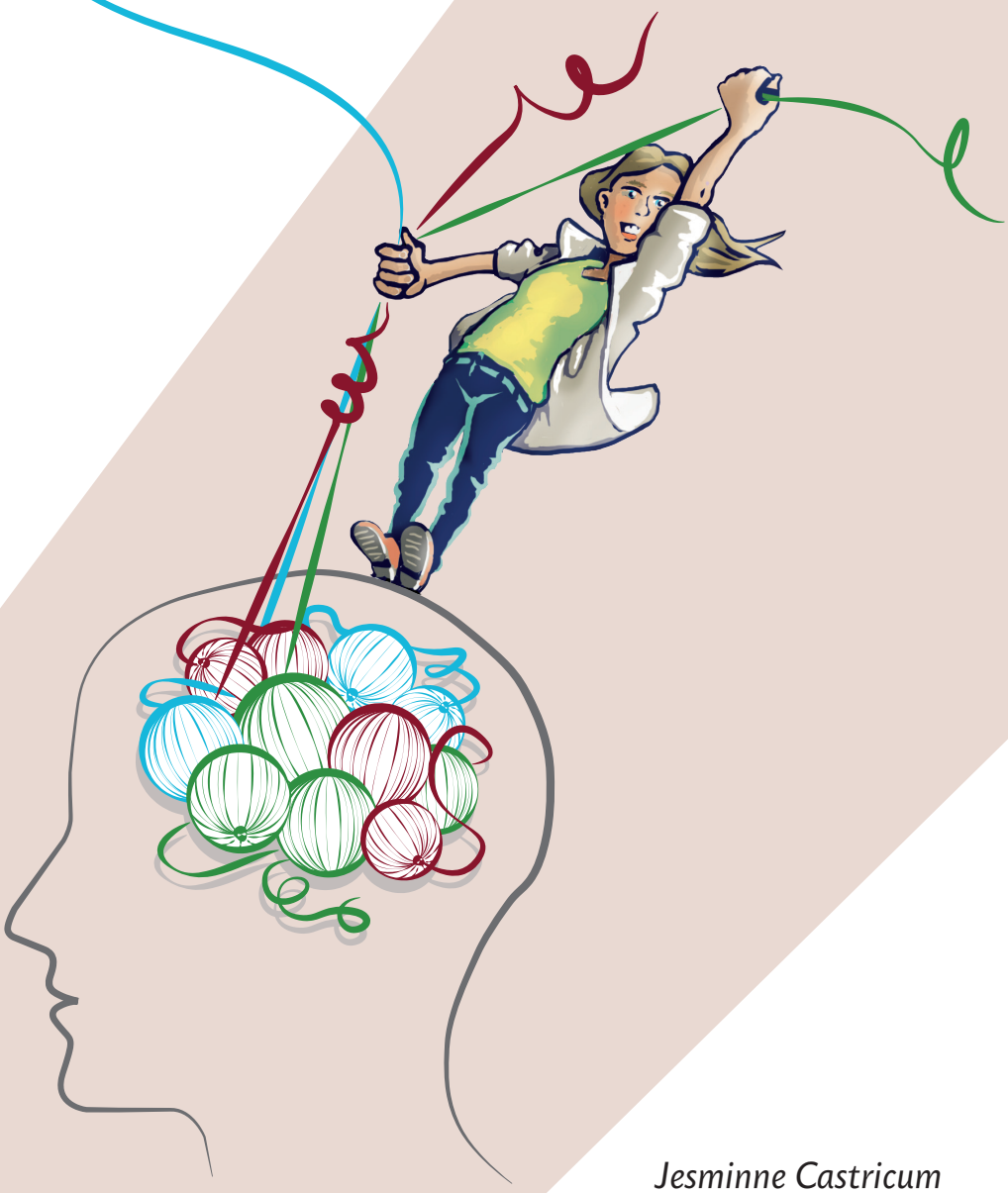
Neurophysiological measures

to assess cognitive functioning in neurofibromatosis type 1



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Neurophysiological Measures to Assess Cognitive Functioning in Neurofibromatosis Type 1

Neurofysiologische uitkomstmaten om cognitieve functies in neurofibromatose type 1 te beoordelen

THESIS

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LIST OF ABBREVIATIONS

NF1	neurofibromatosis type 1
MDD	major depressive disorder
ADHD	attention deficit/hyperactivity disorder
RCFT	rey complex figure test
EEG	electroencephalography
EMG	electromyography
TMS	transcranial magnetic stimulation
LTP	long-term potentiation
NMDA	N-methyl-D-aspartate
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
GABA	gamma-aminobutyric acid
VEP	visual evoked potential
M1	primary motor cortex
iTBS	intermittent theta burst stimulation
MEP	motor evoked potential
SICI	short-interval intracortical inhibition
CSP	cortical silent period
V1	primary visual cortex

Chapter 1

General introduction, aims and outline

GENERAL INTRODUCTION

This thesis is focused on various outcome measures for cognitive deficits to aid future clinical trials to identify treatments for cognitive disorders. We used neuropsychological and neurophysiological approaches to assess cognitive deficits, specifically in adults with neurofibromatosis type 1 (NF1). NF1 is a neurodevelopmental disorder caused by mutations in the NF1 gene (1) and affects approximately one in every 2000 (2,3). Apart from a wide variety of somatic symptoms, many individuals with NF1 suffer from cognitive deficits and learning disabilities that can affect their quality of life. These deficits have been extensively studied in children with NF1, but limited studies have focused on adults with NF1. Furthermore, little is known about the role of the underlying neuropathophysiology of cognitive deficits in patients with NF1. Since all clinical trials in NF1 patients aimed at improving these deficits failed, despite very promising pre-clinical studies, it is important to look for relevant neurophysiological outcome measures. In addition, to expand our knowledge of the use of the neurophysiological measures in disorders with cognitive deficits, it is relevant to establish the specificity of findings by studying other patient groups with cognitive deficits such as patients diagnosed with a major depressive disorder (MDD). The inclusion of relevant and reliable neurophysiological outcome measures in future clinical trials could provide us with more knowledge of the underlying neurophysiological mechanism of cognitive deficits and the efficacy of a treatment in various cognitive disorders.

Cognitive impairment

Cognition represents the mental processes involved in comprehension and information acquisition through experience, thinking, and the sensory organs. These mental processes include multiple cognitive functions, such as attention, learning, and executive functions (4). Cognitive functions are associated with the function of specific brain areas, cortical networks, or neural pathways in the brain. According to the DSM-V (5), they comprise six key cognitive domains with subdomains that process complex attention, learning and memory, perceptual-motor function, executive function, language, and social cognition (Figure 1) (6). Cognitive impairment refers to reduced functioning in one or more of these domains. Cognitive impairment can affect the quality of life by contributing to

a lower socioeconomic status, lower self-esteem, or social isolation in adult life (7). Furthermore, they can also predict problems in mental health (8). Cognitive impairment has been associated with mental disorders, such as obsessive-compulsive disorder, and MDD (9), and neurodevelopmental disorders, such as intellectual disability, attention-deficit/hyperactivity disorder (ADHD), motor disorders including developmental coordination disorder, and NF1. Importantly, several factors could affect cognitive functions including motivation, anxiety, or sensory perception. Furthermore, emotion seems to strongly influence cognitive functions (10), such as (chronic or acute) stress. Stress can both increase or decrease the processes of learning and memory (11).

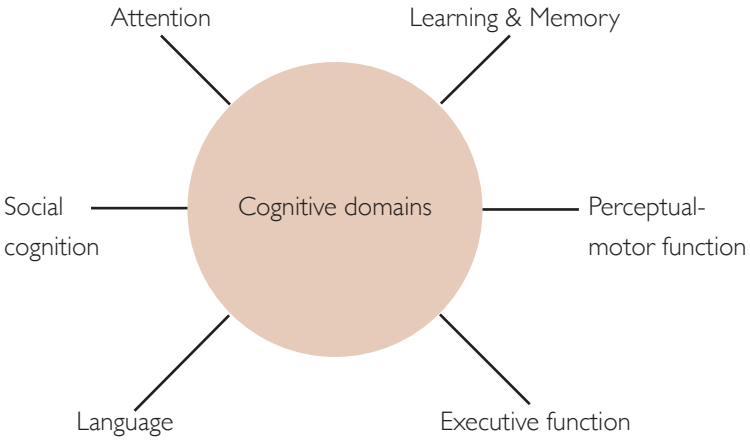

Figure 1. The six key cognitive domains according to the DSM-V, adapted from Sachdev et al. (10).

Neuropsychological outcomes of cognitive impairment

Cognitive impairments in patients with NF1 are wide-ranging (12). Hence, neuropsychological outcome measures are used to assess multiple cognitive domains in patients (Figure 1), including the Rey complex figure test (RCFT) and the sustained attention dots (SAD) task (Figure 2). These measures assess the cognitive domains perceptual-motor function and attention. Interestingly, the RCFT showed the largest alterations in the performance of children with NF1 in a study into the evaluation of cognitive outcome measures in clinical trials (13). Notably, the domain of attention is

most often studied in clinical trials with NF1 patients (14). Previous studies have shown a poorer performance on the SAD, in which it is required to focus for a longer period on changing and unpredictable stimuli (Figure 2) (15–18).

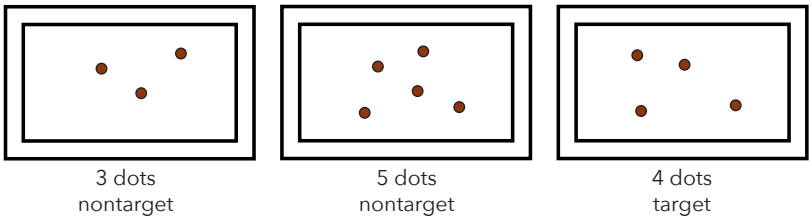

Figure 2. The sustained attention dots (SAD) is a neuropsychological test that assesses sustained attention, adapted from De Sonneville (19). The subject has to keep focus over a longer period. The subject has to press 'YES' if a pattern with four dots (target) is shown, or she/he has to press 'NO' if a pattern with three or five dots (nontargets) is shown. Misses and false alarms are followed by an auditory feedback signal.

