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NEUROMAGNETIC STUDIES ON SOMATOSENSORY FUNCTIONS IN CLN3, CLN5 AND CLN8 FORMS OF NEURONAL CEROID LIPOFUSCINOSES

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Academic Dissertation

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To Professor Pirkko Santavuori

CONTENTS

ABBREVIATIONS

bp	base pair
CC	corpus callosum
CCT	central conduction time
CLN3	juvenile neuronal ceroid lipofuscinosis gene
CLN3	juvenile neuronal ceroid lipofuscinosis, protein encoded by *CLN3*
CLN5	Finnish variant late infantile neuronal ceroid lipofuscinosis gene
CLN5	Finnish variant late infantile neuronal ceroid lipofuscinosis, protein encoded by *CLN5*
CLN8	Northern epilepsy syndrome gene
CLN8	Northern epilepsy syndrome, protein encoded by *CLN8*
CNS	central nervous system
CSF	cerebrospinal fluid
CT	computed tomography
ECD	equivalent current dipole
EEG	electroencephalography, -gram
EPSP	excitatory postsynaptic potential
ER	endoplasmic reticulum
ERG	electroretinogram
IPSP	inhibitory postsynaptic potential
IQ	intelligence quotient
ISI	interstimulus interval
JNCL	juvenile neuronal ceroid lipofuscinosis
kb	kilobase pair
LMN	left median nerve
LINCL	late infantile neuronal ceroid lipofuscinosis
MEG	magnetoencephalography
MPRAGE	magnetization prepared rapid acquisition gradient echo
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
NCL	neuronal ceroid lipofuscinosis
NES	Northern epilepsy syndrome
PET	positron emission tomography
PME	progressive myoclonus epilepsy
PPC	posterior parietal cortex
PSP	postsynaptic potential
RMN	right median nerve
SD	standard deviation
SEF	somatosensory evoked magnetic field
SEP	somatosensory evoked potential
SI	primary somatosensory cortex
SII	secondary somatosensory cortex
SIIc	contralateral SII
SIIi	ipsilateral SII
SPECT	single photon emission computed tomography
SQUID	superconducting quantum interference device
TE	echo time
TR	repetition time
ULD	Unverricht–Lundborg type of PME
VEP	visual evoked potential
VPL	ventral posterolateral nucleus of the thalamus
VPM	ventral posteromedial nucleus of the thalamus
$vLINCL_{Fin}$	Finnish variant late infantile NCL

LIST OF ORIGINAL PUBLICATIONS

This Thesis is based mainly on the following publications, which are referred to in the text by their Roman numerals. Some unpublished results are also included in this Thesis.

I Lauronen L, Heikkilä E, Autti T, Sainio K, Huttunen J, Aronen HJ, Korvenoja A, Ilmoniemi RJ, Santavuori P. Somatosensory evoked magnetic fields from primary sensorimotor cortex in juvenile neuronal ceroid lipofuscinosis. Journal of Child Neurology 1997; 12: 355–360.

II Lauronen L, Munroe PB, Järvelä I, Autti T, Mitchison HM, O'Rawe AM, Gardiner RM, Mole SE, Puranen J, Häkkinen A-M, Kirveskari E, Santavuori P. Delayed classic and protracted phenotypes of compound heterozygous juvenile neuronal ceroid lipofuscinosis. Neurology 1999; 52: 360–365.

III Lauronen L, Santavuori P, Hirvasniemi A, Kirveskari E, Huttunen J, Autti T. Northern epilepsy syndrome (NES, CLN8) — MRI and electrophysiological studies. European Journal of Paediatric Neurology 2001; 5: 167–173.

IV Lauronen L, Huttunen J, Kirveskari E, Wikström H, Sainio K, Autti T, Santavuori P. Enlarged SI and SII somatosensory evoked responses in the CLN5 form of neuronal ceroid lipofuscinosis. Submitted.

1. ABSTRACT

Background: Neuronal ceroid lipofuscinoses (NCLs) are autosomally recessively inherited progressive encephalopathies that are known to occur world-wide. To date, thirteen different forms of NCL have been identified, all of which are characterized by the accumulation of ceroid and lipofuscin like material. The clinical symptoms are caused by neuronal dysfunction and death, the timing and pattern of which vary according to the different forms of the disease. In recordings of somatosensory evoked potentials (SEPs), the infantile form of NCL has been shown to lead to extinction of the responses, whereas the late infantile forms are associated with so-called giant (high amplitude) SEPs. In juvenile forms, the findings have varied from attenuated to enhanced SEPs. Some of this discrepancy may be due to the fact that the progression of the NCL disease causes physical changes (*e.g.*, the amount of cerebrospinal fluid increases, the skull becomes thicker), which will distort the signal between the active brain source and the measuring device when using electroencephalographic (EEG) techniques. Thus, magnetoencephalography (MEG), which records synchronous neuronal activity in the way as EEG, but is insensitive to the different electrical conductivities, would better reflect the underlying neuronal activation in NCL diseases. Furthermore, the neuronal activity originating from the different cortical areas participating in somatosensory processing can be modelled using MEG.

Purpose and methods: We used MEG to examine in detail somatosensory cortical responses to median nerve stimulation in three genetically different forms of NCL in order to study the effects of the NCL gene defects on the functioning of neurons of the somatosensory pathway. The patients included 24 with juvenile NCL (JNCL), 4 with Northern epilepsy syndrome (NES) and 5 with Finnish variant late infantile NCL ($vLINCL_{Fin}$). The somatosensory evoked magnetic fields (SEFs) of the patients were compared with those of healthy controls in the same age range. Altogether 27 healthy subjects, 17 of whom were under 19 years of age, were measured. In patients with JNCL and $vLINCL_{Fin}$, the SEFs, structural brain changes evaluated using magnetic resonance imaging (MRI), and some clinical findings were further investigated for phenotypic differences. In addition, the brain MRIs of NES patients were visually and quantitatively analyzed.

Results: In the normal controls, the responses from the primary somatosensory cortex (SI) were of similar morphology in all subjects throughout the whole age range, and consisted of identifiable N20m, P35m and P60m deflections. The early N20m responses were significantly stronger in older subjects than in younger subjects. A similar trend was seen also for the P35m strength. The height and age of the subject had the greatest influence on the N20m latency. Activity from contra- and ipsilateral secondary somatosensory cortex (SII) was seen in 83% and 72% of the subjects, respectively.

In JNCL, the SEFs from SI were normal in patients between 6–11 years of age. After that the responses became stronger, 68% of the first cortical N20m deflections and 39% of the following P35m deflections being abnormally strong. The different mutations in the *CLN3* underlying classic or delayed classic forms of the disease did not affect the SEF strengths differently. However, the phenotype of the two patients who were compound heterozygous for the major 1.02-kb deletion and a missense mutation, was atypically protracted with milder than expected MRI findings and normal or only slightly enlarged SEFs. A slight prolongation of the N20m latency was seen among all the different phenotypes.

In the patients with NES (gene symbol *CLN8*; age range 26–43 years), the SEFs were mainly within normal limits. Their MRIs showed only mild cerebral atrophy combined with more prominent cerebellar atrophy. In addition, no pathologically altered signal intensities of the brain structures were found, thus distinguishing NES from JNCL and $vLINCL_{Fin}$.

The most pathological findings were observed in patients with $vLINCL_{Fin}$, which is caused by mutations in the *CLN5* gene. All five patients studied, between the ages of 8–16 years, had abnormally strong SI responses with a delayed N20m latency. The strength of the responses was clearly greater than in patients with JNCL. Furthermore, the morphology of the SI deflections differed from that of the normal controls: the extremely strong N20m and P35m were followed by N45m, a deflection that was not seen in the controls. In addition, the normally occurring P60m was not seen in patients. The increased excitability of the somatosensory system was also seen as an enlargement of the SII responses in four patients. The patients with the mutations predicted to result in the most severely truncated proteins showed the strongest responses, indicating that there may be a subtle link between genotype and neurophysiologic phenotype.

Conclusion: The median nerve SEFs, reported for the first time in a relatively large number of children and adolescents, showed that the SEFs in healthy children over 6 years are morphologically similar to those in adults. However, new information about the development of SEFs, *e.g.*, that both the N20m and P35m become stronger with age, was demonstrated. Thus, the need for age-matched control groups is emphasized when children and adolescents are studied.

The present studies demonstrate that the use of MEG is feasible when studying children with a severe degenerative brain disease like NCL. The enlargement of the early SI deflections in JNCL with the progression of the disease and the presence of enlarged SII deflections in $vLINCL_{Fin}$ were demonstrated for the first time, indicating that MEG may uncover new information about defective neuronal function in these diseases. The pathogenesis of JNCL and $vLINCL_{Fin}$ were shown to lead to enlargement of SEFs, suggesting that the diseases disturb the balance between inhibition and excitation of the somatosensory network. In contrast, although inclusions characteristic for NCL diseases are seen in the neurons of patients with NES at autopsy, the well preserved cerebrum and normal SEFs suggest that the underlying *CLN8* gene defect is relatively well tolerated by the neurons of the somatosensory network.

2. INTRODUCTION

Neuronal ceroid lipofuscinoses (NCLs) are autosomally recessively inherited progressive encephalopathies, known to occur world-wide. They are characterized by the accumulation of ceroid and lipofuscin like material in storage cytosomes that leads to neuronal dysfunction and death (Zeman *et al.*, 1970; Santavuori *et al.*, 2000). Clinically, NCL diseases can be classified into 13 different forms that are caused by defects in at least 8 different genes, located on different chromosomes (Mole, 1999). Three of the proteins involved in NCL pathogenesis are known to be associated with lysosomal function, but the reason for neuronal damage is not known. Although the disease affects virtually all neurons in the central nervous system, the pattern and timing of the neuronal dysfunction and death vary between different neuronal populations and between the forms of NCL. Macroscopically, a progressive brain atrophy associated with pathological signal intensities of the brain is detected using magnetic resonance imaging (MRI; Autti *et al*., 1997b).

The functioning of the affected neurons is more difficult to assess; however, one method of objectively evaluating the integrity of the sensory system is to record cortical evoked responses. The infantile form of NCL that leads to the death of virtually all cerebral neurons is associated with the extinction of evoked potentials by the age of 4 years (Vanhanen *et al.*, 1997). However, the progressive death of neurons in the late infantile NCL forms leads to increased amplitudes of visual and somatosensory evoked potentials (VEPs and SEPs, respectively; Pampiglione and Harden, 1977; Santavuori *et al.*, 1991). In juvenile NCL, VEPs are known to become attenuated (Harden and Pampiglione, 1982; Santavuori *et al.*, 1988), but SEPs have been reported to be of decreased (Goebel, 1995) or increased amplitude (Schmitt *et al.*, 1994), or even normal (Cracco *et al.*, 1980; Harden and Pampiglione, 1982). This discrepancy in the findings can be partly due to the fact the studies have been performed at a time when genetic confirmation of the diagnosis was not possible, and thus the patient material may have been heterogeneous. Furthermore, the patients have been studied in different stages of their disease. Since the electroencephalographic (EEG) techniques are influenced by the electrical conductivities of the media between the brain source and the measurement device, the progression of NCL that causes brain matter to decrease, the amount of cerebrospinal fluid to increase and the skull to thicken, can affect the electric potentials measured with EEG and lead to inconsistent results.

The magnetic field caused by an activation within the brain is not altered by the conductivity of the intervening media, and can be measured with magnetoencephalography (MEG). With MEG, neuronal activity that generates tangential currents can in many cases be modelled with equivalent current dipoles (ECDs) and localized in the corresponding brain areas (Hämäläinen *et al.*, 1993). Thus, measuring somatosensory evoked magnetic fields (SEFs; Hari and Forss, 1999) has the advantage that the origin of the responses and the strength of the underlying activity can be accurately assessed.

The aim of this Thesis was to evaluate the integrity of the somatosensory system in three of the NCL forms (juvenile NCL, the Finnish variant late infantile NCL, and Northern epilepsy syndrome) by recording somatosensory evoked responses using MEG.

3. REVIEW OF THE LITERATURE

3.1. NEURONAL CEROID LIPOFUSCINOSES (NCLS)

3.1.1. Overview

The first report of patients most likely affected with neuronal ceroid lipofuscinoses dates back to the beginning of the 19th century (Stengel, 1826), but it was only 1969 when Zeman and Dyken named the disease group as NCL, thus differentiating it from the other "amaurotic familial idiocies" (Zeman and Dyken, 1969). Childhood-onset NCL diseases, inherited in autosomal recessive fashion, form the largest group of inherited progressive encephalopathies world-wide. The common denominator in these diseases is the accumulation of autofluorescent, ceroid and lipofuscin like material in storage cytosomes, leading to neuronal death (Zeman *et al.*, 1970; Rapola, 1993). Clinically, NCLs have been divided into four major types: infantile, late infantile, juvenile and adult forms (Goebel, 1995; Santavuori *et al.*, 2000). At present, altogether eight different gene loci are known to underlie the NCL forms (Table 1); and it has become apparent that a clinically defined patient group can include individual patients with defects in different NCL genes (Das *et al.*, 1998). Furthermore, the individual NCL phenotypes are influenced by the modifying genes and environmental factors (Järvelä *et al.*, 1997; Munroe *et al.*, 1997).

Table 1. Classification of NCL diseases

Gene locus	Chromosome location	Mutations*	Gene product	Clinical type
CLN1	1p32	37	lysosomal palmitoyl protein thioesterase	infantile, late infantile, juvenile
CLN2	11p15	41	lysosomal pepstatin-insensitive peptidase	late infantile
CLN3	16p12	31	(lysosomal) transmembrane CLN3 protein	juvenile
CLN4	not known		not known	adult
CLN5	13q22	4	transmembrane CLN5 protein	Finnish variant late infantile
CLN6	15q21–23		not known	early juvenile/ variant late infantile
CLN7	not known		not known	early juvenile/ variant late infantile
CLN8	8p23	1	endoplasmic reticulum/Golgi protein	Northern epilepsy

* described in NCL mutation home page (http://www.ucl.ac.uk/ncl, accessed on 10/6/2001). See text for references.

The clinical manifestation of NCL is caused by neuronal dysfunction. The characteristic symptoms are epilepsy, mental retardation, motor dysfunction and in most cases blindness. The pattern, timing and severity of these symptoms differ between the different childhood NCL forms

(Santavuori, 1988; Santavuori *et al.*, 2000). A congenital form evident at birth is extremely rare. Infantile form (also known as Santavuori–Haltia disease), caused by mutations in *CLN1*, progresses rapidly leading to total loss of active motor and cognitive skills by the age of 3 years, which is also the time when EEG usually becomes isoelectric. The late infantile phenotypes can be caused by mutations in five different NCL genes (Table 1). The classic late infantile NCL (Jansky–Bielschowsky disease) manifests itself with seizures and progressive dementia between the ages of 2 and 4 years with additional signs of myoclonia and ataxia, and visual failure that becomes apparent later. The juvenile form (Batten or Spielmeyer–Vogt–Sjögren disease), starts with visual deterioration at early school age (6–10 years), and is followed by mental and motor deterioration, progressing slowly until death at mean between the ages of 20 and 30 years. The newest member of NCL is Northern epilepsy syndrome that manifests with epilepsy usually before the age of ten, and leads to mental and motor deterioration, but is not associated with blindness. The adult form (Kufs disease; *e.g.*, Berkovic *et al.*, 1988) is a variable condition appearing around the age of 30 years, and in addition to the recessively inherited forms, dominantly inherited forms are also known. For example, the overall incidence of NCL has been estimated to be 1.28 per 100 000 live births in West Germany (Claussen *et al.*, 1992). In some areas, the incidence of NCL is higher; for example in Finland, 7.7 and 4.8 per 100 000 live births for infantile and juvenile NCLs, respectively (Santavuori, 1988).

The molecular basis underlying NCLs is still unknown, although recent findings implicate impaired degradation in lysosomes. Two soluble lysosomal enzymes, palmitoyl protein thioesterase (PPT), which removes fatty acids from acetylated proteins (Camp and Hofman, 1993; Lu *et al.*, 1996), and tripeptidyl peptidase I (TPP1), which cleaves three amino acids from proteins undergoing degradation within lysosomes (Vines and Warburton, 1999), are known to be defective in CLN1 and CLN2 forms of NCL, respectively. In addition, the CLN3 protein has also been suggested to lie within the lysosomal membrane (Järvelä *et al.*, 1999). Ultrastructurally, the accumulated autofluorescent lipopigments consist of granular, curvilinear, rectilinear and fingerprint patterns, which can be visualized using electron microscopy (Elleder *et al.*, 1999). The main component of the storage material in most NCL forms is subunit c of mitochodrial adenosine triphosphate (ATP) synthase (Hall *et al.*, 1991; Palmer *et al.*, 1992), but in the infantile type the accumulated material consists mainly of saposins A and D (Tyynelä *et al.*, 1993).

There is no curative or preventive treatment available today. The symptomatic treatment includes medication for epilepsy, muscle relaxants, antiparkinson medication, sedative drugs, analgesics, antidepressants and antipsychotics (*e.g.*, Åberg *et al.*, 2000). In addition, some patients with JNCL are thought to benefit from antioxidant therapy (Santavuori *et al.*, 1988). In many patients also psychotrophic medication is needed.

This Thesis deals with three of the NCL forms, namely juvenile, Northern epilepsy syndrome and the Finnish variant late infantile. These three forms are described in detail in the following.

3.1.2. Juvenile NCL (JNCL, CLN3)

General

The first patients, retrospectively believed to be affected by JNCL, were four siblings from Norway (Stengel, 1826). Over the years several eponyms, including late onset Batten disease and Spielmeyer–Sjögren disease, have been associated with JNCL. Although the basic characteristics of JNCL have remained the same over the years, patients born with the disease today have a better prognosis than did the patients at the beginning of the 20th century (Sjögren, 1931).

Although the disease is still fatal, the quality of life for patients has improved significantly, since the disease can be diagnosed early, patients receive rehabilitation and proper symptomatic medication. In Finland, the incidence of JNCL has remained similar over the years, and is 1 per 21 000 live births with a prevalence of 12 per million inhabitants (Uvebrant and Hagberg, 1997).

Clinical Picture

During the first few years of their lives the patients are normal, healthy children. The first sign of the disease, a progressive loss of vision, is noticed usually between the ages of 4 and 8 years. Most children with JNCL attend normal school for the early grades, but as their sight worsens (most patients become blind between the ages of 6 and 13), they switch to special schools. Mental retardation usually becomes evident between the ages of 8 and 12 (Santavuori *et al.*, 2000). The first epileptic seizure occurs in most patients soon after the age of ten (Hofman *et al.*, 1999; Åberg *et al.*, 2000). The timing of a parkinsonian type of extrapyramidal dysfunction, manifesting itself first as slight truncal ataxia, varies markedly between patients (Järvelä *et al.*, 1997). Rigidity is slight at beginning but worsens with time, and there is difficulty in initiating movement. Most patients become non-ambulant between the ages of 15–28 years (Järvelä *et al.*, 1997). Dysarthria develops to anarthria in the majority at the late stage of the disease. A number of the patients have myoclonic jerks during the late stage of their disease, however these myoclonic features can be confused with partial epileptic seizures. Many patients have behavioural problems, and psychoses with hallucinations are not uncommon (Santavuori *et al.*, 1993). The most commonly used antiepileptic drugs to control the seizures are lamotrigine and sodium valproate in monotherapy, combined later with benzodiazepine (Åberg *et al.*, 2000). Parkinsonian symptoms are medicated with L-dopa in combination with bromocriptin. Some patients benefit from baclophen and tizanidine. In addition, most patients with JNCL are on antioxidant therapy (Santavuori *et al.*, 1988).

Within the JNCL disease, three disease courses progressing at different rates can be identified. In classic JNCL, significant mental retardation (IQ level reduced by more than 10 points from the time of diagnosis) takes place before the age of 10 years, parkinsonian signs emerge before the age of 15, and death occurs usually around the age of 20 and not later than 25 years (Sjögren, 1931; Järvelä *et al.*, 1997). The delayed classic JNCL progresses less rapidly: significant mental retardation is seen soon after 10 years, parkinsonian signs after 15, and death usually after 25 years (Järvelä *et al.*, 1997). The third form, called the protracted JNCL, starts typically at an early age with progressive visual loss, but the mental and motor symptoms manifest themselves later, usually in the third or fourth decade of life (Goebel *et al.*, 1976a; Libert *et al.*, 1982; Wisniewski *et al.*, 1998).

Genetics

The gene for juvenile neuronal ceroid lipofuscinosis, *CLN3*, was isolated on chromosome 16p11.2–12.1 (The International Batten Disease Consortium, 1995). It is composed of at least 15 exons and 14 introns (Mitchison *et al.*, 1997a, 1997b), and encodes a protein consisting of 438 amino acids. The major mutation, a 1.02-kb deletion, present in 81% of the disease chromosomes (The International Batten Disease Consortium, 1995), is still further enriched in the isolated population of Finland (Järvelä *et al.*, 1996). This major deletion removes exons 7 and 8 from the transcript, and is predicted to give rise to a truncated protein consisting of the first normal 153 amino acids followed by 28 novel residues. At present day, in addition to the major deletion, thirty different mutations have been described in *CLN3* (http://www.ucl.ac.uk/ncl, accessed on 10/6/2001). These include 7 deletions, 4 insertions, 1 intron change as well as 4 splice site, 7 missense and 7 nonsense mutations. Most of these mutations cause frameshift and/or truncation of the protein. However, the 7 missense mutations change only one amino acid residue and may

thus result in only slight modification of the CLN3 protein. However, it must be noted that in many genes, like for example in CLN1, change of only one amino acid can have detrimental effects (Hellsten *et al.*, 1996).

The function of the CLN3 protein, also called battenin, remains unknown. It has been shown to traffic through the endoplasmic reticulum (ER) and the Golgi apparatus (Haskell *et al.*, 1999), and is most likely located on lysosomal membrane (Järvelä *et al.*, 1998). Recently, a yeast model has implicated that CLN3 protein plays a role in the regulation of lysosomal pH (Pearce *et al.*, 1999).

Genotype-Phenotype Correlation

The patients homozygous for the major 1.02-kb deletion have either the classic or delayed classic disease course (Järvelä *et al.*, 1997). The intrafamiliar variability may be large, and it seems that environmental factors as well as modifying genes, play an important role in the individual phenotype of a JNCL patient (Järvelä *et al.*, 1997; Munroe *et al.*, 1997). Because of the variability of the disease course, even among the patients homozygous for the major 1.02-kb deletion, differences caused by different genotypes, *e.g.*, in patients compound heterozygous with the 1.02-kb deletion and another mutation, are difficult to demonstrate. However, some differences may exist. It seems that the patients homozygous for the major 1.02-kb deletion may have more pathologically altered sleep (Kirveskari *et al.*, 2000) and brain MRI findings (Järvelä *et al.*, 1997). In addition, an extremely protracted course of the disease, with predominantly only ocular symptoms during the first two to three decades, has never been reported in patients homozygous for the 1.02-kb deletion (Järvelä *et al.*, 1997; Munroe *et al.*, 1997; Wisniewski *et al.*, 1998).

Neuropathology

At autopsy, brain weights are reduced to 800–1000 g; the macroscopic appearance of the brain is slightly smaller than normal due to the diffuse cortical atrophy. The skull bones are thickened. Histologically, there is a variable loss of neurons affecting mainly layers II and V (Braak and Goebel, 1978, 1979). In particular, the small stellate neurons in layer II have disappeared at the time of autopsy. Nerve cells are lost in the corpus striatum and the amygdaloid nucleus; also especially the medial parts of the thalamus are affected (Autti *et al.*, 1997a). Atrophy of the cerebellum shows considerable variation: in some patients there is no cerebellar atrophy, whereas in others the cerebellar atrophy may be more advanced than the cerebral atrophy (Zeman *et al.*, 1970; Hofman *et al.*, 1999). Histologically, a reduction of the dendritic trees of Purkinje cells may be conspicuous despite relative preservation of the cells themselves. The granular layer is severely depleted.

