progressive myoclonus epilepsy carriers have normal activation of the somatosensory and motor cortices

In this study we evaluated possible symptoms and neurophysiological findings in ULD carriers who have only one deficient CSTB gene. The neurophysiological findings of symptomatic and asymptomatic ULD carriers were compared with ULD patients and healthy control subjects studied earlier to find out how a defect in one CSTB allele alters sensorimotor cortical processing and whether these alterations would be related to the manifestation of myoclonic jerks in some ULD carriers.

6.5.1. Results

Genetic findings

All symptomatic and asymptomatic subjects included in the study were found to carry an enlarged minisatellite on one of the CSTB allele (Figure 6.5.1). All expansions were within the range in which most alleles in Finland fit (Virtaneva et al. 1997), and unusually large expansions were identified. Five of the symptomatic carriers were either parents or siblings of ULD patients who were homozygous for the minisatellite expansion mutation. As the occurrence of some other CSTB mutation in them would be extremely unlikely, no further mutation analyses were undertaken. In Subject 5, whose uncle had suffered from ULD, an extensive search for other CSTB mutations was done by sequencing the exons and exon-intron boundaries. However, no other mutation was identified.
Clinical findings
All the six symptomatic ULD carriers included in this study had myoclonic symptoms; three of them had occasional myoclonic jerks, two had become symptom-free and one had occasional feeling of cramp in her fingers and toes. On neurological examination the only abnormal findings were slight tremor in hands in Subject 5 and strabismus in Subject 3.

MEG findings
All symptomatic and asymptomatic ULD carriers showed strongest SEFs in the contralateral SI. In contrast with ULD patients and in line with control subjects, no ipsilateral SI responses were observed. Figure 6.5.2 compares the strength of the early cortical SI responses in the contralateral SI about 35 ms after the LMN and RMN stimuli in symptomatic ULD carriers, asymptomatic ULD carriers, healthy control subjects, and ULD patients. In symptomatic and asymptomatic ULD carriers the P35m amplitudes do not differ statistically significantly from control subjects in either hemisphere (32.0 ± 7 nAm and 49.0 ± 11 nAm vs. 45.2 ± 8 nAm for the right, and 39.3 ± 8 nAm and 41.5 ± 4 nAm vs. 34.2 ± 6 nAm in the left hemisphere). In ULD patients, the P35m responses were over 3 times stronger than in both groups of ULD carriers (180.9 ± 37 nAm in the right hemisphere and 191.7 ± 24 nAm in the left hemisphere).

Fig. 6.5.1. Southern blot analysis of the minisatellite expansion mutation. The blot includes PstI-digested genomic DNA from three symptomatic (SC) and asymptomatic carriers (AC) and one ULD patient (P) hybridized with a CS1B cDNA probe. In patient DNA the expected size fragment (E) is absent and two expanded alleles of 3.4 kb and 4.1 kb in size are seen. The carriers have the expected size fragment and one expanded allele. C refers to a constant band present in all samples.

Clinical findings
All the six symptomatic ULD carriers included in this study had myoclonic symptoms; three of them had occasional myoclonic jerks, two had become symptom-free and one had occasional feeling of cramp in her fingers and toes. On neurological examination the only abnormal findings were slight tremor in hands in Subject 5 and strabismus in Subject 3.
Fig. 6.5.2. Strengths (mean ± SEM) of the early cortical S1 responses in the right and left hemisphere in symptomatic carriers, asymptomatic carriers, controls, and ULD patients.

SII responses were found in 3 symptomatic and 3 asymptomatic ULD carriers. The contralateral SII responses peaked on average at 100–105 ms after the stimuli and the ipsilateral SII responses peaked at 112–113 ms after the stimuli.
Figure 6.5.3 demonstrates reactivity of the 20-Hz rhythm over the right sensorimotor cortex after electric LMN stimulation in one symptomatic carrier (Subject 2), one asymptomatic carrier, one healthy control subject, and one ULD patient. The baselevel of the 20-Hz rhythm was about the same in the symptomatic and asymptomatic carriers and in the control subject, whereas in the ULD patient the baselevel is clearly stronger. After the stimulation (time 0) the level of the rhythm transiently increases reflecting the evoked response. Thereafter the 20-Hz rhythm is suppressed for a short time and then strongly enhanced (rebound) in carriers and the control subject. In contrast, no rebound is observed in the ULD patient.