A previous clinical trial showed no treatment effect on selected neuropsychological tests after administration of simvastatin in children with NF1 compared to a placebo group. These tests include full-scale intelligence, behavioral questionnaires, visual-spatial learning tasks, or sustained attention tasks (20). Notably, Van der Vaart et al. (21) reviewed that of 169 clinical studies on cognitive genetic disorders, there are only 2 treatments with clinical impact. The neuropsychological tasks used to assess cognitive function in patients may be sensitive to spontaneous improvements (21). This may be due to a practice effect of repeated tests, a placebo effect, a developmental effect in studies involving children, or regression to the mean. The regression to the mean refers to a shift in high or low scores on outcome measures towards more moderate scores caused by variation over time, which is unrelated to the treatment. These effects could mask a potential treatment effect. Therefore, neurophysiological measures could potentially be a more reliable outcome measure for assessing treatment effects in humans. Moreover, the short duration of most clinical trials may be inadequate to elicit significant improvements of cognitive deficits. Neurophysiological changes in response to treatment may be detected earlier than neuropsychological changes in response to short treatment.

Cognition and neurophysiology

Clinical neurophysiology studies the central and peripheral nervous systems through the recording of bioelectrical activity, which can be spontaneous or stimulated. Non-invasive neurophysiological measures could document changes in cognition and learning (22). The use of these outcome measures could expand our knowledge of the underlying cause of cognitive deficits.

Synaptic plasticity

The underlying cause of cognitive deficits might be due to deficits in synaptic plasticity. Plastic changes in the functional strength of the synaptic connections are hypothesized to underlie learning and memory processes (23). The definition of plasticity is a change in synaptic efficacy dependent on the activity (24). The theory that synaptic plasticity is essential for (at least some aspects of) learning and memory is widely accepted and strongly supported by pre-clinical findings (25,26).

Long-term potentiation (LTP)

The long-lasting strengthening ('potentiation') of synapses is the most studied form of synaptic plasticity. Synaptic plasticity has several properties including associativity, coincidence, and input specificity, which can lead to the induction of long-term potentiation (LTP) (27). First, associativity is the simultaneous stimulation of input neurons that concentrate on the same output neuron. This property of associativity enables a low-frequency stimulus to induce synaptic strengthening through association with a high-frequency stimulus. This mechanism is hypothesized to underlie higher-order cognitive functioning in which we learn to associate events or entities (28). Second, both the pre-and post-synaptic neurons need to fire simultaneously to induce LTP. This characteristic was first postulated in 1949 by Donald Hebb, i.e. the Hebbian principle: "cells that wire together, fire together" (29). Last, input specificity implies that only the active synapses with input activity are strengthened.

The molecular mechanisms supporting these properties include a variety of signaling systems and receptors in different brain regions (24,28,30). LTP has been extensively studied in the hippocampus, which is essential for learning and memory. The mechanism

of LTP in the hippocampus depends on N-methyl-D-aspartate (NMDA) receptors (31). Glutamate is released during normal synaptic transmission and acts on both the NMDA and non-NMDA receptors, such as the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor. The AMPA receptor modulates rapid synaptic transmission in the central nervous system resulting in excitatory postsynaptic potentials by influx of sodium (Na^+) ions and outflow of potassium (K^+) ions in the cell. However, under these conditions, the NMDA-receptor channels show no ion flow, due to the blockade by a magnesium ion (Mg^{2+}). High-frequency stimulation (i.e. a 100 Hz stimulus) of a synapse could induce LTP by evoking both presynaptic glutamate release as well as depolarization of the postsynaptic membrane. Under these conditions, the Mg^{2+} blockade is removed and calcium ions (Ca^{2+}) influx is permitted, which contributes to LTP by activating downstream proteins including Calcium-calmodulin dependent Kinase 2 (CaMK2). It is this selective gating property of the NMDA receptor that underlies Hebbian plasticity. It is important to note that the sensitivity of the NMDA receptors is not changed during non-associative forms of learning such as habituation or sensitization. During habituation, the response to a repetitive stimulus is suppressed. Thereby, plastic changes result in decreased strength of the synaptic connections caused by the decreased number of vesicle releases from the presynapse. During sensitization, the response to a stimulus is enhanced. The enhancement is caused by modulatory interneurons including serotonergic interneurons. Thereby, the plastic changes result in increased strength of the synaptic connections.

Bliss & Lømo, 1973 (32) were the first to describe long-lasting LTP-like properties in an anesthetized animal through high-frequency stimulation of the hippocampus. Many studies attempted to associate the cellular in vitro findings of LTP with the behavioral findings of learning and memory in animal studies (33), but demonstrating this relation has been challenging. Morris et al. (34) were the first to describe the link between learning impairment and blockage of hippocampal LTP induction. Withlock et al. (35) showed changes in hippocampal glutamate receptors induced by learning in rats similar to LTP.

The translation of findings of animal studies to human cellular neurophysiology is complex. In vitro research can be performed with human brain slices from postmortem tissue or neurosurgical procedures to assess synaptic transmission in the human brain (36). LTP has been studied on the human cellular level by using in vitro brain slices (37), which have shown similar features to LTP induction in mice studies. However, It has been a major challenge to study in vivo LTP similar to in vitro LTP. Non-invasive neurophysiological methods might help to understand these mechanisms in awake humans.

Cortical plasticity

Plasticity that depends on the functioning of the NMDA receptor has been observed in various sensory cortices (38,39). The cortical network consists of excitatory and inhibitory cells, with the excitatory cells using glutamate as a neurotransmitter and the inhibitory interneurons using gamma-aminobutyric acid (GABA) as a neurotransmitter. In animals, LTP in the cortex can be induced with repeated stimulation at a theta frequency (5-7 Hz) resembling the spontaneous neural rhythm in the brain, which has been shown ex vivo in mouse models (40,41). Furthermore, in the primary visual cortex of adult rats, the induction of the LTP in the visual cortex enhanced the amplitudes of visual evoked potentials (VEPs) generated with light flash stimulation (42). The cortical response was shown to be dependent on the NMDA receptor; the NMDA antagonist (±)-3-(2-carboxypiperazin-4-yl)-propyl-L-phosphonic acid (CPP) blocked the potentiation effects. Furthermore, it has been shown in mice that VEP modulation has the LTP characteristics (43). Specifically, VEP modulation was dependent on both the NMDA and AMPA receptor in mice determined with NMDA receptor antagonists and AMPA receptor insertion-inhibitor.

Besides NMDA-dependent plasticity, cortical plasticity may also be associated with enhanced attention, metabolic changes, or functions resulting in improved executive, planning, and motor functions. Although findings of cortical plasticity are consistent with LTP inducing properties, there is a lack of evidence that the cortical potentiation is due to synaptic changes. Therefore, in the literature, the term LTP-like plasticity is often used when referring to lasting cortical plasticity.

Neurophysiological outcomes to measure cognitive function

Different types of neurophysiological measures can be used to assess cognitive function in the human brain, including functional magnetic resonance imaging to measure neuronal activity and magnetic resonance spectroscopy to measure the concentration of neurotransmitters in the brain. Observed changes in the neurophysiological outcomes of the human central nervous system could reflect changes in learning and other cognitive functions (22,44).

Non-invasive methods to stimulate the human cortex to produce long-lasting changes in cortical responses, include the neurophysiological outcomes VEPs induced by visual stimulation and motor evoked potentials (MEPs) induced by transcranial magnetic stimulation (TMS). These methods apply non-invasive repeated stimulation in awake humans that show high resemblance with the techniques used in animal studies to induce LTP (40,43,45,46). The potentiation of cortical responses following high-frequency stimulation by visual stimulation or magnetic stimulation resembles the properties of synaptic LTP including the dependency of the functioning of the NMDA receptor (47). The potentiation of cortical responses to stimulation may be due to changes in the synaptic efficacy or changes in the inhibitory network (28).

For our studies, we included various neurophysiological outcomes. To measure cortical plasticity of cortical neuronal networks involved in cognitive functions, we recorded event-related potentials measured with electroencephalography (EEG) or electromyography (EMG) induced by visual stimulation or TMS in the visual and motor cortex respectively. In addition, we recorded eye and hand movement responses using various eye-tracking tasks, which record the activity of both the autonomous and central nervous systems (9,22). This method is related to the cognitive domain of the perceptual-motor function.

Plasticity of visual evoked potentials

Non-invasive neurophysiological methods have been developed that are able to induce plasticity-like changes in the human auditory or visual cortex (48,49). The visual cortex is an area of the brain that is responsible for visual perception. Previous studies hypothesize that a form of synaptic plasticity is reflected in the change of one of the components of

a VEP, which is a type of event-related potential, produced by a large number of cortical neurons. This change in one of the components of the VEP is induced by repeated sensory stimulation and lasts longer than the stimulation itself. Plasticity in the human visual cortex has been studied by recording these VEPs with EEG (50). Forsyth et al. (51) showed that deficits in cortical plasticity in patients with schizophrenia, measured with VEPs induced by high-frequency visual stimulation, were related to dysfunction of the NMDA receptor by using an NMDA receptor agonist (D-cycloserine). Moreover, this form of plasticity is affected in a variety of neurological and mental disorders.

LTP-like plasticity in the human visual cortex can be measured by the change of VEPs after repeated visual stimulation (50,52,53). VEPs can be elicited from the visual cortex via visual stimulation with a flashing light at a high frequency or a modulation block of checkerboard reversals at a low frequency (54,55). In this thesis, VEPs elicited by visual stimulation are measured with EEG in adults with NF1 and used as the main outcome measure (Figure 3).

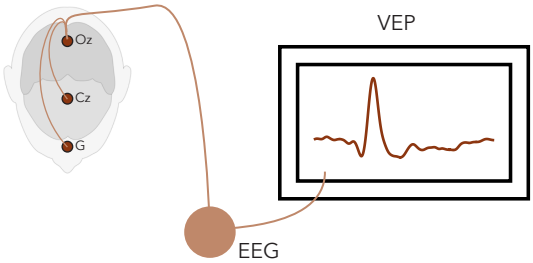

Figure 3. Simplified representation of the measurement of visual evoked potentials (VEP) in the human visual cortex using electroencephalography (EEG). Three electrodes are used, Oz, Cz, and a ground electrode on the forehead (G). The VEPs are shown on a computer screen.