Neuroimaging

Brain computed tomography (CT) is usually normal until the age of about 9 to 10 years (Lagenstein *et al.*, 1981; Raininko *et al.*, 1990). After that the cerebral atrophy usually precedes the cerebellar atrophy (Nardocci *et al.*, 1995). In MRI studies of 30 patients, Autti *et al.* (1996) found cerebral atrophy in all patients over 15 years of age, whereas cerebellar atrophy was seen in patients over 19. Also the brain stem became atrophic with the progression of the disease. The signal intensity ratios of the deep grey and white matter decreased with the progression of the disease, and the pathological finding of dark thalami compared with the basal ganglia on T2-weighted images could be seen after the age of 10 years. However, these pathological deep grey to white matter ratios were seen only in JNCL patients homozygous for the 1.02-kb mutation (Järvelä *et al.*, 1997). Increased signal intensity of the white matter, especially beside the lateral ventricles is another typical, but unspecific, finding and has been demonstrated to be correlated with neuronal loss and gliosis (Autti *et al.*, 1997a).

In agreement with structural MRIs, a proton magnetic resonance spectroscopy (MRS) study of 3 JNCL patients under 12 years of age showed normal metabolic profiles of *N*-acetyl aspartate (NAA), creatine and phosphocreatine (Cr), choline-containing compounds (Cho), myo-inositol (Ins) and lactate (Lac; Brockmann *et al.*, 1996). After 4 years of follow-up, one patient showed reductions of NAA, Ins and Cr of grey matter relative to the first examination, thus indicating neuronal death.

Hypoperfusion of several brain areas, with no clear correlation with age, was typically seen in all JNCL patients (aged 8 to 28 years) studied using single photon emission computed tomography (SPECT) after an injection of technetium-99m labelled hexamethylpropyleneamine oxime (99mTc-HM-PAO; Launes *et al.*, 1996). However, an age-related progression of distinctively decreased metabolic activity was demonstrated in positron emission tomography (PET) with fluorodeoxyglugose: metabolic activity decreased first in the calcarine area and then spread to involve the entire cortex, sparing only the basal ganglia and brainstem (Philippart *et al.*, 1994).

Electrophysiological Findings

EEG findings in JNCL are usually normal until the age of 9 years, after which progressive background abnormalities with spike-and-wave paroxysms appear (Westmoreland *et al.*, 1979; Raininko *et al.*, 1990; Larsen *et al*., 2001). The focal paroxysms become generalized in patients over the age of 12 years. No nocturnal epileptic seizures are observed during polysomnographic (PSG) recordings, occasional paroxysmal activity is seen in light sleep and REM (rapid eye movement) sleep (Kirveskari *et al.*, 2000).

The electroretinogram (ERG) is low or even missing at the time of diagnosis, followed later by the disappearance of the flash-VEP around the age of 13–16 years (Pampiglione and Harden, 1977; Santavuori *et al.*, 1988). SEPs have been described as normal (Cracco *et al.*, 1980; Harden and Pampiglione, 1982) or decreased (Goebel, 1995), however, one patient with enhanced SEPs and bilaterally prolonged central conduction time (CCT) has been reported (Schmitt *et al.*, 1994). Motor and sensory nerve conduction velocities have been reported to be normal (Santavuori *et al.*, 1985) or slightly slow in patients of all ages (Blatt Lyon, 1975).

3.1.3. Northern Epilepsy Syndrome (NES, CLN8)

General

Northern Epilepsy Syndrome, NES, an inherited childhood epilepsy with mental retardation (EPMR), was recently characterized as belonging to the NCL family. This disease has been so far described in 26 patients from the Kainuu region in Finland (Hirvasniemi *et al.*, 1994, 1995).

Clinical Picture

Clinically, NES can be divided into three successive stages. The first stage begins with epilepsy at the mean age of 6.7 years (range 5–10 years); the seizures are typically short generalized tonic-clonic attacks, but complex partial seizures also occur (Hirvasniemi *et al.*, 1995). At first the seizures occur once every month or two; however, despite antiepileptic medication the seizure frequency increases until puberty when one or two attacks per week are common. The antiepileptic drug of choice to control the seizures is clonazepam. Mental deterioration becomes evident two to five years after the onset of epilepsy, and seems to be most rapid at the time of the most frequent epileptic attacks. During the second stage in young adulthood, the frequency of seizures diminishes and the mental decline is slower, but the first signs of clumsiness in fine motor tasks appear. Nearly half of the patients have behavioural problems. The third stage of

permanent disability includes few seizures and continuing retardation, evident clumsiness, balance difficulties and often decreased visual acuity (Hirvasniemi *et al.*, 1994). The life expectancy of NES patients is not at this time known, but it may be slightly shorter than normal (Herva *et al.*, 2000).

Genetics

The NES gene, *CLN8*, was localized to chromosome 8 (Tahvanainen *et al.*, 1994), and encodes a putative transmembrane protein of 286 amino acids (Ranta *et al.*, 1999). So far, one disease causing mutation and two polymorphisms in *CLN8* have been found. Recent evidence suggests that the CLN8 protein is a resident of the endoplasmic reticulum (ER) and recycles between the ER and Golgi (Lonka *et al.*, 2000).

Genotype-Phenotype Correlation

Since all known Finnish NES patients are homozygous for a missense mutation (70C→G, R24G) genotype-phenotype correlation cannot be assessed (Ranta *et al.*, 1999).

Neuropathology

Compared with other childhood-onset NCLs the overall degree of intraneuronal storage, neuronal loss, and secondary astrocytic and microglial reaction are much less pronounced in NES. Although granular cytoplasmic storage material is present in virtually all neurons, the amount of storage material varies greatly within different neuron populations. Most prominent storage occurs in the hippocampus, while slight to moderate storage without evident neuronal loss occurs in the basal ganglia and thalamus. On the cerebral cortex, the pyramidal cells of the deeper parts of lamina III are severely ballooned, while conspicuous axonal enlargements (spindles or meganeurites filled with storage cytosomes) are a frequent feature in the less ballooned nerve cells of the more superficial parts of this layer. (Haltia *et al.*, 1999) In the cerebellum, neuronal death and the accumulation of ceroid and lipofuscin like material are minimal: with a few exceptions the Purkinje cells are intact and the granular cells show only very modest storage material accumulation. However, the dentate, pontine and inferior olivary neurons show moderate storage (Herva *et al.*, 2000).

Neuroimaging

Cerebral atrophy was seen in a CT study in 15 out of 16 patients over 19 years of age. In the patients with cerebral atrophy, epilepsy had lasted more than 12 years (Hirvasniemi and Karumo, 1994). Cerebellar and brainstem atrophy was already evident in the youngest patient at the age of 10 years. However, a MRI of a 10-year-old patient was normal, a 16-year-old patient had slight cerebellar atrophy and 24-year-old had slight cerebral and moderate brainstem and cerebellar atrophy.

Electrophysiology

EEG shows slowing of the background activity, abundant diffuse or intermittent delta and theta activity and scanty epileptiform activity with varying localization and form (Lang *et al.*, 1997). VEPs were abnormal in 44% of the patients and brainstem auditory evoked potentials (BAEPs) in 35%. There are no published reports of SEPs in NES.

3.1.4. Finnish Variant Late Infantile NCL (vLINCL$_{Fin}$, CLN5)

General

Four different forms of late infantile NCL, known also as the Jansky–Bielschowsky disease, are known today (Table 1). The classic LINCL (CLN2) and the so-called CLN6 variant late infantile/early juvenile patients exist in several countries, whereas all the patients reported to have the CLN7 variant have originated from Turkey (Williams *et al.*, 1999). Some patients have a mutation/mutations in CLN2 that results in a disease that manifests abnormally late in childhood (Sleat *et al.*, 1999). In addition, clinical LINCL phenotype can be caused by mutations in the *CLN1* gene (Das *et al.*, 1998). The CLN5 patients come almost exclusively from Finland, and thus the disease is denoted as the Finnish variant LINCL, vLINCL$_{Fin}$. The prevalence of vLINCL$_{Fin}$ in Finland is 2.6 per million inhabitants (Uvebrant and Hagberg, 1997).

Clinical Picture

The first signs of vLINCL$_{Fin}$ are slight clumsiness and muscular hypotonia between 4.5 and 6 years of age. Before that, the children may have had some concentration, visuomotor or learning problems. Visual failure is noticed between 5 and 8 years. Epileptic seizures, which appear around the age of 7 to 10 years, are usually generalized, but partial and complex partial seizures also occur. Broad-based walk, ataxia and myoclonus appear after 7 to 8 (10) years of age. Drug therapy usually includes at first valproate, which may later be combined with lamotrigine and clonazepam. In addition, baclophen and tizanidine are required for preventing myoclonic jerks, dystonia and the formation of contractures. Patients become non-ambulant usually at the age of 9–11 years, although some patients are able to walk supported until the age of 13. Death occurs usually between the ages of 14 and 32. (Santavuori *et al.*, 1982, 1991)

Genetics

vLINCL$_{Fin}$ is caused by mutations in the *CLN5* gene, which is located on chromosome 13 and is suspected of encoding a membrane protein the cellular location of which is as yet unknown (Savukoski *et al.*, 1998). To date, four different mutations in the *CLN5* are known. The Fin major mutation, a 2-bp deletion in exon 4, is detected in 94% of the Finnish disease chromosomes. It is predicted to give rise to a truncated polypeptide of 391 amino acids instead of the expected 407. A nonsense mutation in exon 1 (called Fin minor) causes a truncated polypeptide of only 74 amino acids. One missense mutation has been described in a Dutch patient, and it is predicted to change one residue in exon 4 from aspartic acid (Asp) to asparagine (Asn). The last known mutation of *CLN5,* called Swedish mutation, is a 1-bp insertion in exon 3, expected to cause a truncated protein of 224 of the original amino acids followed by 29 novel amino acids (Holmberg *et al.*, 2000)

Genotype-Phenotype Correlation

Although three of the mutations are predicted to cause a truncated protein and one only to change one residue from aspartic acid (Asp) to asparagine (Asn), all the mutations seem to result in a similar phenotype, when eight patients were studied clinically and with MRI (Holmberg *et al.*, 2000). Thus it seems that all the known *CLN5* mutations result in functional null allele. Thus the location of the missense mutation that changes one residue must be in a functionally important part of the CLN5 protein.

Neuropathology
Autopsy findings have revealed an advanced loss of neurons in the cerebral cortex, often in laminar pattern particularly involving layers III and V (Tyynelä *et al.*, 1997; Santavuori *et al.*, 1999). The remaining cortical neurons have numerous fusiform axonal enlargements (spindles or meganeurites), particularly affected are the neurons in the superficial part of layer III. Also the cerebellum is uniformly and extremely atrophic with almost complete destruction of the granule cells and Purkinje cells, while the few preserved Purkinje cells and their dilated dendrites are loaded with storage granules. The thalamus shows severe loss of neurons but otherwise the subcortical structures have only mild-to-moderate neuronal loss, but moderate-to-pronounced intraneuronal ballooning. The cerebral white matter shows moderate to severe loss of myelin and gliosis, particularly advanced in the periventricular white matter and in the cerebellar folia (Autti *et al.*, 1997a).

Neuroimaging
Cerebellar atrophy is more prominent than cerebral atrophy during the early stages of the disease: in most patients cerebral atrophy can only be seen after the age of 8 years, whereas cerebellar atrophy is already evident on the diagnostic MRI scan, taken usually around the age of 6 (Holmberg *et al.*, 2000). Also the brain stem is atrophic (Autti *et al.*, 1992). Hyperintense white matter zones around the lateral ventricles, decreased signal intensity of the thalamus compared with putamen and nucleus caudatus and a constant hyperintensity of the posterior limb of the capsula interna on T2-weighted images are evident from the age of 6 years. SPECT in $vLINCL_{Fin}$ patients has revealed moderate or severe cerebellar hypoperfusion, and also focal hypoperfusion of one or more of the supratentorial regions (Autti *et al.*, 1992).

Electrophysiology
In EEG, progressive background abnormalities and paroxysmal activity, including spikes and spike and wave paroxysms, are typically noticed between 4–7 years of age (Santavuori *et al.*, 1982). The characteristic electrophysiological findings of posterior spikes in EEG during a low-frequency photic stimulation, detected from the age of 7–10.5 years, and giant visual (around 7–9.5 years) and somatosensory evoked potentials (around 7.5–9.5 years), are of diagnostic significance (Santavuori *et al.*, 1999). ERG disappears around the age of 6–9 years.

3.2. ANATOMY OF THE SOMATOSENSORY SYSTEM

3.2.1. Ascending Somatosensory Pathways

The peripheral somatosensory receptors receive tactile, temperature, pain and proprioceptive stimulation. In the spinal cord, this information is carried towards the somatosensory cortex mainly by dorsal column-lemniscal and anterolateral systems. The sensations of touch, vibration, position and pressure are mediated by large, myelinated fibers in the dorsal columns up to the medulla, where they synapse at the dorsal column nuclei (the cuneate nucleus for upper extremities and gracile nucleus for lower limb and other body parts). The second-order neurons decussate to the opposite side, and carry the signal to the thalamus via the medial lemniscus. The thin fibers of the anterolateral system carry pain, temperature, crude tactile, tickle and itch sensations, and cross to the opposite side shortly after entering the spinal cord. They terminate at several levels of the brain stem and in the thalamus.

Most of the afferent fibers of the somatosensory pathways terminate in the ventrobasal complex of thalamus. The complex is formed by the ventral posterolateral nucleus (VPL), in which the medial lemniscal fibers end; the ventral posteromedial nucleus (VPM), in which the fibers from trigeminal nuclei terminate; and the posterior nuclei, where some fibers from the anterolateral system project. In addition to VPL and VPM, some spinothalamic fibres are also relayed to the intralaminar nuclei. From VPL and VPM the third-order nerve fibers project to the cortex. VPL and VPM receive afferents also from other parts of the thalamus, such as the reticular nucleus, which modulates the signal flow via VPL and VPM to the cortex. (Kahle *et al.*, 1986)

All relay nuclei along the somatosensory pathway preprocess the sensory information and determine whether it is transmitted further towards the cerebral cortex. Divergent presynaptic and convergent postsynaptic connections are repeated in each of the relays, so that sporadic activity and noise is filtered out, whereas strong sequences of repetitive activity from individual sensory fibers and activity transmitted simultaneously by multiple receptors may be enhanced and carried towards the brain. For example, processing in the relay nuclei may enhance the contrast of the sensory information. (Kandel *et al.*, 2000)

3.2.2. Cortical Somatosensory Areas

Several cortical areas participate in the processing of somatosensory information. The primary somatosensory area (SI) receives input from VPL and VPM. The SI lies immediately behind the central sulcus, and comprises Brodmann areas 3a, 3b, 2 and 1. Areas 3b and 1 mainly receive information from cutaneous receptors, while areas 3a and 2 mainly receive proprioceptive information from muscles and joints. These four areas of SI are extensively interconnected, so that both serial and parallel processing are involved in higher-order elaboration of sensory information. The cortical connections show laminar specificity so that layer IV receives most of the input from the thalamus, whereas layer VI projects back to the thalamus and layer V projects to other subcortical structures. Layers II and III project to other cortical regions.

The secondary somatosensory area (SII) is buried deep in the lateral sulcus, in the parietal operculum near the SI face area. Its main input comes from VPL, VPM and SI. In addition, the SI and SII areas send fibers to several other cortical areas, including the posterior parietal association cortex (PPC, Brodmann areas 5 and 7), as well as the primary motor cortex and premotor areas (Brodmann areas 4 and 6, respectively). (Kandel *et al.*, 2000)

3.3. Neural Basis of Evoked Responses

An evoked response means a momentary change in the electrical activity of the brain caused by an external stimulus. Evoked responses can be measured with EEG and MEG. Stimuli of auditory, visual and somatosensory modalities are most often used to evoke the responses. For example, SEPs and SEFs provide the means to non-invasively study the somatosensory system of the peripheral and central nervous systems.

Synaptic activity in cortical pyramidal neurons is believed to be the major contribution to the signal detected by EEG and MEG. Neurons communicate through axodendritic, axosomatic and axoaxonal synapses that can be either inhibitory or excitatory. Most of the excitatory synapses lie

on dendrites, and the majority of inhibitory synapses are localized on the cell body or at the base of the axon, where the action potentials are generated, and can thus be most effectively suppressed (Kahle *et al.*, 1986). When a thalamocortical afferent synapses on a dendrite near the soma of a neuron on layer IV, the discharge of synaptic vesicles leads to changes in the membrane potential of the postsynaptic neuron (Figure 1). In the case of excitatory synapses, current flows into the postsynaptic cell dendrite and the normally negatively charged interior of the cell becomes less negative with respect to the extracellular side, in other words, is depolarized (*e.g.*, Kandel *et al.*, 2000). The site of generation of the so-called excitatory postsynaptic potential, EPSP, is called a current sink. The current loop is closed when the current flows along the dendrite and back out across the membrane at other sites, creating a current source. If the current is measured with an extracellular electrode at the site of the generation of the EPSP (the sink), a current flowing away from the electrode into the cytoplasm would be detected as a negative deflection. In contrast, an extracellular electrode near the source would have the opposite polarity.

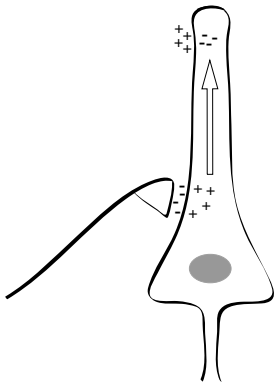

Figure 1. A schematic drawing of a cortical neuron that receives an excitatory afferent. The following local depolarization of the postsynaptic membrane forms the current sink. A current flowing along the apical dendrite closes the current loop. From a distance, the current flowing inside the dendrite looks like a current dipole. One should note, however, that the direction of the current would be opposite if the synaptic input would be to more superficial part of the dendrite.

One postsynaptic current forms a current dipole in the order of 20 fAm (Hämäläinen *et al.*, 1993), and is thus too small to be detected from outside the brain. However, numerous synapses, both inhibitory and excitatory, are active simultaneously on neurons, and synchronous activation of tens of thousands of neurons can in favourable conditions exceed the detection threshold. Because the apical dendrites of pyramidal neurons are aligned in parallel, summated postsynaptic currents flowing in them form so-called electrically open fields, which can be detected at a distance, *e.g.*, by an EEG electrode or an MEG sensor. In contrast, the dendrites of interneurons are oriented randomly and thus the electric fields of PSPs in them tend to cancel each other, and are not detected in EEG or MEG. The detection of activation is facilitated by the temporal summation of PSPs usually lasting tens of milliseconds. Action potentials involve higher voltage fluctuations than PSPs, but still do not contribute significantly to the measured cortical magnetic fields, mainly because they last only for about 1 ms, and are thus poorly temporally summated. Furthermore, an action potential is associated with two current dipoles in opposite directions and thus forms a current quadrupole, whose magnetic field decreases with distance much more rapidly than that of a dipole.

The exact nature of the activated sources can not be deduced from the signals measured from outside the head. For example, an inhibitory postsynaptic potential, IPSP, causes the current to flow out of the postsynaptic neuron, and thus the membrane potential change is opposite to the one created by an EPSP at the same location. Thus, EPSPs in superficial layers and IPSPs in deeper layers both appear as negative potential deflections when measured at the cortical surface.

3.4. MAGNETOENCEPHALOGRAPHY (MEG)

The following description of the MEG technique is based mainly on the extensive review by Hämäläinen *et al.* (1993).

3.4.1. Basic Concepts

MEG records the weak extracranial magnetic fields generated by neuronal currents. These currents are detected with an extremely sensitive piece of equipment, called the SQUID (Superconducting QUantum Interference Device). The SQUID is a superconducting ring, interrupted by one or two Josephson junctions, which operates at temperatures near absolute zero. A flux transformer, consisting of pickup, compensation and signal coils, detects a change in the external magnetic field and converts it into an electric current. Because of mutual inductance between the SQUID and signal coil, the flux sensed by the SQUID is proportional to the original change in magnetic field detected by the pickup coil.

In the analysis of MEG signals, the head is usually approximated by a spherically symmetric conductor, since over many regions of interest the brain does not differ significantly from a sphere. This simplifies the calculation of the sources, since radial primary currents within a sphere do not cause external magnetic fields. Because currents in the apical dendrites mainly flow perpendicularly with respect to cortical surface, currents in the walls of cortical sulci are expected to be tangential, and thus detected with MEG. Because the conducting properties of the tissues between the active brain source and the measurement sensor do not affect the magnetic field patterns, the localization of these sources underlying the cortical tangential currents is less problematic than in EEG.

3.4.2. Recording Equipment

In the present study, measurements were performed in the BioMag Laboratory at the Helsinki University Central Hospital using a Neuromag-122 system (Neuromag Ltd., Helsinki, Finland). The device consists of 122 measuring sensors, positioned in a helmet-shaped configuration (Ahonen *et al.*, 1993). The sensors are planar gradiometers shaped as figure-of-eight and arranged in 61 pairs, each of which measures two orthogonal derivatives of the radial component of the magnetic field. The planar gradiometers are sensitive to nearby sources and detect the maximum signal just above the source. Because the measured magnetic fields are extremely weak, in the order of 50–500 fT, which is about $1/10^9$–$1/10^8$ of the earth's magnetic field, the measurement device is situated inside a magnetically shielded room.

3.4.3. Source Estimation

The major challenge in the evaluation of the MEG data is the so-called inverse problem, *i.e.*, how to use the data measured from outside the head to estimate the sources inside the brain, since an infinite number of primary current distributions can produce a given magnetic field measured from outside the brain. Thus, *a priori* constraints are needed in order to determine the source distributions (Ilmoniemi, 1991). In most MEG measurements, these constraints can be defined on

the basis of knowledge of the underlying physiological phenomena, or, *e.g.,* by using structural MRI to restrict the areas from which activation can arise.

The simplest and most often used source model for MEG studies is the equivalent current dipole (ECD). The ECD represents the location, orientation, and strength of the underlying source, and is calculated by fitting the predicted and measured magnetic field patterns in the least-squares sense. Other analysis methods, which have the advantage of not making as strong *a priori* assumptions of the current distribution, include for example the minimum-norm estimate (Hämäläinen and Ilmoniemi, 1994) and L1-norm minimum-current estimate (Uutela *et al.*, 1999).

3.4.4. Comparison of MEG with EEG

Both MEG and EEG are noninvasive and painless techniques to measure synchronous neuronal activation in millisecond time scale. The primary currents causing the signal are the same for MEG and EEG, but the electric and magnetic field patterns, and thus the directions of highest sensitivity are orthogonal with respect to each other. MEG detects only tangential dipoles, whereas also the radial ones are detected with EEG. However, since approximately 2/3 of the human cortex is embedded in fissures, including large proportions of the primary cortical projection areas, many of the human cortical sources are accessible to MEG. Furthermore, especially in the case of many simultaneous active sources, the analysis of the source location is easier in MEG than in EEG, because the algorithms only have to take tangential currents into consideration.

MEG is not influenced by the changes of conductivity between the active brain source and the measuring device. This makes MEG field patterns more spatially constrained than EEG potential distributions, which means that an easier and more precise estimation of the underlying source location and strength can be carried out with MEG than with EEG.

With EEG, measuring electrodes can be placed along the afferent pathway and impulse transmission followed at many sites from periphery to cortex, whereas the MEG helmets mostly measure cortical activity. However, in MEG measurements, no reference electrodes are needed.

3.5. SOMATOSENSORY EVOKED MAGNETIC FIELDS (SEFS)

3.5.1. SEFs in Normal Subjects

General

The ability to measure SEFs over the SI area with a SQUID device was first demonstrated in 1978 by Brenner and colleagues. These first responses, brought about by a steady-state paradigm and recorded with one measuring channel, succeeded to demonstrate that SEFs to thumb and little finger stimulation originate from different locations in SI (Brenner *et al.*, 1978). Since then MEG devices have evolved to whole-head covering systems that typically incorporate more than one hundred measurement channels (Figure 2), and the somatotopical organization of SEFs along the postcentral cortical strip has been proven by several researchers (*e.g.*, Hari *et al.*, 1984, 1993;

Okada *et al.*, 1984; Huttunen *et al.*, 1987a; Narici *et al.*, 1991; Rossini *et al.*, 1996; Nakagawa *et al.*, 1998; Nakamura *et al.*, 1998; Ishibashi *et al.*, 2000).