![Graph of 20-Hz rhythm](image)

Fig. 6.5.3. Reactivity of the 20-Hz motor cortex rhythm to left median nerve stimulation over right hemisphere in one symptomatic carrier, one asymptomatic carrier, one healthy control subject and one ULD patient. Median nerve is stimulated at time 0.

Figure 6.5.4 shows the MEG–EMG coherence spectra from one channel during left interosseus contraction in one symptomatic ULD carrier (Subject 2), an asymptomatic ULD carrier, a healthy control subject and an ULD patient. Coherence was strongest on this channel over the right sensorimotor cortex with a peak frequency at 21 Hz in the symptomatic carrier, at 19 Hz in the asymptomatic carrier, at 20 Hz in the control subject and at 15 Hz in the ULD patient. Another, weaker peak is observed at about 28 Hz in the symptomatic carrier. The strength of coherence is 0.04 in the symptomatic ULD patient, 0.05 in the asymptomatic ULD patient, 0.05 in the healthy control subject and 0.33 in the ULD patient.

![Graph of coherence spectra](image)

Fig. 6.5.4. Coherence spectra from one channel during left interosseus contraction in one symptomatic ULD carrier, an asymptomatic ULD carrier, a healthy control subject and an ULD patient.
Coherence was found in four symptomatic and four asymptomatic ULD carriers during left hand contraction and in six symptomatic and three asymptomatic ULD carriers during right hand contraction. In one symptomatic carrier, the strength of coherence was remarkably enhanced in the right hemisphere (0.2) and within normal variation (0.05) in the left hemisphere. In the other symptomatic and asymptomatic carriers, the strength of coherence was within normal variation.

6.5.2. Discussion

All of our symptomatic ULD carriers were relatives to ULD patients with homozygous minisatellite expansion mutation and were consequently found to be heterozygous carriers of the same mutation. In symptomatic ULD carriers, the myoclonic jerks did not cause any disability and in 3 subjects the jerks had ceased entirely some years earlier.

In symptomatic, as well as in asymptomatic heterozygous carriers of ULD, the early somatosensory cortical responses were normal in strength.

Cortex–muscle coherence was not enhanced in symptomatic or asymptomatic ULD carriers, suggesting that there are no differences in the cortical drive to isometrically contracting muscle between symptomatic and asymptomatic ULD carriers or healthy control subjects.

Both in symptomatic and in asymptomatic ULD carriers, the reactivity of the ~20-Hz motor cortex rhythm was normal, suggesting that after external stimulus the excitation of the
motor cortex is not prolonged. Further, there was no difference in the reactivity of the ~20-Hz motor cortex rhythm between the symptomatic and asymptomatic ULD carriers.

These findings suggest that the excitability of the somatosensory and motor cortices, and the reactivity of the motor-cortex rhythm to external stimuli appear to be normal in ULD carriers. Therefore it is likely that the generation of myoclonic jerks in some ULD carriers is not directly associated with continuously altered cortical functions of the primary somatosensory and motor cortices.
7. GENERAL DISCUSSION

In this study, the activation of somatosensory and motor cortices was investigated for the first time with a whole-head covering MEG in clinically and genetically verified ULD patients and carriers of ULD. Although ULD is caused by loss-of-function expansion mutations in the gene encoding CSTB (Lafreniere et al. 1997; Lalioti et al. 1997; Virtaneva et al. 1997), the effect of the reduced CSTB levels in the central nervous system has still mainly remained unclear. This etiologically homogeneous patient group and genetically verified carriers of the disease enabled further interpretation of the neurophysiological findings associated to CSTB mutations and discussion about the pathophysiological mechanisms underlying the clinical symptoms in various phenotypes of ULD. Interestingly, the severity of the clinical symptoms in ULD show strong interfamiliar, and also intrafamiliar variability, and the repeat size of the expansion mutation does not correlate with the age of onset or the severity of the disease (Lafreniere et al. 1997; Lalioti et al. 1997; Virtaneva et al. 1997; Lehesjoki 2003).