Plasticity of motor evoked potentials

The non-invasive neurophysiological method TMS can assess cortical excitability in the motor cortex via single-pulse stimulations as well as the modulation of cortical excitability via TMS paradigms (56). TMS can modulate cortical excitability that lasts longer than the stimulation itself, i.e. induce cortical plasticity. The electrical stimulation at theta frequency of mouse brain slices shows a high resemblance to TMS paradigms to induce LTP-like plasticity (40,41,57). TMS makes use of electromagnetic induction. The apparatus consists of a magnetic coil connected to a stimulator which can discharge high currents over the isolated coil in a millisecond time frame. This creates a fast-fluctuating magnetic field. TMS of the human motor cortex induces an electric field in the brain, activating neurons in the primary motor cortex (M1). This will produce measurable MEPs in the hand, measured with EMG (Figure 4). The effect of TMS stimulation on the brain is not only determined by stimulation strength, but also by the frequency and duration of the stimulation pulses. Stimulations are typically applied using a figure-of-8 shaped coil, allowing relative focal stimulations. Different TMS paradigms are presently used to provide non-invasive indices of plasticity and cortical inhibition.

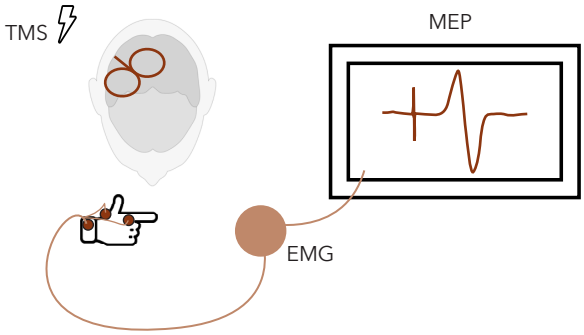

Figure 4. Simplified representation of transcranial magnetic stimulation (TMS) of the human motor cortex. TMS of the human motor cortex induces an electric field in the brain, activating neurons in the primary motor cortex (M1). This will produce measurable motor evoked potentials (MEP) in the hand, measured with electromyography (EMG) and can be shown on a computer screen.

Changes in cortical excitability are reflected in the changes of event-related potentials. Previous studies have shown that these changes are similar to LTP induction (58). Huang et al. (59) have shown that intermittent theta-burst stimulation (iTBS) via TMS induced changes in the MEPs longer than the stimulation itself. Interestingly, iTBS seems to depend on the NMDA receptors (60); the NMDA antagonist memantine blocked the long-lasting changes after iTBS induction. This indicates that it is most likely that iTBS involves changes in plasticity in the human motor cortex. Furthermore, the TMS paradigms Short-Interval intraCortical Inhibition (SICI) and Cortical Silent Period (CSP) are sensitive to changes in GABA-mediated inhibition. Previous studies have shown that the response on the SICI or CSP can be increased by $GABA_A$ or $GABA_B$ receptor agonists (61,62). This indicates that GABA-mediated inhibition deficits in neurocognitive disorders can be measured with the TMS paradigms SICI and CSP. In the present thesis, MEPs elicited by different TMS paradigms are measured with EMG as the main outcome measure. A previous study has shown that stimulation of the M1 with TMS is related to the consolidation of new motor skills in control subjects (63).

Eye and hand movement responses

Eye and hand movement responses could be used as potential neurophysiological outcome measures for neurodevelopmental disorders as NF1, as patients with NF1 show cognitive deficits in visual-spatial and visuomotor function (64). The human brain network responsible for processing visual information is complex (31). The cortical areas have an important role in visual information processing, including visual orientation, recognition, and perception. Visual information is projected from the retina to the primary visual cortex (V1) via the lateral geniculate nucleus. V1 projects to extrastriate visual areas in two parallel pathways: the dorsal pathway to the posterior parietal cortex responsible for visual-spatial perception and visuomotor actions, and the ventral pathway to the inferior temporal cortex responsible for identifying a visual stimulus. The dorsal stream is also described to project to the prefrontal, premotor and medial temporal cortices (65). Previous studies showed that eye movement responses using eye-tracking could indicate deficits in disorders associated with cognitive disabilities (66). Specifically, studies showed that eye and hand movement responses were altered in various disorders with a neurodevelopmental basis, including autism, ADHD, and schizophrenia (67), or in brain

disorders such as Parkinson's disease (PD) and Alzheimer's disease (68–71).

Measuring eye and hand movement responses with the use of eye-tracking (Figure 5) is highly objective to assess the underlying neurophysiology of the visual and motor system, in contrast to the commonly used clinical evaluations and questionnaires. Eye movement responses consist of different types of eye movements, such as saccades and fixation, and different scales of measurements, such as temporal and spatial. Previous studies have shown differences in fixation duration, slower saccadic reaction times, and/or increased error rates in several neurodevelopmental and psychiatric disorders (66). The temporal eye-related measures are most frequently used in studies to learning and provide indirectly the most information of relevant cognitive processes (72).

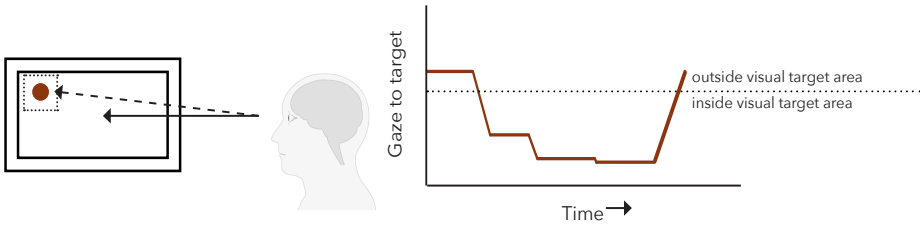

Figure 5. Simplified representation of measuring eye movement responses using an eye-tracking paradigm. Subjects are seated in front of a touch screen on which visual stimuli are shown. They have to shift their gaze from the middle of the screen (solid arrow) to an appearing visual target (dashed arrow). An eye movement trace is represented in degrees from the target area. The horizontal dotted dashed line indicates the border of the visual target area.

Neurofibromatosis type 1

Little is known about the role of the underlying neuropathophysiology of the cognitive deficits in patients with NF1. NF1 is an autosomal dominant single-gene disorder, caused by a mutation in the NF1 gene located on chromosome 17q11.2 (1), which encodes for neurofibromin. NF1 is characterized by café-au-lait spots, axillary freckling, cutaneous neurofibromas, iris hamartomas (Lisch nodules), optic pathway tumors, developmental bony defects, and scoliosis (73). Apart from a wide variety of these somatic symptoms, many individuals with NF1 suffer from cognitive deficits and learning disabilities that can affect their quality of life. These deficits consist of a lower average of the intelligence quotient, deficits in visual-spatial skills, and problems with executive

functions, attention, and motor performance. The cognitive deficits have been studied extensively in children with NF1 (12,13,74–77), but limited studies have focused on adults with NF1. Additionally, NF1 has psychiatric comorbidities such as depression, anxiety, and personality disorders (78).

Using Nf1 mutant mice, it has been shown that reduced neurofibromin activity results in abnormal hyperactivation of RAS signaling in inhibitory interneurons (40,41,79). The RAS hyperactivation causes an increase in inhibition through abnormally high GABA neurotransmission, which leads to a reduction of glutamatergic synaptic plasticity (40,41,79,80). The synaptic plasticity deficits, and learning and attention deficits in these Nf1 mutant mice were rescued by reducing Ras activity (41,81). The mechanism for the before mentioned increased inhibition has been identified based on the Nf1 animal model (40). The hyperpolarization-activated cyclic nucleotide-gated channel (HCN1) is a neurofibromin-interacting protein and underlies the enhanced inhibitory neurotransmission. Omrani et al. (40) have shown that an agonist of the HCN1 channel, lamotrigine, can rescue deficits in inhibition and plasticity in the Nf1 animal model. In addition, lamotrigine rescues the learning and motor learning deficits in Nf1 mouse models.

These preclinical findings have been translated to clinical trials within the ENCORE expertise center in the Erasmus University Medical Center in Rotterdam. To reduce the Ras activity, simvastatin was administered to children with NF1, but the medication did not improve the cognitive deficits in comparison to placebo measured with neuropsychological tasks (20). Furthermore, the HCN1 channel agonist lamotrigine has been administered to adolescents with NF1, although results are not available yet. Interestingly, neurophysiological TMS measures have been added in the study design as secondary outcome measures to assess the effect of lamotrigine on cortical plasticity. This was in part motivated by a report that observed effects on motor cortical plasticity after a single dose of lamotrigine in unaffected controls measured with the TMS paradigm paired associative stimulation (PAS) (82). The consensus for the use of cognitive outcome measures in clinical trials in NF1 is still in development (83).

Major depressive disorder

Patients with a neurocognitive disorder frequently have complex interactions between cognitive deficits and psychiatric symptoms. To extend our knowledge of the use of the neurophysiological measures in NF1, it is relevant to establish the specificity of findings by studying other patient groups with cognitive deficits such as patients diagnosed with MDD. MDD is a severe mental disorder that causes a depressed mood and loss of interest or pleasure in life activities but is also related to cognitive deficits. MDD has a prevalence of 4.7% worldwide (84). The cognitive deficits in MDD include problems with attention, deficits in memory and visual-motor speed (85,86).

The underlying mechanism of these deficits in MDD has been studied with both neuropsychological and neurophysiological measures. Interestingly, the study of VEPs has been used to get a better understanding of the neurophysiology of MDD (55). Visual cortical plasticity using VEPs in response to visual stimulation has been observed in healthy adults, but depressed patients had reduced visual cortical plasticity as indicated by their non-potentiated response to VEP induction. In addition, eye-tracking tasks have been used to understand cognitive deficits in patients with MDD. Eye-tracking studies to visual attention in patients with MDD showed an attentional bias to negative information measured with the fixation duration of the eyes (87–89). Furthermore, this negative attentional bias seemed to also affect other cognitive abilities in patients with MDD, such as memory and information processing.

It is known that GABAergic deficits could play an important role in the modulation of neuronal plasticity in MDD (90,91). GABAergic deficits in MDD have been studied in preclinical and treatment studies, including TMS studies (92). However, TMS findings were inconsistent and further clarification of the presence of underlying changes in neurophysiological functioning in drug-free severely depressed patients is relevant. The before mentioned TMS paradigms iTBS, SICI, and CSP can be used in patients with MDD to study cortical plasticity and cortical inhibition in the pathophysiology of the cognitive deficits in patients with MDD.