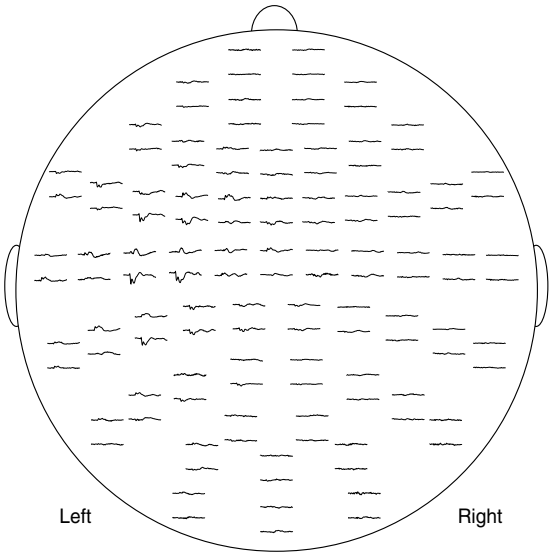

Figure 2. The 122-channel somatosensory evoked responses to right median nerve stimulation. The greatest activation is seen over the left hemisphere, on the contralateral SI hand area.

SEFs from the Contralateral SI

The strongest and earliest cortical activation after peripheral sensory stimulation is seen at the contralateral SI. After electrical median nerve stimulation, usually four deflections (N20m, P35m, N45m and P60m) can be distinguished. The precise area from which the earliest cortical SEFs arise, is believed to be the posterior bank of the central sulcus, mainly area 3b (Wood *et al.*, 1985; Huttunen *et al.*, 1987a; Tiihonen *et al.*, 1989; Ishibashi *et al.*, 2000). The pyramidal neurons in area 3b are located parallel to each other, perpendicular to the cortex. Earliest activation of area 3b occurs approximately 20 ms after peripheral stimulation of the median nerve, when the thalamocortical afferents synapse on the basal dendrites, resulting in current flow in the apical dendrites towards the superficial part of the cortex, which is seen as a forward pointing dipole on magnetic field contour maps. This generation model of the earliest deflection, N20m, was suggested already in the early studies with combined MEG and EEG measurements (Wood *et al.*, 1985), and is now widely accepted. A recent study further analyzed the evolution of the N20m activity, and found that the underlying source moves in a direction orthogonal to the apical dendrites, parallel to the surface of the posterior bank of the central sulcus (Hashimoto *et al.*, 2000).

The generator mechanisms of SI deflections following N20m are still disputed. Over the years, several lines of evidence have suggested that the cortical populations generating P35m differ from the N20m generators. First, the location of the P35m ECD is slightly different from the location of the N20m ECD (Huttunen *et al.*, 1987a; Allison *et al.*, 1989; Tiihonen *et al.*, 1989; Wikström *et al.*, 1996). In some studies, the different location of P35m has been interpreted as evidence that P35m is at least partly composed of activation generated in the anterior bank of central sulcus (Huttunen *et al.*, 1987a; Tiihonen *et al.*, 1989; Baumgartner *et al.*, 1991; Kawamura *et al.*, 1996). However, it was also suggested that this anterior location of P35m could be a modelling error rather than a true difference in location (Huttunen, 1997). In addition, it is

possible that the SI areas 3a, 1 and 2, which also receive somatosensory input, may have some contribution to SEFs. It was suggested that sometimes tangential components could be detected from areas mainly giving rise to radial dipoles (Tiihonen *et al.*, 1989; Baumgartner *et al.*, 1991). A recent study of finger II, III and V stimulation suggested that, unlike the N20m and P60m, the P35m ECDs do not demonstrate somatotopy, but are instead located in clusters, probably in area 1 (Ishibashi *et al.*, 2000). In addition, the different interstimulus interval (ISI) dependencies of N20m and P35m have been interpreted as evidence that P35m is activated by polysynaptic pathways (Tiihonen *et al.*, 1989), and also that P35m reflects inhibitory postsynaptic potentials (Wikström *et al.*, 1996).

SEFs from SII

The characteristic feature of bilateral activation of the parietal opercular sources after somatosensory stimulation was shown using MEG already in the early 1980s (Hari *et al.*, 1983, 1984). The exact location of SII in humans is probably in the upper bank of the lateral sulcus (Sylvian fissure), just below the head representation of SI. Although at least two different loci of somatosensory activation seem to exist within the human perisylvian cortex (Mima *et al.*, 1997; Korvenoja *et al.*, 1999), the opercular responses measured with MEG have been traditionally designated as emanating from the secondary somatosensory cortex (SII).

The SII SEFs demonstrate somatotopic arrangement, but the differentiation between body parts is not as clear as in SI SEFs (Hari *et al.*, 1990, 1993; Maeda *et al.*, 1999). Although the field patterns of the contralateral (SIIc) and ipsilateral (SIIi) secondary somatosensory responses are similar, the SIIc responses seem to be stronger (Hari *et al.*, 1983, 1984; Mima *et al.*, 1998a) and peak earlier (Forss *et al.*, 1994a; Simões and Hari, 1999; Wegner *et al.*, 2000) than the SIIi responses.

Usually, the onset of SII activation has been reported to be after 60–80 ms (Hari *et al.*, 1984; Mauguière *et al.*, 1997b; Wegner *et al.*, 2000). However, two studies have suggested that SII activity may already begin about 20–30 ms after stimulation (Karhu and Tesche, 1999; Korvenoja *et al.*, 1999).

Other Activated Areas

Activation of the above-described SI and SII areas account for most of the SEFs. However, multidipole modelling techniques have shown that at certain time periods, additional sources are needed to satisfactorily explain the data. The presence of a posterior parietal cortex (PPC) source when the median nerve was electrically stimulated was described by Forss *et al.* (1994a). This source is most active between 70–110 ms, but the activation range across individual subjects can be 46–200 ms (Mauguière *et al.*, 1997b). Although PPC has mainly been described as a contralateral response (Forss *et al.*, 1994a; Wikström *et al.*, 1996; Mauguière *et al.*, 1997b) right- and left-side air-puff stimulation consistently activated the right hemisphere PPC (Forss *et al.*, 1994b).

Electrical stimulation of the median nerve has also demonstrated activation of an ipsilateral SI source (Korvenoja *et al.*, 1995), as well as bilateral sources in the ipsi- and contralateral midfrontal or inferior frontal gyri (Mauguière *et al.*, 1997b). However, the ipsilateral SI was seen in only 5 out of 10 subjects, peaking within a time range of 90–290 ms, with a rather weak maximum strength. The sources for frontal activation, seen in 5 out of 8 subjects studied, were active between 65 and 200 ms, and showed rather scattered localization across subjects.

Yet another novel source, originating from the mesial cortex and peaking at 120–160 ms, was demonstrated by its enhancement to selective attention towards both median and ulnar nerve stimulation (Forss *et al.*, 1996).

Interhemispheric Differences

The waveforms of SEFs, at least during the first 100-ms period, within one subject seem to be similar and stable on both hemispheres (Tecchio *et al.*, 2000), suggesting thus that dissimilar waveforms from the two hemispheres could have pathological significance in patients. However, another study on healthy subjects demonstrated that although the response strengths from the two hemispheres are often symmetric, in some subjects there may be marked differences (Wikström *et al.*, 1997). The contralateral SI responses have been found to be stronger in the left than in the right hemisphere (Rossini *et al.*, 1994), but these differences were not confirmed in another study (Wikström *et al.*, 1997). In addition, although there may be no systematic statistical differences between left and right hemisphere SI ECD locations in all populations (Rossini *et al.*, 1994), within individual brains, the cartesian coordinates expressing the locations of the sources may differ between the two hemispheres. In fact, a careful analysis of the SEFs with a whole head covering MEG device showed that the interhemispheric differences in the cartesian coordinates within an individual can be in the order of few centimetres, although at group level the mean is below 5 mm (Wikström *et al.*, 1997).

It has also been suggested that compared with right hemisphere SII responses, left hemisphere SII responses are stronger (Forss *et al.*, 1994a), become active earlier (Forss *et al.*, 1994b) and locate more posteriorly (Simões and Hari, 1999; Lin *et al.*, 2000). However, in other studies, no interhemispheric differences in the SII responses have been observed (Wikström *et al.*, 1997; Wegner *et al.*, 2000), and it has also been found that the SII response latencies are longer in the left than in the right hemisphere (Simões and Hari, 1999).

Influence of Recording Parameters

In clinical situations, it is usually beneficial to optimize the protocol so that the measurement session is short and comfortable for the patient, and yields repeatable and reliable results. Since averaging a sufficient number of responses to reach an adequate signal-to-noise ratio is essential, the ways to shorten the recording time are mainly to reduce the stimuli interval times or to stimulate different nerves alternately or even simultaneously in a single run. However, all these factors significantly change the morphology, and especially the strengths of the responses, as discussed below. Nevertheless, the locations of the ECDs seem to be very stable, since a change of ISI (Wikström *et al.*, 1996; Mertens and Lutkenhöner, 2000), stimulus intensity, frequency or attention (Mima *et al.*, 1998a), or interfering tactile stimulation (Naka *et al.*, 1998) do not seem to affect the ECD locations. However, one study reported that sources activated by air-puff stimulation were located deeper in the wall of the central sulcus than those activated by electrical stimulation (Rossini *et al.*, 1996), but this finding was not validated by others (Forss *et al.*, 1994b).

Stimulus Type

Electrical stimulation of mixed nerves is the most used stimulus method. It has the advantage of producing clear and strong responses, but the disadvantage of the simultaneous activation of a large number of fibers that potentially have different conduction velocities. More natural methods, such as air-puff stimulation that selectively activates cutaneous mechanoreceptors, or electrical stimulation of the sensory part of the nerve, can be used, but the resulting SEFs over SI areas are weaker and peak at longer latencies (Huttunen, 1986; Kaukoranta *et al.*, 1986; Huttunen *et al.*, 1987b; Forss *et al.*, 1994b; Rossini *et al.*, 1996). However, later parts of the responses,

namely SII(c) and PPC, do not seem to be smaller as a result of air-puff stimulation than of electrical stimulation (Forss *et al.*, 1994b).

Stimulus Intensity

Generally, in studies of somatosensory evoked responses, a stimulus exceeding the motor threshold but being below the pain threshold, is believed to cause reliably repeatable responses (*e.g.*, Huttunen, 1995). However, recent studies have demonstrated that the SEFs may be influenced by the stimulus intensity even above the motor threshold. The strength of N20m but not P35m, was reported to increase when the stimulus intensity was changed to cause vigorous movement instead of a small thumb twitch (Jousmäki and Forss, 1998). Similar results of up to a two-fold increase in the N20m amplitudes in some subjects were obtained when stimulus strength was increased from motor threshold to 1.5 times the motor threshold (Tsutada *et al.*, 1999). However, when the stimulus intensity was further increased to twice the motor threshold the N20m amplitudes were not increased. The latency of N20m was not affected when stronger stimuli were applied, whereas the P35m latency shortened (Jousmäki and Forss, 1998). There were no differences in the strengths of SII and PPC responses when the strength of a stimulus above motor threshold was further increased, however, the SII latencies were shorter than in a situation when stimulus was just above sensory threshold.

Interstimulus Interval

Tiihonen and colleagues (1989) noticed that increase of ISI from 0.2 to 0.5 s increases the amplitude of P35m more than that of N20m. In fact, between ISIs of 0.3–5 s, the strength of N20m is rather stable, whereas P35m strength is stable at longer ISIs (from 3 to 5 s), but decreases with the shortening of ISI (Wikström *et al.*, 1996). The other SI responses are also strongly ISI dependent: the N45m dominates the responses at short ISIs (below 1 s), whereas the P60m attenuates towards the shorter ISIs. The change of ISI has not been reported to change the ECD locations (Wikström *et al.*, 1996; Mertens and Lutkenhöner, 2000).

The strengths of the later responses are strongly influenced by the interstimulus interval. Even though the SII responses can be recorded with ISIs as short as 1 s (Wikström *et al.*, 1996), it has been shown that in some subjects the SII response may become stronger up to ISIs of 8 s (Hari *et al.*, 1993). In other studies, the strengths of SII and PPC responses increased when ISI was increased from 1 to 3 s, but remained stable when ISI was further increased to 5 s (Forss *et al.*, 1994a; Wikström *et al.*, 1996).

Alternating, Unilateral or Bilateral Stimulation

Stimulation of the median nerve 20–120 ms after applying a conditioning stimulus on the ipsilateral median or ulnar nerves causes attenuation of most of the SI responses (Huttunen *et al.*, 1992). However, the SI responses remain unchanged if the prior stimulus is on the tibial or contralateral median nerves. Furthermore, the early SI responses seem to be unaffected by simultaneous bilateral stimulation of the median as well as the tibial nerves (Shimojo *et al.*, 1996, 1997), and also by alternating stimulation of the median nerves with ISI of 3 s (Wegner *et al.*, 2000). Thus, it seems that the neural populations causing the early onset SI activity do not have bilateral receptive fields, but are partially the same for ulnar and median nerves that have adjacent innervation areas peripherally (Huttunen *et al.*, 1987a, 1992). Similar findings of greater occlusion and/or surround inhibition from adjacent fingers than from fingers further apart, was suggested by Ishibashi and colleagues, since simultaneous stimulation of the II and III fingers resulted in greater interaction of N20m and P60m deflections than did stimulation of fingers II and V (Ishibashi *et al.*, 2000).

Thus, alternating or simultaneous bilateral stimulation of the median nerves can be chosen when the purpose is to study contralateral SI responses. However, when attempting to study responses outside the SI area, unilateral stimulation of the median nerves may be advisory, since the SII responses are attenuated by both bilateral (Shimojo *et al.*, 1996, 1997) and alternating stimulus, at least when the interstimulus interval is 3 s or shorter (Wegner *et al.*, 2000). A long-lasting modulation of SII activity by prior stimulation was further supported by a recent study that demonstrated that when stimuli to median nerves were presented in pairs of two, the response latencies for the second stimulus were always longer than for the first, despite of the order of the stimulated nerves in the pair (Simões and Hari, 1999).

Modulation of the Responses by Behavioural Factors

Active attention does not seem to affect the SI responses, whereas the SII (Hari *et al.*, 1990; Mauguière *et al.*, 1997c; Mima *et al.*, 1998a) and mesial cortex responses are increased by active attention to the stimulus (Forss *et al.*, 1996). There is some evidence to suggest that SII response strengths could be more vulnerable to reduced vigilance than SI responses, since the SII responses strengths were smaller at the end of the session than early in the session (Wegner *et al.*, 2000).

Interfering tactile stimuli may cause reduced response amplitudes from both SI (Naka *et al.*, 1998) and SII cortices (Huttunen *et al.*, 1996). In contrast, active movement of fingers, contraction of thenar muscles or deltoid muscle on the same side as the median nerve stimulation results in increase of the SII response strengths (Huttunen *et al.*, 1996; Lin *et al.*, 2000). However, the SII response strengths are reduced by contraction of the masseter and the ipsilateral tibial muscle (Lin *et al.*, 2000).

Effect of Maturation and Ageing

The development of SEFs in childhood and adolescence is poorly characterized, because only occasional SEFs of children have been published (Paetau *et al.*, 1994). The only systematic study of the effect of age on SEFs was conducted on adults, and demonstrated that in the age range of 20–73 years, the N20m strength correlates positively with age, whereas the strengths of P35m and P60m do not (Huttunen *et al.*, 1999). However, according to studies on SEPs in children, several developmental changes in the SEFs can be expected with age. First, the latencies of the cortical SEP components are affected by two opposing factors during development. The growth of the body increases the lengths of the pathways and should thus prolong the latencies, while myelination and maturation should decrease the latencies by accelerating the conduction. In fact, in SEP studies, the latencies of the cortical components initially decrease until about the age of 3 years (Bartel *et al.*, 1987; Taylor and Fagan, 1988; Müller *et al.*, 1994), but after that, the latencies become longer with increasing body size (Zhu *et al.*, 1987; Taylor and Fagan, 1988). However, judging from the CCT, which measures the time the impulse travels within the central nervous system, maturation of the afferent central pathways is not completed during the first 5–10 years (Bartel *et al.*, 1987; Taylor and Fagan, 1988; Müller *et al.*, 1994; Boor *et al.*, 1998; Boor and Goebel, 2000), or perhaps even during the first decades of life (Hume *et al.*, 1982; Allison *et al.*, 1983, 1984). It must also be noted that the maturation and development may not affect all components of the cortical responses similarly (Taylor and Fagan, 1988). In addition, some differences that exist in adults may not be seen in children. For example, the latencies in children may be unaffected by gender (Allison *et al.*, 1983, 1984; Mattigk, 1991).

In addition to the above-mentioned changes in latencies, the SEP waveforms and amplitudes are also affected by development (Desmedt *et al.*, 1976; Taylor and Fagan, 1988; Kakigi and Shibasaki, 1991; Mattigk, 1991). Also changes in the stimulus parameters, like the interstimulus interval, can have a stronger effect on the evoked responses in children of different ages (Araki *et*

al., 1999). In clinical SEP recordings, lower stimuli rates are recommended to be used in infants than in adults.

3.5.2 SEFs in Patients with Central Nervous System (CNS) Disorders

Recording of SEPs is routinely used in clinical studies of patients with different cerebral diseases (Chiappa, 1989). Alterations of SEPs are seen, *e.g.*, with impaired conduction properties of peripheral or central parts of the somatosensory pathways. However, although latency abnormalities along the afferent pathways are readily characterized with SEP techniques, the interpretation of slightly altered response amplitudes in SEP recordings is compromised by the distortion of the electric fields by the conductivities of the intervening media. Thus, it seems that recording SEFs could be advantageous in certain clinical situations, which require precise source localization or characterization of the response amplitudes. Indeed, the most established role of MEG in a clinical setting is its use in presurgical evaluation, where SEFs are used to locate the central sulcus (*e.g.*, Alberstone *et al.*, 2000; Kumabe *et al.*, 2000; Mäkelä *et al.*, 2001). In addition, MEG has successfully been employed to determine the sources of epileptic activity (*e.g.*, Minassian *et al.*, 1999; Paetau *et al.*, 1999; Sobel *et al.*, 2000).

Another interesting application of MEG has been the study of the magnetic counterparts of the giant SEPs, seen usually in patients with progressive myoclonus epilepsy (PME; Rothwell *et al.*, 1984; Obeso *et al.*, 1985; Shibasaki *et al.*, 1985; Berkovic *et al.*, 1991). The short-latency SEFs, both normal and enlarged, have been shown to be generated at SI in patients with familial PME (Uesaka *et al.*, 1993), in patients with PME of the Unverricht-Lundborg type (ULD; Karhu *et al.*, 1994; Forss *et al.*, 2001), and in patients with cortical myoclonus of various origins (Mima *et al.*, 1998b). The most significantly enlarged SI deflection in all these patients was the P35m, whereas the N20m was reported normal or slightly enlarged in some patients. N45m deflections, normal or enhanced, were detected in two of the studies (Karhu *et al.*, 1994; Mima *et al.*, 1998b). In addition to being enlarged, P35m was also reported to peak earlier than in normal controls (Uesaka *et al.*, 1993; Karhu *et al.*, 1994; Forss *et al.*, 2001). Abnormal late "repetitive SEFs" at 200 ms from area 3b were observed in three patients with cortical myoclonus (Mima *et al.*, 1998b).

Activation of brain areas outside contralateral SI in PME patients was noticed in the two studies conducted with whole-head covering devices. Ipsilateral activity, most likely from SI, was demonstrated in several patients (Mima *et al.*, 1998b; Forss *et al.*, 2001), and it has been suggested that the appearance of the ipsilateral SI would be associated with the increased risk of generalized seizures. However, SII responses, which were searched for in three of the studies, were found in only two of the altogether 11 ULD patients and in one of the cortical myoclonus patients (Mima *et al.*, 1998b; Forss *et al.*, 2001). In these patients, the SII responses were not abnormally strong.

In paediatrics, MEG has been used in one study to examine SEFs in patients with benign rolandic epilepsy (Kubota *et al.*, 2000). Until the studies described in this Thesis, no data has been available on SEFs in paediatric patients with degenerative brain disorders such as NCL.

4. AIMS

The general aim of this Thesis was to clarify the mechanisms of deterioration of the functioning of the neuronal networks in NCL. We focused on the somatosensory system and recorded SEFs to median nerve stimulation in three genetically different NCL forms, JNCL, NES and $vLINCL_{Fin}$. In order to compare the SEFs in patients with adequate control data, an age-matched control group consisting of healthy subjects was measured for each study. The degree of neuronal dysfunction caused by the disease, demonstrated by the alterations in SEFs, was related to the genotype, clinical findings and the structural brain MRI alterations in individual patients. The specific aims of the Studies I–IV were:

I To examine somatosensory cortical excitability in JNCL by characterizing how the progression of the disease affects the median nerve SEFs

II To evaluate the effect of the genotype of 10 compound heterozygous patients with JNCL to their clinical, neurophysiological and neuroradiological phenotype

III To study somatosensory cortical excitability in patients with NES and to compare their brain MRI findings with the known findings in JNCL and $vLINCL_{Fin}$

IV To investigate excitability of the primary and secondary somatosensory cortex in patients with $vLINCL_{Fin}$

5. METHODS

5.1. STUDY DESIGN

Altogether 33 patients, including 24 with JNCL, 4 with NES and 5 with $vLINCL_{Fin}$, participated in the study (Table 2). SEF recordings were performed on 31 of the patients, 25 of whom also underwent structural brain MRI imaging. At the beginning of these studies, the mutations of patients with JNCL were not yet known and thus Study I included patients with different genotypes. Here, the patients with JNCL are presented in two groups. JNCL–I group includes 14 patients homozygous for the major 1.02-kb deletion, including 6 novel patients in addition to the 8 homozygous patients originally included in Study I. JNCL–II group includes the 10 patients who are compound heterozygous for the major 1.02-kb deletion and for another *CLN3* mutation (Study II).

Table 2. Control subjects and patients

	Controls	**JNCL–I (homozygous for the 1.02-kb deletion)**	**JNCL–II (compound heterozygous for the 1.02-kb deletion and another *CLN3* mutation)**	**NES**	**$vLINCL_{Fin}$**
Study	I–IV	I and unpublished	II	III	IV
No. subjects	27	14	10	4	5
SEFs	27	14	8	4	5
Age range*	6.5–44.0	6.4–21.0	7.3–30.7	26.2–43.4	8.8–16.7
Male/female	13/14	7/7	6/4	2/2	3/2
MRI*	25	12	5	4	4

* at the time of SEF measurements

The study protocol was approved by the ethical committee at the Hospital for Children and Adolescents, University of Helsinki. Informed consent was obtained from all subjects and/or their parents. The healthy control subjects over 18 years gave their informed consent, in younger subjects and all patients the consent was asked also from their parents or legal guardians. The purpose of these investigations was considered important and the results could not be achieved in any other way except by studying these patients. None of the examinations performed (clinical, MRI and SEF studies) caused pain or are considered harmful to the subjects. No anaesthetics were used.

5.2. PATIENTS

The 24 patients with JNCL and 5 patients with $vLINCL_{Fin}$ were recruited by a paediatric neurologist (P. Santavuori) from the outpatient clinic of the Department of Paediatric Neurology

at the Hospital for Children and Adolescents, University of Helsinki during years 1995–1998. Only patients older than 6 years were considered eligible to participate. All the patients with JNCL and $vLINCL_{Fin}$ had been clinically followed up by P. Santavuori for 3–21 years. All patients with JNCL had vacuolated lymphocytes and typical ophthalmologic findings of retinal and macular degeneration (Santavuori, 1988). Fifteen patients with JNCL represented the only case in their family, 3 patients had one affected sibling who also participated, and one had two affected siblings who participated. In patients with JNCL, rectal biopsy had been performed on the oldest affected sibling in the family (altogether 15 patients) and showed the characteristic fingerprint profiles and/or curvilinear bodies (Rapola, 1993). At present, all patients have genetically confirmed diagnoses. Fourteen of the patients were compound heterozygous for the major 1.02-kb deletion that removes exons 7 and 8 from the transcript (Järvelä *et al.*, 1997). In 4 of the compound heterozygous patients, the screening for the major 1.02-kb deletion and delineation of mutations on the other chromosome was reported earlier (The International Batten Disease Consortium, 1995; Munroe *et al.*, 1997), whereas in six of the compound heterozygous patients the other *CLN3* mutation was described in Study II. The mutations and clinical course in relation to phenotype-genotype correlation of all the $vLINCL_{Fin}$ patients was reported earlier (Holmberg *et al.*, 2000). In patients with $vLINCL_{Fin}$, the rectal or skin biopsy showed the characteristic fingerprint profiles or fingerprint profiles and curvilinear bodies. None of the patients included in the study had other neurological diagnoses.