In line with previous studies of patients with cortical myoclonus (Shibasaki and Kuroiwa 1975; Chadwick et al. 1977; Brown et al. 1991; Reutens et al. 1993; Karhu et al. 1994), our ULD patients with generalized tonic-clonic seizures had giant contralateral SI responses 30 ms after electric median nerve stimulation, suggesting pathologic hyperexcitability of the sensorimotor cortex. However, the strength of the SI responses did not correlate to the severity of disability in these patients. N20m was not statistically significantly enhanced, which may reflect normal functions of the thalamocortical projection system.

In five of seven “classical” ULD patients with generalized tonic-clonic seizures, both left and right median nerve stimuli elicited responses in the ipsilateral sensorimotor cortex. In contrast to a previous MEG study on etiologically heterogeneous cortical myoclonus patients (Mima et al. 1998b), the ipsilateral responses were shown to generate in the SI cortex. The about 20-ms time lag between contra- and ipsilateral SI responses suggested spread of activation from contralateral to ipsilateral SI through corpus callosum. The five patients who exhibited ipsilateral SI responses were more disabled and they had more generalized seizures. In addition, ULD patients without generalized tonic-clonic seizures showed no ipsilateral SI activity. These findings support the suggestion that interhemispheric spread of cortical excitation represents an
additional pathophysiological defect and may underlie the generalized seizures in cortical myoclonus patients (Brown et al. 1991).

In five out of seven patients, the SII cortices were bilaterally unresponsive to peripheral stimuli. In the control study with one patient, SII activation was not reliably detected even with an interstimulus interval of 9 seconds, indicating that abnormally long recovery cannot explain the lack of SII responses in these patients. In monkeys, SI has been shown to have a background facilitatory influence on SII (Zhang et al. 1996). Accordingly, modified activation of SI may alter responsivenes of SII in ULD patients. However, ULD patients without generalized tonic-clonic seizures showed normal or only slightly enhanced SI responses and no SII responses suggesting that also other mechanisms may be involved.

Due to its small size, SII is difficult to investigate, and its functional significance in humans is still unclear. In monkeys, SII lesions result in profound defects in tactile learning and especially intermanual transfer of learned skills (Garcha and Etlinger 1978), suggesting participation of SII in tactile memory and bilateral integration of tactile input (Garcha and Etlinger 1978; Burton and Carlson 1986; Friedman and Murray 1986). SII may also serve as an important link conveying somatosensory information to the motor system (Burton 1986; Huttunen et al. 1996; Lin et al. 2000). Our patients with absent SII activation exhibited more severe motor symptoms, such as clumsiness and difficulties in moving. Therefore, lack of activation of SII in ULD patients could reflect disturbed sensorimotor integration, which may contribute to impaired movement coordination in these patients.

ULD patients showed no rebounds of the ~20-Hz motor-cortex rhythm after median nerve stimulation. In addition, their baseline level of the ~20-Hz activity was double compared with that of the controls. Although the ~20-Hz rebounds were absent both in “classical” and in atypical ULD patients without generalized tonic-clonic seizures, it can not be regarded specific to ULD due to the small number of patients in this study and the fact that reactivity of the ~20-Hz motor-cortex rhythm has not been systematically studied in other patient groups.

As the ~20-Hz rebounds after external stimulation are considered to reflect inhibitory state of the motor cortex (Salmelin et al. 1995), lack of ~20-Hz rebounds may reflect prolonged excitation of the motor cortex in ULD patients. On the other hand, if the enhanced baseline level of the ~20-Hz activity is caused by a larger number of active motor cortical neurons or by a maximal
tendency of the neurons to fire synchronously, lack of rebounds could be a sealing phenomenon; the rhythm can not be further enhanced after the stimulus.

Nevertheless, the enhanced baseline level of the ~20-Hz motor-cortex rhythm and the enhanced cortex–muscle coherence may reflect a higher tendency of the motor cortex neurons to fire synchronously both at rest and during contraction in ULD patients. Mechanisms underlying the higher synchrony of the motor-cortex neurons could be searched for from animal models. In monkeys, application of GABA\(_A\) antagonist to the primary motor cortex enhanced both movement-related and spontaneous neuronal activity and increased the number of active neurons; the neuronal changes were accompanied by clumsy movements and muscle jerks that became so frequent that they had to be terminated with barbiturate to prevent generalized seizures (Matsumura et al. 1991; Matsumura et al. 1992). These disturbances closely resemble the clinical symptoms of our ULD patients. Thus our findings may reflect decreased GABA-ergic inhibition in the motor cortex of ULD patients.