Aims and outline of the thesis

This thesis is focused on the use of different neurophysiological approaches in adults with the neurodevelopmental disorder NF1. The inclusion of neurophysiological outcomes could show the effect of treatment on the human brain and provide more information about the optimal duration of treatment. Furthermore, it could still indicate significant neurophysiological changes in the absence of overt neuropsychological changes.

The overall aim of this thesis is to assess the use of outcome measures for cognitive deficits to improve future clinical trials to treat these deficits. The specific aims of the studies were:

1) to determine the cognitive deficits in adult patients with NF1,

2) to investigate potential deficits in motor and visual cortical plasticity related to the cognitive deficits in NF1, and

3) to extend our knowledge of the use of these neurophysiological measures as outcome measures by examining their variability and specificity by including another distinct clinical population with cognitive deficits: MDD.

The cognitive functions of attention, motor learning, and perceptual-motor function were assessed in adult patients with NF1 and compared to a control group (Chapter 2-3). Chapter 2 presents the commonly used neuropsychological approach to cognitive deficits used in children with NF1, but now focused on adults with NF1. In Chapter 3, we objectively assessed the visual-spatial and visuomotor functioning by recording eye and hand movement responses using eye-tracking tasks to assess the underlying neurophysiology of the visual and motor system. In Chapter 4 and Chapter 5, cortical plasticity, hypothesized to underlie cognitive functions, is assessed in the motor and visual cortex by recording evoked potentials in adults with NF1 and unaffected controls. In Chapter 4, we studied VEPs measured with EEG and induced by visual stimulation. In Chapter 5, we investigated MEPs measured with EMG and induced by several TMS paradigms in adults with NF1 (Chapter 5.1) and extended this study by also applying the TMS paradigms to patients with MDD (Chapter 5.2) who also suffer from cognitive deficits. We discuss our overall findings and the future perspectives in Chapter 6.

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Chapter 2

Attention and motor learning in adult patients with neurofibromatosis type 1

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ABSTRACT

Neurofibromatosis type 1 (NF1) is an autosomal dominant genetic disorder that is associated with cognitive disabilities, including attention and motor learning problems. These disabilities have been extensively studied in children with NF1 but limited studies have been performed in adults.

Attention, motor learning and intellectual performance were studied with neuropsychological tasks in 32 adults with NF1 and 32 controls.

The NF1 and control group performed similarly on attention and motor learning tasks, although controls had shorter reaction times than adults with NF1 during the motor learning task ($t(60) = -2.20, p = 0.03$). Measures of attention or motor learning were not significantly associated with reduced intellectual performance in NF1.

In contrast to many studies in children with NF1, our findings did not provide evidence for presence of attention or motor learning problems in adults with NF1 in neuropsychological tasks. Our observations may be of clinical importance to determine treatment focus in adults with NF1.

INTRODUCTION

Neurofibromatosis type 1 (NF1) is an autosomal dominant genetic disorder caused by a heterozygous loss-of-function mutation in the NF1 gene (1). NF1 is frequently associated with cognitive disabilities, in addition to the characteristic somatic features (2). These cognitive disabilities include reduced intelligence and deficits in attention and motor learning, which have been well-documented and assessed more extensively in children with NF1 (3–9). Cognitive impairment has been shown to relate to decreased quality of life in children and adolescents with NF1(10). Although attention and motor learning deficits have been extensively studied in children with NF1, limited studies have focused on adults with NF1.

Attention is the most frequently affected ability in children with NF1, next to learning disabilities and motor problems, with observed attention deficits in 33% to 50% of the children and with an overrepresentation of ADHD (11). The domain of attention is most often studied in NF1 cognitive clinical trials (Walsh et al., 2016) with wide variability in the use of tools to measure attention. To our knowledge, only a few other studies have investigated attention in adults with NF1 with contradictory findings (12-16). In twenty adults with NF1, impairments in attention were shown using a neuropsychological test battery (13), consistent with findings in children with NF1. Moreover, Ferner et al. (15) observed impaired attention in a large cohort of 103 NF1 patients with an age range of 6-75 years, although differences between children and adults were not investigated separately. More recent studies in twenty adults with NF1, showed no deficits in attention, including selective and sustained attention (12) or visual attention (14).

Attention problems may be associated with difficulties in motor learning observed in children with NF1 (4). Children with ADHD showed a high prevalence of disabilities in fine motor skills (17). In addition, previous studies in children with NF1 showed disabilities in fine motor skills, motor speed, and motor performance (4,7,18,19). Neuroimaging studies in children with NF1 showed an association between deficits in cognitive deficits including motor skills and cerebral physiopathology, although the exact link remains unclear (20,21). Motor problems have also been shown in 44 young

adults with NF1 with disabilities in fine motor skills (22) and in 21 adults with NF1 with reduced voluntary muscle force (23). One study investigated motor skill learning in 9 adults with NF1 by using the sequential finger-tapping task and found that motor learning was affected (24). In contrast, an older study observed no specific problems in basic motor speed in twenty adults with NF1(13).

Intelligence in neurotypical controls seems to be strongly associated with neuropsychological functioning in cognitive domains such as attention (25). The distribution of the full-scale intelligence quotient (IQ) of children with NF1 is shifted downward, although the variability in cognitive ability is similar to the general population (5). In neurotypical adults, an association between a lower than average IQ and reduced attention has been demonstrated (25), and reduced intellectual functioning correlated with reduced executive functioning (26). In contrast, previous studies found no association between intelligence and attention in children with NF1 (3) nor between attention and motor learning problems in adults with NF1 (3,24).

Considering the high prevalence of attention and motor learning deficits clinically reported in children with NF1, and the limited and inconsistent findings in adults with NF1, further clarification of the presence or absence of these deficits in adults with NF1 is important. Hence, we examined attention, including alertness and sustained attention, and motor skill learning in adults with NF1 compared to neurotypical controls. We made use of standardized measures that examined alertness and sustained attention (27). These measures have frequently been used in studying attention deficits in various disorders, including ADHD and children with NF1 (27). Additionally, motor skill learning was examined by the sequential visual isometric pinch task (SVIPT) (28,29) and intellectual performance was examined by administering four subtests of the Wechsler Adult Intelligence Scale (WAIS-IV-NL).

METHODS AND MATERIALS

Subjects

In this study, 32 NF1 patients and 32 controls between 18-56 years participated. NF1 patients were recruited from the ENCORE-NF1 expertise center for Neurodevelopmental Disorders at the Erasmus MC upon referral by their treating neurologist or through the Dutch NF patient association (NFVN). The patients had a genetic and/or a clinical diagnosis. Controls matched for age and gender were unaffected peers of the patients or recruited through online advertisements. According to the inclusion and exclusion criteria, the subjects in the control group had no current presence or history of neurological, psychiatric or medical disorders. The subjects in the NF1 group had no current presence or history of neurological or medical disorders other than NF1, or psychiatric disorders except for the comorbidity with ADHD based on clinical diagnosis. Furthermore, subjects with NF1 were excluded if the neurological illness influenced the function of the central nervous system or motor tract, or influenced the function of the peripheral nervous system involving the sensory or motor function of the hands. All subjects had no severe hearing problems and/or visual problems. All subjects were not taking medication at the time of the study (except for contraceptives, and methylphenidate (n_{NF1} = 1)). The Dutch Central Medical Ethics Committee of the Erasmus Medical Center Rotterdam approved the study (MEC-2017-029, NL59730.078.16), which was conducted in accordance with the Declaration of Helsinki (2013). All subjects gave their written informed consent.

Procedure

The subjects were asked to abstain from alcohol and caffeinated beverages 24 hours before the start of the measurements. All subjects completed the tasks in the laboratory between 01:00 PM and 04:00 PM after having a light lunch. The tasks examined intellectual performance, alertness, sustained attention, and motor skill learning.

Intellectual performance

Intellectual performance was examined by administering four subtests of the Wechsler Adult Intelligence Scale (WAIS-IV-NL). The tests included block design, matrix reasoning,

vocabulary, and similarities. This selection has a high correlation with full-scale IQ score (30). Verbal intelligence quotient (VIQ) was estimated based on the subtests vocabulary and similarities. Performance intelligence quotient (PIQ) was estimated based on the subtests block design and matrix reasoning (27). Furthermore, the level of education of the subjects was coded using the international standard classification of education (ISCED, 2011) varying from 'early childhood education' (0) to 'doctoral or equivalent' (8).

Alertness

Alertness is the reaction time to a stimulus without any preparatory cue, and reflects the intensity of attention (32). We measured alertness with the baseline speed task (BS) from the Amsterdam Neuropsychological Tasks (ANT) (27), which is a standardized visual reaction-time task. The ANT has proven to have sufficient psychometric properties, such as validity and test-retest reliability (27). Subjects were in front of a monitor, while holding their index fingers on both the mouse buttons. The subjects had to look at a cross in the middle of the screen. They had to press the mouse button as quickly as possible when the cross changed into a square with a randomized inter-stimulus time interval of 500-2500 ms. We measured the reaction time in ms and the change over time in reaction time, i.e. stability of the reaction time.

Sustained Attention

Sustained attention is the ability to focus for a longer period of time on an unpredictable and changing stimulus. We measured sustained attention with the standardized ANT visual sustained attention dots (SAD) task (27). The subjects had to press 'YES' if a pattern with four dots (target) was displayed, or they had to press 'NO' if a pattern with three or five dots (non-targets) was displayed. Misses and false alarms were followed by an auditory feedback signal. Dot patterns were presented in 50 series of 12 trials with a post response interval of 250 ms. The duration of the task was between 12-15 minutes depending on the reaction time of the subjects. The task requires to remain focus over a longer period of time. Therefore, the actual performance can be assessed with the so called time-on-task (TOT) effects (33–35). We measured the change in performance over time (33). Therefore, we computed five consecutive periods for quantification of reaction time in seconds per series and number of misses; each consisted of ten series of 12 trials.