For Study III, the four patients with NES were recruited by an invitation letter that was distributed by a paediatric neurologist (A. Hirvasniemi). All patients that participated belong to the original NES patient series published earlier (Hirvasniemi *et al.*, 1994, 1995). They all had genetically confirmed diagnoses (Ranta *et al.*, 1999), and a typical clinical picture, neurophysiological and imaging findings. Two of the patients were siblings.

Before each SEF measurement, a paediatric neurologist (P. Santavuori) clinically examined all patients and recorded their motor performance, balance, coordination, speech, visual performance and history of epilepsy, as described in Santavuori *et al.* (1988). The clinical course of the patients with JNCL was determined as classical, delayed classic or protracted. In homozygous JNCL patients this classification was performed at the time of writing this Thesis. The phenotype was determined classic if significant mental retardation (IQ level reduced by more than 10 points from the time of diagnosis) had taken place before the age of 10 years and/or parkinsonian signs emerged before the age of 15. Patients were determined to have delayed classic course if significant mental retardation took place after 10 years of age and/or parkinsonian signs after 15. Protracted JNCL was characterized in patients that had progressive visual loss at an early age, but did not have mental and motor symptoms in the first two decades of life. The medication of the patients, which included antiepileptic drugs, muscle relaxants and antioxidants (only patients with JNCL), was unchanged during the several months prior to the measurements.

5.3. HEALTHY CONTROL SUBJECTS

The 27 control subjects were relatives or friends of the laboratory personnel. Seventeen of them were younger than 19 years. All of the control subjects attended or had graduated from normal school. None of them had a history of neurological illness or head trauma. Altogether 25 controls underwent MRI. All controls from Studies I–IV are presented in Table 2 and in the Results section of this Thesis. However, the patients' values here and in each of the Studies I–IV were

compared with those of specific age-matched control groups. However, because novel patients with JNCL outside the original age range are included in this Thesis, all patients with JNCL are compared to the control group used for Study II that consists of 19 of the control subjects (age range 6–25 years, 8 males, height 125–187 cm). The control group for Study III consists of 8 subjects (age range 25–44 years, 5 males, height 156–191 cm), and that for Study IV consists of 10 controls (age range 8–16 years, 4 males, height 140–175 cm).

5.4. MEG Studies

5.4.1. Stimulation

The median nerves were stimulated in separate runs at the wrist with 0.2-ms constant-current pulses. In 25 patients and 26 controls both the left (LMN) and right (RMN) median nerves were stimulated. In 2 patients with $vLINCL_{Fin}$, in 4 patients with JNCL and in one control only the RMN was stimulated, because of the poor condition of the patient or lack of cooperation. The ISI was 2 s. The stimulus intensity was adjusted to be clearly above the motor threshold, but care was taken not to cause discomfort to the subjects.

5.4.2. Recording

Measurements were performed in a magnetically shielded room (Euroshield Ltd., Finland) with the 122-channel gradiometer system (Neuromag-122™, Neuromag Ltd., Finland; Ahonen *et al.*, 1993) of the BioMag Laboratory at the Helsinki University Central Hospital.

Prior to measurements being taken, three position indicator coils were attached to the subject's head, and the coil locations with respect to anatomical landmarks were found with a 3D digitizer. The preauricular points were used for the determination of the *x*-axis, which pointed to the right. The *y*-axis was oriented towards the nasion and the *z*-axis pointed upwards. During the recordings, the subject sat with his/her head inside the helmet-shaped sensor array. Healthy control subjects and patients with NES watched a self-chosen video film; stories were read to the other patients. All children and patients were accompanied by an adult during the measurements. At the beginning of each measurement run, the exact head position inside the helmet was determined by measuring signals from the three indicator coils. With this information, the MEG and MRI coordinate systems were aligned during data analysis.

Two sets of 200 responses were averaged for each hand, except for patients with $vLINCL_{Fin}$ with whom 100–200 sweeps were taken. The signals were band-pass-filtered between 0.03 and 300 Hz and sampled at 942 Hz. Epochs containing amplitudes exceeding 3000 fT/cm in the MEG channels or 150 μV in the electro-oculogram were automatically discarded. An epoch lasted 350 ms, including a 50–100-ms prestimulus baseline.

5.4.3. Data Analysis

For the analysis of the SEF data, a spherical model of the head was used. The center of the sphere was determined on the basis of the local radius of curvature near the SI, obtained from individual

three-dimensional MPRAGE (Magnetization Prepared Rapid Acquisition Gradient Echo) images. If the MPRAGE was not available (6 patients, 2 control subjects), the MRI of the subject with the same diagnosis at the closest age was used.

In all studies, responses from the contralateral SI were analyzed. Local subsets of channels were chosen to represent the magnetic field pattern over the region of interest. At the SI, a set of at least 28 channels was used. Least-squares searches were then applied to the signals from these subsets to determine the locations, orientations and strengths of the ECDs that best explained the measured signals at the peaks of the main deflections (Hämäläinen *et al.*, 1993). A 50 to 100-ms time period before the occurrence of the stimulus was used as a baseline. The earliest negative deflection, peaking at 15 to 30 ms, was defined to be N20m. The following positive deflection, peaking at 25 to 50 ms, was defined as P35m. In most subjects, the next deflection was positive, peaked at 50 to 140 ms, and was designated as P60m. In some subjects, the deflection following P35m was negative, and was thus called N45m. It was included in the analysis in Study IV, and only in the subjects in whom it crossed the baseline. The signals were in most cases digitally low-pass filtered at 90 Hz before the analysis of the P60m. For each deflection, the ECD with the greatest dipole moment was chosen, dipoles with a goodness-of-fit value over 80% were used (Kaukoranta *et al.*, 1986).

In Studies III and IV, some deflections generated from brain areas outside SI were also modelled. Usually 18–24-channel subsets were chosen to cover the SIIc and SIIi areas, and the ipsilateral SI in Study IV. In several subjects, the signal-space-projection method was used to eliminate the contribution of the SI sources from the SII signals (Uusitalo and Ilmoniemi, 1997). A time-varying multidipole model with one ECD from each activated brain area (usually P35m, SIIc and SIIi) was calculated by taking into account signals from all channels: the locations and orientations of the ECDs were kept constant and the strengths of the ECDs were allowed to vary in order to best explain the whole data set.

The ECD locations were superimposed on the individual MRIs. The latency, location, orientation, and strength (the dipole moment) of each ECD were compared with those of the normal controls in each study. For the classification of ECD strengths, the upper limit for the normal value was set at the control group mean + 2.5 SD. In the Result sections of this Thesis and in Studies III and IV, the peak latencies of the patients' deflections were considered abnormal if they fell outside the range of latencies in the age-matched controls.

5.4.4. Statistical Analyses

In Study I, the significance of the differences in latency and locations of the ECDs between patients and controls were evaluated using paired two-tailed *t*-tests. The sign test for matched pairs was used to test the significance of the difference in the dipole moments between patients and matched controls, because the response strengths were not normally distributed. The Kendall tau coefficient was determined to test whether the dipole moments correlated with age.

For the results section of this Thesis, further statistical analyses were performed. Differences between the deflections to RMN and LMN stimulation in healthy control and patient groups were tested with Wilcoxon matched pairs test. In the other tests the values for right and left hands were tested separately. The significance of the differences of the ECDs between controls and patients with JNCL, as well the differences between the JNCL–I and JNCL–II groups were evaluated

using Mann–Whitney U-tests. The Spearman R correlation coefficient (r_S) was calculated to determine if the latencies and dipole moments correlated with age.

5.5. MRI STUDIES (I–IV)

Altogether 25 patients and 25 controls underwent brain MRI examination (Table 2), in which a sagittal T1-weighted 3D MPRAGE sequence, with time of repetition (TR) 9.7 ms and time of echo (TE) 4 ms was taken to be used in the analysis of the SEF data. In addition, the MPRAGE images of patients were used for the visual grading of the size of the corpus callosum (CC) and cerebellar and cerebral atrophy (Autti *et al.*, 1996). In Study III, in which the age range of the subjects was 26–43 years, the size of cerebral cerebrospinal fluid (CSF) spaces were graded according to the method developed by Salonen *et al.* (1997), in which standard images are used as references to grade the size of CSF spaces into five categories (grade 1 represents the smallest size of CSF spaces, grade 5 represents the most enlarged CSF spaces; grades are based on images of neurologically healthy Finnish subjects (Salonen *et al.*, 1997). In addition, in Study III, the sagittal diameters of the mesencephalon, pons and medulla oblongata, as well as three diameters of the CC, the area of CC and the brain area were measured from the midsagittal MPRAGE image. The CC/brain area ratio was calculated. The normal values were established by measuring the eight normal controls and calculating the normal mean ± 2 SD. These normal values were found agree very well with those established previously (Laissy *et al.*, 1993; Raininko *et al.*, 1994).

In Study III, a 5-mm T2-weighted spin-echo (TR 3000 ms, TE 85/14 ms) and FLAIR (fluid attenuation inversion recovery, TR 9999 ms, TE 105 ms, inversion time (TI) 2500) axial images were taken. The signal intensity ratios of deep grey and white matter structures were evaluated according to method described previously (Autti *et al.*, 1994). In addition, for Study II and this Thesis, previous T2-weighted images of patients with JNCL (11 homozygous patients, age range 6–17 years, and 9 compound heterozygous patients, age range 6–32 years) were retrieved and re-evaluated, and the signal intensity ratios of the deep grey matter structures were measured and compared with established normal values (Autti *et al.*, 1994).

In addition, in Study III, a post-mortem MRI with the same imaging parameters than in the living NES patients was performed on one brain of a patient with NES who had died at the age of 38 (this patient was a sibling to the 40-year-old patient). The brain was cut into 1–2-centimeter slices and stored in a formalin solution; before the MRI the brain was rinsed in distilled water. The brain sections were imaged in a vessel containing distilled water and $MnCl_2$ (Autti *et al.*, 1997a).

6. RESULTS

6.1. SEFs

6.1.1. Responses from the Contralateral SI (I–IV and unpublished data)

Healthy Controls

The response waveforms over the SI area contralateral to stimulation in the 27 control subjects usually consisted of easily identifiable N20m, P35m and P60m deflections (Figure 3). An N45m deflection was seen as an upward deflection between P35m and P60m, but was not included in the analysis if it did not cross the baseline. The field patterns of the responses were dipolar: the N20m ECDs pointed anteriorly and the P35m and P60m ECDs posteriorly. The responses were well reproduced. The ECD locations corresponded to the known location of SI (Table 3).

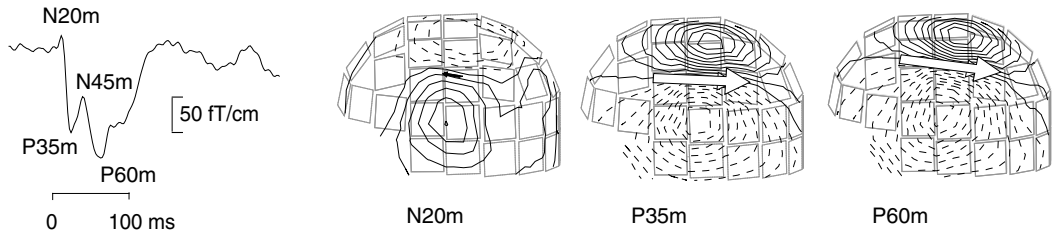

Figure 3. The waveform of the contralateral SI response in a 10-year-old control subject showing the typical N20m, P35m and P60m deflections to RMN stimulation. The isofield contour maps correspond to the peaks of the individual SI deflections, the helmets are pictured from a left lateral view. The contour step is 50 fT, the dashed lines indicate magnetic flux entering the head and solid lines magnetic flux exiting the head. The arrows denote the corresponding ECDs with lengths proportional to the ECD strength.

Table 3. N20m and P35m ECD latency ranges and mean locations (standard deviations in parenthesis) in controls of different age groups. Because there were no significant differences between the left and right hemispheres only data to right median nerve stimulation is shown.

	Age groups	**Height range**		**Latency range**		**Coordinates**	
	[years]	[cm]	*n*	[ms]	*x* [mm]	*y* [mm]	*z* [mm]
N20m	< 11	125–150	6	16–20	–35.7 (4.2)	4.6 (15.0)	92.9 (4.9)
	11–15	148–170	5	18–20	–35.0 (8.2)	9.5 (8.5)	89.7 (8.1)
	16–20	167–187	5	18–22	–36.1 (4.1)	14.1 (8.1)	97.6 (3.4)
	> 21	156–191	9	18–24	–43.5 (6.2)	12.9 (11.8)	95.1 (3.8)
P35m	< 11		6	30–33	–36.6 (6.5)	4.6 (17.2)	93.7 (6.7)
	11–15		7	31–40	–33.8 (4.9)	13.5 (8.2)	96.4 (6.5)
	16–20		5	31–39	–34.1 (4.1)	17.2 (7.4)	100.0 (3.8)
	> 21		9	30–45	–38.5 (5.6)	10.5 (12.0)	99.1 (6.6)

Within the individual control groups for the Studies I–IV the response strengths did not correlate with age. The normal upper limits of the ECD strengths that were used for the different patient groups are shown in Table 4.

Table 4. The mean (SD) values of the N20m and P35m deflections of the control groups for each study, and the calculated upper normal limits for left and right median nerve stimulation.

		Controls for JNCL–I and JNCL–II		Controls for NES		Controls for vLINCL$_{Fin}$	
		Mean (SD) [nAm]	**Normal limit** [nAm]	**Mean (SD)** [nAm]	**Normal limit** [nAm]	**Mean (SD)** [nAm]	**Normal limit** [nAm]
N20m	**LMN**	11.8 (4.8)	24	20.9 (6.8)	38	11.1 (6.1)	26
	RMN	12.6 (3.4)	21	18.2 (6.8)	35	11.6 (3.8)	21
P35m	**LMN**	38.3 (13.6)	72	42.4 (12.6)	74	38.1 (17.5)	82
	RMN	32.8 (11.6)	62	42.6 (12.2)	73	33.0 (12.0)	63

Normal limit = the upper normal limit calculated from the normal mean ± 2.5 SD

However, when all controls, aged from 6 to 44 years, were grouped together it seems that the controls over 25 years of age had stronger N20m responses than the younger subjects (Figure 4). The ranges of N20m and P35m dipole moments were 5–31 and 11–79 nAm, respectively. The dipole moments of N20m and P35m did not differ significantly between males and females.

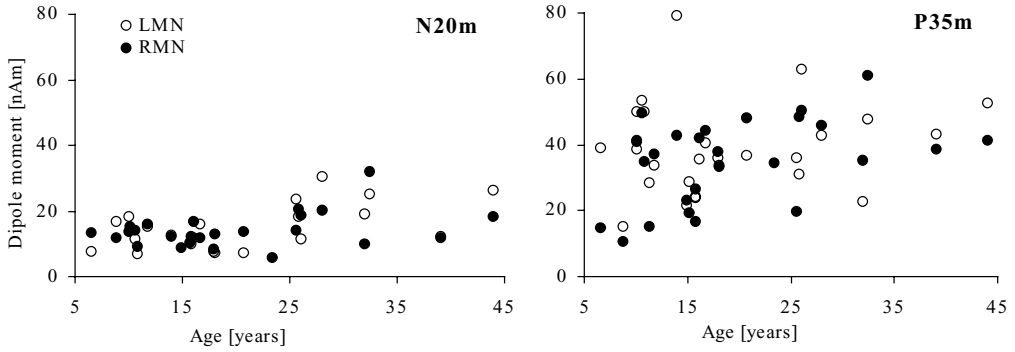

Figure 4. The N20m and P35m dipole moments as a function of age of normal controls to LMN and RMN stimulation. The N20m strength correlated with age to both LMN ($p = 0.02$, $r_S = 0.52$) and RMN ($p = 0.047$, $r_S = 0.39$) stimulation. The P35m strength significantly correlated with age to RMN stimulation ($p = 0.03$, $r_S = 0.43$).

The latency of N20m was longer in older ($p = 0.003$ and $r_S = 0.62$ to LMN stimulation) and taller subjects ($p = 0.002$ and $r_S = 0.64$ to LMN stimulation, $p = 0.047$ and $r_S = 0.39$ to RMN stimulation). The latency of P35m was longer in older ($p = 0.006$ and $r_S = 0.53$) and taller subjects ($p = 0.0008$ and $r_S = 0.62$) with left but not with right side stimulation. The N20m latency was longer in the males than females to both LMN and RMN stimulation ($p = 0.03$ and $p = 0.02$, respectively), which is most likely related to the greater height of the males.

Patients

All patients demonstrated activity originating from the contralateral SI area. In patients with JNCL or NES, the response waveforms were similar to those in controls, being typically separable into N20m, P35m, and P60m deflections (Figure 5). In the older patients with JNCL, the P60m deflection seemed to become less distinguishable. In contrast to the other patients, in vLINCL$_{Fin}$, only the morphology of the two earliest deflections, N20m and P35m, was similar to that in controls. An ensuing N45m deflection was seen in all patients with vLINCL$_{Fin}$, while the P60m deflection was not detected (Figure 5). The responses in patients with vLINCL$_{Fin}$ were extremely strong compared with those of patients with JNCL or NES (Figure 6).

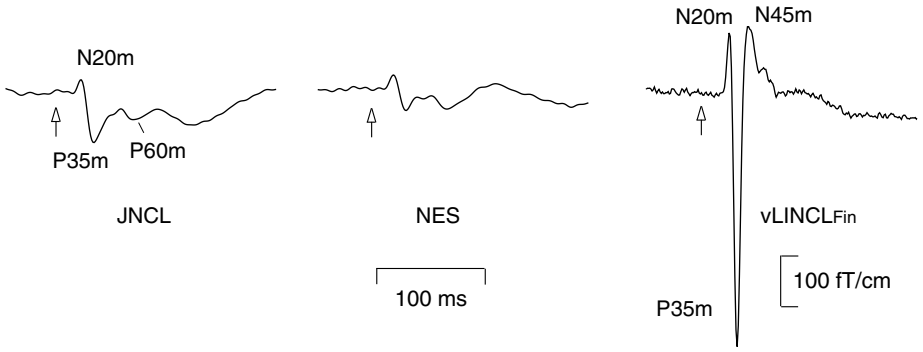

Figure 5. SI response waveforms from an 8.9-year-old patient with JNCL, a 26.2-year-old patient with NES, and a 12.3-year-old patient with vLINCL$_{Fin}$. The arrow depicts the stimulus. The response waveforms in JNCL and NES were generally similar to those of controls, and consisted of N20m, P35m and P60m deflections. In all patients with vLINCL$_{Fin}$, an N45m deflection followed the N20m and P35m deflections, whereas no P60m response was seen.

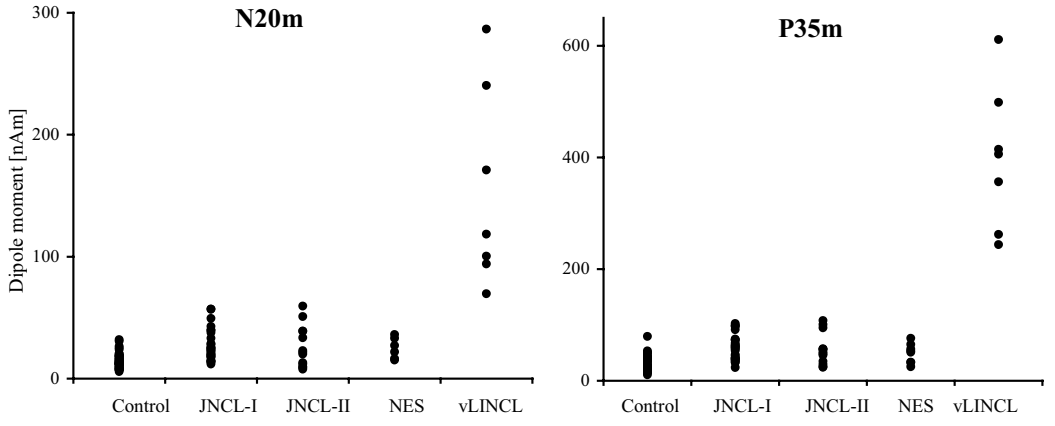

Figure 6. Dipole moments of N20m and P35m in different patient groups to both LMN and RMN stimulation. The P35m dipole moment was always stronger than that of the N20m (note the different scales). Although the responses were extremely strong in patients with vLINCL$_{Fin}$ compared with the other groups, also the responses in patients with JNCL were significantly stronger than in controls.

With JNCL, the N20m responses were stronger than in the age-matched controls ($p = 0.0006$ and $p = 0.0001$, respectively for LMN and RMN stimulation). This enhancement of N20m in patients with JNCL correlated with age and thus with disease duration (Figure 7; $p = 0.01$ and $r_S = 0.59$ for LMN stimulation, and $p = 0.000001$, $r_S = 0.84$ for RMN stimulation). Also the P35m responses in patients with JNCL were stronger than in the controls ($p = 0.001$ and $p = 0.0006$, respectively for LMN and RMN stimulation), but the P35m responses did not correlate with the age of the patient.

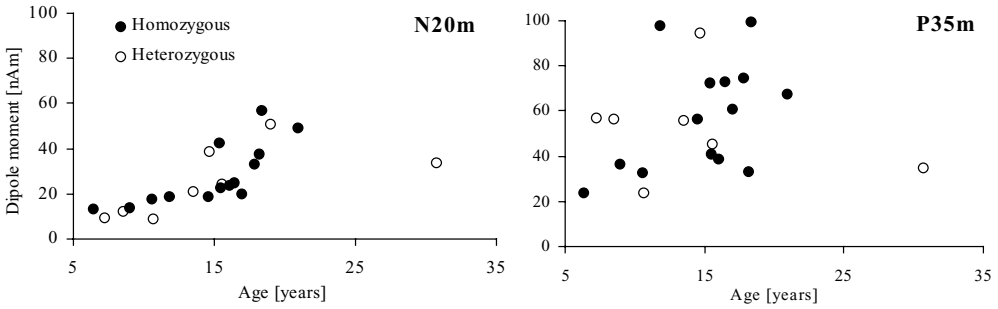

Figure 7. The strengths of N20m and P35m in response to RMN stimulation as a function of age in patients with JNCL. The enhancement of N20m was correlated with age to both LMN and RMN stimulation.

When the dipole moments were compared with the upper normal limits, most patients with JNCL over the age of 11 years had abnormally strong responses (Table 5). The N20m strength was over the upper normal limit more often than the P35m strength. There were no statistically significant differences in the ECDs between the patients homozygous for the 1.02-kb deletion and compound heterozygous patients.

Table 5. The percentage of abnormally strong responses in different patient groups. In parentheses, the number of abnormal responses are given for all the responses evoked by left or right median nerve stimulation.

Age	**Deflection**	**JNCL -I**	**JNCL -II**	**NES**	**vLINCL$_{Fin}$**
< 11.0 years	N20m	0% (0/5)	0% (0/4)	-	100% (2/2)
	P35m	0% (0/5)	0% (0/4)	-	100% (2/2)
> 11.0 years	N20m	68% (15/22)	67% (6/9)	13% (1/8)	100% (5/5)
	P35m	41% (9/22)	33% (3/9)	13% (1/8)	100% (5/5)

For patients with NES, the ECD strengths were generally within normal limits: the only exceptions were a slightly enhanced N20m in one patient and a slightly enhanced P35m in another patient. In vLINCL$_{Fin}$, all patients had enhanced N20m (range 70–287 nAm) and P35m (range 180–611 nAm) deflections to both LMN and RMN stimulation; no age-related correlation of the response strengths was observed. The N45m dipole moment in patients with vLINCL$_{Fin}$

was also strong, being approximately of the same magnitude as that of N20m (range 127–274 nAm, except for one deflection, which was 31 nAm).