All our patients had antiepileptic polytherapy including moderate doses of benzodiazepine, which is known to enhance the “beta band” oscillatory activity in EEG (Lindhardt et al. 2001). The strength of the ~20-Hz motor-cortex rhythm may even double in healthy subjects after diazepam injection, whereas the cortex–muscle coherence may decrease (Baker and Baker 2003). Accordingly, the increased baseline level of the ~20-Hz motor-cortex rhythm may be enhanced by benzodiazepine but it is unlikely that it has enhancing impact on the cortex–muscle coherence in ULD patients.

Our ULD patients were treated also with other antiepileptic drugs than benzodiazepine, and systematic studies monitoring the effects of these antiepileptic drugs on brain functions, such as cortex–muscle coherence, somatosensory evoked responses, ~20-Hz motor-cortex reactivity or transcallosal conduction, have not yet been performed. In addition, further studies on ULD patients and also on other patient groups are needed to assess whether enhanced cortex–muscle coherence is a specific finding of ULD patients.

In addition, cerebellar dysfunction may contribute to the firing of motor cortical neurons in ULD patients. All of our ULD patients in this study suffer from cerebellar symptoms of varying degree. Previous neuropathological studies have shown loss of cerebellar inhibitory Purkinje cells in ULD patients (Haltia et al. 1969; Koskiemi et al. 1974a). A recent MRI study
showed degeneration in cerebellar hemispheres but also in the basis pontis and medulla (Mascalchi et al. 2002).

CSTB has been shown to prevent cerebellar apoptosis in mice (Pennacchio et al. 1998). It is possible that the degenerative changes in the cerebellum in ULD are also directly related to the homogeneous mutation in the CSTB gene. Interestingly, Purkinje cell loss has been demonstrated also in some other patients with cortical myoclonus, and the effect of the cerebellar pathology on the sensorimotor cortical hyperexcitability has been suggested (Tijssen et al. 2000). Transcranial magnetic stimulation (TMS) studies in humans have shown that cerebellum has influence on motor cortex excitability: TMS over the cerebellum elicited suppression in the motor cortex in healthy subjects but not in patients with a lesion in the cerebellum or cerebellothalamocortical pathways (Di Lazzaro et al. 1995; Ugawa et al. 1995; Ugawa et al. 1997). In addition, cerebellum has effect on the cortical rhythms. Stimulation of cerebellar deep nuclei in cats has been shown to influence motor cortical rhythmicity (Steriade 1995). Recent MEG studies have shown that cerebellar cortical stroke abolishes transiently oscillatory cortical drive to contracting hand muscles ipsilateral to the lesion (Pohja et al. 2000). As the Purkinje cells have mainly inhibitory influence on the cerebellar deep nuclei, their loss could lead to enhanced cerebello-thalamo-cortical output, which may affect both the excitability and the oscillatory activity of the motor cortex in ULD patients.

In conclusion, the results of the present thesis show that in ULD patients the balance between excitation and inhibition of the sensorimotor cortical neurons is strongly modified. This change may be related to decreased CSTB levels and it probably also recruits changes in other brain areas such as cerebellum. Changes in sensorimotor cortical functions may alter in different phenotypes of ULD, and careful neurophysiological examination in addition to direct detection of CSTB gene mutations may separate the different phenotypes.
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REFERENCES


Chadwick D, Hallett M, Harris R, Jenner P, Reynolds EH and Marsden CD: Clinical, biochemical, and physiological features distinguishing myoclonus responsive to 5-
hydroxytryptophan, tryptophan with a monoamine oxidase inhibitor, and clonazepam. *Brain* 1977, 100: 455-487.


Lundborg H: *Die progressive Myoclonus Epilepsie (Unverricht's Myoklonie)*. Almqvist and Wiksell, Uppsala, 1903.


