Quantitative Methods for Evaluation of Tremor and Neuromotor Function:

Application in Workers Exposed to Neurotoxic Metals and Patients With Essential Tremor

Gunilla Wastensson

Institute of Medicine at Sahlgrenska Academy
University of Gothenburg

2010

UNIVERSITY OF GOTHENBURG

Photos: The Swedish Shipbuilding Yards History Club in Gothenburg, Gunilla Rydén, Lars Barregård

ISBN 978-91-628-8143-6

Printed by Geson Hylte Tryck, Göteborg, Sweden 2010

MILJÖMÄRKT

Quantitative Methods for Evaluation of Tremor and Neuromotor Function:

Application in Workers Exposed to Neurotoxic Metals and Patients With Essential Tremor

Gunilla Wastensson
Occupational and Environmental Medicine, School of Public Health and Community Medicine,
Institute of Medicine, University of Gothenburg, Gothenburg, Sweden

ABSTRACT

The overall purpose of this thesis was to investigate the usefulness of certain quantitative methods for detecting or quantifying changes in tremor or other neuromotor functions. Tremor and impairment in neuromotor function may be the early signs of adverse effects due to low-level exposure to neurotoxic metals such as mercury and manganese, and are also common features of neurological diseases. One of the most common movement disorders is essential tremor (ET), which is characterized by postural and kinetic tremor usually affecting the arms. Sensitive quantitative tests of tremor, motor speed, manual dexterity, diadochokinesis, eye–hand coordination, and postural stability were administered to a group of chloralkali workers with current mercury exposure, as well as former ship welders with previous manganese exposure. No effects of low-level mercury exposure on tremor amplitude and the ability to perform rapid pointing movements or rapid alternating forearm movements were shown. However, some findings provided support for a decrease in tremor frequency in the non-dominant hand resulting from mercury exposure. Former welders performed less well than referents in a test of manual dexterity and motor speed, and poorer performance was associated with cumulative manganese exposure, which indicates an irreversible adverse effect of long-term exposure to manganese. However, the performance in most of the other neurobehavioral tests was similar between groups. The use of certain quantitative methods in evaluating the efficacy of thalamic deep brain stimulation (DBS) was examined in a group of ET patients, and these methods were compared with traditional clinical tools for tremor assessment. The agreement between clinical rating of postural tremor and tremor intensity as measured by an accelerometer was relatively high (rs=0.74). Moreover, the quantitative system's sensitivity and specificity were estimated at 100% and 100%, respectively. The agreement between clinical rating of kinetic tremor and the main outcome variable from a quantitative test was low (rs=0.34), as was the sensitivity for this test (47%), even if the specificity was high (100%). In general, agreement between clinical tremor rating and quantitative measurements of tremor was low at low tremor amplitudes. In conclusion, no effect of low-level mercury exposure was shown, either on tremor amplitude, or on other certain neuromotor functions. Former welders had poorer performance on a test of motor speed and manual dexterity and this finding is probably caused by previous manganese exposure, even long after cessation of exposure. Quantitative methods may be useful tools for detecting subtle changes in tremor or other neuromotor functions at low-level exposure to neurotoxins; qualitative methods may be too insensitive as tools in this situation. Quantitative methods for measurement of tremor could complement clinical assessment in evaluating the efficacy of DBS in clinical practice.

Key words: Tremor, neuromotor function, neurobehavioral methods, mercury vapor, manganese previous exposure, welding, essential tremor, deep brain stimulation

ISBN 978-91-628-8143-6
http://hdl.handle.net/2077/23133

List of original papers

This thesis is based on the following papers, which are referred to in the text by Roman numerals (**I–IV**):

I. Wastensson G, Lamoureux D, Sällsten G, Beuter A, Barregård L. Quantitative tremor assessment in workers with current low exposure to mercury vapor. *Neurotoxicology and Teratology 2006;28:681–93.*

II. Wastensson G, Lamoureux D, Sallsten G, Beuter A, Barregard L. Quantitative assessment of neuromotor function in workers with current low exposure to mercury vapor. *Neurotoxicology 2008;29:596–604.*

III. Wastensson G, Sallsten G, Bast-Pettersen R, Barregard L. Quantitative assessment of neuromotor function in welders formerly exposed to manganese. *Submitted*.

IV. Wastensson G, Holmberg B, Johnels B, Barregard L. Quantitative methods for evaluating the efficacy of deep brain stimulation in patients with essential tremor. *Manuscript*.

Papers I and II are reproduced with the kind permission of the publishers of the respective journal.

List of abbreviations

CEI	Cumulative exposure index (mAm^2 x years)
CNS	Central nervous system
CV	Coefficient of variation
DBS	Deep brain stimulation
DIADO	Diadochokinesimeter
EKM	Eurythmokinesimeter
EMG	Electromyography
ET	Essential tremor
ETRS	Essential Tremor Rating Scale
FCAW	Flux cored arc welding
Hg^0	Elemental mercury
Hg^{2+}	Mercuric or divalent mercury
Hz	Hertz
IPG	Implantable impulse generator
MIG	Metal inert gas
MMA	Manual metal arc
MMT	Methylcyclopentadienyl manganese tricarbonyl
Mn	Manganese
MPTP	1-methyl-4-phenyl-1,2,3,6-terahydropyridine
MRI	Magnetic resonance imaging
PD	Parkinson’s disease
TIG	Tungsten inert gas
U-Hg	Mercury concentration in urine corrected for creatinine (µg/gC)
U-Hgcum	Cumulative exposure index (years x µg/gC)
U-Hgm5	Mean exposure for the past 5 years (µg/gC)
UPDRS	Unified Parkinson’s Disease Rating Scale
Vim	Ventralis intermedius
WHO	World Health Organization

Contents

1 Introduction 1

1.1 The neuromotor system 1

1.1.1 The pyramidal tract 2

1.1.2 The extrapyramidal system 2

1.2 Tremor 4

1.2.1 Classification of tremors 4

1.2.2 Sources of tremor 5

1.2.3 Physiologic tremor 6

1.2.4 Pathological tremors 6

1.2.5 Animal models of tremor 8

1.3 Methods for measurement of tremor 9

1.3.1 Tremor rating scales 9

1.3.2 Quantitative methods 9

1.4 Methods for evaluation of other neuromotor functions 11

1.5 Mercury vapor 11

1.5.1 Metabolism and distribution 13

1.5.2 Neurotoxic effects 13

1.5.3 Studies of mercury-exposed workers 14

1.6 Manganese 14

1.6.1 Metabolism and distribution 16

1.6.2 Neurotoxic effects 16

1.6.3 Studies of manganese-exposed workers 16

1.6.4 Studies of manganese-exposed welders 17

1.7 Essential tremor 17

2 Aims of the thesis 21

3 Material and methods 23

3.1 Study design and study population 23

3.2 Ethics 25

3.3 Exposure assessment 26

3.3.1 Mercury vapor (papers I and II) 26

3.3.2 Manganese (paper III) 27

3.4 Questionnaires 27

3.5 Clinical examinations and clinical rating scales 28

3.6 Quantitive assessment of tremor and other neuromotor functions 28

3.6.1 Quantitive tremor tests 28

3.6.2 Quantitive assessment of rapid pointing movements (eurythmokinesimetry) 31

3.6.3 Quantitive assessment of rapid alternating movements (diadochokinesimetry) 32

3.6.4 Tests of grip strength, motor speed and coordination 34

3.6.5 Postural stability 36

3.7 Statistical methods 36

4 Results 39

4.1 Effects of low-level mercury exposure on hand tremor (paper I) 39

4.1.1 Clinical examination 39

4.1.2 The laser-based system 39

4.1.3 The CATSYS Tremor Pen 41

4.2 Effects of low-level mercury exposure on certain neuromotor functions (paper II) 43

4.2.1 Quantitative assessment of rapid pointing movements (eurythmokinesimetry) 43

4.2.2 Quantitative assessment of rapid alternating movements (diadochokinesimetry) 43

4.3 Effects of previous manganese exposure on tremor or other neuromotor functions (paper III) 45
4.3.1 Clinical examination 45
4.3.2 Quantitative tests of tremor and other neuromotor functions 45
4.4 Efficacy of deep brain stimulation in patients with ET (paper IV) 47
4.4.1 Clinical assessment of tremor 47
4.4.2 The CATSYS Tremor Pen 47
4.4.3 Quantitative assessment of rapid pointing movements (eurythmokinesimetry) 47
4.4.4 Evaluation of tremor suppression using quantitative tests 48
4.5 Comparisons between methods (papers I, II, III) 49
4.5.1 The CATSYS Tremor Pen 49
4.5.2 The laser-based system 49
5 Discussion **53**
5.1 Validity aspects 53
5.1.1 Subjects 53
5.1.2 Exposure assessment 54
5.1.3 Methods 55
5.1.4 Potential confounders 57
5.1.5 Generalizability 59
5.2 Findings 59
5.2.1 Does low-level mercury exposure affect tremor and neuromotor function? (paper I and II) 59
5.2.2 Does previous Mn exposure affect neuromotor function? (paper III) 62
5.2.3 Could quantitative methods be used to evaluate the effect of thalamic deep brain stimulation on tremor suppression? (paper IV) 64
5.2.4 Comparisons between methods (papers I, II and IV) 66
6 Conclusions **69**
7 Acknowledgements **70**
8 References **71**

1 Introduction

Movement, involuntary as well as voluntary, is produced by contraction of muscle. A muscle that conducts a movement, such as bending of a joint, is called an agonist. Muscles with an opposite effect (stretching of the joint) are called antagonists. The nervous system is involved in all forms of motor function. The nervous system is divided into two main parts: the central nervous system (CNS), which includes the cerebrum, the cerebellum, the brainstem and the spinal cord: and the peripheral nervous system, which consists of the cranial and spinal nerves. The CNS is composed of a large number of excitable nerve cells (neurons) that receive signals through processes called dendrites, and conduct nerve impulses to other cells through their extensions (axons). The neurons are connected via synapses; in these, transmission of information from an axon of one neuron to a dendrite of a second neuron occurs via electrical activity and release of transmitter substances such as acetylcholine and dopamine. The neuron exerts its action in two ways: either by excitation or by inhibition. The spinal nerves consist of bundles of nerve fibers and conduct information from sensory organs to the CNS (afferent pathways), or from the CNS to effector organs such as muscles (efferent pathways). A simplified description of structures and pathways involved in neuromotor function is given in section 1.1 below.

1.1 The neuromotor system

Movement is organized in hierarchical levels according to increasing complexity. Voluntary movements are normally initiated and controlled by the frontal lobes in the cerebrum. The primary motor cortex in the frontal lobe is responsible for the direct production of movements, whereas other areas are responsible for higher order control of motor function. Stereotypic repetitious movements such as walking, swimming, and biking are controlled by neural networks in the spinal cord, brainstem, and cerebellum. Simple reflexes are controlled at the spinal level. A reflex is an involuntary response to a stimulus (Snell, 2006), and is the simplest form of motor behavior. There are several types of reflex arcs; one example is the monosynaptic stretch reflex arc involving only one synapse (Figure 1.1). The arc consists of a

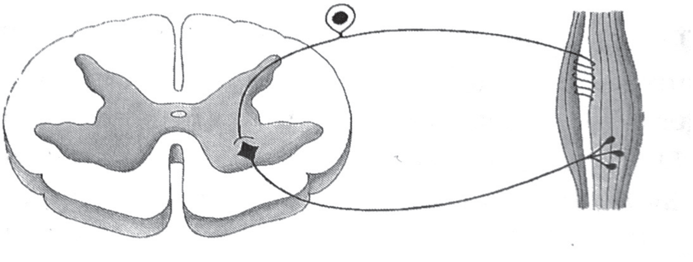


Figure 1.1. *The monosynaptic reflex arc.*

receptor organ in a muscle or tendon, an afferent neuron, an efferent neuron, and the effector organ (muscle). The motor unit is the final common unit and consists of a motor neuron in the anterior column of the spinal cord and all muscle fibers it supplies.

1.1.1 The pyramidal tract

The corticospinal (pyramidal tract) is the pathway most involveded in the performance of voluntary movements, especially rapid and skilled movements (Snell, 2006). Most of the cell bodies of the pyramidal tract arise from the motor cortex in the frontal lobe and carry information to the motor neurons in the brain stem and spinal cord (Figure 1.2). The primary motor cortex has a broadly somatotopic representation of different body parts in an arrangement called a motor homunculus. The motor neuron cell bodies, together with their axons that descend through the brain stem and spinal cord are referred to as upper motor neurons. The dense bundle of corticospinal axons forms a swelling known as the pyramid when the axons reach the medulla oblongata. In the medulla oblongata, most of the fibers cross over to the contralateral (opposite) side (pyramidal decussation). Thus, stimulation of different areas of the primary motor cortex will produce a movement in the corresponding part of the body that the area represents but on the contralateral side of the body. The axons of the upper motor neuron connect, mostly via interneurons, with the lower motor neurons located in the anterior column of the spinal cord. The lower motor neuron axons leave the spinal cord via the anterior roots of the spinal nerves and finally end at the neuromuscular plate thus providing motor innervation for skeletal muscles.

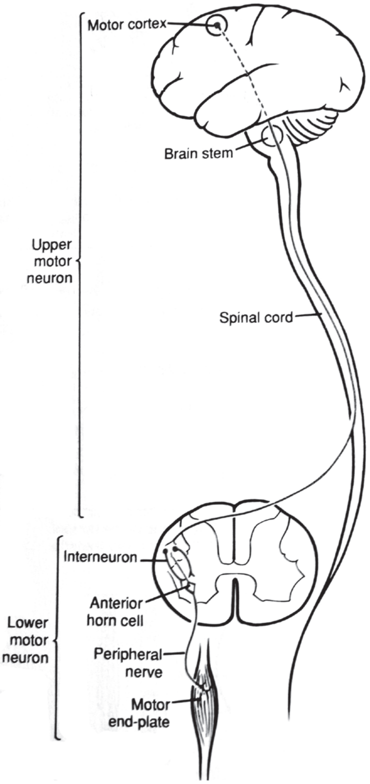


Figure 1.2. *The pyramidal tract. Modified from Waxman, 1996.*

1.1.2 The extrapyramidal system

The extrapyramidal system includes the remaining descending tracts involved in motor function that originate in the midbrain and brain stem regions, as well as other structures such as the basal nuclei and the cerebellum (see below). The system is simply called extrapyramidal to distinguish it from the pyramidal pathway (corticospinal tract) that may directly innervate the motor neurons of the spinal cord or brainstem. The extrapyramidal system acts indirectly by modulation of motor activity without directly innervating the motor neurons.

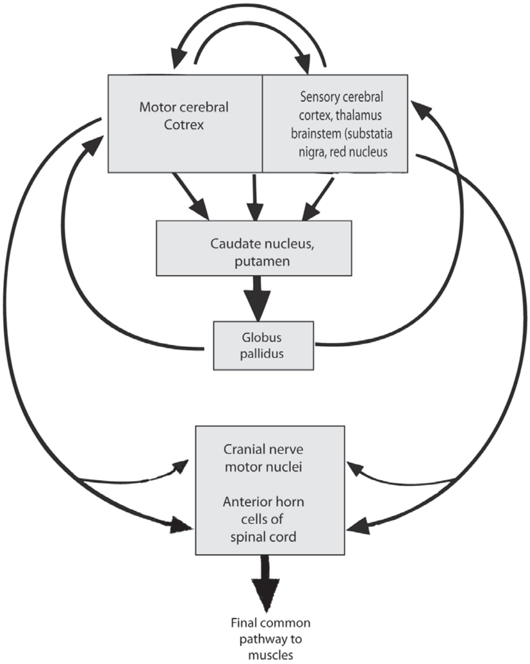


Figure 1.3. *The main functional connections of the basal nuclei. Modified from Snell, 2006.*

The ***basal nuclei*** (or basal ganglia) are a group of nuclei consisting of gray matter symmetrically located deep within each cerebral hemisphere and include the caudate nucleus, putamen, globus pallidus, and amygdala (Fahn & Jankovic, 2007). The caudate nucleus and the putamen are often referred to as striatum. From a functional point of view, other closely connected nuclei such as the subthalamic nucleus and the substantia nigra should also be included (Fahn & Jankovic, 2007). Striatum receives afferent information from the cerebral cortex and the brainstem, including the substantia nigra. Afferent information is also received from the thalamus, an important relay center closely linked to the cerebral cortex. The efferent pathway goes mainly via the globus pallidus, which acts on motor areas in the cerebral cortex or other motor centers in the brainstem (Figure 1.3). The basal nuclei prepare for and assist in the execution of voluntary movements, as well as the learning of motor skills, by direct influence on the cerebral cortex. Disorders of the basal nuclei may manifest in excessive and abnormal movements, slowness of movements (bradykinesia), tremor, and postural instability.

The ***cerebellum*** is a separate structure situated underneath the cerebral hemispheres. The cerebellum receives afferent information from the cerebral cortex, as well as sensory input from muscles, tendons and joints, and thus receives information about ongoing voluntary movements (Snell, 2006). The vestibular nerve sends afferent fibers to the cerebellum concerning balance. The information is processed by the cerebellum and sent back to the areas in the cerebral cortex and brainstem that conduct motor performance. It is believed that the cerebellum continuously compares the outflow from the motor area of the cerebral cortex with sensory input from the site of muscle action, and sends back signals about necessary adjustments of ongoing movements. Cerebellar disease is usually manifested as a complex of motor symptoms called ataxia that includes impairment in manual coordination, alterations in gait and postural control, and speech disturbances. Common findings are a typical intention tremor (see below), an inability to perform alternating movements regularly and rapidly (dysdiadochokinesia), and postural instability.

1.2 Tremor

Tremor is the most common type of involuntary movement (Fahn and Jankovic, 2007). The word tremor originates from the Latin word tremere, which means "to tremble." Tremor is defined as "any involuntary, approximately rhythmic, and roughly sinusoidal movement" (Elble & Koller, 1990, p. 1.). It is produced by alternating or synchronous contractions of antagonist muscles and may involve the limbs, neck, face, trunk, vocal cords, and other body parts. The underlying cause may differ; more than 100 etiologies are known to cause tremor (Deuschl et al., 1996).

1.2.1 Classification of tremors

A slight, barely visible physiologic tremor appears normally in every human being. Tremor is characterized by its frequency, which is the number of cycles per second or Hertz (Hz), and by its amplitude. Regarding frequency, hand tremors may be classified as low: <4 Hz, medium: 4–7 Hz, and high: >7 Hz (Deuschl et al., 1996). Pathological tremors occur in several conditions, either as an isolated phenomenon or together with other neurological signs and symptoms. A tremor frequency in the ranges 1–3 Hz or 11–20 Hz is usually considered to be pathological, and the two ranges are sometimes referred to as "slow" and "fast," respectively (Fahn & Jankovic, 2007). Tremor amplitude is basically a measure of severity of tremor but does not help to distinguish different tremor types; the tremor amplitude could vary widely within the same tremor type.

The current classification of tremors is based entirely on clinical criteria and combines etiologically defined tremors with phenomenologically defined tremor syndromes (Deuschl et al., 1998). Tremor frequency, as well as additional information from medical and family history and findings on the neurological examination, has to be taken into account in clinical assessment of tremor (Deuschl et al., 2001). Furthermore, the conditions that activate tremor are a key factor in recognizing the different types of tremor:

- *Resting tremor* occurs in a body part that is relaxed and supported against gravity.
- *Action tremor* occurs during voluntary muscle activation and includes several types of tremor.
- *Postural tremor* occurs during maintenance of a position against gravity, such as holding the arm outstretched in front of the body.
- *Kinetic tremor* occurs in goal-directed or non–goal-directed movements such as performing the finger–nose test.
- *Intention tremor* means a marked increase in tremor amplitude during the terminal part of a targeted movement.
- *Task-specific tremor* occurs during isolated tasks such as writing and speaking.

1.2.2 Sources of tremor

There are basically four principles of tremor genesis in humans: the mechanical tremor of a body part, reflex activation leading to oscillatory activity, central oscillation, and oscillatory activity resulting from unstable feedforward or feedback systems (Deuschl et al., 2001).

The ***mechanical component*** of tremor is a passive mechanical oscillation of a body part (Deuschl et al., 2001; Elble, 1996). The mechanical properties of most body parts permit damped oscillations in response to cardioballistic vibrations resulting from ejection of blood at cardiac systole, and to normally occurring discontinuities of innervations. The limb will oscillate at a certain resonance frequency that depends on the stiffness of the muscle (K) and the inertia (mass) of the oscillating limb. The resonance frequency will be determined by the following formula:

$$\text{Frequency} = \sqrt{\frac{K}{Inertia}}$$

The resonance frequency is different for different body parts – for example, it is 25 Hz for the fingers and 6-8 Hz for the hand. The resonance frequency may be decreased by adding mass (inertia) or increased by adding stiffness. Usually, a mechanical tremor component is identified by adding mass (load); thus, the frequency will decrease.

Reflexes of the CNS may contribute under certain conditions (Deuschl et al., 2001; Elble, 1996). When the frequencies of the mechanical and reflex oscillations are similar, they might be entrained in a single frequency. The frequency of mechanical reflex tremors is more dependent on reflex loop properties and will therefore be less altered by mechanical loads. Participation of the stretch reflex may be present in enhanced physiologic tremor.

Central oscillation is produced by normal and several pathological oscillators. These may originate from the rhythmic activity of a group of neurons inside a nucleus or be due to oscillations within loops consisting of neuronal populations or different nuclei and their axonal connections (Deuschl et al., 2001). Central oscillators are believed to work independently to peripheral output, but these tremors may be affected by peripheral stretch reflex manipulations under certain conditions, and may resonate with the mechanical tremor in a body part if their natural frequencies are similar (Elble, 1996).

Malfunction of feedforward loops within the CNS may produce tremor (Deuschl et al., 2001). This type of tremor occurs mostly in goal-directed movement. Such a movement consists of the following sequence: first, the agonist initiates the movement, then the antagonist breaks the movement, and finally the second agonist contraction is performing the fine-tuning of the movement. These movements are preprogrammed, but the corticospinal system alone is not sufficient to control the motor sequence. The cerebellum is assumed to be tuning the strength and duration of the first agonist, and the timing and shape of antagonist activation. If this feedforward system is defective, the motor performance will be dependent on delayed information from the periphery, resulting in impaired timing and shape of voluntary muscle activation and thus tremor during goal-directed movements.

1.2.3 Physiologic tremor

A physiologic tremor of high frequency is present in all humans (Deuschl et al., 1996). The mechanical component of tremor usually represents the main frequency component (Deuschl et al., 2001). An enhancement of the mechanical component by participation of the stretch reflex may occur in some cases such as fatigue, anxiety, certain medical conditions (elevated thyroid hormone levels, hypoglycemia), or intake of certain drugs. An enhanced physiologic tremor occurs mainly in the postural condition; the tremor frequency is usually high, but within the normal range (Deuschl et al., 1996). Also, physiologic tremor contains an 8–12 Hz central component that is believed to originate from oscillations in pathways involving the cortex, cerebellum, thalamus and the inferior olives in the brainstem (Elble, 2009). It has been estimated that the 8–12 Hz central component contributes significantly to tremor amplitude in 30% of normal subjects (Deuschl et al., 2001).