The N20m, P35m and P60m ECDs were generally located in the vicinity of the hand representation area of the SI. In patients with vLINCL$_{Fin}$, the location of the N45m ECD was either the same as the ECD locations for N20m and P35m (in 3 hemispheres) or somewhat more posterior (8–22 mm, 4 hemispheres).

With JNCL (Figure 8), the N20m latencies at the group level were slightly delayed compared with those of normal controls' ($p = 0.0019$ and $p = 0.02$, respectively for LMN and RMN stimulation). Interestingly, when compared with the control values, the N20m latencies of especially compound heterozygous patients with JNCL were delayed (Table 6). In all patients with NES, the N20m latencies were normal, whereas in all patients with vLINCL$_{Fin}$, all the N20m latencies were delayed.

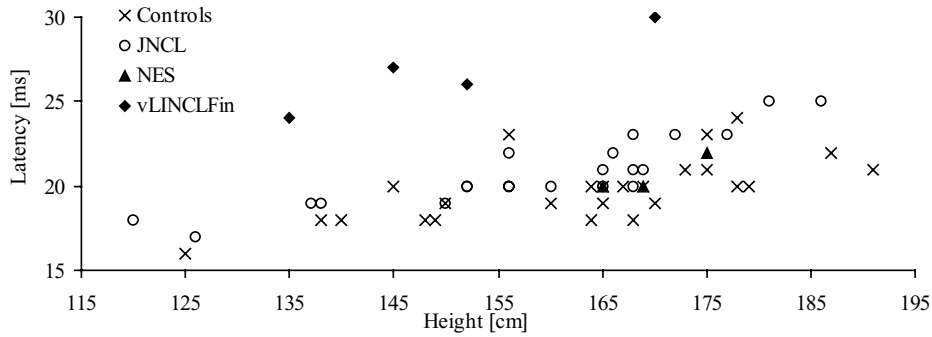

Figure 8. The latency of N20m to RMN stimulation in controls and patient groups. The latencies were clearly delayed in all patients with vLINCL$_{Fin}$. In JNCL, the latencies were slightly delayed at the group level, whereas in NES all latencies were within normal limits.

The P35m latency did not differ significantly between patients with JNCL and the controls. Also in NES and vLINCL$_{Fin}$ patients, the P35m latencies were within the range of latencies of the controls (with one exception, a patient with vLINCL$_{Fin}$ with an abnormally short latency P35m). However, the time interval between N20m and P35m was significantly shorter ($p < 0.05$) in patients with JNCL than the controls. The same effect was especially pronounced in patients with vLINCL$_{Fin}$, in whom the latency difference between N20m and P35m was shorter in all patients (7–9 ms) compared with age-matched controls (12–24 ms).

Table 6. The percentage of abnormal N20m latencies in different patient groups. In parentheses, the number of abnormal responses of all the responses evoked by left or right median nerve stimulation are given.

Age	**JNCL-I**	**JNCL-II**	**NES**	**vLINCL$_{Fin}$**
< 11.0 years	0% (0/5)	0% (0/4)	-	100% (2/2)
> 11.0 years	36% (8/22)	78% (7/9)	0% (0/8)	100% (5/5)

6.1.2. Responses from the Ipsilateral SI (IV)

Healthy Controls
Activity from the ipsilateral SI was found in 6 of the 10 control subjects in Study IV: in one subject in both hemispheres, in 2 in the right hemisphere, and in 3 in the left hemisphere. The latencies varied between 44 and 165 ms and the ECD strengths between 6 and 74 nAm.

Patients
Activity from the ipsilateral SI was examined only in patients with $vLINCL_{Fin}$. Two of the three patients, in whom the LMN was stimulated showed activity at ipsilateral SI; however, only in one patient this could be modelled with an ECD (peak was at 77 ms with a strength of 33 nAm). RMN stimulation did not reveal any ipsilateral SI activity in the patients.

6.1.3. Responses from SII (III and IV)

Healthy Controls
Most of the 18 healthy control subjects studied had activity at the frontoparietal opercular regions; however the strength of the activity was in some cases weak and dipole modelling could not be performed. Thus, the SIIc deflection was modelled in 13 and 15 of the controls after LMN and RMN stimulation, respectively. The SIIi deflection was amenable to modelling in 10 and 13 of the subjects after LMN and RMN stimulation, respectively. The locations of the acceptable ECDs of these responses were in the vicinity of the lateral sulcus near the SII area.

The dipole moments varied between 6–90 and 8–42 nAm for SIIc and SIIi responses, but only the dipole moment of SIIi to RMN stimulation was correlated with age (p = 0.006 and r_S= 0.71, Figure 9). The only significant differences between the strengths of the responses to LMN or RMN stimulation was that the SIIi was stronger to LMN stimulation (p = 0.04). The SIIc deflections for RMN were significantly stronger than the SIIi deflections (p = 0.01), but to LMN stimulation there was no significant differences between the SIIc and SIIi strengths. Gender did not affect the response strengths.

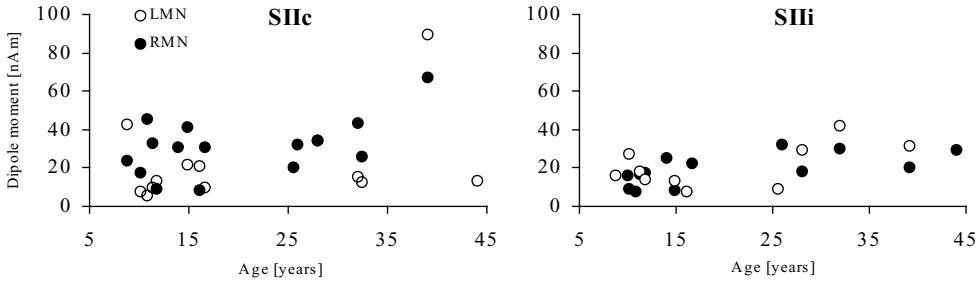

Figure 9. The strengths of SIIc and SIIi ECDs in healthy control subjects as a function of age.

The range of the SIIc peak latencies in all the controls was 91–197 and 79–185 ms to LMN and RMN stimulation, respectively. The range of peak latencies of the SIIi responses was 79–199 and

111–200 ms to LMN and RMN stimulation, respectively. This wide range was due to the fact that if the first deflection of the bi-phasic responses was not clearly detectable, the SII responses were modelled from the second deflection. The latencies of SIIc or SIIi responses for either median nerves did not correlate with age, nor were there significant differences between the deflections to RMN and LMN stimulation. The SIIc deflections to RMN, but not to LMN stimulation, peaked significantly earlier than the SIIi deflections ($p = 0.03$).

Patients

The SII responses were evaluated in $vLINCL_{Fin}$ and NES. The SII response was basically a bi-phasic response. The SII ECDs were generally located in the vicinity of the lateral sulcus (Figure 10). The SII responses seemed to be equally often present in patients with NES than in controls. All patients with $vLINCL_{Fin}$ had SIIc responses, but the SIIi response was missing from 4 out of 7 hemispheres (Table 7).

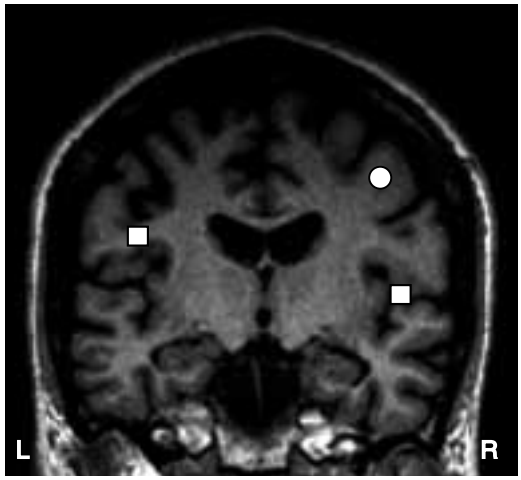

Figure 10. A T1-weighted coronal MRI of 40-year-old patient with NES. The circle denotes the location of P35m and squares the location of SII ECDs to LMN stimulation. L = left, R = right.

The SII dipole moments were within the range of the controls' dipole moments in the patients with NES (25–32 and 14–55 nAm for SIIc and 10–19 and 8–25 nAm for SIIi to LMN and RMN stimulation, respectively). In patients with $vLINCL_{Fin}$ the dipole moment of the SIIc response was enlarged in 4 out of the 5 patients, whereas the SIIi response was enlarged in two of the three patients in whom it was present (Table 7).

Table 7. The latencies and dipole moments of the earliest SII response peaks in patients with vLINCL$_{Fin,}$ and the normal limits established from the values of age-matched controls

Age	SIIc				SIIi			
	LMN		RMN		LMN		RMN	
[years]	Peak [ms]	Peak strength [nAm]	Peak [ms]	Peak strength [nAm]	Peak [ms]	Peak strength [nAm]	Peak [ms]	Peak strength [nAm]
9.1	na	na	79	39	na	na	–	–
12.3	97	**45**	**57**	**118**	–	–	**68**	26
16.7	na	na	**65**	**169**	na	na	–	–
8.8	106	**122**	na	na	**77**	**82**	na	na
12.5	**59**	**229**	105	**218**	–	–	112	**123**
Normal limits	91–197 (n = 8)	0–30	79–185 (n = 9)	0–60	79–199 (n = 6)	0–32	111–200 (n = 7)	0–32

na = data not available, – = no SII responses were detected. The latencies below the range of normal values in controls and the enlarged ECD strengths are in bold typeface.

In patients with NES the SII response latencies fell within the range of latencies of the healthy controls. The peaks of the main SII responses in the patients with vLINCL$_{Fin}$ were generally of short latency compared with those of the controls: 4 patients had peak latencies below the normal range determined from the controls' values (Table 7). When the onset of the SII activity was judged from the multidipole models (Figure 11), both SIIc and SIIi sources were activated 20–35 ms after stimulus in all patients. In contrast, only 2 of the controls had SIIc activity before 35 ms; otherwise the SIIc and SIIi onset ranges in the controls were 40–120 and 60–150 ms, respectively.

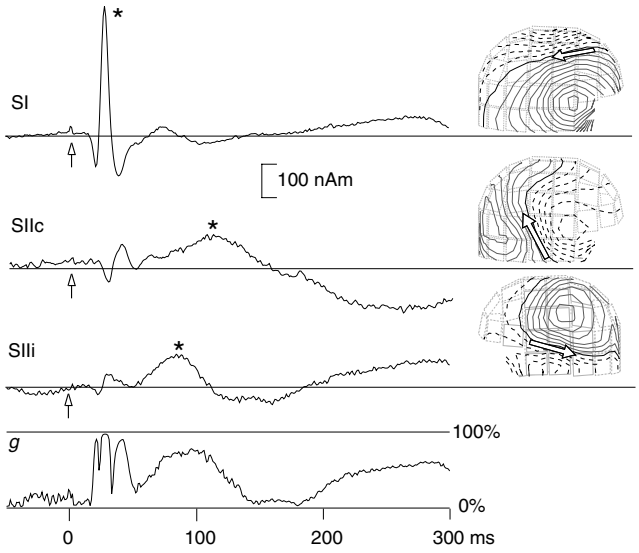

Figure 11. The multidipole model of responses from an 8-year-old vLINCL$_{Fin}$ patient showing the time courses of the SI, SIIc and SIIi ECD strengths. The arrows indicate the occurrence of the stimulus at 0 ms. The goodness-of-fit value of the multidipole model is expressed as g at the bottom. The isofield contour maps of the individual sources, at the latencies specified with the asterisks, are shown on the right. The dashed lines indicate magnetic flux entering and solid lines magnetic flux exiting the head. The contour step is 200 and 50 fT for the SI and SII responses, respectively. SII activity can be seen on the traces starting 25–35 ms after stimulus, however, the main SII peak starts later.

6.2. MRI (I–IV)

6.2.1. Patients with JNCL (I and II)

Atrophic changes of the cerebellum and cerebrum were observed in patients older than 11 years in both JNCL–I and JNCL–II groups (Table 8). In addition, one compound heterozygous patient had slightly dilated lateral ventricles already at the age of 8 years. Analysis of previously taken T2-weighted images of eleven of the homozygous patients showed abnormally high signal intensity of the periventricular white matter in three patients, at the ages of 11, 12, and 14 years. This alteration was seen only in one compound heterozygous patient at the age of 9 years. None of the compound heterozygous patients had abnormally low signal intensity ratios between the deep grey matter structures and the white matter, whereas this alteration was seen in one homozygous patient at the age of 17.

Table 8. MRI findings in 17 patients with JNCL at the time of SEF measurement

	Cerebellar atrophy		Cerebral atrophy	
Age	**JNCL–I**	**JNCL–II**	**JNCL–I**	**JNCL–II**
< 11.0 years	0% (0/3)	0% (0/1)	0% (0/3)	0% (0/1)
> 11.0 years	56% (5/9)	25% (1/4)	89% (8/9)	50% (2/4)

6.2.2. Patients with NES (III)

Cerebellar atrophy with enlarged (> 2 mm) cerebellar fissures was seen in all four patients; the atrophy was graded slight in two patients and moderate in the other two (Table 9). The size of the cerebral central CSF spaces was visually judged to be normal in all patients, whereas peripheral CSF spaces were slightly enlarged in two patients. However, similar grade 2 peripheral CSF spaces are seen in 10–20% of the control population of the same age (Salonen *et al.*, 1997). The midsagittal brain area was within normal limits in all patients. The corpus callosum was graded visually to be normal in the two youngest patients, but the measurements revealed that it was below normal limits in one of them, as it was in the two oldest patients in whom the corpus callosum was visually graded to be thin.

Table 9. MRI results in 4 patients with NES

Age	**Cerebellar atrophy**	**Cerebral atrophy**	**Mesen-cephalon**	**Pons**	**Medulla oblongata**	**Corpus callosum (CC)**				**Brain area**	**CC/ brain area**
						genu	**body**	**splenium**	**area**		
[years]			[mm]	[mm]	[mm]	[mm]	[mm]	[mm]	[cm^2]	[cm^2]	%
26	Slight	Normal	**14**	**19**	11	10	6	10	**5.0**	143	3.5
30	Moderate	Slight	**14**	**20**	11	9	**4**	8	5.8	148	3.9
40	Slight	Normal	16	22	11	**8**	5	8	**4.6**	139	3.3
43	Moderate	Slight	**14**	**20**	**10**	**6**	**3**	**7**	**4.2**	125	3.4
Normal limits			16–19	21–26	11–15	9–15	5–8	8–14	5.1–8.7	125–202	3.3–5.2

Bold typeface = values outside normal limits (calculated from the normal controls' mean ± 2 SD values)

The CC/brain area ratios were within normal limits in all patients indicating that the corpus callosum is of relatively normal size when the size of the brain is taken into account. The measured diameters of the brain stem had values below normal limits in three patients.

The general grey to white matter differentiation was normal, and no focal signal intensity abnormalities were found in patients or in the post-mortem brain.

6.2.3. Patients with vLINCL$_{Fin}$ (IV)

All the four patients that had MRI at the time of the SEF recording showed both cerebellar and cerebral atrophy. Only in the youngest patient, aged 8.8 years, was the cerebral atrophy graded slight, the other patients had moderate to severe atrophy (Figure 12). Cerebellar atrophy was graded moderate to severe in all the four patients. A notable feature was also the thinning of the corpus callosum in all the patients. Abnormal signal intensity changes of the deep grey matter structures on T2-weighted images (abnormally low signal intensity of the thalamus, abnormally high signal intensity of the posterior limb of capsula interna) and abnormally high signal intensity of the periventricular white matter were previously observed in all the patients (Holmberg *et al.*, 2000).

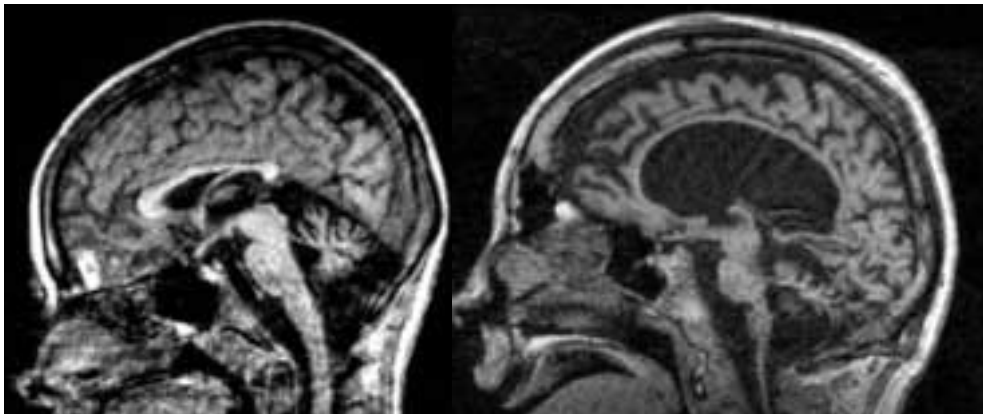

Figure 12. A sagittal T1-weighted MPRAGE image of a 8.8-year-old patient with vLINCL$_{Fin}$ on the left and of a 16.7-year-old patient on the right. In the younger patient, the cerebrum is only slightly atrophic, whereas the corpus callosum is thin and cerebellum moderately atrophic. In the older patient, the cerebrum and cerebellum are extremely atrophic and corpus callosum is extremely thin. Note also the thickening of the skull in the older patient.

6.3. Effect of the Genotype on the Phenotype (I, II, IV)

6.3.1. Patients with JNCL (I and II)

Clinically, the patients with JNCL were classified to have three different types of disease progression (Table 10), the delayed classic course of which was seen most commonly, in altogether 15 of the 24 patients. The classic course was seen in 7 patients, all of whom were homozygous for the major 1.02-kb deletion.

Table 10. Genotype, clinical phenotype, SEF and MRI findings in 24 patients with JNCL

	Mutations	Location	Phenotype	AED	SEFs (LMN/RMN)			MRI	
					N20m latency	N20m [nAm]	P35m [nAm]	Cerebellar atrophy	Cerebral atrophy
1	1.02-kb del/ 1.02 kb del	Intron 6–8/ intron 6–8	Classic (14)	–	19/19 (9)	17/14	40/36	– (9)	–
2*	"	"	" (15)	–	20/20 (10)	15/18	41/33	– (10)	–
3	"	"	" (17)	LTG	**20**/20 (11)	**28**/19	**102/98**	– (11)	–
4*	"	"	" (18)	LTG, VPA	**23**/**23** (14)	23/19	59/56	– (14)	+
5*	"	"	" (19)	LTG	**20**/20 (15)	**26**/**43**	64/**72**	– (14)	++
6	"	"	" (21)	VPA, CZP	19/20 (17)	15/20	59/61	+ (17)	+
7	"	"	" (22)	VPA	21/21 (18)	**40**/**57**	**91**/**100**	+ (18)	++
8	"	"	Delayed classic (12)	–	na/18 (6)	na/13	na/24	– (6)	–
9*	"	"	" (19)	–	**20**/20 (15)	21/**22**	45/41	– (15)	+
10	"	"	" (22)	VPA	20/20 (16)	12/**24**	47/38	+ (16)	+
11	"	"	" (23)	VPA	21/21 (16)	**25**/**25**	63/**73**	+/– (16)	+
12*	"	"	" (21)	VPA	21/21 (17)	**40**/**33**	**97**/**75**	+ (16)	++
13*	"	"	" (24)	VPA	**25**/**25** (18)	**29**/**38**	36/33	– (18)	+
14	"	"	" (26)	VPA	22/**23** (20)	**57**/**49**	54/**67**	+ (21)	+
15	1.02-kb del/ 2.8-kb del[1]	Intron 6–8/ intron 9–13	Delayed classic (7)	–	na			na	
16	"	"	" (7)	–	na/17 (7)	na/10	na/57	– (6)	–
17	"	"	" (8)	–	na/19 (8)	na/12	na/56	– (8)	–
18	"	"	" (12)	LTG	19/19 (10)	13/9	30/24	– (8)	+
19	"	"	" (15)	LTG	na/**22** (14)	na/**39**	na/**95**	– (14)	–
20	"	"	" (15)	LTG	21/**22** (15)	**39**/**24**	48/46	– (15)	+
21	1.02-kb del/ 2.8-kb del	Intron 6–8/ intron 9–13	" (20)	VPA, CZP	**23**/**23** (19)	**60**/**51**	**108**/**101**	+ (18)	–
22	1.02-kb del/ splice site	Intron 6–8/ intron 7	" (15)[2]	VPA	na	na	na	– (9)	–
23	1.02-kb del/ missense	Intron 6–8/ exon 11	Protracted (32)	LTG	**25**/**25** (30)	22/**34**	51/35	++ (30)	+
24	1.02-kb del/ missense	Intron 6–8/ exon 13	Protracted (16)	LTG	**20**/20 (13)	8/21	26/56	– (13)	–

Patients 1–14 form the JNCL–I group (* novel patients) and patients 15–24 form the JNCL–II group. [1]this novel deletion is reported in Study II of this Thesis, [2]the mental decline in this patient resembled that seen in classic JNCL. In parenthesis are the ages (in years) the examinations were done. Bold typeface = delayed N20m response or abnormally strong response. – = not present or normal, + = slight atrophy, ++ = moderate or severe atrophy. AED = antiepileptic medication, CZP = clonazepam, LTG = lamotrigine, VPA = valproate, na = data not available

Among the patients with the delayed classic course, cerebral and cerebellar atrophy on MRI seemed to be present equally often in the patients homozygous for the 1.02-kb deletion than in the compound heterozygotes. In addition, the SEFs were similarly altered in compound heterozygotes as in homozygous patients: most patients over 11 years had abnormally strong N20m responses, and in eight patients the P35m responses were also enlarged. In fact, the age of the patient, and thus the duration of the disease, seemed to be a better predictor for an enlarged response than the genotype.

A clearly more slowly progressing disease, defined as protracted, was seen in the two patients with missense mutations in exons 11 (Gly295Lys) and 13 (Arg334His). The 30-year-patient had only slightly enlarged cerebral sulci in MRI, normal corpus callosum and thalamic signal intensity, but his cerebellar atrophy was moderate. He had only one enlarged SEF deflection (the N20m to RMN stimulation). The other patient had normal SEFs and MRI at the age of 13.

6.3.2. Patients with vLINCL$_{Fin}$ (IV)

The three different genotypes (Table 11) in patients with vLINCL$_{Fin}$ did not cause phenotypic differences in the SEF response waveforms, locations or latencies. However, although all patients had enhanced responses, the lowest SI and SII response strengths were seen in patients homozygous for the 2-bp deletion (the Fin major) mutation, which is predicted to result in a less truncated polypeptide than the other two mutations in the patients of this study.

Table 11. MRI and SEF findings in 5 patients with vLINCL$_{Fin}$

	Mutations	Predicted effect[1]	Age [years]	MRI			SEFs (LMN/RMN)			
				Cerebellar atrophy	Cerebral atrophy	Corpus callosum	N20m [nAm]	P35m [nAm]	SIIc [nAm]	SIIi [nAm]
1	2-bp del/ 2-bp del	Tyr392 Stop	9.1	na	na	na	na/**100**	na/**262**	na/39	na/–
2	"	"	12.3	Moderate	Moderate	Thin	**94**/**70**	**244**/**356**	45/**118**	–/26
3	"	"	16.7	Severe	Severe	Very thin	na/**240**	na/**499**	na/**169**	na/–
4	2-bp del/ 1-bp ins	Glu253 Stop	8.8	Moderate	Slight	Thin	**119**/na	**414**/na	**122**/na	**82**/na
5	Nonsense/ nonsense	Trp75 Stop	12.5	Moderate	Moderate	Thin splenium	**171**/**287**	**406**/**611**	**229**/**218**	–/**123**

[1] of the second mutation, na = not available, – = not detected, dipole moments over the upper normal limits are in bold typeface

7. DISCUSSION

In this Thesis, we were able to demonstrate previously unknown alterations in the somatosensory evoked responses in NCL diseases by using MEG. In Studies I and II, an enlargement of the early SI SEFs with the progression of JNCL was demonstrated in both homozygous and compound heterozygous patients. In Study III, the newest member of the NCL disease group, NES, was found to be associated with extremely mild cerebral MRI and SEF findings. Study IV demonstrated that the increased excitability of the somatosensory system in $vLINCL_{Fin}$ extends also to SII areas. Furthermore, when combined, the SEFs from the control subjects from Studies I–IV form the largest published material of SEFs in healthy children and adolescents.