Motor skill learning

Motor skill learning was measured by the sequential visual isometric pinch task (SVIPT) (28,29). The paradigm is a custom-built in a graphical user interface (GUI) in MatLab (MathWorks). The SVIPT is easy to understand, although it is challenging to perform as it shows improvements over five days in controls (29). This will prevent a ceiling effect in the control group and could be a sensitive task to detect differences between NF1 patients and a control group. Subjects were seated in front of a monitor, while holding a force transducer in their non-dominant hand. The SVIPT was displayed on the monitor consisting of colored targets from left to right (Figure 1). Subjects had to move the cursor between these targets in a predetermined order by squeezing the transducer. The cursor had to be on a target on each beat of a metronome of 1.67 Hz. Logarithmic transformation was applied to the force, to scale cursor movement according to Coxon et al. (28). The farthest target was set at 45% of the maximum force. A total number of 12 blocks consisting of 15 trials were presented. The duration of the task was between 25-30 minutes. Visual feedback was displayed at the end of each block.

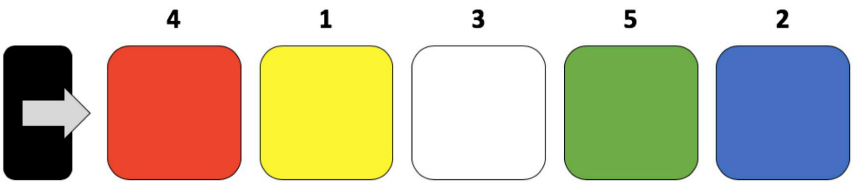


Figure 1. Schematic view of the motor skill learning task. The sequential visual isometric pinch task (SVIPT) was displayed on the monitor consisting of colored targets from left to right. Subjects had to move the cursor (arrow) back and forth from the home-box (black rectangle) to the targets in a predetermined order (1-2-3-4-5) by squeezing the transducer.

Data analysis and statistics

Statistical analyses were performed in IBM Statistics SPSS (version 25). Nonparametric statistical tests were performed when assumptions for parametric statistics were violated. Demographics were compared between controls and NF1 patients with Chi square test, independent t-tests or Mann Whitney U-tests. Reaction time and stability on the BS task were analyzed with independent t-tests using z-scores. Repeated measures

ANOVAs were performed to analyze potential differences in reaction time and number of misses on the SAD between controls and NF1 patients over the five consecutive periods in time. Independent t-tests were performed to analyze the slope of reaction time and error rate on the SVIPT between controls and patients as quantified based on the 12 consecutive blocks in time. Statistical outliers were analyzed and removed if the value exceeded 3 standard deviations from the mean. Correlations were tested between outcome parameters computing Pearson correlation coefficients within the NF1 group. A p-value of $p<0.05$ was considered to indicate a significant difference. The p-values were corrected for multiple testing with the Bonferroni correction.

RESULTS

We measured 64 participants ($n_{control} = 32$ $n_{NF1} = 32$). After the measurements, exclusion of participants was necessary due to technical problems during the attention tasks ($n_{control} = 2$; $n_{NF1} = 1$), insufficient knowledge of the Dutch language ($n_{control} = 1$), IQ recently tested, but not available ($n_{NF1} = 1$), and withdrawal during the motor learning task ($n_{NF1} = 1$). Age ($M_{control} = 35.4 \pm 11.0$, $M_{NF1} = 30.9 \pm 12.0$) and gender were not different between the groups ($t_{age}(57) = 1.08$, $p = 0.28$; $\chi^2_{gender} = 0.167$, $p = 0.68$). Educational attainment was significantly lower in the NF1 group than in the control group ($U_{ISCED} = 303$, $p = 0.006$) (Table 1).

Intellectual performance

Verbal IQ ($M_{control} = 99 \pm 12.9$, $M_{NF1} = 85 \pm 16.6$) and performance IQ ($M_{control} = 98 \pm 19.6$, $M_{NF1} = 87 \pm 15.3$) were significantly lower in the NF1 group than in the control group (Table 1; $t_{VIQ}(59) = 3.66$, $p = 0.001$, $t_{PIQ}(60) = 2.42$, $p = 0.018$). These results indicate a lower intellectual performance in adults with NF1 than controls. Furthermore, the scores on the subtests of the WAIS-IV-NL were all significantly lower in the NF1 group than in the control group (Table 1; $t_{block\ design}(61) = 3.53$, $p = 0.001$, $t_{similarities}(60) = 2.94$, $p = 0.005$, $t_{vocabulary}(60) = 4.17$, $p<0.001$), except for the subtest matrix reasoning. The scores on the subtest matrix reasoning were the same for both groups ($t_{matrix}(61) = -0.82$, $p = 0.41$) (Table 1).

Alertness

There were no significant differences in mean reaction time in ms ($M_{control}$ = 272±26.7, M_{NF1} = 282±40.3) ($t_{z\text{-}score}(59) = -0.99, p = 0.33$) or the stability of reaction time (U_{RT} = 472, p = 0.92) during the BS task between NF1 patients and controls (Table 1). Therefore, alertness tested with the BS task was not different between the groups.

Sustained Attention

Mean reaction time during the SAD task in seconds per series ($M_{control}$ = 8.5±1.6, M_{NF1} = 9.1±2.2) was the same for both groups (U_{RT} = 385, p = 0.34) as well as the variability in time (speed) (U_{speed} = 383, p = 0.33). Therefore, sustained attention measured with the SAD task was similar for both groups. Mean reaction time during the SAD task did also not differ over the five consecutive periods in time (TOT effects) ($F_{RT}(2.29,141.8) = 0.85, p = 0.44$) (Figure 2A). In addition, there was no significant interaction effect between group and consecutive periods. Overall, controls made more misses, but this was nominally significant ($F_{misses}(1,60) = 3.77, p = 0.057$). There was a significant main effect over the five consecutive periods over time for the number of misses: controls made more misses during period 2 and 3 than NF1 patients ($F_{misses}(4,240) = 3.73, p = 0.006$) (Figure 2B). There was no significant interaction effect between group and consecutive periods.

Motor skill learning

Although individuals with NF1 had a similar reaction time as neurotypical controls at the first training sessions, controls had significantly shorter reaction times than adults with NF1 during the motor learning task (slope of the reaction time during the SVIPT ($t_{slopeRT}(60) = -2.20, p = 0.031$); Table 1; Figure 3A). However, there was no significant difference in the slope of the error rate during the task between the groups ($t_{error\ rate}(60) = -1.42, p = 0.16$), indicating that NF1 patients and controls both learned the task equally well (Table 1; Figure 3B).

Correlations

We did not find significant correlations within the NF1 group between estimated IQ and reaction time during the BS task (z-score) (r_{VIQ} = -0.061, p = 0.75, r_{PIQ} = -0.228, p = 0.23), during the SAD task (r_{VIQ} = -0.405, p = 0.02, r_{PIQ} = -0.372, p = 0.04), or during the motor learning task (r_{VIQ} = -0.224, p = 0.24, r_{PIQ} = -0.072, p = 0.71). There

were no significant correlations between estimated IQ and the error rate during motor learning (r_{VIQ} = -0.332, p = 0.07, r_{PIQ} = -0.308, p = 0.10). Furthermore, there were also no significant correlations between reaction time during the motor learning task and reaction time during the BS task (z-score) (r_{BS} = 0.108, p = 0.51) or during the SAD task (r_{SAD} = 0.03, p = 0.87). The p-values were corrected for multiple testing with the Bonferroni correction (α of 0.05 adjusted for 10 comparisons, p<0.01).

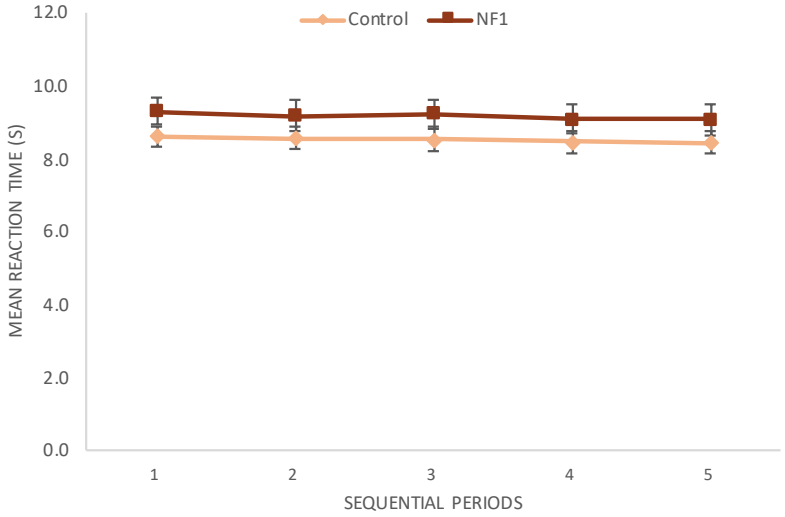
Table 1. Demographics, intellectual performance, attention, and motor learning parameters (mean ± SD) of the NF1 group and the control group separately.

	NF1 group (n = 32)	Control group (n = 32)
Demographics		
age in years	30.9 ± 12.0	35.4 ± 11.0
gender: male in % (#)	41 (13)	50 (16)
educational attainment, median (range)*	4 (1-6)	5 (2-6)
Intellectual performance		
verbal IQ*	85 ± 16.6	99 ± 12.9
Similarities*	7.2 ± 3.1	9.4 ± 2.8
Vocabulary*	7.5 ± 2.8	10.2 ± 2.4
performance IQ*	87 ± 15.3	98 ± 19.6
Block design*	6.7 ± 2.8	9.4 ± 3.6
Matrix reasoning	9.0 ± 2.9	9.7 ± 3.4
Alertness		
BS reaction time (ms)	282 ± 40.3	272 ± 26.7
BS reaction time (z-score)	0.2 ± 1.1	-0.03 ± 0.8
Sustained attention		
SAD reaction time (s per series)	9.1 ± 2.2	8.5 ± 1.6
SAD variability in time (speed, s per series)	1.2 ± 0.7	1.0 ± 0.4
number of misses (# per series), median (range)	15 (2-96)	15 (2-74)
Motor learning		
reaction time (slope)*	-0.8 ± 0.9	-1.2 ± 0.7
error rate (slope)	-1.7 ± 1.1	-2.1 ± 1.0

*significantly different between patients and controls (p-value <.05)

#, number of subjects; BS, baseline speed task; SAD, sustained attention dots task; series, consists of 12 trials with the representation of a dot pattern (in total 50 series of 12 trials).