1.2.4 Pathological tremors

Essential tremor (ET) is the most common type of pathological tremor (see also section 1.7 below). Usually the arms are affected by a postural and/or kinetic tremor, but other body parts may be involved (Elble, 2009). The tremor frequency normally ranges from 4 Hz to 12 Hz (Elble & Deuschl, 2009) and decreases over time (Elble, 2000; Hellwig et al., 2009). The source of oscillation is believed to be located in the thalamocortical and olivocerebellar pathways (Elble, 2009).

Parkinson tremor is the second most common pathological tremor. It occurs at rest in the upper or lower limbs, and is one of the most typical features of Parkinson's disease (PD). James Parkinson first described the disease in 1817 in his paper, An Essay on the Shaking Palsy (Parkinson, 1817/2002). The tremor frequency in PD is low, 3–5 Hz (Deuschl et al., 2001), and the characteristic rest tremor in the hand is often referred to as "pill-rolling tremor." An action tremor with a higher frequency of 5–10 Hz may also be present but is usually not disabling (Elble, 2009). Bradykinesia and rigidity are other hallmark features of PD. The underlying pathology is degeneration of dopaminergic cells within the substantia nigra, leading to dopamine depletion of the striatum (Deuschl et al., 2001). The tremor is of central origin, and believed to be generated within the basal ganglia loop (Deuschl et al., 2001).

Cerebellar tremor is merely an intention tremor of low frequency (<5 Hz) that occurs uni- or bilaterally (Deuschl et al., 2001) following lesions within the cerebellum or the afferent or efferent cerebellar pathways resulting from diseases such as multiple sclerosis, stroke, or traumatic brain injury. The rhythm and amplitude of cerebellar tremor are often irregular (Elble, 2009). The typical intention tremor is believed to be caused by disturbed timing and grading of the activity of antagonist muscles.

Orthostatic tremor is a rhythmic, high-frequency (13–18 Hz) postural tremor that occurs when standing, giving the patient a sense of postural instability (Deuschl et al., 2001; Elble, 2009). The tremor might be palpable in affected muscles, and the diagnosis is confirmed by typical findings in electromyographic (EMG) recordings. Orthostatic tremor is presumed to be of central origin.

Holmes tremor, also known as rubral tremor, is a low-frequency (2–5 Hz) rest, postural, and kinetic tremor occurring in a limb (Elble, 2009). The tremor begins following thalamic or midbrain trauma or stroke, and it is believed that compensatory changes in the CNS function contribute to the tremorogenesis.

Psychogenic tremor may occur as two types: the coherent type and the co-contraction type. The coherent type of psychogenic tremor is a conscious or subconscious rhythmic movement of a joint, usually at a frequency around 6 Hz or lower (Elble, 2009). Performance of voluntary rhythmic movements in the same or contralateral limb will interrupt or affect tremor frequency in the affected limb. In the co-contraction type, tremor is produced by strong simultaneous activation of antagonist muscles around a joint. The tremor will stop when the co-activation is interrupted – for example, during passive manipulation of the affected joint.

Neuropathic tremor is a symptomatic tremor that develops in association with an acquired or inherited peripheral neuropathy (Elble, 2009). The tremors are usually postural and kinetic, with a frequency between 3 Hz and 10 Hz, and affect the upper as well as the lower limbs. The sensory loss and nerve conduction velocity are usually unrelated to the amplitude and frequency of the tremor. It is believed that neuropathic tremor is mainly due to compensatory changes in CNS function.

Drug-induced tremors may occur following the intake of certain drugs. Numerous drugs used in clinical practice may cause tremor, as reviewed by Elble and Koller (1990). The clinical features of tremor may differ according to the drug. The most common type is an enhanced physiologic tremor after intake of beta-adrenergic agonists and antidepressants (Deuschl et al., 2001). Some drugs (neuroleptics) may produce a rest tremor similar to rest tremor in PD (Elble & Koller, 1990). Lithium may produce an action tremor similar to ET (Elble & Koller, 1990).

Toxic tremors are due to intoxications. Tremor following withdrawal of alcohol is believed to be a form of enhanced physiologic tremor, whereas chronic alcoholism with cerebellar damage results in a typical intention tremor (Deuschl et al., 2001). Nicotine exposure increases the amplitude of physiologic tremor (Elble & Koller, 1990). Exposure to several neurotoxins, including pesticides, β-carboline alkaloids, and the neurotoxic metal, lead, may cause tremor (Louis, 2008). Chronic exposure to lead due to gasoline sniffing produces an acute and progressive disease including a prominent action tremor (Louis, 2008). Mercury and manganese are other neurotoxic metals that may cause tremor and are discussed in sections 1.5 and 1.6 below.

1.2.5 Animal models of tremor

Several animal models of tremor are available that entail different approaches to produce tremor in animals, such as application of tremorogenic drugs, experimental central nervous lesions, and study of genetic mutants (Wilms et al., 1999). One of the most well-known tremorogenic drugs is the neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-terahydropyridine). In the 1980s, seven people developed parkinsonism after using a synthetic opoid drug contaminated with MPTP. It was shown that MPTP causes symptoms similar to PD by destroying dopaminergic neurons in the substantia nigra in primates (Langston et al., 1983). MPTP is the most common animal model for rest tremor. Another animal model for PD is the pesticide rotenone, which causes symtoms similar to PD in rats by destroying dopaminergic neurons in the substantia nigra (Greenamyre et al., 2003).

β-Carboline alkaloids are a group of tremor-producing substances that are naturally occurring in the human diet, especially in meats that are cooked at high temperatures for extended periods (Louis, 2008). The blood concentration of these alkaloids has been reported to be higher in patients with ET than in controls, but the meaning of this finding is still unclear (Louis, 2008). The action tremor produced by β-carboline alkaloids resembles ET and is at present the main animal model for the disease.

1.3 Methods for measurement of tremor

1.3.1 Tremor rating scales

Tremor rating scales are commonly used in clinical assessment of tremor severity. In this manner, the examiner makes a subjective evaluation of rest, postural, and kinetic tremor in the hands and other body parts according to a 4- or 5-point grading scale. The motor tasks used are similar to the clinical tests performed in a standard neurological examination – for example, the finger-nose test (Elble & Koller, 1990). Other common tasks for tremor assessment are handwriting, drawing an Archimedean spiral, and pouring water from one cup to another. Most rating scales have quite good reproducibility; however, the sensitivity is usually insufficient to detect small changes in tremor amplitude (Elble & Koller, 1990). Some rating scales include the patient's self-assessment of disability and embarrassment due to tremor.

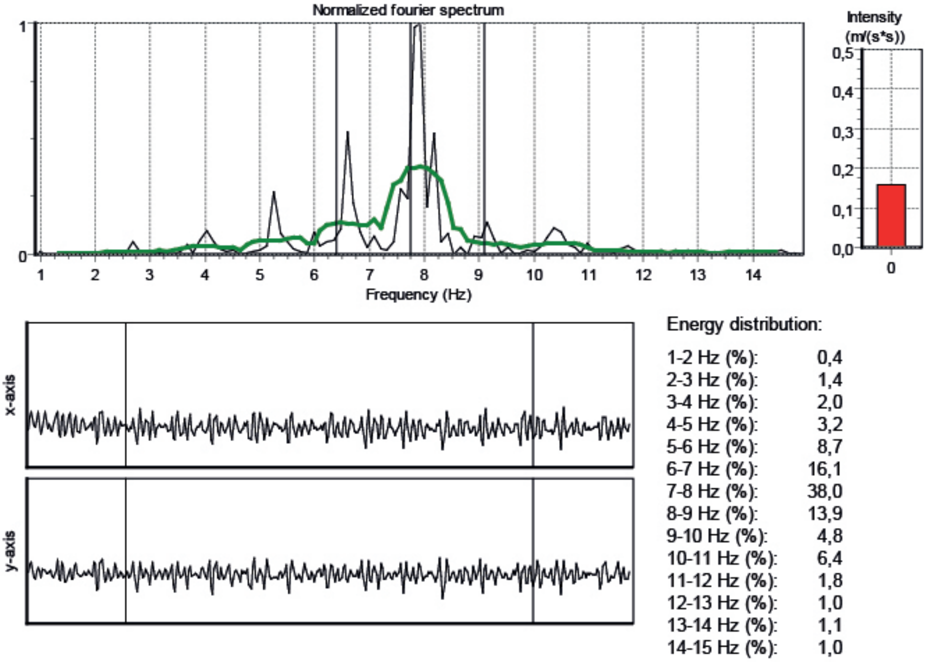
Commonly used rating scales are the Fahn-Tolosa-Marin scale (Fahn et al., 1988), which rates tremor severity from 0 (none) to 4 (severe) by body part, and the rating scale developed by the Washington Heights-Inwood Genetic Study of Essential Tremor (WHIGET; Louis et al., 2001). The Unified Parkinson's Disease Rating Scale (UPDRS) is a common rating scale used for PD and assigns 0–4 points for severity of tremor in rest, postural, and kinetic conditions (Fahn & Elton, 1987).

1.3.2 Quantitative methods

Neurophysiological techniques such as electromyography (EMG) and accelerometry can be useful tools in addition to clinical evaluation of tremor. EMG measures muscle electrical activity in a tremulous body part. It is usually recorded with surface electrodes, but in some cases needles or fine wires are used. As muscles contract, microvolt-level electrical signals can be measured from the skin's surface. EMG is recorded simultaneously from a pair of agonist/antagonist muscles and thus will give information on whether the muscle activity is synchronous or not. The registrations give a measure of the frequency and rhythmicity of tremor. The EMG pattern is pathognomonic for orthostatic tremor, and may be useful in separating tremor from other forms of involuntary movements (Deuschl, 1999).

Several transducers that measure movement (displacement, velocity or acceleration) of an oscillating body part are available (Elble & Koller, 1990). Velocity is the first time derivative of motion, whereas acceleration (m/s^2) is the second time derivative of motion (Elble & Koller, 1990). Lightweight accelerometers are most widely used and have high sensitivity (Deuschl, 1999). Uniaxial accelerometers measure acceleration in one linear direction, but biaxial and triaxial accelerometers are also available. Tremor in writing and drawing can be recorded using a digitizing tablet, which has a surface that is sensitive to the touch of a special pencil (Deuschl, 1999).

Quantitative tremor recordings give an oscillating curve in the time domain. Usually the time series is processed using a mathematical method called Fourier analysis, which gives quantitative values for the amplitude and frequency of tremor (Elble & Koller, 1990). By Fourier transformation, the tremor curve is approximated by a series of sine and cosine waves of various frequencies and amplitudes. Because the variance of a pure sinusoidal wave is equal to one half of its squared peak amplitude, the variance of the sum of the waves can be used as a measure of amplitude. The Fourier analysis also provides a power spectrum that gives quantitative values of amplitude over frequency. The tremor recordings performed with a biaxial accelerometer and the normalized Fourier spectrum in a normal subject are shown in Figure 1.4.



Energy distribution:

1-2 Hz (%):	0,4
2-3 Hz (%):	1,4
3-4 Hz (%):	2,0
4-5 Hz (%):	3,2
5-6 Hz (%):	8,7
6-7 Hz (%):	16,1
7-8 Hz (%):	38,0
8-9 Hz (%):	13,9
9-10 Hz (%):	4,8
10-11 Hz (%):	6,4
11-12 Hz (%):	1,8
12-13 Hz (%):	1,0
13-14 Hz (%):	1,1
14-15 Hz (%):	1,0

Figure 1.4. *The two-axis perpendicular accelerometric tremor recordings over 8.2 seconds are shown in the figure. The normalized Fourier spectrum of tremor frequency is shown by a green line, and the power distribution of tremor in the frequency band 0.9-15 Hz determined by Fast Fourier transformation on the combined signal is provided. The measure of tremor frequency (center frequency, 7.8 Hz) is indicated, as well as the SD of the center frequency (1.3 Hz). The obtained value of amplitude, in this case expressed as intensity (the root mean square of accelerations over frequency), was 0.16 m/s*^{2} *(red bar).*

Tremor frequency may be an important measure for diagnostic purposes; for instance, a tremor frequency <4 Hz is probably due to Holmes tremor or cerebellar tremor (Deuschl, 1999). Moreover, measurement of tremor frequency combined with loading of an extremity will help in differentiating between normal and pathological tremors with a stable central oscillator (Deuschl, 1999). The amplitude of tremor can be used to quantify tremor severity and detect changes in tremor intensity over time, but has no value for diagnostic purposes (Deuschl, 1999). Other features of the tremor curve than the basic measures of amplitude and frequency may be described by applying mathematical techniques of time series analysis. This kind of advanced waveform analysis might be useful in separating different forms of tremor (Beuter & Edwards, 1999; Edwards & Beuter, 2000).

Other quantitative methods that can be used for assessment of tremor severity are hole tremormeters such as the Kløve-Matthews static steadiness test, and the Nine-Hole steadiness test. In these almost identical tests, the subject is required to hold a stylus within successively smaller holes without touching the sides. These types of tests are measuring displacement of motion (Bast-Pettersen & Ellingsen, 2005).

1.4 Methods for evaluation of other neuromotor functions

Several neurobehavioral tests are available for evaluation of different aspects of neuromotor function. Some commonly used tests are listed in Table 1.1.

Table 1.1. *Neurobehavioral tests used for assessment of neuromotor function.*

Motor speed	Finger tapping, Foot tapping, Simple reaction time, Luria-Nebraska motor scale
Hand–eye coordination	Hand–eye coordination (HECT), Orthokinesimeter, eurythmokinesimeter
Manual dexterity	Grooved pegboard, Purdue pegboard, Santa Ana pegboard
Diadochokinesis	Diadochokinesimeter
Postural stability	Postural Sway

1.5 Mercury vapor

Mercury is naturally occurring in the environment, most of it released from the earth's crust and the oceans into the atmosphere. However, a considerable amount is released to the environment by human activities. Mercury occurs as elemental mercury and inorganic and organic mercury compounds (Berlin et al., 2007). Elemental mercury, or quicksilver, is a highly volatile silver-white metal that exists as a liquid or vapor (Hg^0) at room temperature (World Health Organization [WHO], 2003). The chemical symbol, Hg, is an abbreviation of its ancient name hydrargum (Latin) hydrargyros (Greek), meaning water/silver (Clarkson & Magos, 2006). Mercury has been used by mankind since ancient times for such purposes as the preparation of red ink, and for medical purposes (Goldwater, 1972). Occupational use of mercury in mirror making in Venice has been described by Ramazzini in his classic monograph “Diseases of Occupations” (Ramazzini, 1713/1964). In modern time, occupational exposure occurs in mercury mines and chloralkali plants, and in the manufacture of thermometers, fluorescent lightbulbs, and batteries (Berlin et al., 2007). Inhalation of mercury is the most common route of exposure to mercury from occupational sources (WHO, 2003). The main source of non-occupational exposure to mercury vapor is from dental amalgam (Clarkson & Magos, 2006). A question of great concern is the accumulation of organic mercury (methylmercury) in the food chain (especially fish food) owing to transformation from inorganic mercury by microbial activity in polluted areas (Berlin et al., 2007).

At the time of the studies presented in ***papers I and II***, chlorine was produced by the mercury cell method at two chloralkali plants in Sweden (Figure 1.5). In this process, a thin layer of elemental mercury is utilized as a cathode in the bottom of an electrolytic cell (Sällsten et al., 1990). Brine is pumped into the cell, where it is electrolyzed to chlorine and a liquid sodium-mercury amalgam. The amalgam then meets a counter-flow of water to produce caustic soda and hydrogen gas. Mercury is regenerated in the process, but release of mercury vapor into the working environment may occur and thus expose the workers.

Figure 1.5. *A mercury cell in a chloralkali plant. Photo L. Barregård.*

1.5.1 Metabolism and distribution

Inhalation is the main route of entry into the body following exposure to elemental mercury (WHO, 2003). The vapor is readily absorbed through the alveolar membrane into the blood and about 80% is retained (WHO, 2003). Mercury vapor is oxidized to divalent mercury (Hg^{2+}) in the red blood cells and other tissues by the hydrogen peroxide–catalase pathway (Clarkson & Magos, 2006). However, mercury vapor dissolved in the bloodstream may cross the blood–brain barrier before oxidation and thus enter the brain. After exposure, most of the mercury in the brain is cleared with a short half-life, but a fraction may have a much longer half-life of several years (Clarkson & Magos, 2006). Excretion is via urine and feces, with a whole-body half-life of about 60 days (Clarkson & Magos, 2006). Recent mercury exposure is reflected in blood and urine. Blood samples are most useful in short-term exposure at higher levels (WHO, 2003). However, urine samples are considered to be the best indicator of body burden with long-term exposure to elemental mercury (WHO, 2003) and are normally used for biological monitoring of exposed workers. Mercury concentration in urine may be affected by hydration; therefore, it is normally corrected for creatinine and expressed as µg/g creatinine (µg/gC;WHO, 2003). Levels of urinary mercury are expected to be <5 µg/gC in an unexposed population (WHO, 1991).

1.5.2 Neurotoxic effects

Exposure to mercury vapor may cause adverse effects in many organs, and the central nervous system is considered to be a critical organ in humans (Berlin et al., 2007). After entering the brain, mercury vapor is oxidized to Hg^{2+}, which is assumed to be the proximate toxic agent, exerting its action by attaching to thiol groups present in most proteins (Clarkson & Magos, 2006). Even if little is known about the exact pattern of mercury distribution in the CNS in humans (Clarkson & Magos, 2006), the extent of and variety in neuropsychological impairment following mercury exposure suggest that most structures in the CNS are affected. The mechanism of its action on brain function is poorly understood, but it has been demonstrated that exposure to Hg ions induces retrograde degeneration of the neuron membrane in vitro, possibly by interfering with the formation of microtubules (Leong et al., 2001).

The earliest symptoms and signs of mercury poisoning include a neurasthenic syndrome, with unspecific symptoms such as weakness, fatigue, and anorexia, called micromercurialism (Berlin et al., 2007). Another typical sign is a fine tremor interrupted by coarse shaking movements, initially involving the hands (Berlin et al., 2007). The tremor is intentional, but becomes postural in more severe cases (Clarkson & Magos, 2006). Erethism, characterized by severe behavioral and personality changes such as extreme shyness and increased excitability, may finally occur (Berlin et al., 2007). These signs and symptoms will reverse slowly after cessation of exposure, but remaining adverse effects have been reported several decades after exposure has ceased (Clarkson & Magos, 2006).

1.5.3 Studies of mercury-exposed workers

Numerous studies have reported the neuropsychological /neurobehavioral effects of occupational mercury exposure. Meta-analyses performed on studies of mercury-exposed workers have shown a larger impairment in neuromotor performance than in other domains (Meyer-Baron et al., 2004; Rohling & Demakis, 2006). A tendency toward more neurological abnormalities in mercury-exposed workers has been reported in studies of high current Hg exposure, down to U-Hg levels of around 70 µg/gC (Ehrenberg et al., 1991; Miller et al., 1975; Urban et al., 1996). Several studies of high or moderate Hg exposure (>U-Hg 25 µg/gC or µg/L) have reported reduced motor speed (Günther et al., 1996; Langolf et al., 1978; Miller et al., 1975), and impairment in eye–hand coordination (Günther et al., 1996; Langolf et al., 1978; Miller et al., 1975; Piikivi et al., 1984; Roels et al., 1982; Roels et al., 1989; Williamson et al., 1982). However, some studies show a possible effect on motor speed (Echeverria et al., 1998; Liang et al., 1993; Lucchini et al., 2002; Ngim et al., 1992), even at relatively low exposure levels (≤U-Hg 20–25 µg/gC or µg/l).

Tremor is a hallmark feature of excess mercury exposure. Traditionally, the finger–nose test (Clarkson & Magos, 2006) and writing were used in periodic examinations of mercury-exposed workers; however, decreasing exposure levels due to improved hygienic conditions require more precise and sensitive tools. Quantitative tremor measurement has been used in several studies of workers with long-term exposure to mercury, as reviewed by Beuter and de Geoffroy (1996), and in some more recent studies (Biernat et al., 1999; Bittner et al., 1998; Echeverria et al., 1998; Ellingsen et al., 2001; Lucchini et al., 2002; McCullough et al., 2001). Increased tremor amplitude has been reported in studies with high or moderate current Hg^0 exposure (Langolf et al., 1978; Roels et al., 1985; Roels et al., 1989; Williamson et al., 1982; Wood et al., 1973) down to urinary mercury levels about 35 µg/gC (Verberk et al., 1986). Some studies indicate a possible effect on tremor parameters at even lower (U-Hg 20–25 µg/gC or µg/l) exposure levels (Chapman et al., 1990; Fawer et al., 1983; Langworth et al., 1992). Thus, despite several studies having been conducted in this field, a specific no-observed-adverse-effect level has not yet been possible to settle.

1.6 Manganese

Manganese (Mn) is a naturally occurring element, comprising about 0.1% of the earth's crust. It occurs in rocks, soil, water, and food. Manganese exists as inorganic and organic compounds, but the inorganic form is most common in the environment (Santamaria & Sulski, 2010). The main routes of Mn exposure are ingestion and inhalation (WHO, 1999). Food is the main source of exposure in the general population (Santamaria & Sulski, 2010). However, significant Mn exposure may occur by ingestion of contaminated drinking water in some areas (WHO, 1999). In the working environment, exposure to Mn occurs mostly via inhalation of manganese fumes or manganese-containing dust (Šarić & Lucchini, 2007). Occupational exposure occurs in the ferromanganese, iron, steel, dry cell battery, and welding industries, as well as during manganese mining and ore processing (Šarić & Lucchini, 2007). People living near a plant that releases manganese dust into the air may be exposed to Mn above average levels (Šarić & Lucchini, 2007). Some organic compounds of Mn are used as gasoline antiknock additives (methylcyclopentadienyl manganese tricarbonyl [MMT] and fungicides (maneb and mancozeb) in agriculture (Šarić & Lucchini, 2007).

Welders are exposed to manganese by inhalation of welding fumes. Manganese is present in many welding rods and wires, as well as in most kinds of steel, and is released to the air during the welding process (Flynn & Susi, 2010). The most common welding techniques are manual metal arc (MMA or stick), metal inert gas (MIG), tungsten inert gas (TIG), and flux cored arc welding (FCAW). Exposure depends on the type of metal being welded, the welding technique, and the work environment, i.e. ventilation (Santamaria et al., 2007). In the past century, Gothenburg was the site of one of the largest shipyard industries in the world, but most of these industries closed down in the beginning of the 1990s. Welding was a common operation in these industries, mostly metal arc welding, but all types of welding techniques were used. The study presented in ***paper III*** was performed on former ship welders from these industries (Figure 1.6).