7.1. METHODOLOGICAL ISSUES

7.1.1. Patient and Control Groups

Because of the rare nature of NCL diseases, the number of patients was known from the beginning of the studies to be small. Patients were recruited between 1995 and 1998, and some patients with JNCL are included in this Thesis that were not included in the original publications. Thus, we were able to perform statistical evaluation between the JNCL groups as well as between patients with JNCL and controls. Unfortunately, the small number of patients with NES and $vLINCL_{Fin}$ did not allow statistical group comparisons to be carried out.

When the patients with JNCL were measured for Study I the underlying mutations were not known. As the mutations were characterized it became apparent that several different mutations were present in the Finnish JNCL patient group, and that some genotype-phenotype correlation could exist (Järvelä *et al.*, 1997). Thus, when a novel mutation was found in six patients from three Finnish families, Study II was performed in order to evaluate the genotype-phenotype relation in the compound heterozygous patients with JNCL. Because the imaging and neurophysiological data was retrospectively collected, the patients had undergone the different investigations at different ages, which made the evaluation of the data more difficult.

At the beginning of this study, no normative data of SEFs in children was available in the literature. However, based on SEP studies, age-related changes in SEFs were expected in the different age ranges of NCL patients (Allison *et al.*, 1983, 1984; Taylor and Fagan, 1988; Araki *et al.*, 1999). Therefore, an age-matched control group was recorded for each study for comparisons of the SEFs in the specific age groups. Adult controls were measured for Study III, because although there was more knowledge of the SEFs in the age group 26–43 years, our recording parameters differed slightly from those reported, and thus a control group was needed. Within the control groups in Studies I–IV the ECD parameters other than latencies were not found to correlate with age, and thus the same normal limits were used for the whole age range in each study. For this Thesis, all the controls were presented together, thus forming a group of 27 individuals, with an age range of 6.5–44 years. Since the main purpose of this Thesis was to elucidate the alterations caused by NCL diseases, the findings in the control group were not analyzed in detail. However, since novel information about the development of SEFs with age can obviously be deduced from the control group, a thorough evaluation of the SEFs in these

normal subjects will be done in the future. In fact, by measuring some new controls, proper reference limits of SEFs can be set for different age groups and the effect of maturation of SEFs, unknown at present, can be assessed.

7.1.2. SEFs

Whole-head MEG was chosen for evaluating the somatosensory system in NCL diseases for several reasons. Previous reports on patients with JNCL have been inconsistent regarding the SEP amplitudes. They have been described as normal (Cracco *et al.*, 1980; Harden and Pampiglione, 1982), decreased (Goebel, 1995) or enhanced (Schmitt *et al.*, 1994). However, the SEP amplitudes may be affected by the changes of conductivity between the brain source and recording electrodes caused by the disease, which include thickening of the skull, increase of CSF volume, decrease of brain tissue and gliosis (Zeman *et al.*, 1970; Autti *et al.*, 1997a). Since these changes do not affect the magnetic fields measured with MEG, it should give a more accurate estimate of the strengths of the underlying neuronal activation (Hämäläinen *et al.*, 1993). In $vLINCL_{Fin,}$ the SEPs are known to be giant (Santavuori *et al.*, 1982, 1991). However, no source modelling of the responses has been performed, and it is not known whether the giant responses originate only from the SI. Thus, whole-head MEG, which offers a possibility to study the activation of the whole brain simultaneously, and to localize accurately the activity within the brain, seemed well suited for the study of the somatosensory system in NCL.

The young age of some of the subjects brought up some specific difficulties in the MEG recording session. The helmet of the MEG measurement device accommodates most adult size heads, and thus additional cushions were needed to make the child subject's head fit snugly inside the helmet. However, it is not impossible that the subject moved after the determination of the indicator coils used in aligning the MEG and MRI coordinate systems or even during the measurement, and thus the localization accuracy of the ECDs in these subjects was not expected to be as good as for co-operating adults. However, the repeatability of the recordings, which was evaluated by repeating most of the measurement runs, was good. Accordingly, the sources of ECDs, determined from the individual MRIs, were located in the vicinity of the SI and SII areas. In both the control subjects and the patients the modelling and localization of the SII sources was more difficult than those of the SI. This could be due to, *e.g.,* the use of spherical model, since the sphere was defined to approximate primarily the parietal SI areas. In addition, different opercular areas may be active simultaneously (Korvenoja *et al.*, 1999), which can hamper the SII dipole modelling. However, we considered that the most important aspect of the localization of the ECDs was that we could demonstrate that the abnormally enlarged response components in patients originated within or in the proximity of the SI and SII areas.

Although our aim was to stimulate both median nerves of each subject, in six of the patients only one side was stimulated because of restlessness or poor condition of the patient. However, in general the recordings went surprisingly well. Most of the patients sat still and listened to the story that was read to them or watched a video film. However, at the time the recordings were done, sitting was the only position possible for the subjects. Furthermore, the patients' custom-made wheelchairs could not be taken inside the measurement room because of the magnetic artefacts, which made the most severely affected patients feel uncomfortable. Nowadays, significant improvements have been made, as the MEG recordings can now be done in the supine position.

7.1.3. MRI

The MRI findings in patients with JNCL and $vLINCL_{Fin}$ have been recently described and the present study did not aim to repeat these descriptions. However, since 25 of the patients underwent MRI, the atrophic changes were evaluated as described earlier (Autti *et al.*, 1996) in order to relate the SEF findings to the macroscopical atrophic changes. The MRI of patients with NES were described more thoroughly, because the findings had not been described in the context of NCL diseases. However, since the patients with NES were adults, the same normative values that had been used for other NCL patients were not applicable. Thus, with NES, the measured values of some of the brain structures were compared to those 8 controls that underwent SEF studies as controls for NES patients. The normal values established from these controls were found correlate well with the normative data published earlier (Laissy *et al.*, 1993; Raininko *et al.*, 1994). The sizes of the cerebrospinal fluid spaces were compared to those of a previously described large normal material of Finnish adults (Salonen *et al.*, 1997).

7.2. SEFs IN HEALTHY CONTROLS

When all controls from Studies I–IV are compiled, they form a group of 27 healthy subjects, (13 males, 14 females), with an age range of 6–44 years (mean 19 years). Seventeen of the subjects were younger than 19 years, which makes this study the first to publish SEFs of a relative large number of young subjects. The SEF waveforms and ECD locations in the adult controls corresponded well to other published findings in normal subjects (*e.g.*, Wikström *et al.*, 1997). However, some age-related alterations in the SEF strengths and latencies were seen. The controls over 25 years of age had stronger N20m responses than younger subjects, in line with the previous analysis of age-related changes in SEFs of adults (Huttunen *et al.*, 1999). In addition, there was a tendency for the P35m to be stronger in older subjects this correlation being significant, however, only to right median nerve stimulation. In the study of Huttunen *et al.* (1999), there were no age-related changes of the P35m strength, although in SEP studies older subjects may have stronger late SI responses (Kakigi and Shibasaki, 1991). The dipole moments of the deflections studied did not differ significantly between males and females, corroborating the previous SEF study (Huttunen *et al.*, 1999).

The SII responses could be modelled in 72–83% (SIIc) and 56–72% (SIIi) of the subjects. This partial lack of SII responses, and especially the SIIi responses, is similar to those reported in one previous study (Wikström *et al.*, 1996). However, in some other studies the SII responses have been present in all subjects (*e.g.*, Forss *et al.*, 1994a; Mauguière *et al.*, 1997b; Simões and Hari, 1999; Lin *et al.*, 2000). This discrepancy could be due to the relatively short ISI we used, since the SII responses become stronger with increasing ISI up to at least 3 s (Hari *et al.*, 1993; Forss *et al.*, 1994a; Wikström *et al.*, 1996). In addition, although the SII responses seemed to be present as frequently in our older control subjects as in the younger, some age related differences in the presence of SII responses cannot be ruled out. In SEP studies, the evoked responses in children of different ages have been shown to be affected differently by the changes of ISI (Araki *et al.*, 1999). Nevertheless, since the SII responses were not detected in a relatively large number of our control subjects, a similar lack of the SII responses in patients was considered normal.

The interindividual variability in the strengths of the SII dipole moments was large: they were between 6–67 nAm (and in one control even 90 nAm). However, the age of the subject was not a

significant factor in determining the strength of SII responses since only SIIi to RMN stimulation correlated with age. The SIIc deflections were statistically stronger than the SIIi deflections as reported previously (Hari *et al.*, 1983, 1984; Mima *et al.*, 1998a), although statistically significantly to right median nerve stimulation only. The only hemispheric difference found was that the left hemisphere SIIi was weaker than the right hemisphere SIIi. Thus, our results are in line with some previous studies (Wikström *et al.*, 1997; Wegner *et al.*, 2000), but we can not corroborate other studies showing stronger, earlier peaking and more posteriorly located left hemisphere SII responses (Forss *et al.*, 1994a; Simões and Hari, 1999; Lin *et al.*, 2000).

The latency of N20m correlated with age and height, as expected from previous SEP studies (Allison *et al.*, 1983, 1984; Zhu *et al.*, 1987; Taylor and Fagan, 1988). The N20m peaked significantly later in males than in females, this difference most likely reflecting the taller stature of the males. The range of SII latencies in the control subjects was wide, peaking at the earliest around 80 ms, in agreement with previous reports (Hari *et al.*, 1984; Mauguière *et al.*, 1997b; Wegner *et al.*, 2000), but at the latest not until 200 ms. This wide latency range was due to the fact that the responses were modelled either from the first or second deflection of the bi-phasic response, thus making the normal limits for the latencies very wide. However, the previously observed tendency of the SIIc deflections to peak earlier than the SIIi deflections was also demonstrated in the present study (Forss *et al.*, 1994a; Simões and Hari, 1999; Wegner *et al.*, 2000), although it became statistically significant only to right median nerve stimulation.

7.3. SEFs IN PATIENTS WITH JNCL, NES AND vLINCL$_{Fin}$

The most remarkable finding in patients with JNCL and vLINCL$_{Fin}$ was the enlargement of the SI responses compared with healthy controls and patients with NES. Similar enlarged or giant somatosensory evoked responses are usually reported in patients with cortical myoclonus, most often diagnosed with PME (*e.g.*, Rothwell *et al.*, 1984; Obeso *et al.*, 1985; Shibasaki *et al.*, 1985; Berkovic *et al.*, 1991; Karhu *et al.*, 1994; Mima *et al.*, 1998b; Forss *et al.*, 2001). However, enlarged SEPs/SEFs have also been reported in patients without myoclonus, *e.g.,* in benign rolandic epilepsy (Kubota *et al.*, 2000), and in patients with heterogeneous diagnoses (Micheloyannis *et al.*, 1989; Schmitt *et al.*, 1994). Out of these groups our patients have much in common with PME (Berkovic *et al.*, 1986). In patients with vLINCL$_{Fin}$, frequent myoclonus is seen from the age of 8–10 years, whereas the myoclonus in patients with JNCL is a later feature of the disease, and may be subtle or even undetected. Although the patients with JNCL in this study did not have clinically evident myoclonus, the increased excitability of SI may precede clinical myoclonus. This assumption is supported by the findings of giant SEPs in patients with other NCL forms that classically have myoclonus, including other LINCL forms and some patients with the adult (Kufs) form of NCL (Harden and Pampiglione, 1982; Vercruyssen *et al.*, 1982). On the other hand, SEFs were normal in patients with NES that is not associated with myoclonus.

Compared with the previously published enlarged SEPs/SEFs in other CNS disorders, the most striking feature in JNCL and vLINCL$_{Fin}$ was the enlargement of the earliest cortical deflection, N20m. In the previous reports of patients with enlarged or giant responses, the 20-ms deflections (N20/N20m) have been normal or only slightly enhanced (Rothwell *et al.*, 1984; Obeso *et al.*, 1985; Shibasaki *et al.*, 1985; Uesaka *et al.*, 1993; Karhu *et al.*, 1994; Mima *et al.*, 1998b; Forss *et al.*, 2001). The 30–40-ms deflections (P30/P35m), which were usually most increased, were also

enlarged in the patients with $vLINCL_{Fin}$, but only in one third of the patients with JNCL. These findings suggest that the enlargement of N20m reflects pathophysiological events specific for NCLs. Since MEG measures postsynaptic currents at the population level, the enlargement of the N20m (and possibly also that of P35m) could result from increased synchronous thalamic input to area 3b, which could originate at several levels of the afferent pathway. For example, the responses could be hypersynchronized at the thalamic level: after destruction of a part of thalamic neurons, the remaining neurons could sprout to innervate a greater than normal amount of cortical neurons. In $vLINCL_{Fin}$, thalamic abnormality is constantly seen on MRIs from 6 years onward, and in several patients with JNCL from 10 years onward (Autti *et al.*, 1992, 1996; Holmberg *et al.*, 2000). In histopathological analysis, the thalamus shows severe loss of neurons in $vLINCL_{Fin}$, whereas especially the medial parts of the thalamus are affected in patients with JNCL (Autti *et al.*, 1997a; Tyynelä *et al.*, 1997).

Two recent studies suggest that increased excitability of the sensorimotor cortex can be a distant effect of cerebellar pathology (Kolodziejak *et al.*, 2000; Tijssen *et al.*, 2000). This proposition is also intriguing with respect to some NCL diseases, since late infantile NCL forms have prominent cerebellar atrophy (Dunn, 1987; Autti *et al.*, 1992; Wisniewski *et al.*, 1993; Petersen *et al.*, 1996; Holmberg *et al.*, 2000) and are associated with especially large somatosensory cortical responses (the present study; Harden and Pampiglione, 1982). In juvenile NCL, both the cerebellar atrophy and enhancement of the SEFs manifest themselves later and are not so severe as in late infantile NCL (the present study; Autti *et al.*, 1996; Järvelä *et al.*, 1997). In addition, the lack of NCL specific storage material in the cerebellum of patients with NES (Haltia *et al.*, 1999; Herva *et al.*, 2000) and the normal SEFs found in this study would support this hypothesis.

At the cortical level, the enlarged response strengths could also result from defective presynaptic inhibition or from altered membrane or cable properties of the pyramidal cells. The possible effect of the altered neuronal structure cannot be ruled out in NCL, since in addition to the advanced loss of neurons in the cerebral cortex in $vLINCL_{Fin}$ and JNCL, the remaining cortical neurons have prominent storage (Zeman *et al.*, 1970; Tyynelä *et al.*, 1997). In studies of NCL animal models, defective GABAergic (γ-aminobutyric acid) inhibition has been suggested to underlie neuronal death (March *et al.*, 1995; Walkley *et al.*, 1995). Furthermore, a recent study in mnd mice, a naturally occurring NCL animal model, has shown that subpopulations of interneurons are affected differently (Cooper *et al.*, 1999). According to a model of SEF generators proposed by Wikström *et al.* (1996), the missing P60m deflection in patients with $vLINCL_{Fin}$ may reflect decreased inhibition due to a missing subpopulation of interneurons. This lack of inhibition could leave secondary EPSPs unmasked, and thus allow the N45m deflection to emerge.

An interesting finding in $vLINCL_{Fin}$ was that the responses from SII were also enlarged in four out of five patients. However, SIIi responses were not detected in some patients despite enhanced SIIc responses. This relative lack of SIIi responses could be due to diminished transcallosal connections, since all patients had a significantly thinned corpus callosum. Deficient transcallosal signalling could also explain the fact that responses from the ipsilateral SI, which has been detected in several other patients with PME (Mima *et al.*, 1998b; Forss *et al.*, 2001), were seen only in one of our patients.

In addition to the enlargement of the deflections, neuronal dysfunction was demonstrated in the N20m response latencies. All patients with $vLINCL_{Fin}$ had moderately prolonged N20m latency. In patients with JNCL, the latencies of N20m were slightly prolonged, and in patients with NES the latencies were within normal limits. Similar latency prolongations have been reported in some

patients with PME (Mervaala *et al.*, 1984; Karhu *et al.*, 1994), but not in all of them (Uesaka *et al.*, 1993; Mima *et al.*, 1998b; Forss *et al.*, 2001). In other NCL patients, the latencies have been reported to be normal or slightly delayed (Westmoreland *et al.*, 1979; Vercruyssen *et al.*, 1982). Although peripheral neuropathy is not a usual finding in these NCL forms, the latency prolongation in the patients we studied, may partially reflect slower conduction in the peripheral nerves that show mild changes in axons and myelin (Blatt Lyon, 1975; Goebel *et al.*, 1976b; Elze *et al.*, 1978). Because MEG only measures the cortical responses, the level of conduction delay cannot be determined from MEG results only. Simultaneous recording of SEPs or studies of peripheral nerve conduction with MEG could in the future help to determine whether the latency prolongation is due to peripheral or central nervous system damage. However, it seems that in our patients a significant part of the latency prolongation is due to slower conduction in the CNS, since the white matter is pathologically altered in MRI. This alteration, seen as increased signal intensity on the T2-weighted MRIs, is seen especially in the periventricular areas and the posterior limb of capsula interna in $vLINCL_{Fin}$ patients (Holmberg *et al.*, 2000). Similar findings of white matter rims around the lateral ventricles are also seen in patients with JNCL (Autti *et al.*, 1996), especially in patients homozygous for the major 1.02-kb deletion (Järvelä *et al.*, 1997). At autopsy, the loss of CNS myelin, especially in the periventricular white matter, is prominent in $vLINCL_{Fin}$ and JNCL (Autti *et al.*, 1997a; Tyynelä *et al.*, 1997), and is believed to reflect demyelination secondary to neuronal death (Haltia *et al.*, 1973). In NES, the normal N20m latencies with no white matter alterations in the MRI, are in line with the finding that the white matter seems to be intact at autopsy (Herva *et al.*, 2000).

The time course of the SII activity in patients with $vLINCL_{Fin}$ was different from that of the control subjects. The main SII peaks were generally earlier in patients than in controls, and in addition, the activity onset, determined from the multidipole models, was 20 to 35 ms after the stimulus in patients, often only shortly after the SI activation. This SII activation at short latency most likely reflects the fact that the enhanced activation strength exceeds the detection threshold generally earlier than in controls. Previously similar short-latency SII activation has been suggested by studies using special analysis methods in healthy subjects (Karhu and Tesche, 1999; Korvenoja *et al.*, 1999).

The medication of the patients was not changed because of the measurements, and it is thus possible it had an effect on the SEFs in some of the patients. However, it seems that most of the antiepileptic drugs the patients used do not affect somatosensory evoked responses significantly (Borah and Matheshwari, 1985; Mervaala *et al.*, 1987; Mauguière *et al.*, 1997a; Klamt and Posner, 1999). However, at least some benzodiazepines seem to prolong N20 latency and lower its amplitude (Sloan *et al.*, 1990; Lauer *et al.*, 1994), although not all studies have observed this amplitude change (Koht *et al.*, 1988). In our study, clonazepam was used by one homozygous and one compound heterozygous patient with JNCL, two $vLINCL_{Fin}$ and all NES patients. The N20m latencies in patients with NES were within normal limits, but the effect of clonazepam on the SEF strengths can not be excluded since the strengths were mainly within normal limits. However, at least in the patients with JNCL and $vLINCL_{Fin}$, the use of clonazepam was associated with similarly enlarged SEFs as with the other patients. In fact, the two $vLINCL_{Fin}$ patients on clonazepam had enlarged SEFs with delayed N20m latency as did the patients without clonazepam. It is possible that some of the latency prolongation in them may have resulted from the medication. The compound heterozygous patient with JNCL had delayed N20m latency and increased strength of N20m and P35m, whereas the homozygous patient with JNCL had a normal N20m latency and strength. Thus, although the medication used, especially clonazepam, may have had some effects on the evoked responses, it is likely that the results are not significantly influenced by the patients' medication.

7.4. MRI FINDINGS

The MRIs of patients with JNCL and $vLINCL_{Fin}$ showed features typical for these diseases, as reported earlier (Autti *et al.*, 1992, 1996; Holmberg *et al.*, 2000). This was the first study to report the MRI findings of patients with NES in the context of NCL diseases. The major differences in MRI findings between NES and other NCL forms were the relatively well preserved cerebrum and normal grey to white matter differentiation seen in NES. In two patients, aged 26 and 40 years, there was no atrophy, and this was neither reported in the two previously described patients who were under 16 (Hirvasniemi and Karumo, 1994). Furthermore, the slight enlargement of cerebrospinal fluid spaces seen in the two male patients, at the ages of 30 and 43 years, is similar to that reported in 10–20% of healthy controls of the same age groups (Salonen *et al.*, 1997) and can thus not be considered abnormal or even to be necessarily related to the disease. On the contrary, all patients with $vLINCL_{Fin}$ have cerebral atrophy at least from 9 years onward (Autti *et al.*, 1992; Holmberg *et al.*, 2000), and in JNCL cerebral atrophy is seen in most patients after 15 years of age (Autti *et al.*, 1996; Järvelä *et al.*, 1997). In addition, no signal intensity alterations were seen in patients with NES, in contrast to JNCL and $vLINCL_{Fin}$, which show impressions of pathologically dark thalami after 10 and 6 years, respectively (Autti *et al.*, 1992, 1996; Järvelä *et al.*, 1997; Holmberg *et al.*, 2000). Thus, the normal MRI findings, in addition to the normal SEFs, support the autopsy findings that the overall degree of intraneuronal storage, neuronal loss, and secondary astrocytic and microglial reactions are much less pronounced in NES than in other childhood onset NCL forms (Herva *et al.*, 2000).

However, a slight discrepancy was seen in the cerebellar findings compared with histopathology. At autopsy, the cerebellum of patients with NES is relatively well preserved, showing only modest storage in granular cells (Herva *et al.*, 2000). However, in our MRIs and in previous CT studies, all patients over 10 years had cerebellar atrophy (Hirvasniemi and Karumo, 1994). Similar early and prominent cerebellar atrophy is typical also in patients with $vLINCL_{Fin}$ but not in patients with JNCL (Autti *et al.*, 1992, 1996; Järvelä *et al.*, 1997; Holmberg *et al.*, 2000). However, it is possible that in NES the cerebellar atrophy in MRI reflects the antiepileptic medication (Iivanainen *et al.*, 1977), which in the past has included phenytoin for several years (Hirvasniemi and Karumo, 1994).

7.5. CORRELATION OF THE GENOTYPE WITH THE PHENOTYPE

After the introduction of genetic diagnosis of NCL diseases, it has become apparent that fairly similar clinical phenotypes can be caused by mutations in different genes (Das *et al.*, 1998). All patients here have genetically confirmed diagnoses.

Of the patients with JNCL, 14 were homozygous for the major 1.02-kb deletion that removes introns 6–8 from the transcript and leads to a truncated protein (The International Batten Disease Consortium, 1995), and 10 were compound heterozygous for the major mutation and another *CLN3* mutation. A novel mutation, a 2.8-kb genomic deletion that removes exons 10–13, was described in 6 patients of three families in this study. A distinct, previously described mutation that leads to deletion of exons 10–13, was seen in one patient (The International Batten Disease Consortium, 1995). These mutations are predicted to result in truncated proteins, as is the splice site mutation located in intron 7 that results in a number of aberrantly spliced transcripts. The

phenotypes of the patients with these different mutations resembled each other and also the one that was seen in half of the Finnish patients homozygous for the major 1.02-kb deletion (Järvelä *et al.*, 1997). Thus, clinically the compound heterozygous patients with mutations predicted to lead to truncated proteins had a similar delayed classic disease course as a half of the homozygous patients. Also the MRI findings were similar in these two groups, including normal cerebrum or slight cerebral atrophy in the younger patients and cerebellar atrophy manifestation in older patients (Autti *et al.*, 1996; Järvelä *et al.*, 1997). However, the cerebral atrophy and brain tissue signal intensity alterations may manifest themselves later in the compound heterozygous patients than in the homozygous patients (Järvelä *et al.*, 1997). In contrast to these structural alterations, in the present study the timing and severity of the SEF abnormality did not differ between these two genetically different groups, suggesting that the onset of neuronal dysfunction is not markedly different between them. Similarly, the earlier report showed that the onset of epilepsy and blindness were highly concordant among these groups (Järvelä *et al.*, 1997). Furthermore, the environmental factors and modifying genes have been demonstrated to play an important role in the individual JNCL phenotypes, since the patients homozygous for the 1.02-kb deletions may have the classic or delayed classic disease course and even the intrafamiliar variability is large (Järvelä *et al.*, 1997). These observations suggest that although all mutations, which are predicted to result in truncated proteins, resulted in the delayed classic phenotype in this study, it may still be that at the cellular level, the mutations lead to similar dysfunction of the CLN3 protein as the major 1.02-kb deletion.