A



B

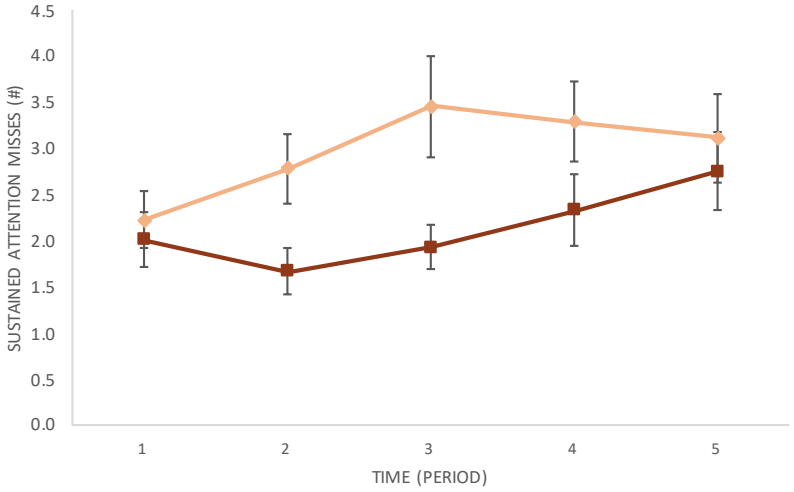


Figure 2. Sustained attention parameters on the sustained attention dots task (SAD) of the NF1 (red) and control group (blue). A. The mean series reaction time in seconds ± SEM. There was no significant difference in mean reaction time between the groups ($F(2.29,141.8) = 0.85$, $p = 0.44$). B. The mean number of misses ± SEM. There was a nominally significant difference in the number of misses between the groups ($F(1,60) = 3.77$, $p = 0.057$). There was a significant main effect over the five consecutive periods in time for the number of misses ($F(4,240) = 3.73$, $p = 0.006$). There were no significant interaction effects.

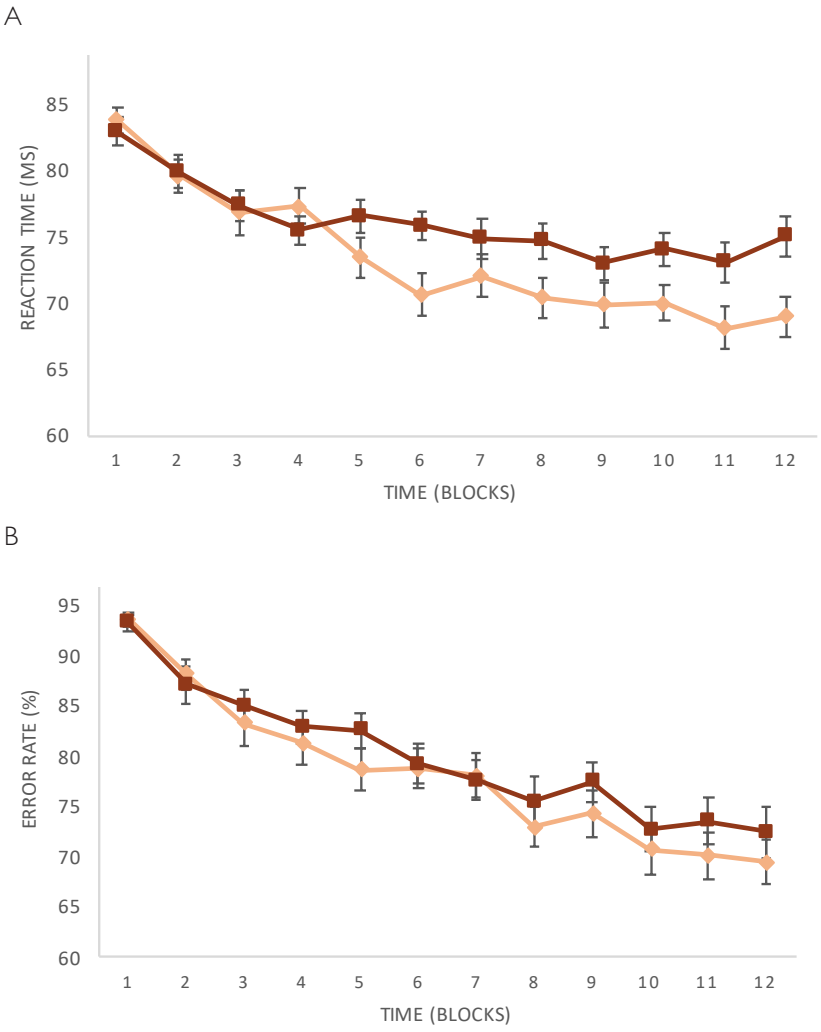


Figure 3. Motor learning parameters on the sequential visual isometric pinch task (SVIPT) of the NF1 (red) and control group (light orange). A. The mean reaction time ± SEM. There was a significant difference in the slope of the mean reaction time between the groups ($t(60) = -2.20$, $p = 0.031$) B. The mean error rate ± SEM. There was no significant difference in the slope of the error rate between the groups ($t(60) = -1.42$, $p = 0.16$).

DISCUSSION

Attentional and motor learning problems have been frequently observed in children with NF1 (3), but less is known of the prevalence of these problems in adults with NF1. Based on the previous studies, we hypothesized that we would observe reduced alertness and sustained attention, as well as reduced motor learning in adults with NF1. However, these attention measures and this motor skill learning task did not provide convincing evidence for attention and motor learning problems in adults with NF1, although controls reached a faster reaction time compared to adults with NF1 in the motor learning task.

Attention

Although attention is the most frequently affected ability in children with NF1 (36), our findings did not provide evidence for the presence of attention deficits in adults with NF1. The absence of a difference in performance in the alertness and sustained attention tasks in adults with NF1 is in contrast to previous studies in children with NF1 (37–40). In two NF1 studies, the same measure of alertness showed diminished alertness in children with NF1 compared to controls (41,42). Furthermore, sustained attention was affected in 63% of children with NF1 (3). The first reason for the lack of attention differences between adults with NF1 and controls could be due to developmental changes from childhood to adulthood (12,40). The delay in the development of attention components (43) could reflect the attention deficits mainly seen in children with NF1 (40). It would be interesting to take the maturation process into consideration in future prospective longitudinal studies. The second reason could be the low incidence of clinically diagnosed ADHD in our sample (n = 1), which could indicate a potential recruitment bias. Mautner et al. (16) showed that comorbidity of ADHD symptoms in NF1 patients persists during adulthood. However, attention problems should also be present in NF1 patients without ADHD symptoms according to Ribeiro et al. (44). They showed that attention deficits are linked to a specific increase in the amplitude of alpha oscillations, which have been observed in children with NF1 without ADHD at rest and during visual stimulation (44). Interestingly, this increased alpha was associated with a similar performance in children with NF1 and controls during a visual detection task,

indicating that the aberrant alpha rhythm might still be functional. Therefore, it would be interesting to study alpha oscillations related to attention in adults with NF1 in future research. Finally, the reason for the lack of attention deficits in adults could be the result of using only quantitative performance-based measures of attention. Although these measures are objective and have been used in previous NF1 studies including measures of alertness and sustained attention (37–40), Biotteau et al. (45) advised the use of both observer-rated questionnaires and performance based assessments.

Despite the fact that we did not see attention deficits in our NF1 cohort, it is known that adults with NF1 experience attention problems in their daily lives that affects their quality of life (46). In addition, learning disabilities and attention problems could predict problems in mental health in NF1 adults (47). In the present study, attention deficits were not observed in an experimental setting, but keeping attention levels high, could be associated with increased fatigue in NF1 patients. Rietman et al. (48) noted that fatigue has a large effect on the daily life of NF1 adult patients and that it also limited the coping skills of patients. Although patients did not express fatigue during the measurements or at the final evaluation in the present study, we still recommend including an objective measure of fatigue in the future studies. Additionally, our patients were highly motivated, which could have contributed to the significantly lower number of misses over time during the SAD task in the NF1 patients than in controls.

Motor learning

The observed overall slower reaction times in the NF1 group on the motor learning task is consistent with previous studies suggesting a slower information processing overall in NF1 (40,42,49). However, one study in adults with NF1 showed no specific problems in basic motor speed measured with a finger tapping test in 30 adults with NF1 (13). Another explanation for the slower reaction time may be due to reduced maximal voluntary muscle force in NF1 (23). NF1 patients are known to have reduced maximum voluntary muscle strength (23) required to successfully perform the motor learning task by reaching the most distal target (28). However, in our study, the most distal target was set to (only) 45% of their individual maximal muscle force needed to reach all the targets. Since the accuracy on the motor learning task was not significantly different

between groups, potential reduced muscle force in adults with NF1 was unlikely to affect the performance on the motor learning task.

Our findings suggest that adults with NF1 and controls performed similarly on the motor learning task. This finding is in contrast to a previous study in 9 adults with NF1 that assessed a similar motor learning task over five consecutive days (24). That study indicated a relative inability to perform the motor learning task as well as controls, which was already evident at the first day of training. This difference was not observed in our study using the SVIPT, even though the duration of the measurement was three times longer in our study to make the task more challenging to perform and more sensitive to detect differences (29).

Intellectual performance

We found no association between intelligence and attention and motor learning problems in NF1 patients, which is consistent with previous studies in children and adults (3,24). The estimated verbal and performance intelligence score of the NF1 patients are in line with previous studies (3,5,9). Ottenhoff et al. (5) showed in 497 children with NF1 significantly lower IQ-scores, whereas the variability in IQ was similar to the general population. In our study with adults, most subscale IQ scores were significantly lower in NF1 patients than in controls, although patients had no diminished performance in matrix reasoning. Matrix reasoning measures visual-spatial functioning similar to the subtest block design, but is, in contrast with block design, independent of a time constraint. Subscale scores on matrix reasoning have not yet been described in NF1 patients. Possibly, deficits in processing speed were not addressed with matrix reasoning (50).