Figure 1.6. *Welders at a shipyard in Gothenburg in the 1940s. Photo kindly provided by the Swedish Shipbuilding Yards History Club in Gothenburg.*

1.6.1 Metabolism and distribution

Manganese is an essential trace element, required for several functions such as energy metabolism, nervous system function, and protection from damage resulting from free radicals (Šarić & Lucchini, 2007; WHO, 1999). The amount of Mn absorbed across the gastrointestinal tract after ingestion is around 1–5% (Santamaria & Sulski, 2010). There is metabolic interaction between manganese and iron, and iron deficiency increases the absorption of Mn (WHO, 1999). Inhaled particles deposited in the lower airways are absorbed from the alveolar lining, whereas particles deposited in the upper airways are swallowed and might be absorbed from the gastrointestinal tract (Šarić & Lucchini, 2007; WHO, 1999). Manganese crosses the blood–brain barrier and accumulates in the brain, predominantly in the globus pallidus and midbrain (Kim et al., 1999). More manganese reaches the brain following inhalation than following ingestion given comparable doses (WHO, 1999). Manganese exposure is reflected by an increased magnetic resonance imaging (MRI) signal intensity in the globus pallidus, which is present in about 75% of asymptomatic welders (Kim et al., 1999). In healthy humans, homeostatic mechanisms regulate absorption and excretion rates in order to maintain normal physiologic ranges and to avoid deficiency as well as intoxication (WHO, 1999). Biological whole-body half-life is 2–5 weeks, but the half-life in the brain is much longer (Šarić & Lucchini, 2007). Excretion is mainly via the bile (Šarić & Lucchini, 2007; WHO, 1999).

1.6.2 Neurotoxic effects

The main target organs following long-term exposure to manganese dust are the lungs and the central nervous system (WHO, 1999). Chronic exposure to high levels of airborne Mn (>1 mg/m^3) may cause manganism, a debilitating neurological disease resembling Parkinson’s disease (Santamaria & Sulski, 2010; WHO, 1981). In 1837, Couper reported the first cases among workers employed in grinding manganese dioxide ore (Couper, 1837). The clinical features of manganism include psychiatric disturbances (“manganese madness”) and motor deficits, such as bradykinesia and rigidity (Calne et al., 1994). Other features are the characteristic “cock walk” and a tendency to fall backward. Tremor is less common and more often postural than resting in nature (Calne et al., 1994). Neuropathological studies have shown selective damage to the globus pallidus in manganism, but not to the substantia nigra pars compacta, in contrast to Parkinson’s disease (Perl & Olanow, 2007). Potentially adverse effects on the CNS, such as mood changes and impairment in cognition and neuromotor function, have been described even at low exposure levels (Santamaria et al., 2007; Šarić & Lucchini, 2007).

1.6.3 Studies of manganese-exposed workers

Poorer performance and/or associations with exposure in neuromotor tests evaluating motor speed, eye–hand coordination, manual dexterity, and rapid alternating movements have been reported in several studies among workers employed in the ferroalloy industry, as well as baggers, miners, smelters and foundry workers (Beuter et al., 1994b; Bouchard et al., 2007; Chia et al., 1993; Hochberg et al., 1996; Hua et al., 1991; Iregren, 1990; Lucchini et al., 1995; Lucchini et al., 1997; Lucchini et al., 1999; Mergler et al., 1994; Roels et al., 1987b; Roels et al., 1992; Roels et al., 1999; Wennberg et al., 1991). Impairment in hand steadiness and alterations in tremor parameters are also common findings (Bast-Pettersen et al., 2004; Crump & Rousseau, 1999; Hochberg et al., 1996; Lucchini et al., 1999; Mergler et al., 1994; Roels et al., 1987b; Roels et al., 1992; Roels et al., 1999), and one study has reported increased postural instability following Mn exposure (Chia et al., 1995). Some studies are, however; essentially negative (Gibbs et al., 1999; Myers et al., 2003a; Myers et al., 2003b). It is still unclear whether some of these effects will persist for a long time after cessation of exposure, as some studies have indicated is a possibility (Beuter et al., 1994b; Bouchard et al., 2007; Hochberg et al., 1996; Roels et al., 1999).

1.6.4 Studies of manganese-exposed welders

Subtle effects on the CNS have been reported at 0.1–0.3 mg/m^3 in air, an exposure level that is common in welding; however, relatively few studies have been performed on welders (Bowler et al., 2003; Bowler et al., 2006; Bowler et al., 2007; Chang et al., 2009; Ellingsen et al., 2008; Sjögren et al., 1996). The neurobehavioral effects reported among welders with current Mn exposure are decreased grip strength (Bowler et al., 2003; Bowler et al., 2006), reduced performance in tests of fine manual dexterity, motor speed, and coordination (Bowler et al., 2003; Bowler et al., 2007; Chang et al., 2009; Ellingsen et al., 2008; Sjögren et al., 1996), and increased tremor or alterations in tremor parameters (Bowler et al., 2007; Chang et al., 2009). While most studies have focused on workers with ongoing exposure, only two studies of welders with previous Mn exposure have been performed, both reporting impairment mainly in motor speed and manual dexterity (Bowler et al., 2006; Ellingsen et al., 2008). Both studies examined workers a relatively few years after cessation of exposure. The question whether a slight affection of the CNS due to manganese exposure will persist many years after cessation of exposure is still to be settled.

1.7 Essential tremor

ET is the most common tremor disease. The prevalence of ET varies from 0.4% to 3.9% in population-based studies, and increases with age, as does its incidence (Louis, 2005). In typical cases, the upper limbs are affected by postural tremor and/or kinetic tremor (Elble & Deuschl, 2009). Recent research has raised the question whether ET is a potentially reversible disturbance in neuronal oscillation, or a heterogeneous neurodegenerative disease (Deuschl & Elble, 2009). Although evidence indicates a genetic basis for ET, no specific gene has been found (Deng et al., 2007). However, a family history of ET is present in about 50% of patients, and certain environmental factors, such as β-carboline alkaloids, lead, and pesticides, which might contribute to the etiology of ET, are presently under investigation (Louis, 2008).

The diagnosis is entirely based on clinical criteria (Deuschl et al., 1998). ET is progressive in nature, and with longer disease duration, the tremor amplitude increases and other body parts than the upper limbs may be affected, most often the head (Louis, 2005). In patients with more severe ET, an intentional tremor component in voluntary movements and other motor signs such as difficulty with tandem gait may occur, indicating involvement of the cerebellum (Elble & Deuschl, 2009). Furthermore, recent studies have shown difficulties in eye–hand coordination (Trillenberg et al., 2006) and impaired rhythm generation (Avanzino et al., 2009; Farkas et al., 2006) in ET patients, probably because of cerebellar dysfunction. Rest tremor may be present in patients having severe disease and long disease duration (Cohen et al., 2003).

Difficulties with basic daily activities are common with ET, and >90% of patients who come to medical attention report disability (Louis, 2005). Most patients with ET report a prominent but temporary effect on tremor following ethanol intake (Elble & Koller, 1990). This specific effect of ethanol on tremor has been shown in several studies; traditionally, ethanol was used for treatment of ET (Louis, 2005). These days, the treatment of ET primarily involves pharmacotherapy with propranolol or primidone, which have proven to be equally efficient (Elble & Deuschl, 2009). However, pharmacotherapy is successful in only about 50% of ET patients (Elble & Deuschl, 2009), and for those patients who do not respond to or tolerate medication, neurosurgery might be an alternative.

At present, continuous deep brain stimulation (DBS) in the ventrolateral thalamus (ventralis intermedius [Vim] of the thalamus is the most common surgical approach for ET patients with medication-resistant, disabling tremor (Elble, 2009). DBS has proven to be effective in reducing hand tremor by 50% to 91% in several studies with follow-up times varying from 1 to 7 years (Lyons & Pahwa, 2008). It is believed that thalamic DBS interrupts resonant tremorogenic oscillation in the thalamocortical loop, but the mechanism of action is not fully understood (Elble, 2009). The device consists of an electrode lead connected to an implantable pulse generator (IPG) by an extension wire. The implantation of the electrode lead in the optimal position is the most critical moment during surgery; accuracy of lead position is directly related to stimulation efficacy (Dowling, 2008). The surgeon uses a coordinate system linked to MRI for guidance, while the lead is targeted stereotactically. The patient is normally awake at this stage of the surgical procedure, which allows for testing of stimulation efficacy. Once the lead electrode has been implanted, general anesthesia is given to the patient while the extension wire and the IPG are implanted. The IPG is normally placed in a subclavicular position (Figure 1.7).

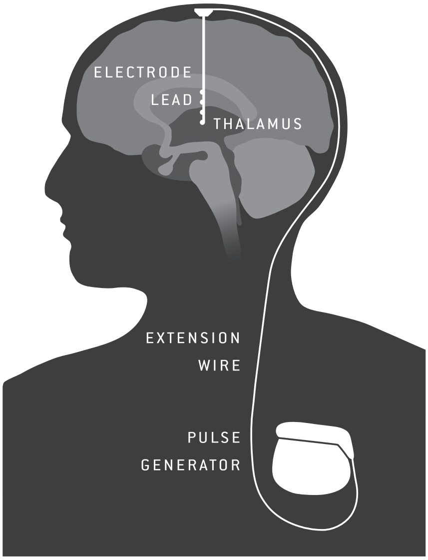


Figure 1.7. *Deep brain stimulation.*

Following implantation of DBS, the system requires programming; in this process, the electrode configuration and stimulation parameters are set. The electrode lead contains four contacts, and the IPG can stimulate through any combination of positive and negative contacts. The stimulation parameters can be varied regarding amplitude, pulse width, and frequency. Selection of optimal stimulus parameters is necessary for successful tremor suppression with a minimum of side effects, and may prolong battery life. Even if some general guiding principles can be given (Kuncel et al., 2006), the selection of stimulus parameters is usually performed ad hoc, which may require several sessions and be uncomfortable for the patient. In clinical practice, different combinations of stimulation parameters on tremor suppression are evaluated using clinical tests – for example, patients are asked to hold their hands outstretched, or to touch the tip of their nose or the examiner’s finger with their index finger. However, quantitative methods for tremor assessment could also be useful tools in this situation.

2 Aims of the thesis

The overall purpose of this thesis was to investigate the usefulness of certain quantitative methods for detecting or quantifying changes in tremor or other neuromotor functions.

The specific aims were to:

- Investigate whether effects of low-level mercury exposure on hand tremor could be detected using sensitive quantitative methods for tremor measurements (***paper I***).

- Investigate whether effects of low-level mercury exposure on the ability to perform rapid pointing movements or rapid alternating forearm movements could be detected using sensitive quantitative tests (***paper II***).

- Investigate whether effects of previous manganese exposure on tremor or other neuromotor functions could be detected using sensitive quantitative tests (***paper III***).

- Investigate whether the efficacy of deep brain stimulation in patients with essential tremor could be assessed using sensitive quantitative tests (***paper IV***).

- Compare different qualitative and quantitative methods for measuring tremor (***papers I, II, and IV***).

3 Material and methods

3.1 Study design and study population

Papers I and II

The studies presented in ***papers I and II*** were cross-sectional and comprised the same population of workers from two chloralkali plants located in the southwestern region of Sweden. All workers in the plants were regularly monitored for mercury in urine (U-Hg) by their local occupational health service units. In all, 83 mercury-exposed workers who were working at these two plants were identified, and among these, the 60 workers with the highest concentrations of U-Hg during the previous few months were selected. A non-exposed referent group consisting of 42 blue-collar workers, and at the same age interval as that of the exposed workers, was collected from five other departments at the same two plants. Only workers who had been employed at the plant for at least 1 year and who had not been exposed to other heavy metals or organic solvents were included in the study. In total, 102 potential participants (60 mercury-exposed workers and 42 referents) were contacted and asked to participate in the study. Among these, 58 mercury-exposed workers (97%) and 35 referents (83%) agreed to participate in the study. Participants with diseases, medication, or other circumstances that could affect their performance in the neurobehavioral tests were excluded. After exclusions, 65 subjects (43 exposed and 22 referents) remained for further analysis. All subjects were male. The selection procedure is described in Figure 3.1. The mercury-exposed workers and the referents were similar regarding the relevant background variables (Table 3.1).

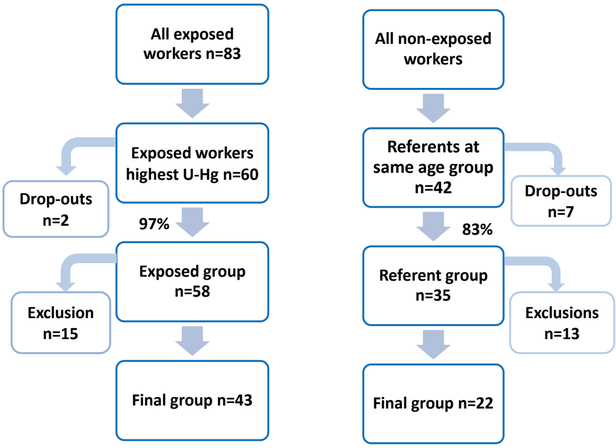


Figure 3.1. *The recruitment process and exclusions of mercury-exposed workers and non-exposed workers at two chloralkali plants (**papers I and II**). The participation rates are shown as percentages.*

Table 3.1. *Background characteristics of 43 mercury-exposed workers and 22 referents.*

	Exposed subjects (n=43)	Referents (n=22)
Mean age, yrs (range)	41 (25–65)	40 (21–61)
Shift work, % (n)	65 (28)	68 (15)
Smokers, % (n)	26 (11)	27 (6)
All tobacco use, % (n)	53 (23)	32 (7)
Amalgam fillings, % (n)	86 (37)	95 (21)
Eating fish >2 times/week, % (n)	5 (2)	14 (3)

Paper III

The study in ***paper III*** was designed as a retrospective cohort study. The subjects were drawn from an original cohort of 451 shipyard workers. All former ship welders aged 75 years or below (n=47) were listed. The reference population consisted of all filers and electricians in the same age category (n=51). All subjects were male, and had previously worked at the same shipyards. Seven of the welders were deceased, nine had emigrated or moved to other parts of Sweden, and two were not found in the national registration, leaving 29 potential participants. In the referent group, nine were deceased, four had emigrated or moved to other parts of Sweden, and one was not found in the national population registries. Thus, 66 potential participants (29 former welders and 37 referents) were contacted by telephone and asked to participate in the study. Three of the former ship welders were not included because of current exposure to welding fumes. Of the remaining 63 subjects (26+37), 19 former welders (73%) and 26 referents (70%) agreed to participate in the study. Exclusion criteria were known or suspected alcohol abuse (alcohol consumption >300 grams per week), diabetes mellitus, and other diseases that might affect neuromotor functions but are not known to be related to Mn exposure. The relevant background characteristics of the remaining 17 former ship welders and 21 referents are given in Table 3.2.

Table 3.2. *Background characteristics of 17 formerly manganese-exposed welders and 21 referents.*

	Welders (n=17)	**Referents (n=21)**
Mean age, yrs (range)	68.9 (59–76)	66.1 (59–75)
Mean body mass index (range)	26.1 (20.1–32.4)	26.5 (20.7–32.4)
Current smokers, % (n)	12 (2)	10 (2)
Current nicotine users, % (n)	18 (3)	19 (4)
Self-reported alcohol consumption, g/week (range)	56.4 (0–268)	67.8 (0–165)
Self-reported use of medication, % (n)	41 (7)	38 (8)
Hypertension, self-reported, % (n)	29 (5)	29 (6)

Paper IV

The study population in ***paper IV*** comprised all patients who had undergone DBS surgery for ET during the previous 10 years at Sahlgrenska University Hospital, Sweden, and had had ongoing treatment with DBS for at least 6 months. Before surgery, these patients had been evaluated and diagnosed with ET by a neurologist with experience in movement disorders. In all, 26 potential participants were contacted, and of these, one patient was excluded owing to concurrent neurological disease (Charcot-Marie-Tooth disease), and two patients were unable to participate in the study owing to other diseases (cancer and lumbago-ischias). One patient had moved to another part of Sweden and was not possible to trace. The remaining 22 subjects (11 males and 11 females) aged from 33 to 78 years were included in the study. All 22 patients had unilateral implants in the Vim of the thalamus, contralateral to the dominant hand. The mean duration of treatment with DBS was 5.9 years. One patient had undergone DBS surgery twice: the first time 14 years earlier and the second time 5 years before this study. Eight patients had current treatment with propranolol. The background characteristics of the 22 patients are summarized in Table 3.3.

Table 3.3. *Background characteristics of 22 patients with essential tremor.*

Age, in years, mean (range)	64 (33–78)
Sex, no. of females/males	11F/11M
Right-handedness, % (n)	91 (20)
Heredity for tremor, % (n)	73 (16)
Use of β-blockers, % (n)	36 (8)
Duration of treatment with DBS in years, mean (range)	5.9 (2–14)

3.2 Ethics

The studies presented in ***papers I–IV*** were all approved by the Research Ethics Committee at the University of Gothenburg (***papers I and II***: approval number 349-00; ***paper III***: approval number 177-08; ***paper IV***: approval number 370-09). All participants gave written informed consent.

3.3 Exposure assessment

3.3.1 Mercury vapor (papers I and II)

The exposure assessment was performed by an occupational hygienist, and was based on current and historical biological samples of mercury in urine and blood (for details, see also ***paper I***). For assessment of the current mercury exposure, a first morning urine sample was taken at home in a mercury-free polyethylene bottle. The samples were first stored in refrigerators, and then frozen until analysis. The total mercury concentration was determined using an atomic fluorescence technique after preconcentration on a gold trap (Bergdahl et al., 1995). Each sample was analyzed in duplicate, with a coefficient of variation (CV) of about 5%. The detection limit was 0.3 nmol/L (0.06 µg/L). External quality control samples (Quebec Toxicology Centre, Quebec, Canada) were analyzed together with the samples to check for accuracy. The results, 25 nmol/L (SD 5.5, n=8), 221 nmol/L (SD 0, n=2), and 389 nmol/L (SD 0, n=2), were in accordance with the target values of 25 nmol/L, 229 nmol/L, and 399 nmol/L, respectively. The concentration of U-Hg was expressed in µg/gC to correct for differences in urinary flow rate. Creatinine was determined using a modified kinetic Jaffé method (Lustgarten & Wenk, 1972).

Data on exposure time (years) were collected in a questionnaire. Based on data from previous concentrations of mercury in blood or urine (Sällsten et al., 1990; and chloralkali plant records), a cumulative exposure index (U-Hgcum) was calculated for each exposed subject by summing up the mean yearly levels. If both urine and blood samples were available for an individual for a certain year, the urinary mercury levels were used. The concentration of U-Hg, corrected for creatinine, had been determined at the two plants for 10 and 12 years, respectively. If only blood mercury or uncorrected urinary mercury levels were available, these were transformed into U-Hg µg/gC from published data on these relations (Roels et al., 1987a; Symanski et al., 2000). Mean exposure for the past 5 years (U-Hgm5) was also calculated for each individual.

The mean exposure time for the 43 exposed workers was 15 years (median 13 years, range 2–32 years). As expected, the current mercury level was significantly higher in exposed workers (median 5.9 µg/gC, mean 7.7 µg/gC, range 1.3–25 µg/gC) than among referents (median 0.7 µg/gC, mean 0.9 µg/gC, range 0.2–4.1 µg/gC). The median cumulative exposure index in the exposed group was 161 years x µg/gC (mean 266 years x µg/gC, range 8–1,440 years x µg/gC), while the median value for U-Hgm5 was 6.8 µg/gC (n=40, mean 10.9 µg/gC, range 2.1–37 µg/gC). A significant correlation was found between U-HgC and U-Hgm5 (r_s=0.77), but there were no significant correlations between current mercury exposure and either exposure time or U-Hgcum. Excluded individuals (n=15 among exposed workers, n=13 among referents) did not differ significantly in indices of mercury exposure from those included in the final group.

3.3.2 Manganese (paper III)

The exposure assessment was performed by an occupational hygienist, and focused on previous Mn exposure (for details, see also ***paper III***). Each subject completed a questionnaire about work in different occupations over the years. All of the welders had worked in the shipyard industry for the majority of their working lives. The total number of years of exposure to welding fumes was 28.1 years (mean), with a range of 14 to 45 years. Five former welders had started welding work around 1950, nine in the 1960s, and the remaining three at the beginning of the 1970s. Most of them had stopped working as welders in the 1980s or at the beginning of the 1990s. On average, the time since cessation of welding was 18 years (range 3–27 years).

Results from measurements of iron load in the lungs performed at the beginning of the 1980s were also available to calculate a cumulative exposure index (CEI) for each welder. These earlier investigations were performed with the non-invasive magnetopneumography technique in this industry (Högstedt et al., 1995). For all welders in these investigations, two values of the magnetic moment (mAm^2) from the years 1980 and 1983 were available. To calculate a CEI for each individual, the average value of the magnetic moment multiplied by the exposure time up to 1984. Since the exposure was estimated to decrease over time, half of this average was used for the years after that. The CEI ranged from 135 to 10470 (median 615) mAm^2 x years, and was used to classify the former welders into a high-exposure group (n=10, median CEI 2015, range CEI 476–10470) and a low-exposure group (n=7, median CEI 195, range CEI 135–235). The welder with the lowest CEI had an average magnetic moment of 7.3 mAm^2. The same dataset contained measurements for 14 electricians, none of whom had an average magnetic moment above this figure, as well as measurements for 17 filers, including four (24%) with a value slightly above 7 mAm^2 (the highest was 12 mAm^2). A significant correlation was found between years of exposure and CEI (r_s=0.79).

3.4 Questionnaires

All participants included in ***papers I–IV*** were asked to complete a short questionnaire about previous and current diseases, medication, and self-reported current alcohol and tobacco consumption. The participants in ***papers I and II*** (chloralkali workers) had additional questions about fish consumption and amalgam fillings. They were also asked to complete the Q16 questionnaire that has been used for screening purposes in workers exposed to organic solvents as well as neurotoxic metals (Lundberg et al., 1997).

3.5 Clinical examination and clinical rating scales

The participants underwent a physical examination (***papers I–IV***). The clinical examination included tests of sensory function, deep tendon reflexes, gait, motor strength and tone, tremor (including the finger–nose test), diadochokinesis, and the knee–heel test and Romberg's test. All clinical tests were assessed as normal or abnormal, except for tremors (rest, postural, and kinetic tremors), which were graded as absent, slight (barely noticeable), or moderate (obvious, noticeable tremor, but <2 cm excursions).

The ***Essential Tremor Rating Scale*** (ETRS; Fahn et al., 1988) was used for clinical assessment of tremor in ***paper IV***. The patient was seated in a chair while the severity of tremor (rest, postural, kinetic) was evaluated on a 5-point (0–4) rating scale. In addition, the patient's performance in line drawing and spiral drawing was assessed.

3.6 Quantitative assessment of tremor and other neuromotor functions

3.6.1 Quantitative tremor tests

A ***laser-based system*** was used for tremor recording in ***paper I***. The lasers are analog output sensors that use the optical triangulation range measurement to record displacement of the index finger, as described in detail by Beuter et al. (1994a). At the beginning of each recording day, the lasers were calibrated using a microinch, while recordings were taken at three predetermined distances. Once seated for testing, the subjects had their arms attached to supports. A light splint (<7 g) was fixed to the index finger so that the sensor beam was positioned at 10 cm from the metacarpophalangeal joint. Postural finger tremor without visual feedback was recorded twice, simultaneously in both hands. Each recording period lasted 30 seconds and was separated from the next recording period by a 15-second pause.