The proteins affected by the missense mutations may have some residual function, at least enough to prolong the course of the disease. The missense mutation in exon 11 resulting in a change of one amino acid (Gly295Lys) seems to cause an extremely mild disease course among the patients affected with JNCL. Our 30-year-old patient as well as two previously reported siblings of German origin (Wisniewski *et al.*, 1998) have been blind from the ages of 12–13 years, but otherwise clinically asymptomatic until at least their forties. The MRI of our patient showed slight cerebral atrophy, no signal intensity changes and moderate cerebellar atrophy. In SEFs, he had only a slightly enlarged N20m deflection. The other missense mutation (Arg334His in exon 13) seemed to result in a protracted disease in our patient, although in three previously reported patients (Munroe *et al.*, 1997) it resulted in classic JNCL phenotype. However, our patient was only 13 years old, and it is not impossible that he will develop motor and mental symptoms later. His MRI and SEF findings were normal, which is, however, common in JNCL patients at this age. However, the phenotypic heterogeneity between these patients with an identical compound heterozygous mutation in the *CLN3* gene may be due to genes that modify the phenotype, but the effect of early symptomatic medication and rehabilitation should not be overlooked.

In $vLINCL_{Fin}$, no genotype-phenotype correlation has been found between the different combinations of the known mutations, and it has thus been suggested that each mutation severely disturbs the normal function of the CLN5 protein (Holmberg *et al.*, 2000). In the present study, the patients with the mutations predicted to result in the most truncated proteins had the most enlarged responses, indicating thus a subtle link between genotype and phenotype in $vLINCL_{Fin}$. However, the small number of patients with different genotypes prevents any definitive conclusions on the effect of the genotype on the phenotype. Nevertheless, as expected, it was shown that none of the mutations had left the function of neurons on the somatosensory system intact. Further studies, using a larger cross-sectional patient group or a longitudinal study of the same patients, could clarify whether any significant neurophysiological phenotype-genotype correlations exist.

8. CONCLUSIONS

The present study reports for the first time median nerve SEFs in a relatively large number of children and adolescents, indicating that the SEFs in healthy children over 6 years of age are morphologically similar to those in adults. Novel information about the development of SEFs, *e.g.*, that both the N20m and P35m may become stronger with age was demonstrated. Further studies of SEFs in a large number of healthy children and adolescents may help to clarify the process of maturation of the peripheral and central nervous systems in humans.

The enlarged SEFs in JNCL and $vLINCL_{Fin}$ suggest that these diseases lead to a disturbed balance between inhibition and excitation in the somatosensory network. Furthermore, this increased excitability of the somatosensory system extended to the opercular SII areas in patients with $vLINCL_{Fin}$.

The *CLN3* gene defects predicted to give rise to truncated proteins resulted in classic or delayed classic JNCL. Both phenotypes were associated with age-related increase in SEF strengths, indicating that all underlying mutations similarly affect the functions of the neurons of the somatosensory system. However, the patients who were compound heterozygous for the 1.02-kb deletion and two different missense mutations had an atypically protracted disease course, indicating that these mutations allow CLN3 protein with some preserved function to be encoded.

In NES, the normal SEFs, the well-preserved cerebrum and normal signal intensities of the brain matter on T2-weighted MRIs suggest that the underlying *CLN8* gene defect does not have as detrimental effects on the neurons of the somatosensory network as the *CLN3* and *CLN5* gene defects.

We conclude that studying evoked responses with MEG can bring new information about the neuronal processing in a degenerative brain disorder like NCL, the progression of which is associated with alterations in the conductivities of the structures between the active brain area and the measurement device. The novel information about the altered neuronal processing in NCL obtained with MEG can, in addition to the findings of the ongoing cell biological and animal model studies, help to clarify the pathogenesis underlying NCL.

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10. REFERENCES

Åberg LE, Bäckman M, Kirveskari E, Santavuori P. Epilepsy and antiepileptic drug therapy in juvenile neuronal ceroid lipofuscinosis. Epilepsia 2000; 41: 1296–1302.

Ahonen AI, Hämäläinen MS, Kajola MJ, Knuutila JET, Laine PP, Lounasmaa OV, Parkkonen LT, Simola JT, Tesche CD. 122-channel SQUID instrument for investigating the magnetic signals from the human brain. Physica Scripta 1993; T49: 198–205.

Alberstone CD, Skirboll SL, Benzel EC, Sanders JA, Hart BL, Baldwin NG, Tessman CL, Davis JT, Lee RR. Magnetic source imaging and brain surgery: presurgical and intraoperative planning in 26 patients. J Neurosurg 2000; 92: 79–90.

Allison T, Hume AL, Wood CC, Goff WR. Developmental and aging changes in somatosensory, auditory and visual evoked potentials. Electroencephalogr Clin Neurophysiol 1984; 58: 14–24.

Allison T, McCarthy G, Wood CC, Darcey TM, Spencer DD, Williamson PD. Human cortical potentials evoked by stimulation of the median nerve. I. Cytoarchitectonic areas generating short-latency activity. J Neurophysiol 1989; 62: 694–710.

Allison T, Wood CC, Goff WR. Brain stem auditory, pattern-reversal visual, and short-latency somatosensory evoked potentials: latencies in relation to age, sex, and brain and body size. Electroencephalogr Clin Neurophysiol 1983; 55: 619–636.

Araki A, Takada A, Yasuhara A, Kobayashi Y. The effects of stimulus rates on the amplitude of median nerve somatosensory evoked potentials: the developmental change. Brain Dev 1999; 21: 118–121.

Autti T, Raininko R, Launes J, Nuutila A, Santavuori P. Jansky-Bielschowsky variant disease: CT, MRI, and SPECT findings. Pediatr Neurol 1992; 8: 121–126.

Autti T, Raininko R, Santavuori P, Vanhanen SL, Poutanen VP, Haltia M. MRI of neuronal ceroid lipofuscinosis. II. Postmortem MRI and histopathological study of the brain in 16 cases of neuronal ceroid lipofuscinosis of juvenile or late infantile type. Neuroradiology 1997a; 39: 371–377.

Autti T, Raininko R, Vanhanen SL, Kallio M, Santavuori P. MRI of the normal brain from early childhood to middle age. I. Appearances on T2- and proton density-weighted images and occurrence of incidental high-signal foci. Neuroradiology 1994; 36: 644–648.

Autti T, Raininko R, Vanhanen SL, Santavuori P. MRI of neuronal ceroid lipofuscinosis. I. Cranial MRI of 30 patients with juvenile neuronal ceroid lipofuscinosis. Neuroradiology 1996; 38: 476–482.

Autti T, Raininko R, Vanhanen S-L, Santavuori P. Magnetic resonance techniques in neuronal ceroid lipofuscinoses and some other lysosomal diseases affecting the brain. Curr Opion Neurol 1997b; 10: 519–524.

Bartel P, Conradie J, Robinson E, Prinsloo J, Becker P. The relationship between median nerve somatosensory evoked potential latencies and age and growth parameters in young children. Electroencephalogr Clin Neurophysiol 1987; 68: 180–186.

Baumgartner C, Sutherling WW, Di S, Barth DS. Spatiotemporal modeling of cerebral evoked magnetic fields to median nerve stimulation. Electroencephalogr Clin Neurophysiol 1991; 79: 27–35.

Berkovic SF, Andermann F, Andermann E, Carpenter S, Wolfe L. Kufs disease: clinical features and forms. Am J Med Gen 1988; 5: 105–109.

Berkovic SF, Andermann F, Carpenter S, Wolfe LF. Progressive myoclonus epilepsies: specific causes and diagnosis. New Eng J Med 1986; 5: 296–305.

Berkovic SF, So NK, Andermann F. Progressive myoclonus epilepsies: clinical and neurophysiological diagnosis. J Clin Neurophysiol 1991; 8: 261–274.

Blatt Lyon B. Peripheral nerve involvement in Batten–Spielmeyer–Vogt's disease. J Neurol Neurosurg Psych 1975; 38: 175–179.

Boor R, Goebel B. Maturation of near-field and far-field somatosensory evoked potentials after median nerve stimulation in children under 4 years of age. Clin Neurophysiol 2000; 111: 1070–1081.

Boor R, Goebel B, Doepp M, Taylor MJ. Somatosensory evoked potentials after posterior tibial nerve stimulation — normative data in children. Eur J Paed Neurol 1998; 2: 145–152.

Borah NC, Matheshwari MC. Effect of antiepileptic drugs on short-latency somatosensory evoked potentials. Acta Neurol Scand 1985; 71: 331–333.

Braak H, Goebel HH. Loss of pigment-laden stellate cells: A severe alteration of the isocortex in juvenile neuronal ceroid-lipofuscinosis. Acta neuropathol (Berl) 1978; 42: 53–57.

Braak H, Goebel HH. Pigmentoarchitectonic pathology of the isocortex in juvenile neuronal ceroid-lipofuscinosis: Axonal enlargements in layer IIIab and cell loss in layer V. Acta neuropathol (Berl) 1979; 46: 79–83.

Brenner D, Lipton J, Kaufman L, Williamson SJ. Somatically evoked magnetic fields of the human brain. Science 1978; 199: 81–83.

Brockmann K, Pouwels PJW, Christen H-J, Frahm J, Hanefeld F. Localized proton magnetic resonance spectroscopy of cerebral metabolic disturbances in children with neuronal ceroid lipofuscinosis. Neuropediatrics 1996; 27: 242–248.

Camp LA, Hofman SL. Purification and properties of a palmitoyl-protein thioesterase that cleaves palmitate from H-Ras. J Biol Chem 1993; 268: 22566–22574.

Chiappa KH. Evoked Potentials in Clinical Medicine, Raven Press Ltd, New York, 1989.

Claussen M, Heim P, Knispel J, Goebel HH, Kohlschütter A. Incidence of neuronal ceroid-lipofuscinoses in West Germany: variation of a method for studying autosomal recessive disorders. Am J Med Gen 1992; 42: 536–538.

Cooper JD, Messer A, Feng AK, Chua-Couzens J, Mobley WC. Apparent loss and hypertrophy of interneurons in a mouse model of neuronal ceroid lipofuscinosis: evidence for partial response to insulin-like growth factor-1 treatment. J Neurosci 1999; 19: 2556–2567.

Cracco JB, Bosch VV, Cracco RQ. Cerebral and spinal somatosensory evoked potentials in children with CNS degenerative disease. Electroencephalogr Clin Neurophysiol 1980; 49: 437–445.

Das AK, Becerra CHR, Yi W, Lu J-Y, Siakotos AN, Wisniewski KE, Hofmann SL. Molecular genetics of palmitoyl-protein thioesterase deficiency in the U.S. J Clin Invest 1998; 102: 361–370.

Desmedt JE, Brunko E, Debecker J. Maturation of the somatosensory evoked potentials in normal infants and children, with special reference to the early N_1 component. Electroencephalogr Clin Neurophysiol 1976; 40: 43–58.

Dunn DW. CT in ceroid lipofuscinosis. Neurology 1987; 37: 1025–1026.

Elleder M, Lake BD, Goebel HH, Rapola J, Haltia M, Carpenter S. Definitions of the ultrastructural patterns found in NCL. In: Goebel HH, Mole SE, Lake BD, eds. The Neuronal Ceroid Lipofuscinoses (Batten Disease). IOS Press: Amsterdam, 1999: 5–7.

Elze K-L, Koepp P, Lagenstein I, Steinhausen H-C, Colmant HJ, Schwendemann G. Juvenile type of generalized ceroid-lipofuscinosis (Spielmeyer-Sjögren syndrome). Neuropädiatrie 1978; 9: 3–27.

Forss N, Hari R, Salmelin R, Ahonen A, Hämäläinen M, Kajola M, Knuutila J, Simola J. Activation of the human posterior parietal cortex by median nerve stimulation. Exp Brain Res 1994a; 99: 309–315.

Forss N, Merlet I, Vanni S, Hämäläinen M, Mauguière F, Hari R. Activation of human mesial cortex during somatosensory target detection task. Brain Res 1996; 734: 229–235.

Forss N, Salmelin R, Hari R. Comparison of somatosensory evoked fields to airpuff and electric stimuli. Electroencephalogr Clin Neurophysiol 1994b; 92: 510–517.

Forss N, Silèn T, Karjalainen T. Lack of activation of human secondary somatosensory cortex in Unverricht-Lundborg type of progressive myoclonus epilepsy. Ann Neurol 2001; 49: 90–97.

Goebel HH. The neuronal ceroid-lipofuscinoses. J Child Neurol 1995; 10: 424–437.

Goebel HH, Pilz H, Gullotta F. The protracted form of juvenile neuronal ceroid-lipofuscinosis. Acta Neuropath (Berl) 1976a; 36: 393–396.

Goebel HH, Zeman W, Pilz H. Ultrastructural investigations of peripheral nerves in neuronal ceroid-lipofuscinoses (NCL). J Neurol 1976b; 213: 295–303.

Hall NA, Lake BD, Dewji NN, Patrick AD. Lysosomal storage of subunit c of mitochondrial ATP synthase in Batten's disease (ceroid-lipofuscinosis). J Biochem 1991; 275: 269–272.

Haltia M, Rapola J, Santavuori P. Infantile type of so-called neuronal ceroid-lipofuscinosis. Histological and electron microscopic studies. Acta Neuropathol (Berl) 1973; 26: 157–170.

Haltia M, Tyynelä J, Hirvasniemi A, Herva R, Ranta US, Lehesjoki A-E. CLN8 Northern Epilepsy. In: Goebel HH, Mole SE, Lake BD, eds. The Neuronal Ceroid Lipofuscinoses (Batten Disease). IOS Press: Amsterdam, 1999: 117–121.

Hämäläinen M, Hari R, Ilmoniemi RJ, Knuutila J, Lounasmaa OV. Magnetoencephalography — theory, instrumentation, and applications to noninvasive studies of the working human brain. Rev Mod Phys 1993; 65: 414–497.

Hämäläinen MS, Ilmoniemi RJ. Interpreting magnetic fields of the brain: minimum norm estimates. Med Biol Eng Comput 1994; 32: 35–42.

Harden A, Pampiglione G. Neurophysiological studies (EEG/ERG/VEP/SEP) in 88 children with so-called neuronal ceroid lipofuscinosis. In: Armstrong D, Koppang N, Rider JA, eds. Ceroid-lipofuscinosis (Batten's disease). Elsevier Biomedical Press: Amsterdam, 1982: 61–68.

Hari R, Forss N. Magnetoencephalography in the study of human somatosensory cortical processing. Phil Trans R Soc Lond B 1999; 354: 1145–1154.

Hari R, Hämäläinen H, Hämäläinen M, Kekoni J, Sams M, Tiihonen J. Separate finger representations at the human second somatosensory cortex. Neurosci 1990; 37: 245–249.

Hari R, Hämäläinen M, Kaukoranta E, Reinikainen K, Teszner D. Neuromagnetic responses from the second somatosensory cortex in man. Acta Neurol Scand 1983; 68: 207–212.

Hari R, Reinikainen K, Kaukoranta E, Hämäläinen M, Ilmoniemi R, Penttinen A, Salminen J, Teszner D. Somatosensory evoked cerebral magnetic fields from SI and SII in man. Electroencephalogr Clin Neurophysiol 1984; 57: 254–263.

Hari R, Karhu J, Hämäläinen M, Knuutila J, Salonen O, Sams M, Vilkman V. Functional organization of the human first and second somatosensory cortices: a neuromagnetic study. Eur J Neurosci 1993; 5: 724–734.

Hashimoto I, Kimura T, Sakuma K, Iguchi Y, Saito Y, Terasaki O, Fukushima T. Dynamic mediolateral activation of the pyramidal cell population in human somatosensory 3b area can be visualized by magnetic recordings. Neurosci Lett 2000; 280: 25–28.

Haskell RE, Derksen TA, Davidson BL. Intracellular trafficking of the JNCL protein CLN3. Mol Gen Metab 1999; 66: 253–260.

Hellsten E, Vesa J, Olkkonen VM, Jalanko A, Peltonen L. Human palmitoyl protein thioesterase: evidence for lysosomal targeting of the enzyme and disturbed cellular routing in infantile neuronal ceroid lipofuscinosis. EMBO J 1996; 15: 5240–5245.

Herva R, Tyynelä J, Hirvasniemi A, Syrjäkallio-Ylitalo M, Haltia M. Northern epilepsy: a novel form of neuronal ceroid-lipofuscinosis. Brain Pathol 2000; 10: 215–222.

Hirvasniemi A, Herrala P, Leisti J. Northern epilepsy syndrome: clinical course and the effect of medication on seizures. Epilepsia 1995; 36: 792–797.

Hirvasniemi A, Karumo J. Neuroradiological findings in the northern epilepsy syndrome. Acta Neurol Scand 1994; 90: 388–393.

Hirvasniemi A, Lang H, Lehesjoki A-E, Leisti J. Northern epilepsy syndrome: an inherited childhood onset epilepsy with associated mental deterioration. J Med Genet 1994; 31: 177–182.

Hofman I, Kohlschütter A, Santavuori P, Gottlob I, Goebel HH, Lake BD, Schutgens RBH, Greene NDE, Leung K-Y, Mitchison HM, Munroe PB, Taschner PEM. CLN3 Juvenile NCL. In: Goebel HH, Mole SE, Lake BD, eds. The Neuronal Ceroid Lipofuscinoses (Batten Disease). IOS Press: Amsterdam, 1999: 55–76.

Holmberg V, Lauronen L, Autti T, Santavuori P, Savukoski M, Uvebrant P, Hofman I, Peltonen L, Järvelä I. Phenotype-genotype correlation in eight patients with Finnish variant late infantile NCL (CLN5). Neurology 2000; 55: 579–581.

Hume AL, Cant BR, Shaw NA, Cowan JC. Central somatosensory conduction time from 10 to 79 years. Electroencephalogr Clin Neurophysiol 1982; 54: 49–54.

Huttunen J. Magnetic cortical responses evoked by tactile stimulation of the middle finger in man. Pflugers Archiv - Eur J Physiol 1986; 407: 129–133.

Huttunen J. Effects of stimulus intensity on frontal, central and parietal somatosensory evoked potentials after median nerve stimulation. Electromyograph Clin Neurophysiol 1995; 35: 217–223.

Huttunen J. Does the P35m SEF deflection really come from the motor cortex? Electroencephalogr Clin Neurophysiol 1997; 104: 101–102.

Huttunen J, Ahlfors S, Hari R. Interaction of afferent impulses in the human primary sensorimotor cortex. Electroencephalogr Clin Neurophysiol 1992; 82: 176–181.

Huttunen J, Hari R, Leinonen L. Cerebral magnetic responses to stimulation of ulnar and median nerves. Electroencephalogr Clin Neurophysiol 1987a; 66: 391–400.

Huttunen J, Kaukoranta E, Hari R. Cerebral magnetic responses to stimulation of tibial and sural nerves. J Neurol Sci 1987b; 79: 43–54.

Huttunen J, Wikström H, Korvenoja A, Seppäläinen A-M, Aronen H, Ilmoniemi RJ. Significance of the second somatosensory cortex in sensorimotor integration: enhancement of sensory responses during finger movements. Neuroreport 1996; 7: 1009–1012.

Huttunen J, Wikström H, Salonen O, Ilmoniemi RJ. Human somatosensory cortical activation strengths: comparison between males and females and age-related changes. Brain Res 1999; 818: 196–203.

Iivanainen M, Viukari M, Helle E-P. Cerebellar atrophy in phenytoin-treated mentally retarded epileptics. Epilepsia 1977; 18: 375–386.

Ilmoniemi RJ. Estimates of neuronal current distributions. Acta Otolaryngol (Stockh) 1991; 491: 80–87.

Ishibashi H, Tobimatsu S, Shigeto H, Morioka T, Yamamoto T, Fukui M. Differential interaction of somatosensory inputs in the human primary sensory cortex: a magnetoencephalographic study. Clin Neurophysiol 2000; 111: 1095–1102.

Järvelä I, Autti T, Lamminranta S, Åberg L, Raininko R, Santavuori P. Clinical and magnetic resonance imaging findings in Batten disease: analysis of the major mutation (1.02-kb deletion). Ann Neurol 1997; 42: 799–802.

Järvelä I, Lehtovirta M, Tikkanen R, Kyttälä A, Jalanko A. Defective intracellular transport of CLN3 is the molecular basis of Batten disease (JNCL). Hum Mol Gen 1999; 8: 1091–1098.

Järvelä I, Mitchison HM, Munroe PB, O'Rawe AM, Mole SE, Syvänen AC. Rapid diagnostic test for the major mutation underlying Batten disease. J Med Gen 1996; 33: 1041–1042.

Järvelä I, Sainio M, Rantamäki T, Olkkonen VM, Carpén O, Peltonen L, Jalanko A. Biosynthesis and intracellular targeting of the CLN3 protein defective in Batten disease. Hum Mol Gen 1998; 7: 85–90.

Jousmäki V, Forss N. Effects of stimulus intensity on signals from human somatosensory cortices. Neuroreport 1998; 9: 3427–3431.

Kahle W, Leonhardt H, Platzer W. Color atlas and textbook of human anatomy. Volume 3: Nervous System and Sensory Organs, Georg Thieme Verlag, Stuttgart, 1986.

Kakigi R, Shibasaki H. Effects of age, gender and stimulus side on scalp topography of somatosensory evoked potentials following median nerve stimulation. J Clin Neurophysiol 1991; 8: 320–330.

Kandel ER, Schwartz JH, Jessell TM. Principles of Neural Science, McGraw Hill, New York, 2000.

Karhu J, Hari R, Paetau R, Kajola M, Mervaala E. Cortical reactivity in progressive myoclonus epilepsy. Electroencephalogr Clin Neurophysiol 1994; 90: 93–102.

Karhu J, Tesche CD. Simultaneous early processing of sensory input in human primary (SI) and secondary (SII) somatosensory cortices. J Neurophysiol 1999; 81: 2017–2025.

Kaukoranta E, Hämäläinen M, Sarvas J, Hari R. Mixed and sensory nerve stimulations activate different cytoarchitectonic areas in the human primary somatosensory cortex SI. Exp Brain Res 1986; 63: 60–66.

Kawamura T, Nakasato N, Seki K, Kanno A, Fujita S, Fujiwara S, Yoshimoto T. Neuromagnetic evidence of pre- and post-central cortical sources of somatosensory evoked responses. Electroencephalogr Clin Neurophysiol 1996; 100: 44–50.

Kirveskari E, Partinen M, Salmi T, Sainio K, Telakivi T, Hämäläinen M, Larsen A, Santavuori P. Sleep alterations in juvenile neuronal ceroid-lipofuscinosis. Pediatr Neurol 2000; 22: 347–354.

Klamt JG, Posner J. Effects of lamotrigine on pain-induced chemo-somatosensory evoked potentials. Anaesthesia 1999; 54: 774–777.

Koht A, Schütz W, Schmidt G, Schramm J, Watanabe E. Effects of etomidate, midazolam, and thiopental on median nerve somatosensory evoked potentials and the additive effects of fentanyl and nitrous oxide. Anesth Analg 1988; 67: 435–441.

Kolodziejak A, Dziduszko J, Niechaj A, Tarnecki R. Influence of acute cerebellar lesions on somatosensory evoked potentials (SEPs) in cats. J Physiol Pharmacol 2000; 51: 41–55.

Korvenoja A, Huttunen J, Salli E, Pohjonen H, Martinkauppi S, Palva JM, Lauronen L, Virtanen J, Ilmoniemi RJ, Aronen HJ. Activation of multiple cortical areas in response to somatosensory stimulation: combined magnetoencephalographic and functional magnetic resonance imaging. Hum Brain Mapping 1999; 8: 13–27.