Strengths and limitations

This study has three key strengths: the use of a relatively large sample size, the use of a representative NF1 sample, and the use of standardized test measures to assess attention. A large sample size might help to avoid bias in recruitment of patients. It is important to note that patients were free of any psychoactive medication, except for 1 patient receiving methylphenidate, that could affect the outcome measures. Additionally, patients were not receiving mental health care. The estimated IQ of adults with NF1

in our study suggests that our sample was cognitively affected in the same way as overall present in the NF1 population. Furthermore, it could be that the NF1 samples in previous studies were not representative for the population due to specific recruitment of only patients with academic problems (51) or only patients with high education (12,24). Although educational attainment was significantly different between the groups, it did not predict the outcome measures in the present study as expected. Furthermore, age and gender were similar for the NF1 and control group. Last, in the present study we used standardized measures of alertness and sustained attention frequently used in studies to measure attention deficits in various disorders (27).

To conclude, the present study shows a similar performance on attention and motor learning tasks in a representative NF1 adult sample in an experimental design, despite potential problems in these cognitive domains seen in the NF1 population. Overall, our NF1 patients seemed highly motivated to perform the tasks. Their similar performance on these tasks compared to controls may reflect this and may be related to the increased fatigue or other associated complaints in NF1 patients in their daily life. Research into attention in adults with NF1 has important clinical implications to determine treatment focus. It would be interesting to validate our findings by performing a prospective longitudinal study controlling for both the maturation process from childhood to adulthood and the heterogeneous cognitive phenotype.

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Chapter 3

Visual-spatial and visuomotor functioning in adults with neurofibromatosis type 1

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In preparation

ABSTRACT

Neurofibromatosis type 1 (NF1) is a neurodevelopmental genetic disorder associated with various somatic symptoms and cognitive deficits. These cognitive deficits include visual-spatial and visuomotor deficits, although this has mostly been studied in children with NF1. We assessed visual-spatial and visuomotor functioning in adults with NF1 by measuring eye and hand movement responses.

In 22 adult patients with NF1 and 31 controls, visual-spatial functioning was assessed by measuring reaction time to fixation and fixation duration of the eyes during the Visual Threshold Task. Subsequently, visuomotor functioning was assessed by measuring eye latency, hand latency, and hand accuracy during the Trajectory Prediction Task and 3 Tapping Tasks: pro-, anti-, and memory-tapping.

Visual-spatial functioning showed no differences between the NF1 and control groups. The NF1 group had a significantly faster primary eye latency than the control group in the pro-and memory-tapping tasks, and a faster decisive eye latency in the Trajectory Prediction task. In the pro-and anti-tapping tasks, these faster eye movement responses were associated with a significantly reduced hand accuracy in the NF1 group. Hand latency was not significantly different between the 2 groups in the visuomotor tasks.

In contrast to previous neuropsychological findings, our findings suggest no alterations in primary visual-spatial information processing in adult patients with NF1 compared to controls. However, the faster eye movement responses and associated changes in eye-hand coordination in the patients are in line with the comorbid symptoms of NF1 such as hyper-reactivity and motor problems. Impairments in eye movement responses and hand accuracy during specific visuomotor tasks can indicate deficits in visuomotor functioning in adult patients with NF1.

INTRODUCTION

Neurofibromatosis type 1 (NF1) is a neurodevelopmental genetic disorder associated with various somatic symptoms and cognitive disabilities (1). Cognition can refer to learning, but also the development of cognitive abilities including perception and motor skills (2). Cognitive disabilities in NF1 include deficits in attention, lower than average intelligence quotient, motor problems, and visual-spatial deficits (3–7). The visual-spatial and motor deficits have been extensively studied in children with NF1 using neuropsychological tasks, but limited studies have been performed in adult patients with NF1. Adult patients with NF1 experience several problems in performing daily cognitive activities which affect their quality of life; these problems may in part be caused by deficits in the integration of the visual and motor system (8–10).

The visual-spatial function includes cognitive processes to identify a visual stimulus, its location, and visual and spatial relationships between objects. In the human brain, the parietal-occipital region processes visual-spatial information (11–13). Impairment in visual-spatial function has been shown in children and adults with NF1 using paper-based neuropsychological tests including the judgment of line orientation (JLO) and Rey complex figure test (RCFT) (7,14). The performance on the RCFT showed the largest deficits in children with NF1 in a study that evaluated cognitive outcome measures in clinical trials (7). In adults patients with NF1, deficits in visual-spatial function, including performance on the JLO and RCFT have also been reported (15–18).

Visual-spatial information is processed and translated to the visuomotor network to perform eye and hand movements (19). The human visuomotor network integrates the sensory, attentional, executive, and motor systems (20). Visuomotor performance can be affected by disruption of the integration of these different domains in the brain. The visuomotor network plays an important role in the daily effortful cognitive activities. Moreover, motor problems have been shown in children and adults with NF1 in their disabilities in motor learning (4,21), fine motor skills (6,22), and voluntary muscle force (23). Therefore, adult patients with NF1 may suffer from deficits in the integration of the visual and motor system.

Thus far, the visual-spatial and visuomotor deficits in NF1 patients have only been assessed by using a diversity of paper-based neuropsychological tests. Eye and hand movement responses are related to the activity of the nervous system and could indicate deficits in the underlying neurophysiology of cognitive deficits in NF1 (24). Interestingly, Sailer et al. (25) showed that eye and hand movement responses changed while learning a new visuomotor task. Visual-spatial and visuomotor functioning can be quantitatively assessed by measuring eye and hand movement responses based on eye-tracking tasks (26–28). These measures have several advantages over the more conventional paper-based assessments. First, the measures can objectively assess the underlying neurophysiology of the visual and motor system. Secondly, the measures are easy to perform and no task instructions are needed. Furthermore, the measures can be collected in a short time duration of 5 to 20 minutes. Lastly, it has been shown that participants are often unaware of their visual behavior including eye movement responses (29).

Visual-spatial function can be assessed with a Visual Threshold Task showing different visual stimuli of varying difficulty of detection (28). During this task, the reaction time to fixation (RTF) and fixation duration (FD) of the eye movement response can be measured (28). Visual stimuli can differ in contrast, form, or motion to the background. Visuomotor function is often assessed with multiple repetitive eye-hand coordination tasks (30). Eye-hand coordination tasks can include the Trajectory Prediction Task and various Tapping Tasks (31,32). During these tasks, the eye latency (EL) of the eye movement response, and the hand latency (HL) and accuracy (i.e. error rate, HE) of the hand movement response could indicate deficits in the integration of the visual and motor system. In studies of cognitive processes, the commonly used time-dependent eye movement responses provide information related to cognitive processes related to learning (2).

Previous studies showed that eye movement responses were altered in various disorders with a neurodevelopmental basis, including autism, attention-deficit/hyperactivity disorder (ADHD), and schizophrenia (33,34). Munoz et al. (35) showed increased eye latencies and higher error rates using the anti-saccade task in patients with ADHD and schizophrenia compared to unaffected controls. Moreover, Tseng et al. (36) showed

distinctive features of eye movement responses with eye-tracking of various disorders such as Parkinson's disease and ADHD for potential use as a behavioral biomarker. Anderson et al. (37) reviewed the characteristics of eye movement responses in neurodegenerative diseases, and claimed that these responses are highly clinically relevant in the diagnosis of the progress and severity of the disease.

Additionally, deficits in eye and hand movement responses were observed in disorders as Parkinson's disease (PD), PD dementia, and Alzheimer's disease (AD) (30–32,38–40). Muilwijk et al. (32) assessed the pro-tapping and anti-tapping tasks in 15 patients with PD compared to controls. Interestingly, they found a significantly faster initiation of eye movements in the anti-tapping task in PD patients than in controls, but there were no differences in the pro-tapping task. This might indicate that PD patients have problems with inhibition of eye movements in an intrinsic goal-directed task. Furthermore, Verheij et al. (31) reported deficits in eye-hand coordination tasks in AD, as measured in 16 AD patients compared to 18 controls. These studies showed that measuring eye and hand movement responses could indicate deficits in disorders associated with cognitive disabilities. Since eye and hand movement responses have a strong bidirectional relation, it is important to measure both to assess visuomotor integration (41).

We considered measures of eye and hand movement responses as potential neurophysiological outcome measures for NF1 patients. To our knowledge, eye and hand movement responses have not yet been assessed in patients with NF1. The study aims to quantitatively assess visual-spatial and visuomotor functioning in NF1 patients measuring eye and hand movement responses. Based on previous studies, we expect alterations in the eye and hand movement responses during the visual-spatial assessments (i.e. the Visual Threshold Task) and the visuomotor assessments (i.e. the Trajectory Prediction Task and Tapping Tasks) in adult patients with NF1 compared to controls.

METHODS & MATERIALS

Subjects

This study enrolled 22 patients with NF1 and 31 controls between 18-55 years old. According to the in- and exclusion criteria, all subjects had no severe visual problems and used no psychoactive medication at the time of the study. All subjects had no neurological, psychiatric disorders, medical disorders, or ocular pathology. Patients with NF1 had no neurological problems that involve the sensory function of the eyes or neurological disorders other than NF1. Patients had a genetic or clinical diagnosis of NF1 and were outpatients from the ENCORE NF1 expertise center for neurodevelopmental disorders at the Erasmus Medical Center Rotterdam. Controls were unaffected unrelated peers of the patients or were recruited via online advertisements. The Dutch Central Medical Ethics Committee of the Erasmus Medical Center Rotterdam approved the study following the Declaration of Helsinki (2013).

Procedures

The measures were executed in the afternoon between 12 PM and 5 PM at the Erasmus University Medical Center in Rotterdam. We measured the eye and hand movement responses using a keyboard in combination with a touch screen. Subjects were seated in front of the touch screen at a distance of arm's length. The Tobii Pro X3-120 Eye Tracker (Tobii Technology, Sweden) with infrared cornea reflection and pupil tracking was connected below the screen on the monitor. Eye movements were recorded with Tobii Studio software after automatic correction of head movements. Before every task, a standardized calibration procedure was performed to verify a clear vision of the targets. The touch screen automatically recorded each touch or release from the hand by using a custom-made MATLAB script (MATLAB R2019b, MathWorks). Subjects performed several tasks in a fixed order taking a total duration of ca. 20 min (see also experimental procedures; Figure 1). Participants practiced the visuomotor tasks at least two times directly before the task. The procedure included tasks that tested the observation of a specific visual stimulus and the goal-directives of visually guided motor action. Additionally, prior to the eye-tracking procedure, we measured the level of sleepiness using the Karolinska sleepiness scale (KSS), a self-report questionnaire on a

nine-point Likert scale (42), and we scored the level of education following the ISCED (43).