Using the same experimental setup, postural finger tremor with visual feedback (static tremor) was first recorded on the right side and then on the left side. Visual feedback was given through an oscilloscope placed about 80 cm from the subject's eyes. Tremor was recorded twice during an uninterrupted, 90-second recording session, divided into three 30-second periods. During the first and the last 30-second recording period, while looking at the oscilloscope screen, the subject had to maintain a line corresponding to his finger's position superimposed onto a fixed reference line appearing on the oscilloscope screen. The two static recording periods were separated by a kinetic tremor episode that consisted of a tracking task. In this task (kinetic tremor), the subject had to follow as precisely as possible with his index finger a reference line that moved up and down on the oscilloscope for a period of 30 seconds. The recordings were finally transformed into calculated measures used to characterize tremor recorded with the laser system (Table 3.4). The means of the recordings (two recordings of postural tremor without visual feedback, two trials of static tremor in each recording, and two recordings of kinetic tremor) were used in the statistical analyses.

The ***CATSYS Tremor Pen®*** (Danish Product Development, 2000) was used in ***papers I, III, and IV***. The equipment consists of a biaxial micro-accelerometer embedded in a low-mass stylus (12 cm x 0.8 cm). The subject was asked to sit down in a chair without armrest and hold the stylus as one would hold an ordinary pen with the elbow bent at an angle of 90^0 (Figure 3.2). The stylus was held horizontally and at approximately 10 cm in front of the navel, parallel to the abdomen. The subject was asked to look at the tip of the stylus and to breathe normally during recording. The default test time of 10.2 seconds (2 seconds for stabilization, and 8.2 seconds for data recording) was used in ***paper I***. Three recordings were performed; the median values were used for the statistical analyses. A longer recording time (16.4 seconds) was used in ***papers III and IV***, and only one trial was done. The tremor registrations were displayed in real time on a time axis plot on the computer screen. A fast Fourier transformation was used to determine the power distribution normalized across a frequency band varying from 0.9 Hz to 15 Hz on the combined signal from the two perpendicular accelerometers. The equipment was individually calibrated with a calibration file. Four measures calculated by the CATSYS software were used in ***papers III and IV***: tremor intensity, center frequency, frequency dispersion, and harmonic index (Table 3.5). In ***paper I***, the harmonic index and a fifth measure (tremor index) were recalculated according to Edwards and Beuter (1999). Normative data on 150 healthy subjects from 20 to 70 years and including both sexes are available (Després et al., 2000).

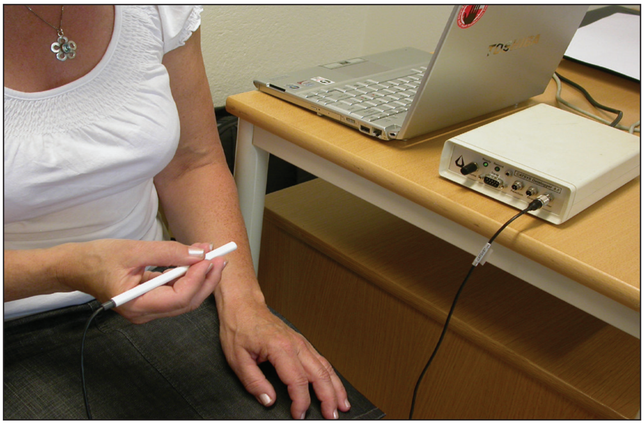


Figure 3.2. *The CATSYS Tremor Pen. Photo: G. Rydén*

Table 3.4. *Definitions of measures used to characterize postural, static, and kinetic tremor recorded with the laser system.*

Characteristics[a]	**Definitions**
Amplitude (mm/s)	Amplitude was calculated as the standard deviation (SD) of each filtered recording. A high-pass filter was applied, at 2 Hz, to the displacement series. The SD is the root mean square, because the mean becomes zero. Abnormal scores are expected to be larger.
Amplitude fluctuations	Amplitude fluctuations are measured by quantifying the variability in an envelope around tremor oscillations. First, a high-pass filter is applied (ramping between 1.5 and 2 Hz to remove any drift), the results are squared, and a low-pass filter (ramping between 1.5 and 2 Hz) is applied to remove the oscillations themselves. The square root of the result is multiplied by $\sqrt{2}$ and normalized with respect to the SD of tremor. Pathological tremors often have more fluctuations in amplitude over time. Abnormal scores are expected to be larger.
Positive/negative asymmetry	The positive/negative asymmetry is the product of two ratios: the ratio of duration of periods with positive values to that of negative values in the time series, and the ratio of amplitudes of the negative and positive parts of the time series. Values much larger or smaller than 1 indicate asymmetry.
Wobble	Wobble is the ratio of the number of extrema in acceleration to the number of extrema in displacement, or, equivalently, the number of zero crossings in their derivatives. Abnormal scores are expected to be larger.
Dispersion about median frequency (Hz)	The width of an interval centered at the median frequency, which contains 68% of the power in the spectrum. Abnormal scores are expected to be smaller.
Harmonic index	This is a measure of how close the spectrum is to a single narrow peak, normalized to the height of the highest peak. Abnormal scores are expected to be larger.
Median frequency (Hz)	Frequency, below which lies 50% of the power in the spectrum, and above which lies the other 50%. Abnormal scores are expected to be smaller.
Proportional power in the 3–4 Hz range	Proportion of the spectrum's power contained in the 3–4 Hz range. Regardless of the sharpness or exact location of peaks in this range, this quantifies how much this frequency range contributes to the tremor. Abnormal scores are expected to be larger.
Proportional power in the 4–6 Hz range	Proportion of the spectrum's power contained in the 4–6 Hz range. Regardless of the sharpness or exact location of peaks in this range, this quantifies how much this frequency range contributes to the tremor. Abnormal scores are expected to be larger.
Proportional power in the 7–12 Hz range	Proportion of the spectrum's power contained in the 7–12 Hz range. Normal physiologic tremor is supposed to be concentrated in the 8–12 Hz range. In the absence of any low-frequency pathological tremor, the value of this characteristic should be great.
Mean tracking error[b]	Mean absolute value of the difference between the reference signal and the finger signal.
Reaction time[b](sec)	The delay in movement reaching midpoint, compared with the reference signal.

[a] Definitions of characteristics from Beuter and Edwards (1999), and Edwards and Beuter (2000).

[b] Characteristics used only in kinetic tremor. Definitions from Beuter and Edwards (1998).

Table 3.5. *Definitions of measures used to characterize postural arm tremor recorded with the CATSYS Tremor Pen.*

Characteristics[a]	**Definitions**
Tremor intensity (m/s^2)	Root mean square of accelerations recorded in the 0.9–15 Hz band. Larger values indicate more tremor.
Center frequency (Hz or s^{-1})	Mean frequency of the accelerations in the 0.9–15 Hz band: 50% of the area under the spectrum is at frequencies above the center frequency, and 50% is below. Abnormal scores are expected to be smaller.
Frequency dispersion (Hz)	The standard deviation of the center frequency indicates the degree of irregularity of the tremor. Sixty-eight percent of the power is dissipated within the center frequency ±SD. A regular tremor has a small frequency dispersion, indicating that most of the area is within a narrow frequency band. Abnormal scores are expected to be smaller.
Harmonic index	This index compares the tremor frequency pattern with the pattern of a single harmonic oscillation, which has a harmonic index of 1.00. The harmonic index decreases when the tremor is composed of many oscillations. Abnormal scores are expected to be larger.
Tremor index	An overall summary index, which incorporates the four previous measures and a fifth component, a measure of dispersion of the harmonic index. Abnormal scores are expected to be larger.

[a] Definitions of characteristics from Danish Product Development (2000).

The ***Kløve-Matthews static steadiness test*** (Matthews & Kløve, 1964) is a stylus-and-hole apparatus that was used in ***paper III***. The test measures displacement of movement and requires the subject to hold a stylus for 15 seconds in nine successively smaller holes without it touching the sides, and without any arm support. Both hands were tested, beginning with the dominant hand. The cumulative number and duration of contacts between stylus and base plate for each hand were used for the statistical analyses.

3.6.2 Quantitative assessment of rapid pointing movements (eurythmokinesimetry)

The ***eurythmokinesimeter (EKM)*** was used in ***papers II–IV*** (Beuter et al., 1999b). The equipment measures rapid and precise proximo-distal movements in a pointing task (Figure 3.3). The apparatus is composed of one distal and one proximal target, each divided into three electrically isolated concentric areas, and a pointer. The centers of the target were kept at a fixed distance of 25 cm. Before starting the test, the patient was asked to sit down in front of the apparatus and hold the pointer like a pen. The rod with the mounted targets was inclined at a 30º angle, and adjusted to such a distance that the patient's arm was only slightly bent when the pointer was touching the distal target. The patient was asked to alternately touch the center of each target, as precisely and quickly as possible, always beginning with the proximal target. Each recording period lasted 30 seconds and was repeated twice with both hands alternating, with a 15-second pause between each recording. The recordings were transformed to nine calculated measures used to characterize the performance, as described in Table 3.6. For the statistical analyses, four values (two trials and two targets) were obtained for each characteristic and averaged to a mean for each hand separately.

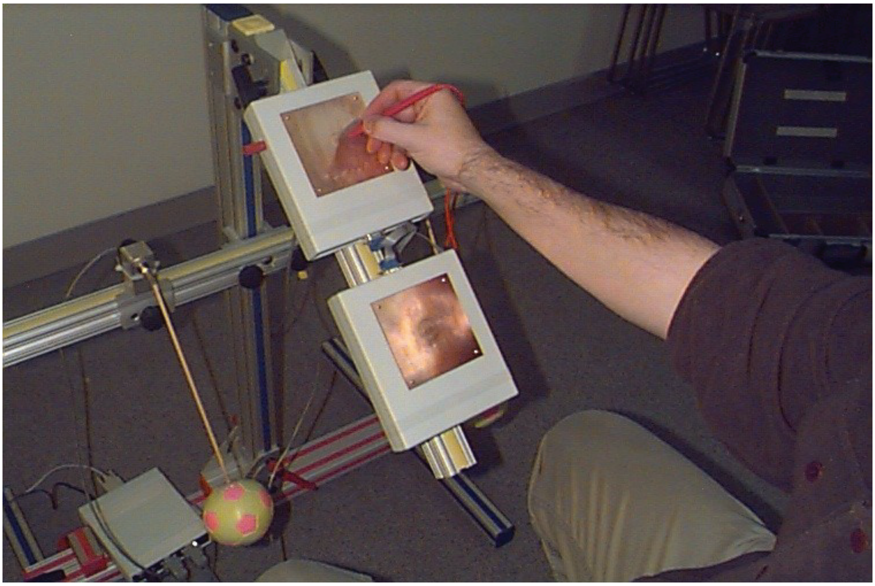


Figure 3.3. *The eurythmokinesimeter. Photo: L. Barregård.*

3.6.3 Quantitative assessment of rapid alternating movements (diadochokinesimetry)

The ***diadochokinesimeter (DIADO)*** was used in ***papers II and III*** (Beuter et al., 1994b; Beuter et al., 1999a). The apparatus measures the performance of rapid alternating movements of the forearms (Figure 3.4). The subject was asked to sit down in front of the system and firmly hold a soft sphere in each hand. The spheres were fixed to flexible rods mounted on a rod and connected to optical encoders by bendable joints. Before testing, the subject's position was adjusted so that the elbows were flexed at a 90º angle and were free from any obstacles. The subject was instructed to hold the spheres so that the palms of the hands were facing each other, and then execute alternating movements of the forearms as rapidly as possible. Each recording lasted 5 seconds and was repeated twice, with a 15-second pause between

Table 3.6. *Definitions of measures used to characterize rapid pointing movements, recorded with a eurythmokinesimeter.*

Characteristics[a]	**Definitions**
Speed	The number of times the target was struck (the number of events on target), divided by the sum of the times taken to reach the target before each event (in events per second). Larger scores indicate faster performance.
Precision	The proportion of events involving a strike on target area A. Larger scores indicate a more precise performance.
Imprecision	The proportion of events involving a strike on target area B, C, or D. Larger scores indicate a more imprecise performance.
Unsureness	The average number of contacts per event. Smaller scores indicate lower disposition to sideslip across target areas or multiple contacts in one target area, and, therefore, better performance.
Tremor	The number of contacts, less the number of target areas contacted (averaged over events). Measurement of the number of extra contacts after the initial contact when there are multiple contacts on a target area. Smaller scores indicate less tremor interfering with the performance.
Transit duration	The average duration of transportation of the hand from one target to another. Smaller scores indicate better performance.
Contact duration	The average total duration of contacts on the target. Smaller scores indicate shorter contact and, therefore, better performance.
Fitts' Law constant	The constant, k, is calculated as the average over events of $k = t/\log(2A/W)$, where t = the transit time to the target, A = the distance between the two target centers = 25 cm, and W = the approximate distance between the location of the contact(s) and the target center (for events with contacts on only one target area, the midpoint of the minimum and maximum distances of points in the area from the center was used; for events with contacts in two adjacent target areas, the distance of the separator between the two areas was used). This constant k should be a measure of inherent ability, independent of the subject's choice in the speed/accuracy trade-off. Smaller scores indicate better performance.
Irregularity	The standard deviation of intervals between events. Events from both targets and trials were used together to calculate a single SD. Smaller scores indicate more regular and, consequently, better performance.

[a] Definitions of characteristics from Beuter et al. (1999b).

each trial. Six measures used to describe the performance were calculated (Table 3.7). For the statistical analyses, the mean for each hand over the two trials for each characteristic was used.

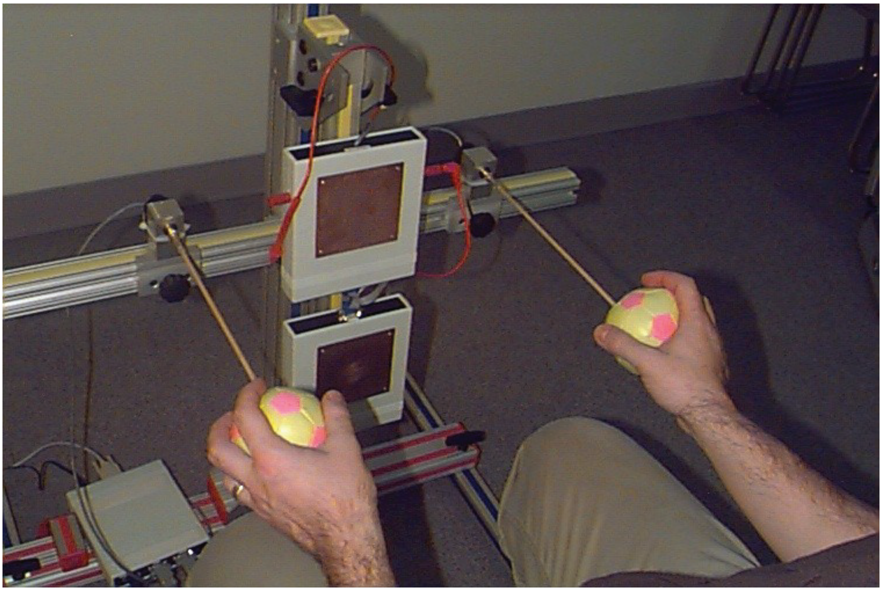


Figure 3.4. *The diadochokinesimeter. Photo: L. Barregård.*

3.6.4 Tests of grip strength, motor speed, and coordination

The ***Martin Vigorimeter*** was used to determine grip strength in ***paper II***. It is a dynamometer with a rubber balloon that is compressed in the hand. The air pressure within the balloon is registered in kp/cm^2 on a manometer connected via a rubber tube. Normative data from 450 Swedish men and women aged 21–65 years are available (Thorngren & Werner, 1979).

The ***Jamar dynamometer*** was used to measure grip strength in ***paper III***. The subject was instructed to hold the dynamometer in the palm of the hand and to squeeze the stirrup with the fingers as hard as possible. The amount of pressure in kg was recorded three times for each hand, and the mean value of the three trials was calculated. Normative data are available for adults aged 21–75+ years (Mathiowetz et al., 1985).

The manual version of ***the finger tapping test*** was used in ***paper III*** for assessment of simple motor speed. The subject was asked to press a tapping key with the index

Table 3.7. *Definitions of measures used to characterize rapid alternating movements of the forearms, recorded with a diadochokinesimeter.*

Characteristics[a]	**Definitions**
Duration	Mean duration of an oscillation (in seconds). Larger values are considered worse in fast cadence conditions.
Range	Mean of total angular displacement (pronation and supination) per cycle (in degrees). Larger values indicate greater range, and, therefore, better performance.
Velocity	Mean velocity for each cycle, averaged over all cycles (in degrees per second). Larger values are better in fast cadence conditions.
Smoothness	From the second differences of the signal, the sum of all absolute values of negative accelerations, where the velocity generally increases from zero to its maximum value, and all positive accelerations, where the velocity generally decreases from its maximum value to zero. The larger the number, the more irregular the performance.
Sharpness	The mean absolute value of velocity relative to peak velocity for ascending and descending segments, and averaged across all cycles. The larger the number, the less difficulty there is at the turn.
Maximum slope	Maximum slope of a regression line, fitted to seven successive data points and averaged over each ascending and descending part of all the cycles (in degrees per sampling point, multiplied by 200 to obtain degrees per second). Larger values are better in fast cadence conditions.

[a] Definitions of characteristics from Beuter et al. (1999a)

finger as rapidly as possible during 10 seconds (Reitan & Wolfson, 1985). The median of four trials for each hand was used for the statistical analyses (Bast-Pettersen et al., 2004).

The ***grooved pegboard test*** was used in ***paper III*** for assessment of motor speed and manual dexterity (Lezak et al., 2004; Matthews & Kløve, 1964). The subject was asked to insert 25 pegs with a ridge along one side on a board with a set of 5 x 5 slotted holes angled in different directions, as rapidly as possible. The time of completion (in seconds) for each hand was used for further calculations.

3.6.5 Postural stability

The ***CATSYS postural sway test*** (Danish Product Development, 2000) was used in ***paper III*** for assessment of postural stability. It consists of a platform with three orthogonal strain-gauge devices. The subjects were asked to stand erect on the platform with their feet about 1 cm apart, and arms hanging loosely at their sides. Postural sway was measured twice, for 60 seconds each time, first with eyes open and then with eyes closed. The outcome variables calculated by the CATSYS software for each condition were mean sway, transversal sway, sagittal sway, sway area, sway intensity, and sway velocity. Normative data on 150 healthy subjects from 20 to 70 years of age and including both sexes are available (Després et al., 2000).

3.7 Statistical methods

The Student's *t*-test or Wilcoxon's rank sum test (n <20, or skewed data) was used for group comparisons (quantitative outcome variables) in ***papers I–III***. For nominal data, the chi-squared test or Fisher's exact test was used (***papers I–III*). In ***paper IV***, the efficacy of DBS was evaluated by comparing the "on" and "off" conditions using the Wilcoxon signed rank test.

Spearman's correlation coefficients were used to evaluate associations between the outcome variables in the quantitative tests, mercury exposure (***papers I and II***) or years of welding and cumulative exposure index (***paper III***), and potential confounders. Multiple linear regression analysis was used to examine associations between the outcome variables in the quantitative tests and exposure adjusted for potential confounders (***papers I–III***). The exposure indices were included separately in the model.

In ***paper II***, the intraindividual variability in the EKM and DIADO tests was assessed by the coefficient of variation (CV = relative standard deviation). Agreement between clinical rating of tremor and quantitative measurements was assessed using

Spearman’s correlations coefficients (***papers I and IV***), while Pearson’s correlation coefficients were used to examine agreement between quantitative methods (***paper I***).

In ***paper IV***, the sensitivity and specificity for the CATSYS Tremor Pen and the EKM test were estimated after classification of the patients into those who were considered as showing a “true” improvement in the “on” condition, and those in whom no clear-cut improvement could be shown. The sensitivity was defined as the ability of the test to correctly classify the patients who had a true improvement in the “on” condition, whereas the specificity was defined as the ability to identify those who did not improve between conditions.

The reported p-values were two-sided throughout all studies (***papers 1–IV***); p-values of <0.05 were considered statistically significant.

4 Results

4.1 Effects of low-level mercury exposure on hand tremor (paper I)

4.1.1 Clinical examination

The prevalence of tremor was similar in exposed workers and referents. Postural tremor was found in 12% of exposed workers compared with 14% in the referent group. Likewise, the prevalence of kinetic tremor was similar between groups (12% vs. 9%). One exposed subject had a slight resting tremor in one hand. No associations between clinical tremor and current or cumulative mercury exposure, age, smoking habits, or work schedule were found. Few abnormal findings were found on other clinical tests (see ***paper II***).

4.1.2 The laser-based system

The exposed workers (n=43) and referents (n=22) were compared regarding their results from measurements of postural, static, and kinetic tremor with the laser-based system. There were no differences in tremor amplitude between exposed subjects and referents in any condition. However, exposed workers had significantly lower proportional power in the 7–12 Hz range in the non-dominant hand than the referents had in the postural condition (Table 4.1). Moreover, an inverse correlation between proportional power in the 7–12 Hz range for the non-dominant hand and U-Hg was found, but the correlation with U-Hg was not significant in the exposed group (Table 4.1). The exposed workers had slightly higher proportional power in the 3–4 Hz and 4–6 Hz ranges in the non-dominant hand, but the differences were not statistically significant. However, there was a significant correlation between current mercury exposure and proportional power in the 4–6 Hz range in the non-dominant hand in the entire group, as well as among exposed workers.

Multiple regression analyses were performed that allow for taking possible confounders into account. The proportional power in the 7–12 Hz range in the non-dominant hand was inversely associated with U-Hg (p=0.004) in the entire group (n=65) in a multiple regression analysis with age, shift work, and smoking included in the model. The association did not remain significant in the exposed group (p=0.06), but there was a similar association with U-Hgm5 (p=0.03). Moreover, significant associations were found between current Hg^0 exposure, U-Hgm5, and proportional power in the 3–4 Hz and 4–6 Hz ranges in the non-dominant hand in multivariate analyses with age, shift work, and smoking included in the model. However, these

Table 4.1. *Results from measurement of postural tremor with the laser-based system in the non-dominant hand.*

	Group comparisons					**Correlation coefficients (Spearman's)**			
	Exposed (n=41)		**Referents (n=22)**			**All (n=63)**	**Exposed (n=41)**		
Characteristics[a]	**Mean**	**SD**	**Mean**	**SD**	**p-value**[b]	**U-Hg**	**U-Hg**	**U-Hgcum**	**U-Hgm5**[c]
Amplitude (mm/s)	0.00749	0.004	0.00868	0.01	0.31	–0.11	0.01	0.08	–0.06
Amplitude fluctuations	0.388	0.15	0.365	0.09	0.45	–0.03	–0.06	–0.14	–0.14
Pos./neg. asymmetry	1.02	0.12	1.02	0.11	0.92	0.07	0.22	–0.03	0.25
Wobble	1.54	0.11	1.50	0.08	0.13	0.16	–0.06	0.16	–0.02
Harmonic index	0.85	0.05	0.871	0.05	0.09	–0.17	–0.07	–0.05	0.00
Median frequency (Hz)	8.71	1.38	8.63	0.84	0.76	0.01	0.08	–0.13	–0.02
Dispersion (med. freq.) (Hz)	4.67	1.14	4.18	1.29	0.12	0.10	–0.05	0.11	–0.05
Proportional power (3–4 Hz)	0.0830	0.04	0.0721	0.03	0.21	0.22	0.28	0.06	0.22
Proportional power (4–6 Hz)	0.125	0.06	0.108	0.04	0.21	**0.26***	**0.33***	0.12	0.25
Proportional power (7–12 Hz)	0.396	0.13	0.485	0.13	**0.01**	**–0.35***	–0.26	**–0.34***	**–0.36***

[a] Mean of both recordings.