Korvenoja A, Wikström H, Huttunen J, Virtanen J, Laine P, Aronen HJ, Seppäläinen A-M, Ilmoniemi RJ. Activation of ipsilateral primary sensorimotor cortex by median nerve stimulation. Neuroreport 1995; 6: 2589–2593.

Kubota M, Takeshita K, Sakakihara Y, Yanagisawa M. Magnetoencephalographic study of giant somatosensory evoked responses in patients with rolandic epilepsy. J Child Neurol 2000; 15: 370–379.

Kumabe T, Nakasato N, Inoue T, Yoshimoto T. Primary thumb sensory cortex located at the lateral shoulder of the inverted omega-shape on the axial images of the central sulcus. Neurol Med Chir 2000; 40: 393–403.

Lagenstein I, Schwendemann G, Kühne D, Koepp P, Stahnke N, Sternowsky H-J. Neuronal ceroidlipofuscinosis: CCT findings in fourteen patients. Acta Paediatr Scand 1981; 70: 857–860.

Laissy JP, Patrux B, Duchateau C, Hannequin D, Hugonet P, Ait-Yahia H, Thiebot J. Midsagittal MR measurements of the corpus callosum in healthy subjects and diseased patients: a prospective survey. AJNR 1993; 14: 145–154.

Lang AH, Hirvasniemi A, Siivola J. Neurophysiological findings in the northern epilepsy syndrome. Acta Neurol Scand 1997; 95: 1–8.

Larsen A, Sainio K, Åberg L, Santavuori P. Electroencephalography in juvenile neuronal ceroid lipofuscinosis: visual and quantitative analysis. Eur J Paed Neurol 2001; 5: 179–183.

Lauer K, Munshi C, Larson S. The effect of midazolam on median nerve somatosensory evoked potentials. J Clin Monit 1994; 10: 181–184.

Launes J, Heiskala H, Nikkinen P, Santavuori P. Brain perfusion SPECT in juvenile neuronal ceroid lipofuscinosis. Neuropediatrics 1996; 27: 84–87.

Libert J, Martin J-J, Ceuterick C. Protracted and atypical forms of ceroid-lipofuscinosis. In: Armstrong D, Koppang N, Rider JA, eds. Ceroid-lipofuscinosis (Batten's disease). Elsevier, Biomedical Press: Amsterdam, 1982: 45–59.

Lin Y-Y, Simões C, Forss N, Hari R. Differential effects of muscle contraction from various body parts on neuromagnetic somatosensory responses. Neuroimage 2000; 11: 334–340.

Lonka L, Kyttälä A, Ranta S, Jalanko A, Lehesjoki A-E. The neuronal ceroid lipofuscinosis CLN8 membrane protein is a resident of the endoplasmic reticulum. Hum Mol Gen 2000; 9: 1691–1697.

Lu J-Y, Verkruyse LA, Hofmann SL. Lipid thioesters derived from acylated proteins accumulate in infantile neuronal ceroid lipofuscinosis: correction of the defect in lymphoblasts by recombinant palmitoyl-protein thioesterase. Proc Natl Acad Sci 1996; 93: 10046–10050.

Maeda K, Kakigi R, Hoshiyama M, Koyama S. Topography of the secondary somatosensory cortex in humans: a magnetoencephalographic study. Neuroreport 1999; 10: 301–306.

Mäkelä JP, Kirveskari E, Seppä M, Hämäläinen M, Forss N, Avikainen S, Salonen O, Salenius S, Kovala T, Randell T, Jääskeläinen J, Hari R. Three-dimensional integration of brain anatomy and function to facilitate intraoperative navigation around the sensorimotor strip. Hum Brain Mapping 2001; 12: 180–192.

March PA, Wurzelmann S, Walkley SU. Morphological alterations in neocortical and cerebellar GABAergic neurons in a canine model of juvenile Batten disease. Am J Med Gen 1995; 57: 204–212.

Mattigk G. Die Medianus-evozierten Potentiale bei gesunden Kindern und Jugendlichen: Normverte. Z EEG-EMG 1991; 22: 147–151.

Mauguière F, Chauve P, Dewailly J, Dousse N, the PMS Study Multicenter Group. No effect of long-term vigabatrin treatment on central nervous system conduction in patients with refractory epilepsy: results of a multicenter study of somatosensory and visual evoked potentials. Epilepsia 1997a; 38: 301–308.

Mauguière F, Merlet I, Forss N, Vanni S, Jousmäki V, Adeleine P, Hari R. Activation of a distributed somatosensory cortical network in the human brain. A dipole modelling study of magnetic fields evoked by median nerve stimulation. Part I: Location and activation timing of SEF sources. Electroencephalogr Clin Neurophysiol 1997b; 104: 281–289.

Mauguière F, Merlet I, Forss N, Vanni S, Jousmäki V, Adeleine P, Hari R. Activation of a distributed somatosensory cortical network in the human brain: a dipole modelling study of magnetic fields evoked by median nerve stimulation. Part II: effects of stimulus rate, attention and stimulus detection. Electroencephalogr Clin Neurophysiol 1997c; 104: 290–295.

Mertens M, Lütkenhöner B. Efficient neuromagnetic determination of landmarks in the somatosensory cortex. Clin Neurophysiol 2000; 111: 1478–1487.

Mervaala E, Keränen T, Tiihonen P, Riekkinen P. The effects of carbamazepine and sodium valproate on SEPs and BAEPs. Electroencephalogr Clin Neurophysiol 1987; 68: 475–478.

Mervaala E, Partanen JV, Keränen T, Penttilä M, Riekkinen P. Prolonged cortical somatosensory evoked potential latencies in progressive myoclonus epilepsy. J Neurol Sci 1984; 64: 131–135.

Micheloyannis J, Samara C, Liakakos T. Giant somatosensory evoked potentials in children without myoclonic epilepsy. Acta Neurol Scand 1989; 79: 146–149.

Mima T, Ikeda A, Nagamine T, Yazawa S, Kunieda T, Mikuni N, Taki W, Kimura J, Shibasaki H. Human second somatosensory area: subdural and magnetoencephalographic recording of somatosensory evoked responses. J Neurol Neurosurg Psych 1997; 63: 501–505.

Mima T, Nagamine T, Nakamura K, Shibasaki H. Attention modulates both primary and second somatosensory cortical activities in humans: a magnetoencephalographic study. J Neurophysiol 1998a; 80: 2215–2221.

Mima T, Nagamine T, Nishitani N, Mikuni N, Ikeda A, Fukuyama H, Takigawa T, Kimura J, Shibasaki H. Cortical myoclonus: sensorimotor hyperexcitability. Neurology 1998b; 50: 933–942.

Minassian BA, Otsubo H, Weiss S, Elliott I, Rutka JT, Snead OC, 3rd. Magnetoencephalographic localization in pediatric epilepsy surgery: comparison with invasive intracranial electroencephalography. Ann Neurol 1999; 46: 627–633.

Mitchison HM, Munroe PB, O'Rawe AM, Taschner PEM, de Vos N, Kremmidiotis G, Lensink I, Munk AC, D'Arigo KL, Anderson JW, Lerner TJ, Moyzis RK, Callen DF, Breuning MH, Doggett NA, Gardiner RM, Mole SE. Genomic structure and complete nucleotide sequence of the Batten disease gene, *CLN3*. Genomics 1997a; 40: 346–350.

Mitchison HM, Taschner PEM, Kremmidiotis G, Callen DF, Doggett NA, Lerner TJ, Janes RB, Wallace BA, Munroe PB, O'Rawe AM, Gardiner RM, Mole SE. Structure of the CLN3 gene and predicted structure, location and function of CLN3 protein. Neuropediatrics 1997b; 28: 12–14.

Mole SE. Batten's disease: eight genes and still counting? Lancet 1999; 354: 443–445.

Müller K, Ebner B, Hömberg V. Maturation of fastest afferent and efferent central and peripheral pathways: no evidence for a constancy of central conduction delays. Neurosci Lett 1994; 166: 9–12.

Munroe PB, Mitchison HM, O'Rawe AM, Anderson JW, Boustany R-M, Lerner TJ, Taschner PEM, de Vos N, Breuning MH, Gardiner RM, Mole SE. Spectrum of mutations in the Batten disease gene, *CLN3*. Am J Hum Genet 1997; 61: 310–316.

Naka D, Kakigi R, Koyama S, Xiang J, Suzuki H. Effects of tactile interference stimulation on somatosensory evoked magnetic fields following tibial nerve stimulation. Electroencephalogr Clin Neurophysiol 1998; 109: 168–177.

Nakagawa H, Namima T, Aizawa M, Uchi K, Kaiho Y, Yoshikawa K, Orikasa S, Nakasato N. Somatosensory evoked magnetic fields elicited by dorsal penile, posterior tibial and median nerve stimulation. Electroencephalogr Clin Neurophysiol 1998; 108: 57–61.

Nakamura A, Yamada T, Goto A, Kato T, Ito K, Abe Y, Kachi T, Kakigi R. Somatosensory homunculus as drawn by MEG. Neuroimage 1998; 7: 377–386.

Nardocci N, Verga ML, Binelli S, Zorzi G, Angelini L, Bugiani O. Neuronal ceroid-lipofuscinosis: a clinical and morphological study of 19 patients. Am J Med Gen 1995; 57: 137–141.

Narici L, Modena I, Opsomer RJ, Pizzella V, Romani GL, Torrioli G, Traversa R, Rossini PM. Neuromagnetic somatosensory homunculus: a non-invasive approach in humans. Neurosci Lett 1991; 121: 51–54.

Obeso JA, Rothwell JC, Marsden CD. The spectrum of cortical myoclonus. From focal reflex jerks to spontaneous motor epilepsy. Brain 1985; 108: 193–224.

Okada YC, Tanenbaum R, Williamson SJ, Kaufman L. Somatotopic organization of the human somatosensory cortex revealed by neuromagnetic measurements. Exp Brain Res 1984; 56: 197–205.

Paetau R, Granström M-L, Blomstedt G, Jousmäki V, Korkman M, Liukkonen E. Magnetoencephalography in presurgical evaluation of children with the Landau-Kleffner Syndrome. Epilepsia 1999; 40: 326–335.

Paetau R, Hämäläinen M, Hari R, Kajola M, Karhu J, Larsen TA, Lindahl E, Salonen O. Magnetoencephalographic evaluation of children and adolescents with intractable epilepsy. Epilepsia 1994; 35: 275–284.

Palmer DN, Fearnley IM, Walker JE, Hall NA, Lake BD, Wolfe LS, Haltia M, Martinus RD, Jolly RD. Mitochondrial ATP synthase subunit c storage in the ceroid-lipofuscinoses (Batten disease). Am J Med Gen 1992; 42: 561–567.

Pampiglione G, Harden A. So-called neuronal ceroid lipofuscinosis. Neurophysiological studies in 60 children. J Neurol Neurosurg Psych 1977; 40: 323–330.

Pearce DA, Ferea T, Nosel SA, Das B, Sherman F. Action of *BTN1*, the yeast orthologue of the gene mutated in Batten disease. Nature Genet 1999; 22: 55–58.

Petersen B, Handwerker M, Huppertz H-I. Neuroradiological findings in classical late infantile neuronal ceroid-lipofuscinosis. Pediatr Neurol 1996; 15: 344–347.

Philippart M, Messa C, Chugani HT. Spielmeyer–Vogt (Batten, Spielmeyer–Sjögren) disease. Distinctive patterns of cerebral glucose utilization. Brain 1994; 117: 1085–1092.

Raininko R, Autti T, Vanhanen SL, Ylikoski A, Erkinjuntti T, Santavuori P. The normal brain stem from infancy to old age. A morphometric MRI study. Neuroradiology 1994; 36: 364–368.

Raininko R, Santavuori P, Heiskala H, Sainio K, Palo J. CT findings in neuronal ceroid lipofuscinoses. Neuropediatrics 1990; 21: 95–101.

Ranta S, Zhang Y, Ross B, Lonka L, Takkunen E, Messer A, Sharp J, Wheeler R, Kusumi K, Mole S, Liu W, Soares MB, Bonaldo MF, Hirvasniemi A, de la Chapelle A, Gilliam TC, Lehesjoki A-E. The neuronal ceroid lipofuscinoses in human EPMR and *mnd* mutant mice are associated with mutations in CLN8. Nature Genet 1999; 23: 233–236.

Rapola J. Neuronal ceroid-lipofuscinoses in childhood. In: Landing BH, Haust MD, Bernstein J, Rosenberg HS, eds. Genetic Metabolic Diseases. Perspect Pediatr Pathol. Basel, Karger, 1993; 17: 7–44.

Rossini PM, Deuschl G, Pizzella V, Tecchio F, Pasquarelli A, Feifel E, Romani GL, Lücking CH. Topography and sources of electromagnetic cerebral responses to electrical and air-puff stimulation of the hand. Electroencephalogr Clin Neurophysiol 1996; 100: 229–239.

Rossini PM, Narici L, Martino G, Pasquarelli A, Peresson M, Pizzella V, Tecchio F, Romani G-L. Analysis of interhemispheric asymmetries of somatosensory evoked magnetic fields to right and left median nerve stimulation. Electroencephalogr Clin Neurophysiol 1994; 91: 476–482.

Rothwell JC, Obeso JA, Marsden CD. On the significance of giant somatosensory evoked potentials in cortical myoclonus. J Neurol Neurosurg Psych 1984; 47: 33–42.

Salonen O, Autti T, Raininko R, Ylikoski A, Erkinjuntti T. MRI of the brain in neurologically healthy middle-aged and elderly individuals. Neuroradiology 1997; 39: 537–545.

Santavuori P. Neuronal ceroid-lipofuscinoses in childhood. Brain Dev 1988; 10: 80–83.

Santavuori P, Heiskala H, Westermarck T, Sainio K, Moren R. Experience over 17 years with antioxidant treatment in Spielmeyer-Sjögren disease. Am J Med Gen 1988; 5: 265–274.

Santavuori P, Lauronen L, Kirveskari E, Åberg L, Sainio K, Autti T. Neuronal ceroid lipofuscinoses in childhood. Neurol Sci 2000; 21: 35–41.

Santavuori P, Linnankivi T, Jaeken J, Vanhanen S-L, Telakivi T, Heiskala H. Psychological symptoms and sleep disturbances in neuronal ceroid-lipofuscinoses (NCL). J Inherit Metab Dis 1993; 16: 245–248.

Santavuori P, Rapola J, Haltia M, Tyynelä J, Peltonen L, Mole SE. CLN5 Finnish variant late infantile NCL. In: Goebel HH, Mole SE, Lake BD, eds. The Neuronal Ceroid Lipofuscinoses (Batten Disease). IOS Press: Amsterdam, 1999: 91–101.

Santavuori P, Rapola J, Nuutila A, Raininko R, Lappi M, Launes J, Herva R, Sainio K. The spectrum of Jansky-Bielschowsky disease. Neuropediatrics 1991; 22: 92–96.

Santavuori P, Rapola J, Sainio K, Raitta C. A variant of Jansky-Bielschowsky disease. Neuropediatrics 1982; 13: 135–141.

Santavuori P, Westermarck T, Rapola J, Pohja P, Moren R, Lappi M, Vuonnala U. Antioxidant treatment in Spielmeyer-Sjögren's disease. Acta Neurol Scand 1985; 71: 136–145.

Savukoski M, Klockars T, Holmberg V, Santavuori P, Lander ES, Peltonen L. *CLN5*, a novel gene encoding a putative transmembrane protein mutated in Finnish variant late infantile neuronal ceroid lipofuscinosis. Nature Genet 1998; 19: 286–288.

Schmitt B, Thun-Hohenstein L, Molinari L, Superti-Furga A, Boltshauser E. Somatosensory evoked potentials with high cortical amplitudes: clinical data in 31 children. Neuropediatrics 1994; 25: 78–84.

Shibasaki H, Yamashita Y, Neshige R, Tobimatsu S, Fukui R. Pathogenesis of giant somatosensory evoked potentials in progressive myoclonic epilepsy. Brain 1985; 108: 225–240.

Shimojo M, Kakigi R, Hoshiyama M, Koyama S, Kitamura Y, Watanabe S. Intracerebral interactions caused by bilateral median nerve stimulation in man: a magnetoencephalographic study. Neurosci Res 1996; 24: 175–181.

Shimojo M, Kakigi R, Hoshiyama M, Koyama S, Watanabe S. Magnetoencephalographic study of intracerebral interactions caused by bilateral posterior tibial nerve stimulation in man. Neurosci Res 1997; 28: 41–47.

Simões C, Hari R. Relationship between responses to contra- and ipsilateral stimuli in the human second somatosensory cortex SII. Neuroimage 1999; 10: 408–416.

Sjögren T. Die juvenile amaurotische Idiotie. Klinische und erblichkeitsmedizinische Untersuchungen. Hereditas (Lund) 1931; 14: 197–426.

Sleat DE, Gin RM, Sohar I, Wisniewski K, Sklower-Brooks S, Pullarkat RK, Palmer DN, Lerner TJ, Boustany R-M, Uldall P, Siakotos AN, Donnelly RJ, Lobel P. Mutational analysis of the defective protease in classic late-infantile neuronal ceroid lipofuscinosis, a neurodegenerative lysosomal storage disorder. Am J Hum Genet 1999; 64: 1511–1523.

Sloan TB, Fugina ML, Toleikis JR. Effects of midazolam on median nerve somatosensory evoked potentials. Br J Anaesth 1990; 64: 590–593.

Sobel DF, Aung M, Otsubo H, Smith MC. Magnetoencephalography in children with Landau-Kleffner syndrome and acquired epileptic aphasia. AJNR 2000; 21: 301–307.

Stengel C. Beretning om et maerkeligt Sygdomstilfoelde hos fire Sødskende i Naerheden af Røraas. Eyr et medicinsk Tidscrift 1826: 1; 347–352.

Tahvanainen E, Ranta S, Hirvasniemi A, Karila E, Leisti J, Sistonen P, Weissenbach J, Lehesjoki A-E, de la Chapelle A. The gene for a recessively inherited human childhood progressive epilepsy with mental retardation maps to the distal short arm of chromosome 8. Proc Natl Acad Sci 1994; 91: 7267–7270.

Taylor MJ, Fagan ER. SEPs to median nerve stimulation: normative data for paediatrics. Electroencephalogr Clin Neurophysiol 1988; 71: 323–330.

Tecchio F, Pasqualetti P, Pizzella V, Romani G, Rossini PM. Morphology of somatosensory evoked fields: inter-hemispheric similarity as a parameter for physiological and pathological neural connectivity. Neurosci Lett 2000; 287: 203–206.

The International Batten Disease Consortium. Isolation of a novel gene underlying Batten disease, *CLN3*. Cell 1995; 82: 949–957.

Tiihonen J, Hari R, Hämäläinen M. Early deflections of cerebral magnetic responses to median nerve stimulation. Electroenceph Clin Neurophysiol 1989; 74: 290–296.

Tijssen MAJ, Thom M, Ellison DW, Wilkins P, Barnes D, Thompson PD, Brown P. Cortical myoclonus and cerebellar pathology. Neurology 2000; 54: 1350–1356.

Tsutada T, Tsuyuguchi N, Hattori H, Shimada H, Shimogawara M, Kuramoto T, Haruta Y, Matsuoka Y, Hakuba A. Determining the appropriate stimulus intensity for studying the dipole moment in somatosensory evoked fields: a preliminary study. Clin Neurophysiol 1999; 110: 2127–2130.

Tyynelä J, Palmer DN, Baumann M, Haltia M. Storage of saposins A and D in infantile neuronal ceroid-lipofuscinosis. FEBS Lett 1993; 330: 8–12.

Tyynelä J, Suopanki J, Santavuori P, Baumann M, Haltia M. Variant late infantile neuronal ceroid-lipofuscinosis: pathology and biochemistry. J Neuropathol Exp Neurol 1997; 56: 369–375.

Uesaka Y, Ugawa Y, Yumoto M, Sakuta M, Kanazawa I. Giant somatosensory evoked magnetic field in patients with myoclonus epilepsy. Electroencephalogr Clin Neurophysiol 1993; 87: 300–305.

Uusitalo MA, Ilmoniemi RJ. Signal-space projection method for separating MEG or EEG into components. Med Biol Eng Comput 1997; 35: 135–140.

Uutela K, Hämäläinen M, Somersalo E. Visualization of magnetoencephalographic data using minimum current estimates. Neuroimage 1999; 10: 173–180.

Uvebrant P, Hagberg B. Neuronal ceroid lipofuscinoses in Scandinavia. Epidemiology and clinical pictures. Neuropediatrics 1997; 28: 6–8.

Vanhanen S-L, Sainio K, Lappi M, Santavuori P. EEG and evoked potentials in infantile neuronal ceroid-lipofuscinosis. Dev Med Child Neurol 1997; 39: 456–463.

Vercruyssen A, Martin JJ, Ceuterick C, Jacobs K, Swerts L. Adult ceroid-lipofuscinosis: diagnostic value of biopsies and of neurophysiological investigations. J Neurol Neurosurg Psych 1982; 45: 1056–1059.

Vines DJ, Warburton MJ. Classical late infantile neuronal ceroid lipofuscinosis fibroblasts are deficient in lysosomal tripeptidyl peptidase I. FEBS Lett 1999; 443: 131–135.

Walkley SU, March PA, Schroeder CE, Wurzelmann S, Jolly RD. Pathogenesis of brain dysfunction in Batten disease. Am J Med Gen 1995; 57: 196–203.

Wegner K, Forss N, Salenius S. Characteristics of the human contra- versus ipsilateral SII cortex. Clin Neurophysiol 2000; 111: 894–900.

Westmoreland BF, Groover RV, Sharbrough FW. Electrographic findings in three types of cerebromacular degeneration. Mayo Clin Proc 1979; 54: 12–21.

Wikström H, Huttunen J, Korvenoja A, Virtanen J, Salonen O, Aronen H, Ilmoniemi RJ. Effects of interstimulus interval on somatosensory evoked magnetic fields (SEFs): a hypothesis concerning SEF generation at the primary sensorimotor cortex. Electroencephalogr Clin Neurophysiol 1996; 100: 479–487.

Wikström H, Roine RO, Salonen O, Aronen HJ, Virtanen J, Ilmoniemi RJ, Huttunen J. Somatosensory evoked magnetic fields to median nerve stimulation: interhemispheric differences in a normal population. Electroencephalogr Clin Neurophysiol 1997; 104: 480–487.

Williams RE, Topçu M, Lake BD, Mitchell W, Mole SE. CLN7 Turkish variant late infantile NCL. In: Goebel HH, Mole SE, Lake BD, eds. The neuronal ceroid lipofuscinoses (Batten disease). IOS Press: Amsterdam, 1999: 114–116.

Wisniewski KE, Kida E, Connell F, Elleder M, Eviatar L, Konkol RJ. New subform of the late infantile form of neuronal ceroid lipofuscinosis. Neuropediatrics 1993; 24: 155–163.

Wisniewski KE, Zhong N, Kaczmarski W, Kaczmarski A, Kida E, Brown WT, Schwarz KO, Lazzarini AM, Rubin AJ, Stenroos ES, Johnson WG, Wisniewski TM. Compound heterozygous genotype is associated with protracted juvenile neuronal ceroid lipofuscinosis. Ann Neurol 1998; 43: 106–110.

Wood CC, Cohen D, Cuffin BN, Yarita M, Allison T. Electrical sources in human somatosensory cortex: identification by combined magnetic and potential recordings. Science 1985; 227: 1051–1053.

Zeman W, Donahue S, Dyken P, Green J. The neuronal ceroid-lipofuscinoses (Batten-Vogt syndrome). In: Vinken PJ Bruyn GW, eds. Handbook of clinical neurology: Amsterdam, North Holland, 1970: 588–679.

Zeman W, Dyken P. Neuronal ceroid-lipofuscinosis (Batten's disease): relationship to amaurotic family idiocy? Pediatrics 1969; 44: 570–583.

Zhu Y, Georgesco M, Cadilhac J. Normal latency values of early cortical somatosensory evoked potentials in children. Electroencephalogr Clin Neurophysiol 1987; 68: 471–474.