[b] T-test, adjusted for unequal variance.

[c] N=38.

*P-value <0.05.

associations were no longer significant when a single outlier was excluded. Except from an inverse correlation between proportional power in the 7–12 Hz range for the non-dominant hand and U-Hgcum (Table 4.1), no associations between cumulative Hg^0 exposure and tremor frequency were found.

Smokers had higher proportional power in the 3–4 Hz range (p=0.03) and the 4–6 Hz range (p=0.01) in the non-dominant hand than non-smokers in the referent group had. Therefore, the analysis was stratified by smoking habits. In the group of non-smokers, exposed subjects (n=31) had higher proportional power in the 3–4 Hz (p=0.05) and 4–6 Hz ranges, and lower proportional power in the 7–12 Hz range in the non-dominant hand than the referents (n=16) had (Table 4.2). However, no differences were found between exposed subjects (n=10) and referents (n=6) among smokers, but the groups were small (Table 4.2).

4.1.3 The CATSYS Tremor Pen

There were no statistically significant group differences between exposed workers (n=41) and referents (n=21) in any of the outcome variables derived from the CATSYS system (see Table 8 in ***paper I***). Furthermore, no correlations were found with any of the exposure indexes. In the multivariate analyses, significant associations between current Hg exposure and the outcome variables intensity and tremor index were found in the non-dominant hand, as well as an inverse, though insignificant, association with center frequency in the dominant hand. However, these associations did not remain significant when a single outlier was removed.

Smokers had lower center frequency (p=0.02) in the non-dominant hand than non-smokers in the referent group had. Consequently, an analysis stratified by smoking habits was performed. Non-smoking referents had the highest center frequency, followed by exposed non-smokers, smoking referents, and exposed smokers (Figure 4.1). In the non-smoking group, the exposed workers had significantly lower center frequency in the non-dominant hand than the referents had (p=0.02), and there was also a similar tendency in the dominant hand (p=0.1), as shown in Table 4.2.

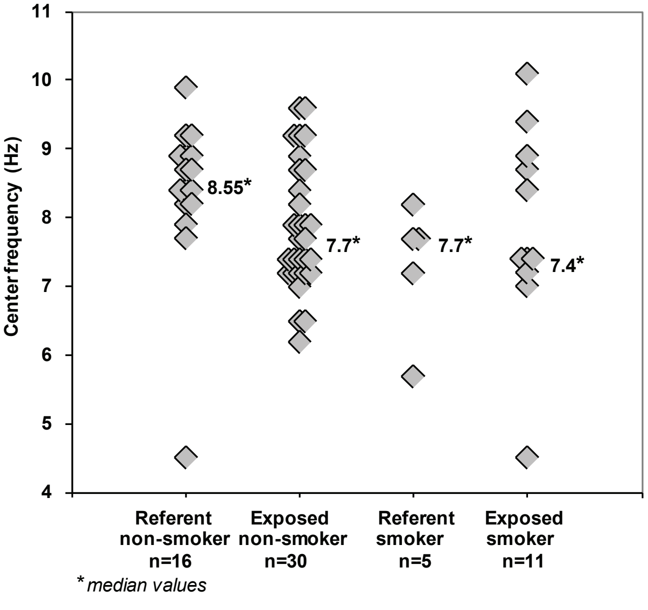


Figure 4.1. *Center frequency stratified by smoking and mercury exposure in the non-dominant hand.*

Table 4.2. *Results from tremor measurements on tremor frequency, stratified by smoking habits, in the laser system and the CATSYS system.*

	Non-smokers			**Smokers**		
	Exposed (n=31)	**Referents (n=16)**		**Exposed (n=10)**	**Referents (n=6)**	
Dominant hand						
Laser test	**Median**	**Median**	**p-value**[b]	**Median**	**Median**	**p-value**[b]
Median frequency (Hz)	8.40	8.60	0.39	9.30	8.55	0.32
Prop. power (3–4 Hz)	0.0830	0.0795	0.95	0.0755	0.0760	1.00
Prop. power (4–6 Hz)	0.129	0.110	0.87	0.111	0.114	0.71
Prop. power (7–12 Hz)	0.413	0.384	0.83	0.307	0.425	0.49
CATSYS system[a]						
Center frequency (Hz)	7.70	7.90	0.10	7.20	7.90	1.00
Non-dominant hand						
Laser test						
Median frequency (Hz)	8.30	8.60	0.33	9.10	8.15	0.09
Prop. power (3–4 Hz)	0.0770	0.0635	0.05	0.0795	0.0910	0.43
Prop. power (4–6 Hz)	0.115	0.100	**0.04**	0.117	0.135	0.46
Prop. power (7–12 Hz)	0.402	0.454	**0.03**	0.330	0.439	0.23
CATSYS system[a]						
Center frequency (Hz)	7.70	8.55	**0.02**	7.40	7.70	0.54

[a] Non-smokers (exposed, n=30; referents, n=16) and smokers (workers n=11; referents, n=5).

[b] Calculated using Wilcoxon's rank sum test.

4.2 Effects of low-level mercury exposure on certain neuromotor functions (paper II)

4.2.1 Quantitative assessment of rapid pointing movements (eurythmokinesimetry)

No significant group differences between the exposed workers and referents were found in any of the characteristics used to describe the ability to perform rapid pointing movements, as measured by the EKM system (see Table 6 in ***paper II***). In the multivariate analyses, unsureness was significantly associated with U-HgC and U-Hgm5 among exposed subjects, with age, work schedule, and smoking included in the model. In the dominant hand, these associations were, however, due to a single outlier. In the non-dominant hand, these findings were supported by a similar significant association in the entire group, with age, work schedule, and smoking included in the model. Tremor in both hands was significantly associated with U-HgC and U-Hgm5 in the multivariate analyses, but in the dominant hand, all associations were due to the same outlier mentioned above. In the non-dominant hand, no association between tremor and U-HgC was found in the entire group, and in fact the exposed workers had less tremor than the referents had. After taking outliers into account, no significant associations were found between previous Hg exposure (U-Hgcum) and any of the outcome variables from the EKM test.

4.2.2 Quantitative assessment of rapid alternating movements (diadochokinesimetry)

There were no significant overall group differences between exposed subjects and referents in any of the outcome variables from the test of rapid alternating movements of the forearms (Table 4.3). There were, however, some inverse associations between Hg exposure, on the one hand, and duration, velocity, sharpness, and range, on the other. In the multivariate analyses, an inverse association was found between velocity in the dominant hand and Hg exposure (U-HgC and U-Hgm5) among exposed subjects, and it remained statistically significant with age and work schedule included in the model. Furthermore, the ability to make fast turns (sharpness) was inversely associated with U-HgC and U-Hgm5 among exposed subjects in the dominant hand, and was close to significant with age and shift work included in the model. Finally, range in the dominant hand was inversely associated with U-HgC and U-Hgm5, but these associations disappeared when a single outlier was removed. No significant associations were found between previous Hg exposure and the outcome variables from the DIADO test in the multivariate analyses.

Table 4.3. *Results of measurement of rapid alternating movements, using a diadochokinesimeter.*

	Group comparisons					**Correlation coefficients[b] (Spearman's)**			
	Exposed subjects (n = 43)		**Referents (n = 22)**			**All (n = 65)**	**Exposed subjects (n = 43)**		
Characteristics[a]	**Mean**	**SD**	**Mean**	**SD**	**p-value[b]**	**U-HgC**	**U-HgC**	**U-Hgcum**	**U-Hgm5[c]**
Dominant hand									
Duration	4.76	1.45	5.22	2.10	0.37	0.05	0.23	0.35*	0.26
Range	333.76	54.34	347.77	63.99	0.36	–0.25*	–0.24	0.19	–0.11
Velocity	75.93	20.06	74.29	22.26	0.77	–0.19	–0.40*	–0.24	–0.29
Smoothness (x10^{-2})	2.81	0.13	0.75	0.54	0.30	0.01	0.06	–0.03	0.00
Sharpness	0.49	0.08	0.49	0.10	0.98	–0.19	–0.29	–0.21	–0.26
Maximum slope	7.61	1.42	7.50	1.70	0.77	–0.02	–0.12	–0.11	–0.06
Nondominant hand									
Duration	4.78	1.44	5.20	1.99	0.33	0.07	0.25	0.37*	0.26
Range	333.06	55.99	335.03	54.79	0.89	–0.04	–0.03	0.22	0.03
Velocity	74.85	18.38	71.22	19.58	0.46	–0.08	–0.20	–0.23	–0.08
Smoothness (x10^{-2})	0.58	0.45	0.77	0.93	0.37	–0.15	–0.01	–0.17	–0.19
Sharpness	0.50	0.08	0.50	0.09	0.77	–0.09	–0.19	–0.32*	–0.18
Maximum slope	7.31	1.36	7.05	1.59	0.50	0.07	–0.00	0.00	0.08

[a] Mean of two recordings.

[b] T-test, adjusted for unequal variance.

[c] N=40.

*P-value <0.05.

4.3 Effects of previous manganese exposure on tremor or other neuromotor functions (*paper III*)

4.3.1 Clinical examination

Former welders had slightly more kinetic tremor at clinical examination than the referent group (47% vs. 29%), but slightly less postural tremor (12% vs. 19%). No differences between the former welders and the referents were found on other clinical tests such as hyporeflexia (24% vs. 24%), impaired sensation (24% vs. 14%), or sense of vibration (6% vs. 14%). Performance on tests of diadochokinesis, knee–heel, and gait, and on Romberg's test was assessed as normal in all subjects.

4.3.2 Quantitative tests of tremor and other neuromotor functions

The performance on the quantitative tests was compared between the former welders and their referents. Former welders had significantly poorer motor speed and poorer manual dexterity in the dominant hand than their referents had (p=0.04), as evaluated with the grooved pegboard test (see Table 4.4, and Table 2 in ***paper III***). Performance in simple motor speed, as assessed with the finger tapping test, did not differ between the groups. There were no group differences in postural forearm tremor as evaluated with the Tremor Pen of the CATSYS system, except for a tendency toward a lower tremor frequency among former welders (see Table 4.4, and Table 3 in ***paper III***). On the other hand, former welders tended to perform slightly better than their referents on the static steadiness test (see Table 4.4, and Table 3 in ***paper III***). Moreover, former welders tended to have slightly higher precision, and were less imprecise in their performance (dominant hand) than their referents, as evaluated with the EKM test (see Table 5 in ***paper III***). Former welders and referents performed similarly on the test of rapid alternating movements in the forearms (see Table 6 in ***paper III***). Likewise, former welders and referents performed similarly on the postural sway test; the results were similar between groups irrespective of the recording condition used (see Table 4 in ***paper III***).

The multiple regression analyses performed on the entire group (n=38), taking age and smoking habits into account, showed associations between exposure (being a former welder) and poorer performance on the grooved pegboard test (p=0.09), but also between exposure and better precision (p=0.04) and less imprecision (p=0.01) in the dominant hand on the EKM test. When number of welding years of exposure was included in the model together with age and smoking habits, working many years as a welder was found to be associated with poorer performance on the grooved pegboard test (p=0.06) and better precision (p=0.05) and less imprecision (p=0.02) in the dominant hand. Finally, a significant association was found between CEI and impaired performance on the grooved pegboard test (p=0.03) in the dominant hand, taking age and smoking habits into account. Multiple regression analyses performed in the former welder group alone (n=17) showed no significant associations between the exposure indices and the results from the quantitative tests, except for postural sway; CEI was significantly associated with poorer performance in transversal sway with eyes open, and with mean sway, transversal sway, sagittal sway, and sway area when blindfolded. All these associations disappeared, however, when a single outlier was removed.

Table 4.4. *Results from tests of neuromotor function and tremor/hand steadiness.*

	Welders (n=17)		Referents (n=21)		
Characteristics[a]	**Mean**	**SD**	**Mean**	**SD**	**p-value**[b]
Grooved pegboard test, dominant hand (s)	85.9	16.5	76.0	11.0	0.04
Grooved pegboard test, nondominant hand (s)	95.3	20.8	90.1	16.5	0.40
Static steadiness test, number, dominant hand	146.6	128.9	159.3	79.8	0.35
Static steadiness test, number, non-dominant hand	183.0	156.6	215.0	156.8	0.32
Static steadiness test, duration (s), dominant hand	7.0	4.2	9.70	8.22	0.30
Static steadiness test, duration (s), non-dominant hand	8.0	4.9	9.52	5.29	0.44
CATSYS Tremor Pen					
Tremor intensity (m/s^2), dominant hand	0.129	0.07	0.136	0.05	0.33
Tremor intensity (m/s^2), non-dominant hand	0.133	0.08	0.140	0.05	0.20
Center frequency (Hz), dominant hand	6.50	0.86	7.04	1.22	0.06
Center frequency (Hz), non-dominant hand	6.98	1.27	7.78	1.64	0.09
Frequency dispersion (Hz), dominant hand	2.59	0.82	3.03	0.76	0.11
Frequency dispersion (Hz), non-dominant hand	2.95	1.18	3.41	0.95	0.19

[a] Non-dominant hand, welders, n=16; referents, n=19.

[b] Wilcoxon's rank-sum test.

4.4 Efficacy of deep brain stimulation in patients with ET (paper IV)

4.4.1 Clinical assessment of tremor

All patients were evaluated in two conditions: with the stimulator activated (stimulation "on") and with it deactivated (stimulation "off"). According to the clinical evaluation of *postural* tremor, 14 patients improved when the stimulators were activated, no change between conditions was found for three patients, and three patients did not have a detectable postural tremor (score=0) in either the "on" or the "off" condition (see Figure 1A in ***paper IV***). Two patients had a higher tremor score in the "on" condition than in the "off" condition. Most patients (n=16) had less *kinetic* tremor (finger–nose test) in the "on" condition, but six patients were unchanged. Of these, 2/6 patients had no kinetic tremor (score=0) in any condition (see Figure 1B in ***paper IV***). Improvement in spiral drawing and line drawing was found among 18 patients when the stimulators were activated. Significantly lower scores for postural (p=0.002) and kinetic tremors were found in the "on" condition than in the "off" condition. The effect of DBS was most pronounced regarding kinetic tremor (p <0.0001), spiral drawing (p <0.0001), and line drawing (p <0.0001).

4.4.2 The CATSYS Tremor Pen

In the CATSYS test, 20/22 patients showed improvement (lower tremor intensity) in the dominant (stimulated) hand in the "on" condition, whereas 2/22 patients showed higher tremor intensity when the stimulators were activated (see Figure 3 in ***paper IV***). Tremor intensity was significantly lower in the dominant hand when the stimulators were activated (p <0.0001). The median ratio of tremor intensity in the "on" vs. "off" condition (n=22) was 0.11. The frequency dispersion was significantly higher (p=0.006) and the harmonic index significantly lower (p=0.0004) in the "on" condition (see Table 4 in ***paper IV***). However, there was no significant change in center frequency between conditions.

4.4.3 Quantitative assessment of rapid pointing movements (eurythmokinesimetry)

The performance on the EKM test in the "off" condition and the "on" condition was compared for all patients. Three patients were unable to touch any target in the "off" condition owing to severe tremor. The missing values for these patients were replaced with the values for worst performance in the other 19 patients. In total, 19/22 patients had lower scores in Fitts' Law constant, which is an overall measurement of a patient's performance on the EKM test, when the stimulators were activated (see Figure 4 in ***paper IV***). Significant changes between conditions in the expected direction (better performance in the “on” condition) were found for most outcome variables, except unsureness and tremor (see Table 5 in ***paper IV***). However, contact duration was significantly longer when the stimulators were activated (p=0.0008). For the Fitts’ Law constant, the median on/off ratio was 0.77 (p <0.0001).

4.4.4 Evaluation of tremor suppression using quantitative tests

In ***paper IV***, an investigation was performed with the aim of evaluating which test of postural tremor (ETRS or CATSYS system) was the more sensitive in detecting a “true” improvement in the “on” condition. In order to evaluate which method better detects a “true” improvement in the “on” condition, the subjects were classified in this respect (see also ***paper IV***). Either a lower score in clinical assessment of postural tremor in the “on” condition than in the “off” condition, or an unchanged clinical score but a substantial improvement in the CATSYS test was assumed to be a true improvement. Thus a combination of the two methods was used as a gold standard. A substantial improvement in the CATSYS test was defined as an on/off ratio <0.5 in tremor intensity, or a change in tremor intensity between conditions from abnormal to normal, the latter defined as a value below the upper reference limit of the general population. Using these criteria, 15 subjects were classified as having a “true” improvement, while this could not be shown for seven patients. Since 14/15 improved according to the clinical assessment, the sensitivity was 93% and the specificity 100% for the clinical test. Using the strict criteria for improvement in the CATSYS test mentioned above, all 15 subjects were found to improve, and thus the test’s sensitivity and specificity were 100%, and 100%, respectively.

Likewise, an investigation was performed in order to evaluate which test of kinetic tremor (ETRS or EKM test) was the more sensitive in detecting a “true” improvement in the “on” condition. Either a lower score in clinical assessment of kinetic tremor in the “on” condition than in the “off” condition and no substantial impairment in Fitts’ Law constant, or an unchanged clinical score and on/off median ratio <0.75 in Fitts’ Law constant, as measured by the EKM system, was a assumed to be a “true” improvement. Seventeen subjects fulfilled these criteria, and among these, 15/17 improved according to the clinical assessment of kinetic tremor. The sensitivity for the clinical test was thus 88% and the specificity was 80%. Using the criteria above for an improved Fitts’ Law constant resulted in “true” improvement in 17 patients, and the sensitivity and the specificity for the EKM test was 47% and 100%, respectively.

4.5 Comparisons between methods (papers I, II and III)

4.5.1 The CATSYS Tremor Pen

The intensity of tremor as measured by the CATSYS Tremor Pen was compared with the results from the clinical ratings of postural tremor in ***paper I***. The measurements of all subjects were used, except those with missing values (n=89). First, the results of the clinical ratings were dichotomized into "tremor: yes" (slight and moderate tremor) or "no". Clinical tremor (slight or moderate tremor) was found in 10 (dominant hand) and 14 (non-dominant hand) subjects who were also examined with the CATSYS Tremor Pen. Subjects classified as having clinical tremor had significantly higher tremor intensity in both hands (dominant hand, p=0.006; non-dominant hand, p=0.0002). The agreement between tremor intensity and clinical evaluation of postural tremor (three grades) was assessed using Spearman's correlation coefficients. The associations between tremor intensity and clinical rating of tremor were modest: dominant hand, r_s=0.31 (p=0.004), and non-dominant hand, r_s=0.41 (p <0.0001).

The agreement between clinical rating of postural tremor as assessed by the ETRS and tremor intensity as measured by the CATSYS system was investigated in patients with ET (n=22) in ***paper IV***. The association in the dominant hand between the clinical test and the quantitative test was relatively high (r_s=0.74, p <0.0001), in the "off" condition. However, the association was low and was insignificant in the "on" condition (r_s=0.18).

4.5.2 The laser-based system

Amplitude of postural tremor, as assessed with the laser-based system, was compared with clinical rating of postural tremor in ***paper I***. The measurements from the entire group were used in the analyses, but subjects who had the two tests on different occasions were not included, leaving 78 subjects for the analyses. Clinical tremor (slight or moderate postural tremor) was found in nine (dominant hand) and 12 (non-dominant hand) subjects who also took part in the laser test. Subjects classified as having clinical tremor had significantly higher tremor amplitude in both hands (dominant hand, p=0.004; non-dominant hand, p=0.01) compared with those without clinical tremor. The agreement between tremor amplitude, as assessed with the laser-based system, and clinical evaluation of tremor (three grades) was low: dominant hand, r_s=0.34 (p=0.002), and non-dominant hand, r_s=0.30 (p=0.007). Tremor amplitude related to clinical tremor grade in the non-dominant hand is shown in Figure 4.2.

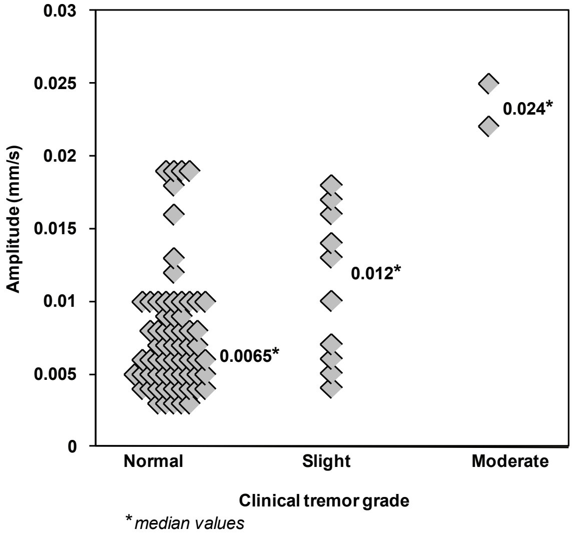


Figure 4.2. *Amplitude (laser-based system) related to clinical tremor grade in the non-dominant hand (n=78).*

A comparison between the laser-based system (postural condition) and the CATSYS test was performed in ***paper I***. The correlation coefficients (Pearson's) between tremor amplitude as measured by the laser system and intensity as measured by the CATSYS system were r=0.35 (p=0.002) and r=0.50 (p <0.0001) for the dominant and non-dominant hand, respectively. The association between the two tests in the non-dominant hand is shown in Figure 4.3.

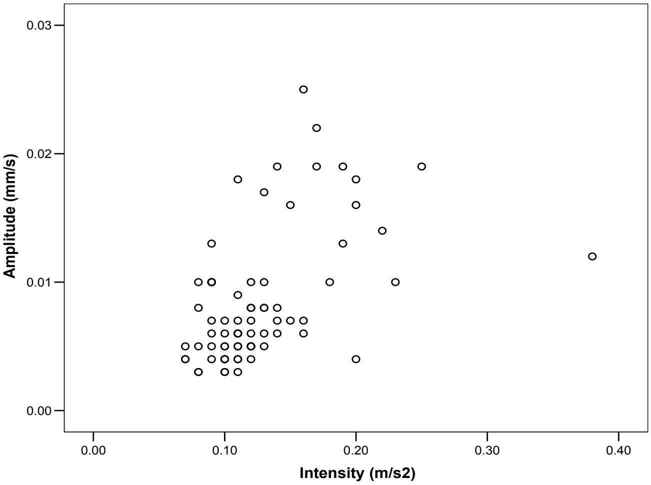


Figure 4.3. *Association between amplitude (laser-based system) and intensity (CATSYS system) in the non-dominant hand (n=74).*

4.5.3 Quantitative assessment of rapid pointing movements (eurythmokinesimetry)

The agreement between kinetic tremor and the outcome variables in the EKM test was investigated in ***paper II***. As for postural tremor, the measurements of all subjects who completed the test were used, except those with missing values (n=85). Kinetic tremor (slight or moderate tremor) was found in 13 (dominant hand) and 16 (non-dominant hand) subjects who were also examined with the EKM test. The agreement between the outcome variables from the EKM test and clinical evaluation of kinetic tremor (three grades) was assessed using Spearman's correlation coefficients. The associations were insignificant for all outcome variables: for example, the associations between Fitt's Law constant and clinical rating of tremor were r_s=0.17 (p=0.21) in the dominant hand and r_s=0.12 (p=0.26) in the non-dominant hand.

The agreement between kinetic tremor as assessed by the ETRS and the main outcome variable from the EKM test (Fitt's Law constant) was investigated in ***paper IV***. The association was low to moderate and did not reach statistical significance (r_s=0.34, p=0.12) in the "off" condition, and was low in the "on" condition (r_s=0.23, p=0.31).

5 Discussion

5.1. Validity aspects

5.1.1 Subjects

Selection of subjects

Epidemiological studies may be affected by selection bias, i.e. a distortion of the estimated effects that depends on the way the subjects are selected for the study or other factors that might affect study participation (Rothman & Greenland, 1998). A specific situation, referred to as "healthy worker effect," is when subjects have acquired a disease resulting from the exposure being studied, and have left work before the study begins. How these potential problems were addressed in ***papers I–III*** is discussed below.

The exposed subjects and referents in ***papers I and II*** were all blue-collar workers employed at the same plants. Important background characteristics, as well as other sources that may give a small contribution to mercury exposure, i.e. amalgam fillings or high fish consumption, were similar among exposed workers and referents. The participation rates were high in both groups (mercury-exposed workers 97% and referents 83%). The relatively long mean time of employment (15 years) and the low turnover rate among the exposed workers speak against selection bias resulting from the "healthy worker effect."

The former welders and their referents in ***paper III*** were former blue-collar workers from the same shipyards. The participation rates were relatively high (former welders 73% and referents 70%). The groups were comparable with respect to important background characteristics, although the former welders were on average 2.8 years older. Great care was taken in finding an appropriate referent group for the welders. Electricians and filers were chosen as referents, as they were considered to have had the most similar working demands in the past. The former welders tended to perform better than the referents in the static steadiness test. Similar findings have been demonstrated in other welding studies (Bast-Pettersen et al., 2000; Ellingsen et al., 2008). The occupation of a welder makes great demands on hand steadiness, and probably a subject with impairment in this function will either not choose the welding occupation, or leave the occupation if that type of problem, which might be related to exposure, occurs (selection into or out of the occupation). Furthermore, former welders tended to perform better than the referents in the eye–hand coordination test, but conversely tended to perform less well in the finger–nose test. The discrepancy in results between these tests is possibly due to a training effect from former working tasks: the EKM system has some similarities with welding, whereas the finger–nose test does not. Consequently, aside from selection into or out of the occupation of a welder, these findings might also be due to a training effect from former working tasks.

Exclusions

Since the effects following low exposure to mercury vapor and manganese are expected to be small, other factors that might affect neuromotor function in the population under study need to be taken into account. In ***papers I–III***, all subjects with diseases and other conditions that might affect performance in the neurobehavioral tests were excluded, in order to reduce "noise" and to be able to detect more subtle effects possibly resulting from the exposure under study. Blood tests for hemoglobin, hematocrit, red blood cell indices, and C-reactive protein, as well as liver function tests, and methylmalonate, glucose, and thyroid function tests, were available in the welding study (***paper III***).

All subjects taking medication known to cause tremor or to suppress tremor, i.e. beta-blockers were excluded in the chloralkali studies (***papers I and II***). The study in ***paper III*** was performed on an older age group, where the prevalence of certain diseases as well as medication was expected to be common. Thus, the self-reported use of medication was quite high (around 40%) and was similar for exposed subjects and referents. In ***paper IV***, the efficacy of DBS was evaluated by comparing the results in the "off" and "on" conditions in each subject. Patients with concurrent neurological diseases were excluded, because the evaluation of the efficacy of DBS was restricted to tremor in ET patients. The aim was to investigate and compare different tests for evaluating the efficacy of DBS on tremor in a situation as similar as possible to common clinical practice; thus the patients were asked to continue their medication as usual. Eight patients were treated with propranolol, which may have involved a reduction of tremor amplitude among these patients.

Acute effects of alcohol, may affect performance in several neurobehavioral tests (Williamson & Feyer, 2000), as well as excessive alcohol abuse (Lezak et al., 2004). However, there are uncertainties regarding at which level of chronic alcohol consumption the performance on neurobehavioral tests will be affected. A consumption>280 g of pure alcohol per week for men has been proposed as a lower threshold for an elevated risk of alcohol-related health effects, particularly liver cirrhosis (Saunders & Lee, 2000). All subjects in ***papers I–III*** were questioned regarding their alcohol habits and consumption. Alcohol consumption was quantified and reported in ***paper III***. Two subjects (one welder and one referent) were excluded because of alcohol abuse in ***paper III***. The self-reported alcohol consumption among those included in the study was well below the limit where health effects might be expected. Moreover, all subjects included in ***paper III*** had normal tests of liver function.

5.1.2 Exposure assessment

The exposure assessment in ***papers I and II*** was based on biological samples of mercury in urine and blood on an individual basis. Previous studies with long-term occupational mercury exposure have shown a close correlation between time-weighted air concentrations and urinary excretion rates of mercury (WHO, 1991). An intraindividual variation of urinary concentration of mercury is present and may be minimized by using the first morning sample and correcting for creatinine (Berlin et al., 2007). A cumulative exposure index was calculated for each of the exposed workers. Some uncertainties in exposure classification might have been present. This would have caused a possible association between exposure and effect to be biased toward the null. Another important aspect is that although the mercury concentration in urine is a fairly good biomarker of body burden of mercury, there is still no adequate biomarker available for one of the most sensitive target organs, the brain.

In ***paper III***, no airborne measurement data available, since the industry closed down at the beginning of the 1990s. However, measurements from shipyards in Sweden and in other countries have shown manganese levels of about 0.1–0.3 mg/m^3 (Chang et al., 2009; Ellingsen et al., 2008; Fored et al., 2006; Järvisalo et al., 1992). The exposure assessment was based on number of years of welding, and most of the exposure time was from welding in the shipyard industry. The results from measurements of iron load in the lungs were used to calculate a CEI for each welder. A relatively high correlation (r=0.83) has been shown between airborne manganese and iron exposure among welders (Flynn & Susi, 2010), and iron load in the lungs might therefore reflect the manganese exposure. Improvements in ventilation were taken into account when estimating the CEI (for details, see ***paper III***). There was a high contrast in CEI between the high-exposure and low-exposure welding groups (a factor of 10 between the average CEIs). As discussed above, if a misclassification with respect to exposure is present, a possible association between exposure and effect will be weakened. The referents, especially the filers, might have been exposed to some welding fumes as well, but at much lower levels. The exposure to welding fumes among the electricians in this study was considered negligible.

5.1.3 Methods

Procedures

The participants were examined at the same premises, either at their ordinary workplaces (***papers I and II***), or at the Department of Occupational and Environmental Medicine, Sahlgrenska University Hospital (***paper III***). The participants were tested either in the morning or in the afternoon, and the percentages of exposed subjects and referents tested at different periods of time in the day were similar. Different test batteries were used in ***papers I–IV***, respectively. The tests were administered in the same order to the participants throughout each study. However, 11 participants were examined with the laser test on a later occasion owing to technical failure (***paper I***). The tests were administered twice in ***paper IV***, with the stimulators either activated or deactivated (“on” or “off”). The order of conditions was randomized, and the evaluators of ETRS were blinded to whether the stimulators were activated or not, in order to minimize biased assessments. The ET patients were also blinded to the present stimulation condition. Another reason to randomize the order of conditions was to take into account any learning effect between the first and second evaluation sessions. The quantitative tests in ***papers I and II*** were conducted by a neuropsychologist who was blinded with respect to the participants' exposure status. The author conducted the clinical examinations together with another physician in ***papers I and II***, the clinical and quantitative tests in ***paper III***, and the quantitative tests in ***paper IV***.

Validity/reliability of methods

Clinical tests, such as evaluation of tremor, are routinely used in daily clinical practice. It has been indicated by Notermans et al. (1994) that these types of clinical tests are usually not sufficiently sensitive to detect subclinical changes in neuromotor function, and contain a considerable degree of subjectivity when criteria are applied (McKeown-Eyssen et al., 1990). In addition, marked interobserver variation has been reported in the assessment of signs of neurological abnormalities (McKeown-Eyssen et al., 1990). Several clinical rating scales, such as the ETRS, have been developed with the aim of assisting in assessment of tremor severity and evaluation of treatment efficacy. The ETRS has been evaluated in patients with ET, and the intrarater reliability in assessment of tremor in the upper limb was found to be very good; however, it was not as good in assessment of writing and drawing (Stacy et al., 2007). The interrater reliability was moderately good in upper limb tremor assessment, but not satisfactory in writing and drawing.

The CATSYS Tremor Pen has been validated in a study by Edwards & Beuter (1997); high associations between different variables were shown when simultaneous recordings were performed with a highly sensitive laser system. The system has proven to have a high degree of reproducibility in one study (Orsnes et al., 1998). However, Edwards & Beuter (1997) found the harmonic index to be less stable between trials, and they have presented a recalculated index (Edwards & Beuter, 1999).

The test–retest reliability of the EKM system has been reported to be good for most outcome variables (Beuter et al., 1999b). In ***paper II***, the intraindividual variability expressed as coefficient of variation was examined for the EKM test, and it was found to be low for most outcome variables (CV ranging from 9–31%) except for tremor and irregularity. Thus, two trials may be insufficient for these characteristics. In the DIADO test, the recordings were made in fast cadence, with both hands moving simultaneously. This condition is anticipated to exacerbate any subclinical adverse effects on the CNS more than other conditions (Beuter et al., 1994b); also, the characteristics used have been shown to be consistent between trials in fast cadence (Beuter et al., 1999a). The test–retest reliability for the DIADO test was examined in ***paper II***. The intraindividual variability, expressed as the CV, ranged from 6% to

12% for most characteristics. However, the variability in smoothness between trials was very high, indicating that the number of trials (or the duration of the test) was insufficient for this variable.

5.1.4 Potential confounders

A confounder is supposed to be associated with both the exposure and the outcome under study (Rothman & Greenland, 1998). The presence of potential confounders has to be especially considered in studies with low exposures and anticipated small effects, because the effect of a potential confounder could be large compared with the effect of exposure. Potential confounders and the way they were addressed in ***papers I–III*** are discussed below.

Age

The normal aging process involves a gradual decline in several neurobehavioral functions, such as motor function, coordination, and balance (Mahant & Stacy, 2001). Age effects are reported for most of the neurobehavioral tests that were used in ***papers I–III,*** such as slower performance in the finger tapping test and the grooved pegboard test, and reduced grip strength by advancing age (Lezak et al., 2004). Tremor frequency slowly decreases with age (Deuschl et al., 1996), but amplitude is not necessarily affected.

In order to obtain good comparability between exposed subjects and their referents, only referents in the same age group as the exposed subjects were invited. Mean age was similar between chloralkali workers and their referents (***papers I and II***). However, former welders were on average 2.8 years older than their referents (***paper III***). Age was considered a potential confounder and thus included in the model as a covariate in the multivariate analyses. In ***paper III***, subjects >75 years were not included after considering the dramatically increased prevalence of neurodegenerative disease, such as movement disorders (Mahant & Stacy, 2001) and Alzheimer's disease, among the elderly.

A general impairment by age in performance on the EKM test and longer cycle durations and more difficulties with turns in the DIADO test have been described in one study (Beuter et al., 1999c). In ***paper II***, increasing age was found to be related to a slower but more precise performance in the EKM test, but the performance in general was not affected. In the DIADO test, older subjects had more difficulties with the turns in the non-dominant hand, but no other age effects were found (***paper II***). However, the subjects in ***paper II*** consisted of a selected working population in contrast to the study by Beuter et al. (1999c), which comprised a large sample from the general population.

Smoking habits

Smoking habits have recently been pointed out as an important potential confounder in epidemiological studies in which the effect on tremor is studied (Ellingsen et al., 2006). Nicotine exposure increases tremor amplitude in humans (Elble & Koller, 1990). Exposure to nicotine is mainly via cigarette smoking, but the use of wet snuff is not uncommon in Sweden. Experimental studies have demonstrated that exposure to cigarette smoke induces an increase in postural tremor amplitude (Lippold et al., 1980; Maykoski et al., 1976; Shiffman et al., 1983). However, not only postural tremor might be affected; it has been shown that current smokers have more kinetic tremor than non-smokers during a variety of hand activities such as the finger-nose test, spiral drawing, and use of a spoon (Louis, 2007).

Current smokers and non-smokers were similarly distributed between exposed subjects and referents in ***papers I–III***, but there were more current users of all kinds of tobacco among the exposed subjects than among the referents ***in papers I and II*** (53% vs. 32%). Smoking habits and/or all kinds of tobacco use were considered as potential confounders and included as a covariate in the multiple regression analyses in ***papers I, II and III***. Another strategy to handle a potential confounder is to use stratification. Stratification for smoking habits and mercury exposure was used in ***paper I***. An effect of mercury exposure on tremor frequency was seen in the non-smoking group, but not in the smoking group. One explanation could be that smoking obscures the effect of mercury on tremor frequency.

In ***paper II***, smokers had more tremor and more tendency to sideslip across target areas on the EKM test than nonsmokers had. This finding is in accordance with other occupational studies that have reported increased tremor related to smoking habits (Bast-Pettersen et al., 2004; Bast-Pettersen et al., 2005; Ellingsen et al., 2001). Smokers had lower tremor frequency in the referent group, but not in the exposed group (***paper I***). In accordance with these findings, lower tremor frequency among smokers was reported in one study (Beuter et al., 1999c), whereas other studies showed no effect on tremor frequency (Bast-Pettersen et al., 2004; Bast-Pettersen et al., 2005). The finding of a possible effect of smoking habits on range using the DIADO test has to be confirmed by other studies (see ***paper II***).

Shift work

Shift work may affect the performance in neurobehavioral tests mainly because of sleep deprivation (Williamson & Feyer, 2000). Most of the workers in ***papers I and II*** were shift workers, but shift work was similar among exposed subjects and referents (65% vs. 68%). Shift workers had lower current mercury exposure than daytime workers (see ***paper I***). Shift work was considered a potential confounder and was included as a covariate in the multivariate analyses in ***papers I and II***. The effect of shift work on performance on the EKM and DIADO tests was investigated in ***paper***

II, but the investigation could not confirm any impairment resulting from shift work on these tests; on the contrary, shift workers were faster than daytime workers on the DIADO test.

5.1.5 Generalizability

Some restrictions were applied regarding the potential participants' availability for the studies. In ***papers I–III***, only blue-collar workers were invited. Furthermore, only men were included; very few women worked in the chloralkali industry, and none were among the former shipyard workers. Moreover, some restrictions were applied to age. The purpose of these restrictions was to obtain greater comparability between the exposed subjects and the referents; on the other hand, the generalizability of the results from the studies is limited to these groups. In ***paper IV***, only patients with a confirmed diagnosis of ET were included; thus the results from the study are limited to patients with ET.

5.2 Findings

5.2.1 Does low-level mercury exposure affect tremor and neuromotor function? (papers I and II)

Current mercury exposure

In the study of chloralkali workers with current low-level exposure to mercury reported in ***paper I***, no effect of mercury exposure was seen on tremor amplitude, irrespective of the method used (clinical rating of tremor, quantitative assessment with the laser system, or the CATSYS Tremor Pen). An increase in tremor amplitude has consistently been reported in studies with high or moderate current mercury exposure down to exposure levels around 30 µg/gC (WHO, 1991). In accordance with the findings in ***paper I***, no significant effects on tremor amplitude were seen in several other studies with low (U-Hg up to 25 µg/gC or µg/l) current exposure levels (Chapman et al., 1990; Echeverria et al., 1998; Ellingsen et al., 2001; Fawer et al., 1983; Langworth et al., 1992; Lucchini et al., 2002), some of which used the CATSYS system (Echeverria et al., 1998; Lucchini et al., 2002), except for a possible effect in a study of gold traders (Biernat et al., 1999).

The main finding in the quantitative tremor tests was a slight decrease of tremor frequency in the non-dominant hand in exposed workers (***paper I***). It was demonstrated by a shift in the proportional power from the 7–12 Hz range to the 4–6 Hz range among exposed subjects in the laser test (postural condition). The group difference was supported by an inverse association between proportional power in the 7–12 Hz range and current mercury exposure. Furthermore, a significantly lower center frequency was found, using the CATSYS system, in the exposed non-smokers than in their non-smoking referents. The results from previous studies on mercury exposure and tremor frequency are contradictory. In studies with high or moderate current exposure, tremor frequency was found to increase (Miller et al., 1975; Verberk et al., 1986; Wood et al., 1973), remain unaltered (Bittner et al., 1998; McCullough et al., 2001; Roels et al., 1985; Schuckmann, 1979), or decrease (Langolf et al., 1978). In contrast to the findings in ***paper I***, some studies with low exposure (U-Hg 20–25 µg/gC or µg/l) indicated increasing tremor frequency following current mercury exposure (Chapman et al., 1990; Fawer et al., 1983). Two studies using the CATSYS system (Echeverria et al., 1998; Lucchini et al., 2002) found no effects on tremor frequency at exposure levels around U-Hg 10 µg/gC or lower. Thus, previous studies do not support the finding in ***paper I*** that tremor frequency may decrease following mercury exposure. The laser system is, however, more sensitive to the high-frequency components in the frequency spectrum than the accelerometer is (Edwards & Beuter, 1999), and there are no previous studies of low mercury exposure using the laser system. The effect on tremor frequency was found only in the non-dominant hand, in contrast to other studies using quantitative methods, where an effect of mercury exposure on tremor parameters was seen in both hands (Biernat et al., 1999; Langolf et al., 1978; Miller et al., 1975; Roels et al., 1985, Roels et al., 1989; Verberk et al., 1986; Williamson et al., 1982; Wood et al., 1973). However, in some studies, tremor was only measured in the right or dominant hand (Chapman et al., 1990; Fawer et al., 1983). Significant differences in physiologic tremor between hands have been demonstrated with the laser system in a study of healthy female subjects (Beuter et al., 2000). The possibility that an effect of mercury exposure is expressed differently in the dominant and non-dominant hands cannot be excluded. The fact that several of the findings on tremor frequency point in the same direction (a decrease) and were consistently found in the same (non-dominant) hand, irrespective of method, speaks in favor of a true effect.

In general, no significant adverse effects of long-term, low-level mercury exposure on certain aspects of neuromotor function (the ability to perform rapid pointing movements, and the ability to perform rapid alternating movements in the forearms) were found in the study of chloralkali workers reported in ***paper II***. Some findings in the EKM test, however, indicate that current mercury exposure may have an effect on eye–hand coordination (unsureness, tremor) in the non-dominant hand. The results from other studies with low exposure levels (≤U-Hg 25 µg/gC or µg/L) regarding motor speed and coordination are somewhat contradictory. One study of chloralkali workers found no differences in finger tapping or eye–hand coordination between exposed subjects and referents (Langworth et al., 1992). The performance in these tests was, however, negatively correlated with earlier peak exposures. In another study of chloralkali workers, no effect on eye–hand coordination was found and, surprisingly, the exposed workers performed better in finger tapping (Piikivi & Hänninen, 1989). By contrast, reduced performance in finger tapping was shown among exposed workers in a study of lamp factory workers with similar exposure levels (Liang et al., 1993). Some studies have shown an association between occupational mercury exposure and reduced motor speed, as evaluated with the finger tapping test (Echeverria et al., 1998; Lucchini et al., 2002; Ngim et al., 1992) at very low exposure levels (U-Hg ≤10 µg/gC) in contrast to the absence of such associations in another study of chloralkali workers with low exposure levels (Ellingsen et al., 2001). Neither have any effects of mercury exposure been reported on other tests of motor speed and manual dexterity, such as the grooved pegboard test (Ellingsen et al., 2001; Ngim et al., 1992). Thus, the findings of a possible effect on eye–hand coordination in ***paper II*** have little support from other studies with similar exposure levels. Impaired performance of rapid alternating movements has not been described at lower exposure levels, and quantitative measurement of these movements has not previously been applied in subjects exposed to mercury. Consequently, the finding of a possible effect on velocity in the dominant hand in the DIADO test must be confirmed by other studies.

Current vs. previous mercury exposure

An important question is whether an observed effect on tremor or other neuromotor functions is due to current or historical mercury exposure. Thus, a possible explanation for discrepancies in observed effects among currently exposed workers is different historical mercury exposure in the study populations. Several studies among previously exposed workers have shown an excess of abnormal clinical findings (Albers et al., 1988; Andersen et al., 1993; Frumkin et al., 2001; Letz et al., 2000; Powell, 2000), impairment in finger tapping (Frumkin et al., 2001; Kishi et al., 1994), eye–hand coordination and manual dexterity (Frumkin et al., 2001; Kishi et al., 1994; Mathiesen et al., 1999; Powell, 2000), even long after cessation of exposure. Moreover, several studies on workers with previous mercury exposure have reported increased hand tremor (Albers et al., 1988; Andersen et al., 1993; Frumkin et al., 2001; Powell, 2000), and this finding is supported by other studies showing that increased hand tremor is more related to past than current mercury exposure (Fawer et al., 1983; Langolf et al., 1878; Roels et al., 1985). However, the historical exposure levels were generally high in most of these studies.

On the laser test, a significant association between previous exposure (U-Hgcum) and amplitude was found in the dominant hand, in the postural condition (***paper I***). However, no relation between cumulative mercury exposure and increased hand tremor was seen in another study of chloralkali workers with similar cumulative Hg exposure to that of the workers in ***papers I and II*** (Ellingsen et al., 2001). Proportional power in the 7–12 Hz range was inversely correlated to U-Hgcum, and the group differences in proportional power were most pronounced between workers with the highest U-Hgcum value and the referents (***paper I***). Consequently, there is a possibility that a lowering of frequency may be a persistent effect of previous exposure to mercury. However, no alteration in tremor frequency was shown when the original group in the study by Ellingsen et al. (2001) was re-examined using the CATSYS system 5 years after cessation of exposure (Bast-Pettersen et al., 2005).

Neither were any effects on motor speed and coordination reported (Bast-Pettersen et al., 2005), which is in accordance with the findings in ***paper II***.

5.2.2 Does previous Mn exposure affect neuromotor function? (paper III)

Former welders performed less well with the dominant hand on the grooved pegboard test than their referents did, and poorer performance was associated with CEI, although the association was not significant in the welder group alone. The grooved pegboard test is a test of motor speed and manual dexterity, so this finding was expected and is in accordance with several studies of workers with current Mn exposure, that have shown decreased performance in fine motor function (Bowler et al., 2003; Chang et al., 2009; Chia et al, 1993; Sjögren et al., 1996), some of them also using the grooved pegboard test (Bowler et al., 2003; Chang et al., 2009). Moreover, high pallidal index on MRI has been reported to be associated with poorer dominant hand performance in the grooved pegboard test (Chang et al., 2009). Furthermore, decreased performance on the grooved pegboard test has been shown among welders, even after cessation of exposure (Bowler et al., 2006; Ellingsen et al., 2008).

However, former welders and referents performed similarly on most of the other neurobehavioral tests. Impaired performance on the finger tapping test is a common finding in workers exposed to Mn (Bowler et al., 2003; Bowler et al., 2006; Chang et al., 2009; Chia et al., 1993; Ellingsen et al., 2008; Iregren, 1990; Lucchini et al., 1999), and has also been reported among welders with previous exposure (Bowler et al., 2006; Ellingsen et al., 2008). This finding was not supported by the results in the welding study in ***paper III***. However, the results are in accordance with those of Bouchard et al. (2007), who reported normal performance in finger tapping among former Mn alloy workers 14 years after cessation of exposure.

Several studies on Mn-exposed workers have shown impairment in hand steadiness (Bast-Pettersen et al., 2004; Crump & Rousseau, 1999; Hochberg et al., 1996; Mergler et al., 1994; Roels et al., 1987b; Roels et al., 1992), and irreversible effects despite decreased Mn exposure have been reported in one study (Roels et al., 1999). Impairment in eye–hand coordination in Mn-exposed workers has been reported by some authors (Hochberg et al., 1996; Roels et al., 1987b; Roels et al., 1992), and improvement in eye–hand coordination following decreasing Mn exposure has been shown in one study (Roels et al., 1999). Some authors have reported negative results (Bast-Pettersen et al., 2004; Iregren, 1990; Sjögren et al., 1996). However, former welders tended to perform better than the referents, both in the static steadiness test and in the EKM test, probably because of selection bias out of or into the occupation of a welder, and/or a training effect from former working tasks. Thus, a possible effect of Mn exposure on these functions is hard to evaluate, but probably there is no major adverse effect.

Impaired ability to perform rapid alternating movement in the forearms (dysdiadochokinesis) may be an early sign of dysfunction in the extrapyramidal system, and has been indicated in some studies among Mn-exposed workers (Beuter et al., 1994b; Wennberg et al., 1991). The fastest cadence was used, i.e. the subjects were asked to perform the movements as rapidly as possible, in order to stress the neuromotor system. However, no significant differences between former welders and referents were found, in accordance with another welding study (Sjögren et al., 1996).

Postural tremor was measured using the CATSYS system, and similar results were found in welders and referents. Some studies have reported alterations in tremor parameters among Mn-exposed subjects using the CATSYS system (Bast-Pettersen et al., 2004; Bowler et al., 2007; Chang et al., 2009; Lucchini et al., 1999), whereas others have not (Ellingsen et al., 2008), in accordance with the findings in ***paper III***. Tremor naturally fluctuates over time, and so a negative finding might be explained by too short a recording time of 8.2 seconds, which is the default time set by the CATSYS system. Thus, a longer testing time has been recommended (Bast-Pettersen & Ellingsen, 2005), and a recording time of 16.4 seconds was used in ***paper III*** Furthermore, the CATSYS system (which measures acceleration) has been shown to be less effective than the static steadiness test (which measures displacement) in discriminating between Mn-exposed subjects and referents (Bast-Pettersen & Ellingsen, 2005).

A tendency to fall backward when displaced is a typical feature in manganism. Impairment in postural stability has been indicated among Mn-exposed workers (Bowler et al., 2007; Chia et al., 1995; Ellingsen et al., 2008), while Chang et al. (2009) found no such effect of Mn exposure. In ***paper III,*** associations between CEI and several outcome variables in the postural sway test were found, but all associations were due to the same outlier, a former welder with the highest CEI (10470 mAm^2 x years). A true effect of high Mn exposure cannot be excluded; on the other hand, some of the referents had similar results, and the comparison between welders and referents pointed in the opposite direction, making it less likely that it is a true causal association.

A battery of highly sensitive neurobehavioral tests was used for evaluating the effects of Mn exposure on neuromotor function in former welders. Manganese accumulates in the basal ganglia, especially in the globus pallidus, which has an important role in regulating voluntary movements. A recent meta-analysis of performance effects resulting from occupational Mn exposure has shown that certain neurobehavioral tests such as those measuring motor speed were among the most affected (Meyer-Baron et al., 2009). Thus, the finding of a decreased performance in the grooved pegboard test is a result that could be expected. However, former welders and referents performed similarly on most of the other neurobehavioral tests, including other tests of simple motor speed such as the finger tapping test. A possible explanation is differences in exposure; however, the welders had long exposure times, and previous Mn exposure was documented on an individual basis. To my knowledge, this study examined the longest time period since cessation of exposure. Since significant time had passed since cessation of Mn exposure, previous adverse effects on the neuromotor system might have ceased. Furthermore, the participants were quite old, and a decrement in neuromotor function due to normal aging processes in both groups might have disguised a slight effect caused by previous Mn exposure. The poorer performance in the grooved pegboard test among former welders suggests a remaining adverse effect of long-term Mn exposure.

5.2.3 Could quantitative methods be used to evaluate the effect of thalamic deep brain stimulation on tremor suppression? (paper IV)

The effect of DBS on tremor suppression was prominent irrespective of the method used. The median score for kinetic tremor as evaluated by the ETRS was higher than the median score for postural tremor in the "off" condition, which is in accordance with findings in other studies where the amplitude of kinetic tremor is usually greater than that of the postural component (Brennan et al., 2002). Thalamic deep brain stimulation affects postural as well as kinetic tremor, but the reduction is believed to be greater for postural tremor (Earhart et al., 2007). However, according to the clinical rating, the effect of DBS was most pronounced on the kinetic tremor component in the ET patients. Two patients worsened in postural tremor when the stimulator was activated in the ETRS test but not in the CATSYS test, and there is no plausible explanation for this finding.

The CATSYS Tremor Pen was used for quantitative assessment of postural tremor. Tremor intensity showed an improvement of 89% in the "on" condition. Two patients had slightly higher tremor intensity (17% and 22%, respectively) when the stimulator was activated, but these small changes were considered as having no clinical significance. The tremor frequency in ET decreases over time and is inversely related to age (Elble, 2000); thus the relatively low tremor frequency (median 4.5 Hz) among the ET patients was probably due to the high average age in this group. Tremor frequency did not change when the stimulator was activated, in accordance with another study (Earhart et al., 2007).

Kinetic tremor is most disabling for the ET patient, and is often accompanied by an intentional component in more advanced cases (Elble & Deuschl, 2009). The EKM test was chosen for quantitative assessment of kinetic tremor because it is supposed to be similar to the clinical finger–nose test. As expected, the ET patients were faster and had greater precision on the EKM test when the stimulator was activated. Thus the Fitts' Law constant, an overall measure of performance, improved 23% between conditions, independently of the subjects' choice in the speed/accuracy trade-off. Interestingly, the patients had a more regular performance when the stimulator was activated, as reflected by a 37% improvement in irregularity, and by longer contact with the targets, which implies better control. Recent studies have shown that difficulties in eye–hand coordination in ET are not only due to intention tremor in the target phase, but also due to defective regulation in the early phase of hand movement, probably as a result of cerebellar dysfunction (Trillenberg et al., 2006). Moreover, impaired rhythm generation has been shown in ET patients (Avanzino et al., 2009; Farkas et al., 2006).

The aim of the study was to examine the use of certain quantitative methods in evaluating the efficacy of thalamic DBS and to compare these methods with traditional clinical tools for tremor assessment. Since no gold standard was available for measuring an improvement in tremor amplitude in the "on" condition, a combination of the two methods (clinical and quantitative) was used and thus defined a "true" improvement in both postural and kinetic tremor (see also the Results section and ***paper IV***). For the CATSYS test, a >50% reduction in tremor intensity was considered to be a clinically significant improvement in postural tremor. All patients who improved in clinical score between conditions (n=14) also had >50% reduction in tremor intensity; therefore, the choice of cut-off point seems reasonable. Moreover, among those who had no postural tremor (score=0) in any condition, two patients had a tremor intensity below the upper reference limit in both conditions. However, tremor intensity for the third patient decreased from 1.93 to 0.13, a 93% reduction. The CATSYS system identified all ET patients with a "true" improvement, and the sensitivity and the specificity for the CATSYS system were excellent, at 100%, and 100%, respectively.

The Fitts' Law constant was chosen for comparison with the clinical rating of kinetic tremor because this measure is considered to be an overall measure of performance and is independent of the patient's choice to perform rapidly but less precisely, or slowly but more precisely. Although 17/17 patients improved on the Fitts' Law constant compared with 15/17 on the clinical test, the sensitivity was not satisfying (47%), even if the specificity was high (100%). One explanation may be that the clinical test (finger–nose test) and the EKM test are not entirely comparable. The finger–nose test is performed with the eyes closed, whereas the EKM test involves the visual pathways. The EKM test measures different aspect of the performance, but the clinical test is focused on tremor and dysmetria only. The EKM test therefore includes other domains of function that may be affected in ET, such as eye–hand coordination and rhythm regulation. The association between these different aspects of motor function and the outcome variables from the EKM test needs further exploration.

5.2.4 Comparisons between methods (papers I, II and IV)

Amplitude of tremor measured by the laser system in the postural condition, and tremor intensity measured by the CATSYS system were compared with clinical rating of postural tremor in ***papers I and IV***. The agreement between clinical tremor evaluation and the quantitative tremor measurements was generally low to moderate, in accordance with other studies (Beuter & Edwards, 1998; Edwards & Beuter, 2000). There may be several reasons for the agreement between the methods not being perfect. First, tremor is naturally fluctuating over time, and a plausible explanation for the low agreement is the time delay between the clinical examination and the quantitative tests. In another study (Gerr et al., 2000), in which the quantitative tremor measurements were obtained immediately after the clinical examination, moderately good agreement was reported between clinical tremor and tremor intensity measured by the CATSYS system. Second, in clinical assessment, tremor is assessed visually while subjects are asked to hold their arms against gravity, a condition presumed to affect displacement more than acceleration, which is measured by the CATSYS system (Edwards & Beuter, 1997). However, the agreement between clinical tremor rating and the laser system (which measures displacement) was low (***paper I***). Possibly, subtle changes in displacement detected by the lasers are not visible at clinical examination. Third, methods such as the CATSYS system give a quantitative value averaged over a time period, while the clinician may take certain qualitative aspects of tremor into account. Fourth, different hand positions in different tests may exacerbate or decrease tremor. Finally, different examiners may also have affected the results in the tremor tests.

Other aspects may need to be considered when data from clinical tremor rating are compared with quantitative measurements of tremor. When the agreement between clinical rating of postural tremor and tremor intensity as measured by the CATSYS system was examined in ET patents, a relatively high association (r_s=0.74) was found in the “off” condition (***paper IV***). Thus, the complete lack of association in the “on” condition (r_s=0.18) indicates that the clinical tremor scoring is not sufficiently discriminative at low tremor amplitudes. Only eight of 22 ET patients had postural tremor in the “on” condition, six of them with a score of 1 on the ETRS. Furthermore, clinical tremor rating is quite crude and has a “floor and ceiling” effect: for example, a patient with no tremor and a patient with discrete tremor will both score 0, and a patient with severe tremor cannot score >4. Moreover, data from a variety of studies show a logarithmic relationship between 4- and 5-point (0–4) rating scales and tremor amplitude (Elble et al., 2006); this relationship must be taken into account when clinical assessment is compared with quantitative measurements of tremor. However, the rank correlation that was used to describe the agreement between these tests ought to also represent the association adequately when the distribution of tremor amplitude is skewed.

The agreement between the Fitts' Law constant from the EKM test and the clinical rating of kinetic tremor was examined in ET patients in ***paper IV***. The association between kinetic tremor as assessed by the ETRS, and the Fitts' Law constant was low to moderate in the "off" condition (r_s=0.34) and is in accordance with other studies (Beuter et al., 1999b). The quantitative test measures several distinct aspects of the performance and is, in contrast to the clinical evaluation, a continuous variable. Another explanation may be the time delay between the tests (about 10 minutes). Moreover, the clinical test (finger–nose test) and the EKM test may not be entirely comparable.

Two quantitative systems for measuring tremor were compared: an accelerometer (the CATSYS system) and a highly sensitive laser system recording displacement (***paper I***). The agreement between these two methods was low to moderate. One possible explanation is that the laser system records in one axis only, while the CATSYS system records in two. Moreover, differences between the two tests in the recording procedures, e.g. duration of tests and support of forearms, may also be of importance. High agreement was shown between different variables including amplitude when simultaneous recordings with the two systems were performed (Edwards & Beuter, 1997). The systems, however, produced significantly different results when the recordings were not simultaneous, indicating that the time factor is of great importance.

6 Conclusions

- No effect of low-level mercury exposure on tremor amplitude was shown irrespective of the methods used (qualitative or sensitive quantitative methods). Limited support was provided for a decrease in tremor frequency in the non-dominant hand resulting from mercury exposure.

- No effect of low-level mercury exposure was shown on certain neuromotor functions, such as the ability to perform rapid pointing movements or rapid alternating forearm movements, using sensitive quantitative tests.

- Former welders had poorer performance in a test of motor speed and manual dexterity, which may have been caused by previous manganese exposure, even a long time after cessation of exposure. Selection bias or training effects must be considered when assessing hand steadiness and eye–hand coordination in welders.

- The efficacy of thalamic deep brain stimulation on suppression of hand tremor in patients with ET was confirmed using sensitive quantitative tests. Quantitative methods for measurement of hand tremor could complement clinical assessment in evaluating the efficacy of DBS in clinical practice.

- Agreement between clinical tremor rating and quantitative measurement of tremor was generally low at low tremor amplitudes. Qualitative methods may be too insensitive as tools for detecting subtle changes in tremor or other neuromotor functions at low-level exposure to neurotoxins; in this situation sensitive quantitative methods may be useful.

7 Acknowledgements

Det är en lång resa att skriva en avhandling och det känns otroligt att det äntligen är klart! Jag skulle vilja tacka alla personer som på olika sätt gett mig stöd, hjälp och uppmuntran under resans gång och då särskilt:

Lars Barregård, min huvudhandledare, som med stort engagemang och oändligt tålamod lotsat mig fram mot resans mål. Jag är mycket tacksam för att jag har fått ta del av dina stora kunskaper som du så generöst delat med dig av till mig och för allt stöd och uppmuntran jag fått under arbetet. Speciellt tack för att du alltid varit tillgänglig för både stora och små frågor.

Bo Johnels, min bihandledare och medförfattare. Tack för att du generöst delat med dig av dina stora kunskaper om neurologiska sjukdomar och allt stöd och uppmuntran under arbetet.

Rita Bast-Pettersen, min bihandledare och medförfattare. Tack för det stora engagemang du visat för mig och mitt arbete med stöd, uppmuntran och stimulerande vetenskapliga diskussioner. Speciellt tack för trevlig samvaro under gemensamma konferensresor.

My other co-authors Anne Beuter, Björn Holmberg, Daniel Lamoureux and Gerd Sällsten are acknowledged for fruitful collaboration. Ett speciellt tack till Gerd Sällsten för ditt stora engagemang och att du alltid ställer upp och läser så noggrant, inget undgår dina falkögon!

Eva M Andersson, som bidragit med goda råd och hjälp angående statistiska analyser och på ett mycket pedagogiskt sätt förklarat avancerade statistiska analyser så att även jag förstår!

Alla studiedeltagare som ställde upp och gav av sin tid för att medverka i studierna.

Annika Claesson och Kristina Wass för mycket gott samarbete i arbetet med svetsarstudien och hjälp med provtagning mm.

Gunnel Garsell för ovärderlig hjälp med allt administrativt kring studierna och all hjälp jag fått med figurer, referenser, mm.

Gunilla Rydén för engagerat arbete med att få till bra foton och figurer och skicklig hjälp med layout.

Alla medlemmar i Tox-forskargruppen för intressanta diskussioner, många goda råd och praktisk hjälp samt inte minst trevlig samvaro på våra resor till internationella konferenser. Övriga kolleger på AMM för gott samarbete, vänligt stöd och uppmuntran.

Min älskade familj, Ola, Viktoria och Josephine. Tack för ert tålamod när jag arbetat sena kvällar och varit helt uppslukad av skrivandet och ibland trött och stressad. Ni är de viktigaste i mitt liv och jag ser fram emot att få mer tid tillsammans med er framöver!

Tack till Forskningsrådet för Arbetsliv och Socialvetenskap (FAS) samt Göteborgs Läkaresällskap som bidragit med finansiellt stöd.

8 References

Albers JW, Kallenbach LR, Fine LJ, Langolf GD, Wolfe RA, Donofrio PD, Alessi AG, Stolp-Smith KA, Bromberg MB. Neurological abnormalities associated with remote occupational elemental mercury exposure. Ann Neurol 1988;24:651–9.

Andersen A, Ellingsen DG, Mørland T, Kjuus H. A neurological and neurophysiological study of chloralkali workers previously exposed to mercury vapour. Acta Neurol Scand 1993;88:427–33.

Avanzino L, Bove M, Tacchino A, Ruggeri P, Giannini A, Trompetto C, Abbruzzese G. Cerebellar involvement in timing accuracy of rhythmic finger movements in essential tremor. Europ J Neurosci 2009;30:1971–79.

Bast-Pettersen R, Ellingsen DG. The Kløve-Matthews static steadiness test compared with the DPD TREMOR. Neurotoxicology 2005;26:331–42.

Bast-Pettersen R, Skaug V, Ellingsen D, Thomassen Y. Neurobehavioral performance in aluminum welders. Am J Ind Med 2000;37:184–92.

Bast-Pettersen R, Ellingsen DG, Hetland SM, Thomassen Y. Neuropsychological function in manganese alloy plant workers. Int Arch Occup Environ Health 2004;77:277–87.

Bast-Pettersen R, Ellingsen DG, Efskind J, Jordskogen R, Thomassen Y. A neurobehavioral study of chloralkali workers after the cessation of exposure to mercury vapor. Neurotoxicology 2005;26:427–37.

Bergdahl IA, Schütz A, Hansson GÅ. Automated determination of inorganic mercury in blood after sulphuric acid treatment using cold vapour atomic absorption spectrometry and inductive heated gold traps. Analyst 1995;120:1205–09.

Berlin M, Zalups RK, Fowler BA. Mercury. In: Nordberg GF, Fowler BA, Nordberg M, Friberg LT, editors. Handbook on the Toxicology of Metals, 3rd ed. Burlington (MA): Academic Press Inc; 2007, pp. 675–729.

Beuter A, de Geoffroy A. Can tremor be used to measure the effects of chronic mercury exposure in human subjects? Neurotoxicology 1996;17:213–28.

Beuter A, Edwards R. Tremor in Cree subjects exposed to methylmercury: A preliminary study. Neurotoxicol Teratol 1998;20:581–89.

Beuter A, Edwards R. Using frequency domain characteristics to discriminate physiologic and parkinsonian tremors. J Clin Neurophysiol 1999;16:484–94.

Beuter A, de Geoffroy A, Cordo P. The measurement of tremor using simple laser systems. J Neurosci Methods 1994a;53:47–54.

Beuter A, Mergler D, de Geoffroy A, Carrière L, Bélanger S, Varghese L, Sreekumar J, Gauthier S. Diadochokinesimetry: A study of patients with Parkinson’s disease and manganese exposed workers. Neurotoxicology 1994b;15:655–64.

Beuter A, de Geoffroy A, Edwards R. Analysis of rapid alternating movements in Cree subjects exposed to methylmercury and in subjects with neurological deficits. Environ Res 1999a;80:64–79.

Beuter A, de Geoffroy A, Edwards R. Quantitative analysis of rapid pointing movements in Cree subjects exposed to methylmercury and in subjects with neurological deficits. Environ Res 1999b;80:50–63.

Beuter A, Edwards R, de Geoffroy A, Mergler D, Hudnell K. Quantification of neuromotor function for detection of the effects of manganese. Neurotoxicology 1999c;20:355–66.

Beuter A, Edwards R, Boucher A. Physiological tremor: Does handedness make a difference? Int J Neurosci 2000;101:9–19.

Biernat H, Ellias SA, Wermuth L, Cleary D, de Oliveira Santos EC, Jørgensen PJ, Feldman RG, Grandjean P. Tremor frequency patterns in mercury vapor exposure, compared with early Parkinson’s disease and essential tremor. Neurotoxicology 1999;20:945–52.

Bittner AC Jr., Echeverria D, Woods JS, Aposhian HV, Naleway C, Martin MD, Mahurin RK, Heyer NJ, Cianciola M. Behavioral effects of low-level exposure to Hg^0 among dental professionals: A cross-study evaluation of psychomotor tests. Neurotoxicol Teratol 1998;20:429–39.

Bouchard M, Mergler D, Baldwin M, Panisset M, Bowler R, Roels HA. Neurobehavioral functioning after cessation of manganese exposure: A follow-up after 14 years. Am J Ind Med 2007;50:831–40.

Bowler RM, Gysens S, Diamond E, Booty A, Hartney C, Roels HA. Neuropsychological sequelae of exposure to welding fumes in a group of occupationally exposed men. Int J Hyg Environ Health 2003;206:517–29.

Bowler R. M, Gysens S, Diamond E, Nakagawa S, Drezgic M, Roels HA. Manganese exposure: Neuropsychological and neurological symptoms and effects in welders. Neurotoxicology 2006;27:315–26.

Bowler RM, Roels HA, Nakagawa S, Drezgic M, Diamond E, Park R, Koller W, Bowler RP, Mergler D, Bouchard M, Smith D, Gwiazda R, Doty RL. Dose-effect relationships between manganese exposure and neurological, neuropsychological and pulmonary function in confined space bridge welders. Occup Environ Med 2007;64:167–77.

Brennan KC, Jurewicz EC, Ford B, Pullman SL, Louis ED. Is essential tremor predominantly a kinetic or a postural tremor? A clinical and electrophysiological study. Mov Disord 2002;17:313–16.

Calne DB, Chu NS, Huang CC, Lu CS, Olanow W. Manganism and idiopathic parkinsonism: Similarities and differences. Neurology 1994;44:1583–86.

Chang Y, Kim Y, Woo ST, Song HJ, Kim SH, Lee H, Kwon YJ, Ahn JH, Park SJ, Chung IS, Jeong KS. High signal intensity on magnetic resonance imaging is a better predictor of neurobehavioral performances than blood manganese in asymptomatic welders. Neurotoxicology 2009;30:555–63.

Chapman LJ, Sauter SL, Henning RA, Dodson VN, Reddan WG, Matthews CG. Differences in frequency of finger tremor in otherwise asymptomatic mercury workers. Br J Ind Med 1990;47:838–43.

Chia SE, Foo SC, Gan SL, Jeyaratnam J, Tian CS. Neurobehavioral functions among workers exposed to manganese ore. Scand J Work Environ Health 1993;19:264–70.

Chia SE, Gan SL, Chua LH, Foo SC, Jeyaratnam J. Postural stability among manganese exposed workers. Neurotoxicology 1995;16:519–26.

Clarkson TW, Magos L. The toxicology of mercury and its chemical compounds. Crit Rev Toxicol 2006;36:609–62.

Cohen O, Pullman S, Jurewicz E, Watner D, Louis ED. Rest tremor in patients with essential tremor: Prevalence, clinical correlates, and electrophysiologic characteristics. Arch Neurol 2003;60:405–10.

Couper J. On the effects of black oxide of manganese when inhaled into the lungs. Br Ann Med Pharmacol 1837;1:41–2.

Crump KS, Rousseau P. Results from eleven years of neurological health surveillance at a manganese oxide and salt producing plant. Neurotoxicology 1999;20:273–86.

Danish Product Development. CATSYS 2000 User's Manual. Snekkersten, Denmark: Danish Product Development Ltd; 2000.

Deng H, Weidong L, Jankovic J. Genetics of essential tremor. Brain 2007;130:1456–64.

Després C, Lamoureux D, Beuter A. Standardization of a neuromotor test battery: The CATSYS system. Neurotoxicology 2000;21:1–11.

Deuschl G. Neurophysiological tests for the assessment of tremors. In: Stern GM, editor. Parkinson's Disease : Advances in Neurology, vol 80. Philadelphia (PA): Lippincott Williams & Wilkins;1999, pp. 57–65.

Deuschl G, Elble R. Essential tremor – Neurodegenerative or nondegenerative disease towards a working definition of ET. Mov Disord 2009;24:2033–41.

Deuschl G, Krack P, Timmer J. Clinical neurophysiology of tremor. J Clin Neurophys 1996;13:110–21.

Deuschl G, Bain P, Brin M. Consensus statement of the Movement Disorder Society on Tremor: Ad Hoc Scientific Committee. Mov Disord 1998;13:2–23.

Deuschl G, Raethjen J, Lindemann M, Krack P. The pathophysiology of tremor. Muscle Nerve 2001;716–35.

Dowling J. Deep brain stimulation: Current and emerging indications. Mo Med 2008;105:424–8.

Earhart GM, Hong M, Tabbal SD, Perlmutter JS. Effects of thalamic stimulation frequency on intention and postural tremor. Exp Neurol 2007;208:257–63.

Echeverria D, Aposhian HV, Woods JS, Heyer NJ, Aposhian MM, Bittner AC Jr., Mahurin RK, Cianciola M. Neurobehavioral effects from exposure to dental amalgam Hg^0: New distinctions between recent exposure and Hg body burden. FASEB J 1998;12:971–80.

Edwards R, Beuter A. Sensitivity and specificity of a portable system measuring postural tremor. Neurotoxicol Teratol 1997;19:95–104.

Edwards R, Beuter A. Indexes for identification of abnormal tremor using computer tremor evaluation. IIEE Trans Biomed Eng 1999;46:895–8.

Edwards R, Beuter A. Using time domain characteristics to discriminate physiologic and parkinsonian tremors. J Clin Neurophysiol 2000;87–100.

Ehrenberg RL, Vogt RL, Smith AB, Brondum J, Brightwell WS, Hudson PJ, McManus KP, Hannon WH, Phipps FC. Effects of elemental mercury exposure at a thermometer plant. Am J Ind Med 1991;19:495–507.

Elble RJ. Central mechanisms of tremor. J Clin Neurophys 1996;13:133–44.

Elble RJ. Essential tremor frequency decreases with time. Neurology 2000;55:1547–51.

Elble RJ. Tremor: Clinical features, pathophysiology, and treatment. Neurol Clin 2009;27:679–95.

Elble RJ, Deuschl G. An update on essential tremor. Curr Neurol Neurosci Rep 2009;9:273–7.

Elble RJ, Koller WC. Tremor. Baltimore (MD): John Hopkins University Press; 1990.

Elble RJ, Pullman SL, Matsumoto JY, Raethjen J, Deuschl G, Tintner R; Tremor Research Group. Tremor amplitude is logarithmically related to 4- and 5-point tremor rating scales. Brain 2006;129:2660–6.

Ellingsen DG, Bast-Pettersen R, Efskind J, Thomassen Y. Neuropsychological effects of low mercury vapor exposure in chloralkali workers. Neurotoxicology 2001;22:249–58.

Ellingsen DG, Bast-Pettersen R, Efskind J, Gjølstad M, Olsen R, Thomassen Y, Molander P. Hand tremor related to smoking habits and the consumption of caffeine in male industrial workers. Neurotoxicology 2006;27:525–33.

Ellingsen DG, Konstantinov R, Bast-Pettersen R, Merkurjeva L, Chashchin M, Thomassen Y, Chashchin V. A neurobehavioral study of current and former welders exposed to manganese. Neurotoxicology 2008;29:48–59.

Fahn S, Elton RL. Members of the UPDRS Development Committee: The Unified Parkinson's Disease Rating Scale. In Fahn S, Marsden CD, Calne DB, Goldstein M, editors: Recent Developments in Parkinson's Disease, vol 2. Florham Park, NJ, Macmillan Healthcare Information: 1987, pp. 153–163, 293–304.

Fahn S, Jankovic J. Principles and Practice of Movement Disorders. Philadelphia (PA): Churchill Livingstone Elsevier; 2007.

Fahn S, Tolosa E, Marin C. Clinical rating scale for tremor. In: Jankovic J, Tolosa E, editors. Parkinson's Disease and Movement Disorders. Baltimore (MD): Urban & Schwarzenberg; 1988, pp. 225–34.

Farkas Z, Szirmai I, Kamondi A. Impaired rhythm generation in essential tremor. Mov

Disord 2006;21:1196–99.

Fawer RF, De Ribaupierre Y, Guillemin MP, Berode M, Lob M. Measurement of hand tremor induced by industrial exposure to metallic mercury. Br J Ind Med 1983;40:204–8.

Flynn MR, Susi P. Manganese, iron, and total particulate exposures to welders. J Occup Environ Hyg 2010;7:115–26.

Fored CM, Fryzek JP, Brandt L, Nise G, Sjögren B, McLaughlin JK, Blot WJ, Ekbom A. Parkinson's disease and other basal ganglia or movement disorders in a large nationwide cohort of Swedish welders. Occup Environ Med 2006;63:135–40.

Frumkin H, Letz R, Williams PL, Gerr F, Pierce M, Sanders A, Elon L, Manning CC, Woods JS, Hertzberg VS, Mueller P, Taylor BB. Health effects of long-term mercury exposure among chloralkali plant workers. Am J Ind Med 2001;39:1–18.

Gerr F, Letz R, Green RC. Relationships between quantitative measures and neurologist's clinical rating of tremor and standing steadiness in two epidemiological studies. Neurotoxicology 2000;21:753–60.

Gibbs JP, Crump KS, Houck DP, Warren PA, Mosley WS. Focused medical surveillance: A search for subclinical movement disorders in a cohort of U.S. workers exposed to low levels of manganese dust. Neurotoxicology 1999;20:299–313.

Goldwater LJ. Mercury: A History of Quicksilver. Baltimore (MD): York Press; 1972.

Greenamyre JT, Betarbet R, Sherer T. The rotenone model of Parkinson's disease: Genes, environment and mitochondria. Parkinsonism Relat Disord 2003;9:559–64.

Günther W, Sietman B, Seeber A. Repeated neurobehavioral investigations in workers exposed to mercury in a chloralkali plant. Neurotoxicology 1996;17:605–14.

Hellwig B, Mund P, Schelter B, Guschlbauer B, Timmer J, Lücking CH. A longitudinal study of tremor frequencies in Parkinson's disease and essential tremor. Clin Neurophysiol 2009;120:431–5.

Hochberg F, Miller G, Valenzuela R, McNelis S, Crump KS, Covington T, Valdivia G, Hochberg B, Trustman JW. Late motor deficits of Chilean manganese miners: A blinded control study. Neurology 1996;47:788–95.

Högstedt P, Kadefors R, Näslund PE. Comparison of lung radiography and counting of exposure time to magnetopneumography for estimation of total lung dust retention in arc welders. Technol Health Care 1995;3:15–21.

Hua MS, Huang CC. Chronic occupational exposure to manganese and neurobehavioral function. J Clin Exp Neuropsychol 1991;13:495–507.

Iregren A. Psychological test performance in foundry workers exposed to low levels of manganese. Neurotoxicol Teratol 1990;12:673–5.

Järvisalo J, Olkinura M, Kiilunen M, Kivistö H, Ristola P, Tossavainen A, Aitio A. Urinary and blood manganese in occupationally nonexposed populations and in manual metal arc welders of mild steel. Int Arch Occup Environ Health 1992;63:495–501.

Kim Y, Kim KS, Yang JS, Park IJ, Kim E, Jin Y, Kwon KR, Chang KH, Kim JW, Park SH, Lim HS, Cheong HK, Shin YC, Park J, Moon Y. Increase in signal intensities on T1-weighted magnetic resonance images in asymptomatic manganese-exposed workers. Neurotoxicology 1999;20:901–8.

Kishi R, Doi R, Fukuchi Y, Satoh H, Satoh T, Ono A, Moriwaka F, Tashiro K, Takahata N, Sasatani H, Shirakashi H, Kamada T, Nakagawa K. Residual neurobehavioral effects associated with chronic exposure to mercury vapour. Occup Environ Med 1994;51:35–41.

Kuncel AM, Cooper SE, Wolgamuth BR, Clyde MA, Snyder SA, Montgomery EB, Rezai AR, Grill WM. Clinical response to varying the stimulus parameters in deep brain stimulation for essential tremor. Mov Disord 2006;11:1920–8.

Langolf GD, Chaffin DB, Henderson R, Whittle HP. Evaluation of workers exposed to elemental mercury using quantitative tests of tremor and neuromuscular function. Am Ind Hyg Assoc J 1978;39:976–84.

Langston JW, Ballard PA, Tetrud JW, Irwin I. Chronic parkinsonism in humans due to a product of meperidine-analog synthesis. Science 1983;219:979–80.

Langworth S, Almkvist O, Söderman E, Wikström BO. Effects of occupational exposure to mercury vapour on the central nervous system. Br J Ind Med 1992;49:545–55.

Leong CC, Syed NI, Lorscheider FL. Retrograde degeneration of neurite membrane structural integrity of nerve growth cones following in vitro exposure to mercury. NeuroReport 2001;26:733–7.

Letz R, Gerr F, Cragle D, Green RC, Watkins J, Fidler AT. Residual neurologic deficits 30 years after occupational exposure to elemental mercury. Neurotoxicology 2000;21:459–74.

Lezak MD, Howieson DB, Loring DW. Neuropsychological Assessment, 4th ed. Oxford, England: Oxford University Press; 2004.

Liang YX, Sun RK, Sun Y, Chen ZQ, Li LH. Psychological effects of low exposure to mercury vapor: Application of a computer-administered neurobehavioral evaluation system. Environ Res 1993;60:320–7.

Lippold OCJ, Williams EJ, Wilson CG. Finger tremor and cigarette smoking. Br J Clin Pharmacol 1980;10:83–6.

Louis ED. Essential tremor. Lancet Neurol 2005;4:100–10.

Louis ED. Kinetic tremors: Differences between smokers and non-smokers. Neurotoxicology 2007;28:569–75.

Louis ED. Environmental epidemiology of essential tremor. Neuroepidemiology 2008;31:139–49.

Louis ED, Barnes L, Wendt KJ, Ford B, Sangiorgio M, Tabbal S, Lewis L, Kaufmann P, Moskowitz C, Comella CL, Goetz CC, Lang AE. A teaching videotape for the assessment of essential tremor. Mov Disord 2001;16:89–93.

Lucchini R, Selis L, Folli D, Apostoli P, Mutti A, Vanoni O, Iregren A, Alessio L. Neurobehavioral effects of manganese in workers from a ferroalloy plant after cessation of exposure. Scand J Work Environ Health 1995;21:143–9.

Lucchini R, Bergamaschi E, Smargassi A, Festa D, Apostoli P. Motor function, olfactory threshold, and hematological indices in manganese-exposed ferroalloy workers. Environ Res 1997;73:175–80.

Lucchini R, Apostoli P, Perrone C, Placidi D, Albini E, Migliorati P, Mergler D, Sassine M-P, Palmi S, Alessio L. Long term exposure to “low levels” of manganese oxides and neurofunctional changes in ferroalloy workers. Neurotoxicology 1999;20:287–98.

Lucchini R, Cortesi I, Facco P, Benedetti L, Camerino D, Carta P, Urbano ML, Zaccheo A, Alessio L. Neurotoxic effect of exposure to low doses of mercury. Med Lav 2002;93:202–14.

Lundberg I, Högberg M, Michélsen H, Nise G, Hogstedt C. Evaluation of the Q16 questionnaire on neurotoxic symptoms and a review of its use. Occup Environ Med 1997;54:343–50.

Lustgarten JA, Wenk RE. Simple, rapid kinetic method for serum creatinine measurement. Clin Chem 1972;18:1419–22.

Lyons KE, Pahwa R. Deep brain stimulation and tremor. Neurotherapeutics 2008;5:331–8.

Mahant PR, Stacy MA. Movement disorders and normal aging. Neurol Clin 2001;19:553–63.

Mathiesen T, Ellingsen DG, Kjuus H. Neuropsychological effects associated with exposure to mercury vapor among former chloralkali workers. Scand J Work Environ Health 1999;25:342–50.

Mathiowetz V, Kashman N, Volland G, Weber K, Dowe M, Rogers S. Grip and pinch strength: Normative data for adults. Arch Phys Med Rehabil 1985;66:69–74.

Matthews CG, Kløve H. Instruction Manual for the Adult Neuropsychology Test Battery. Madison (WI): University of Wisconsin Medical School; 1964.

Maykoski KA, Rubin MB, Day AC. Effects of cigarette smoking on postural muscle tremor. Nurs Res 1976; 25:39–43.

McCullough JE, Dick R, Rutchik J. Chronic mercury exposure examined with a computer-based tremor system. J Occup Environ Med 2001;43:295–300.

McKeown-Eyssen GE, Ruedy J, Hogg S, Read Guernsey J, Woods I. Validity and reproducibility of a screening examination for neurological abnormality in persons exposed to methylmercury. J Clin Epidemiol 1990;43:489–98.

Mergler D, Huel G, Bowler R, Iregren A, Bélanger S, Baldwin M, Tardif R, Smargassi A, Martin L. Nervous system dysfunction among workers with long-term exposure to manganese. Environ Res 1994;64:151–80.

Meyer-Baron M, Schaeper M, Van Thriel C, Seeber A. Neurobehavioral test results and exposure to inorganic mercury: In search of dose-response relations. Arch Toxicol 2004;78:207–11.

Meyer-Baron M, Knapp G, Schäper M, Van Triel C. Performance alterations associated with occupational exposure to manganese. Neurotoxicology 2009;30:487–96.

Miller JM, Chaffin DB, Smith RG. Subclinical psychomotor and neuromuscular changes in workers exposed to inorganic mercury. Am Ind Hyg Assoc J 1975;36:725–33.

Myers JE, teWaterNaude J, Fourie M, Zogoe HB, Naik I, Theodorou P, Tassel H, Daya A, Thompson ML. Nervous system effects of occupational manganese exposure on South African mineworkers. Neurotoxicology 2003a;24:649–56.

Myers JE, Thompson ML, Ramushu S, Young T, Jeebhay MF, London L, Esswein E, Renton K, Spies A, Boulle A, Naik I, Iregren A, Rees DJ. The nervous system effects of occupational exposure on workers in a South African manganese smelter. Neurotoxicology 2003b;24:885–94.

Ngim CH, Foo SC, Boey KW, Jeyaratnam J. Chronic neurobehavioural effects of elemental mercury in dentists. Br J Ind Med 1992;49:782–90.

Notermans NC, Van Dijk GW, Van der Graaf Y, Van Gijn J, Wokke JHJ. Measuring ataxia: Quantification based on the standard neurological examination. J Neurol Neurosurg Psychiatry 1994;57:22–6.

Orsnes GB, Sorensen PS. Evaluation of electronic equipment for quantitative registration of tremor. Acta Neurol Scand 1998;97:36–40.

Parkinson J. An Essay on the Shaking Palsy. London, England: Sherwood, Neely, Jones; 1817. Reprinted in: J Neuropsychiatri Clin Neurosci 2002;14:223–36.

Perl DP, Olanow CW. The neuropathology of manganese-induced parkinsonism. J Neuropathol Exp Neurol 2007;66:675–82.

Piikivi L, Hänninen H. Subjective symptoms and psychological performance of chlorine-alkali workers. Scand J Work Environ Health 1989;15:69–74.

Piikivi L, Hänninen H, Martelin T, Mantere P. Psychological performance and long-term exposure to mercury vapors. Scand J Work Environ Health 1984;10:35–41.

Powell TJ. Chronic neurobehavioral effects of mercury poisoning on a group of Zulu chemical workers. Brain Inj 2000;14:797–814.

Ramazzini B. Disease of Workers. 1713. Reprint (Wright WC, transl). New York: Hafner Publishing Co; 1964.

Reitan RM, Wolfson D. The Halstead-Reitan Neuropsychological Test Battery: Theory and Clinical Implication. Tucson (AZ): Neuropsychology Press; 1985.

Roels H, Lauwerys R, Buchet JP, Bernard A, Barthels A, Oversteyns M, Gaussin J. Comparison of renal function and psychomotor performance in workers exposed to elementary mercury. Int Arch Occup Environ Health 1982;50:77–93.

Roels H, Gennart JP, Lauwerys R, Buchet JP, Malchaire J, Bernard A. Surveillance of workers exposed to mercury vapor: Validation of a previously proposed biological threshold limit value for mercury concentration in urine. Am J Ind Med 1985;7:45–71.

Roels H, Abdeladim S, Ceulemans E, Lauwerys R. Relationships between the concentrations of mercury in air and in blood or urine in workers exposed to mercury vapour. Ann Occup Hyg 1987a;31:135–45.

Roels H, Buchet JP, Ceulemans E, Lauwerys R, Genet P, Sarhan MJ, Hanotiau I, de Fays M, Bernard A, Stanescu D. Epidemiological survey among workers exposed to manganese: Effects on lung, central nervous system, and some biological indices. Am J Ind Med 1987b;11:307–27.

Roels H, Abdeladim S, Braun M, Malchaire J, Lauwerys R. Detection of hand tremor in workers exposed to mercury vapor: A comparative study of three methods. Environ Res 1989;49:152–65.

Roels HA, Ghyselen P, Buchet JP, Ceulemans E, Lauwerys RR. Assessment of the permissible exposure level to manganese in workers exposed to manganese dioxide dust. Br J Ind Med 1992;49:25–34.

Roels HA, Ortega Eslava MI, Ceulemans E, Robert A, Lison D. Prospective study on the reversibility of neurobehavioral effects in workers exposed to manganese dioxide. Neurotoxicology 1999;20:255–72.

Rohling ML, Demakis GJ. A meta-analysis of the neuropsychological effects of occupational exposure to mercury. Clin Neuropsychol 2006;20:108–32.

Rothman KJ, Greenland S. Modern Epidemiology, 2nd ed. Philadelphia (PA): Lippincott Williams & Wilkins; 1998.

Sällsten G, Barregård L, Järvholm B. Mercury in the Swedish chloralkali industry – An evaluation of the exposure and preventive measures over 40 years. Ann Occup Hyg 1990;34:205–14.

Santamaria AB, Sulsky SI. Risk assessment of an essential element: Manganese. J Toxicol Environ Health A 2010;73:128–55.

Santamaria AB, Cushing CA, Antonini JM, Finley BL, Mowat FS. State-of-the-science review: Does manganese exposure during welding pose a neurological risk? J Toxicol Environ Health B Crit Rev 2007;10:417–65.

Šarić M, Lucchini R. Manganese. In: Nordberg GF, Fowler BA, Nordberg M, Friberg LT, editors. Handbook on the Toxicology of Metals, 3rd ed. Burlington (MA):

Academic Press Inc; 2007, pp. 645–74.

Saunders JB, Lee NK. Hazardous alcohol use: Its delineation as a subtreshold disorder, and approaches to its diagnosis and management. Compr Psychiatry 2000;41:95–103.

Schuckmann F. Study of preclinical changes in workers exposed to inorganic mercury in chloralkali plants. Int Arch Occup Environ Health 1979;44:193–200.

Shiffman SM, Gritz ER, Maltese J, Lee MA, Schneider NG, Jarvik ME. Effects of cigarette smoking and oral nicotine on hand tremor. Clin Pharmacol Ther 1983;33:800–5.

Sjögren B, Iregren A, Frech W, Hagman M, Johansson L, Tesarz M, Wennberg A. Effects on the nervous system among welders exposed to aluminium and manganese. Occup Environ Med 1996;53:32–40.

Snell RS. Clinical Neuroanatomy, 6th ed. Philadelphia (PA): Lippincott Williams & Wilkins; 2006.

Stacy MA, Elble RJ, Ondo WG, Wu SC, Hulihan J; TRS Study Group. Assessment of interrater and intrarater reliability of the Fahn-Tolosa-Marin Tremor Rating Scale in essential tremor. Mov Disord 2007;22:833–838.

Symanski E, Sällsten G, Barregård L. Variability in airborne and biological measures of exposure to mercury in the chloralkali industry: Implication for epidemiological studies. Environ Health Perspect 2000;108:569–73.

Thorngren KG, Werner CO. Normal grip strength. Acta Orthop Scand 1979;50:255–9.

Trillenberg P, Führer J, Sprenger A, Hagenow A, Kömpf D, Wenzelburger R, Deuschl G, Heide W, Helmchen C. Eye-hand coordination in essential tremor. Mov Disord 2006;21:373–79.

Urban P, Lukas E, Benicky L, Moscovicova E. Neurological and electrophysiological examination of workers exposed to mercury vapors. Neurotoxicology 1996;17:191–6.

Verberk MM, Sallé HJ, Kempler CH. Tremor in workers with low exposure to metallic mercury. Am Ind Hyg Assoc J 1986;47:559–562.

Wennberg A, Iregren A, Struwe G, Cizinsky G, Hagman M, Johansson L. Manganese exposure in steel smelters a health hazard to the nervous system. Scand J Work Environ Health 1991;17:255–62.

World Health Organization. Environmental Health Criteria 17. Manganese. Geneva, Switzerland: WHO; 1991.

World Health Organization. Environmental Health Criteria 118. Inorganic Mercury. Geneva, Switzerland: WHO; 1991.

World Health Organization. Concise International Chemical Assessment Document 12. Manganese and Its Compounds. Geneva, Switzerland: WHO; 1999.

World Health Organization. Concise International Chemical Assessment Document 50. Elemental Mercury and Inorganic Mercury Compounds: Human Health Aspects. Geneva, Switzerland: WHO; 2003.

Waxman SG. Correlative Neuroanatomy, 23th ed. Stamford (CT): Appleton & Lange; 1996.

Williamson AM, Feyer AM. Moderate sleep deprivation produces impairments in cognitive and motor performance equivalent to legally prescribed levels of alcohol intoxication. Occup Environ Med 2000;57:649–55.

Williamson AM, Teo RKC, Sanderson J. Occupational mercury exposure and its consequences for behaviour. Int Arch Environ Health 1982;50:273–86.

Wilms H, Sievers J, Deuschl G. Animal models of tremor. Mov Disord 1999;14:557–71.

Wood RW, Weiss AB, Weiss B. Hand tremor induced by industrial exposure to inorganic mercury. Arch Environ Health 1973;26:249–52.