Experimental procedures

The following tasks were presented in a fixed order (Figure 1):

The Visual Threshold Task: the Visual Threshold Task assessed visual-spatial functioning and measured gaze responses (28). The threshold task consisted of various visual stimuli focused on motion coherence, form coherence, motion detection, and contrast-detection. The visual stimuli for motion coherence, form coherence, and contrast detection were presented with varying thresholds to increase the difficulty in one of the four screen quadrants. Visual stimuli consisted of abstract forms or a cartoon. The visual stimuli were shown for 4 seconds in each of the four quadrants with a radius of 6°. No practice trials or verbal instructions were given. We measured the RTF and FD in ms of the eye movement responses during the Visual Threshold Task.

The Trajectory Prediction Task: during this visuomotor task, on the screen, a ball fell linearly in the direction of one of six baskets (44). The ball disappeared halfway of the trajectory. Subjects had to predict in which basket the ball would have fallen. They had to touch the correct basket as fast and accurately as possible. Participants were able to practice three trials after written and verbal instructions were given. We measured the primary and decisive EL in ms of the eye movement responses, HL in ms, and HE in degrees of the hand movement responses. The primary EL is the time between the start of a trial and the primary saccade, and the decisive EL is the time between the start of the trial and the decisive saccade towards the target. HL is the time between the start of a trial and the finger release from the monitor. HE is the distance of the finger touch on the monitor from the area of interest (target). The performance was the percentage of incorrectly performed trials, i.e. subjects indicated the wrong basket.

Tapping Tasks: these visuomotor tasks include a reflex-based tapping task (i.e. pro-tapping), a planning-based tapping task (i.e. anti-tapping), and a memory-based tapping task (i.e. memory- tapping) (31,32). In these tasks, participants had to start by fixating on a black circle in the middle of the screen and be as fast and accurate as possible.

Participants had to click on the red circle that appeared on the screen (pro-tapping), or on the opposite side of the screen where the red circle appeared (anti-tapping), or memorize the position of the red circle and then click on the screen where the red circle had appeared (memory-tapping). We measured the EL in ms of the eye movement responses, HL in ms, and HE in degrees of the hand movement responses. During the anti-tapping and memory-tapping tasks, the performance was measured with the percentage of incorrectly performed trials, i.e. subjects made a reflexive eye movement towards the stimulus.

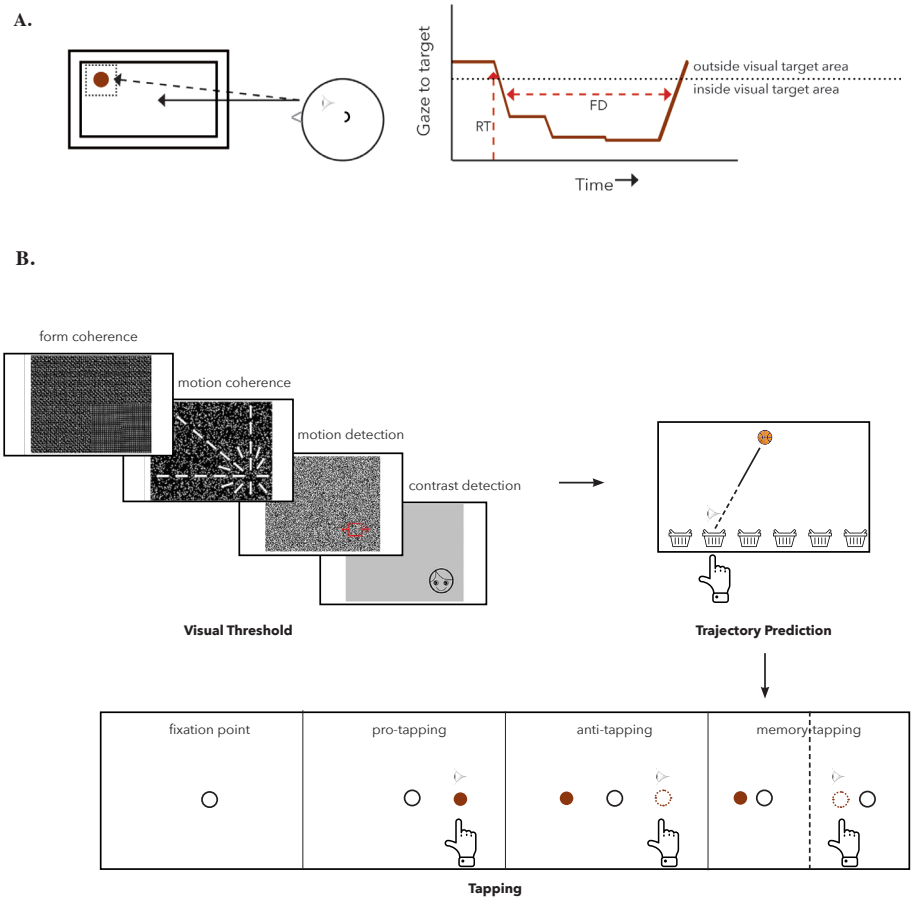


Figure 1. Schematic overview of the experimental procedures. A. Left: Subjects were seated in front of the touch screen at a distance of arm's length. Visual stimuli were shown for 4 seconds in each of the four quadrants with a radius of 6°. Right: visualization of reaction time to fixation (RTF) and fixation duration (FD). An eye movement trace is represented in degrees from the target area (visual stimulus). The horizontal dashed line is the border of the visual stimulus. B. The visual threshold, trajectory prediction, and eye-hand coordination tasks. The order is indicated with arrows. The Visual Threshold Task consisted of various visual stimuli including motion coherence, form coherence, motion detection, and contrast detection. The visual stimuli were presented with varying thresholds to increase the difficulty. During the Trajectory Prediction Task, a ball fell linearly from the top of the screen into one of the six baskets on the bottom of the screen. The ball disappeared halfway of the trajectory. Subjects had to predict in which basket the ball would have fallen. The Tapping Tasks include a pro-tapping, anti-tapping, and memory-tapping task. Participants had to start by fixating on the black circle in the middle of the screen and click on the red dot that appeared on the screen (pro-tapping) or on the opposite side of the screen where the red dot appeared (anti-tapping), or memorize the position of the red dot (memory-tapping) and click on the screen where the red dot had appeared (after vertical dashed line).

Statistical analyses

Recordings of eye and hand movements were analyzed using a custom-made MATLAB script (MATLAB R2019b, MathWorks). Trials were excluded if the eye-tracking was poorly recorded (i.e. invalid data), if no eye movements were made, or if the visual target was not seen (i.e. invalid performance). Data points were excluded from further analyses if the values were more than ± 2SD of the mean and showed an invalid performance after revision of the analyses. Additionally, participants were excluded from further analyses if less than two valid responses were measured to one of the visual stimuli of the Visual Threshold Task.

Statistical analyses were performed using IBM Statistics SPSS (version 25). All variables were tested for normality using the Kolmogorov-Smirnov test. Variables that had positive or negative skewness were transformed with a square root, or reflect and square root transformation, respectively. The control and patient group were tested for significant differences in age, sleepiness, and level of education using the non-parametric Mann-Whitney U test. Differences in gender between groups were tested using the chi-square test. If one of these variables was significantly different between both groups, the variable was added as a covariate in further analyses. Differences between the groups in the eye and hand movement responses of each task were analyzed with multivariate analyses of variance (MANOVA). In the MANOVAs for the Visual Threshold Task, the dependent variables were RTF and FD and the independent variable was group (patient or control). In the MANOVAs for the Trajectory Prediction and Tapping tasks, the independent variables were the EL, HL and HE, and the independent variable was group (patient or control) (31,32). Multivariate and univariate main effects were reported. Correlations between age, educational attainment, and the outcome measures were evaluated using the nonparametric Spearman's correlation coefficients, and p-values were corrected for multiple testing with the Bonferroni correction. A p-value < 0.05 was statistically significant.

RESULTS

We included 22 patients with NF1 and 31 controls in our study. We identified 3.3% of the data as outlier. Furthermore, 2 participants were excluded for the Visual Threshold Task due to lack of sufficient valid data ($n_{control} = 1$, $n_{NF1} = 1$). Patients and controls did not significantly differ in age ($M_{NF1} = 28.9 \pm 11.0$; $M_{control} = 32.9 \pm 11.1$; $U_{age} = 246.5$, $p = 0.09$), gender ($\chi^2_{gender} = 0.03$, $p = 0.87$) or in sleepiness prior to the experiment ($U_{KSS} = 162$, $p = 0.16$). However, patients showed a significant lower level of education than controls ($U_{education} = 139$, $p < 0.001$) (Table 1). Therefore, the transformed level of education was added as covariate in the further analyses.

Table 1. The demographics per group for controls and adult patients with NF1.

Demographics	Control (n = 31)	NF1 (n = 22)
Age in years (mean ± SD)	32.9 ± 11.1	28.9 ± 11.0
Gender: Male in % (N)	38.7 (12)	40.9 (9)
Sleepiness (median, range)	2.0, 1-7	2.0, 1-6
Level of education (median, range)	6, 4-7	5, 1-6

The Visual Threshold Task

Overall, no significant differences were found in RTF and the transformed FD between the control and patient groups in the Visual Threshold Task (Table 2). This indicates that visual-spatial functioning was similar in both groups.

The Trajectory Prediction Task

The patient group did significantly differ from the control group during the Trajectory Prediction task ($F(4,38) = 4.4$, $p = 0.005$, Wilk's $\Lambda = 0.69$) (Table 2; Figure 2). Univariate tests showed that the patient group had a significantly faster decisive EL than the control group ($F(1,44) = 10.4$, $p = 0.002$, $eta^2 = 0.2$), but no differences were found between the 2 groups in the transformed primary EL, HL and HE. The performance on the trajectory prediction task did not differ between the groups ($Errorrate_{NF1} = 13.8\% \pm 21.0$; $_{control} = 11.0\% \pm 19.8$).

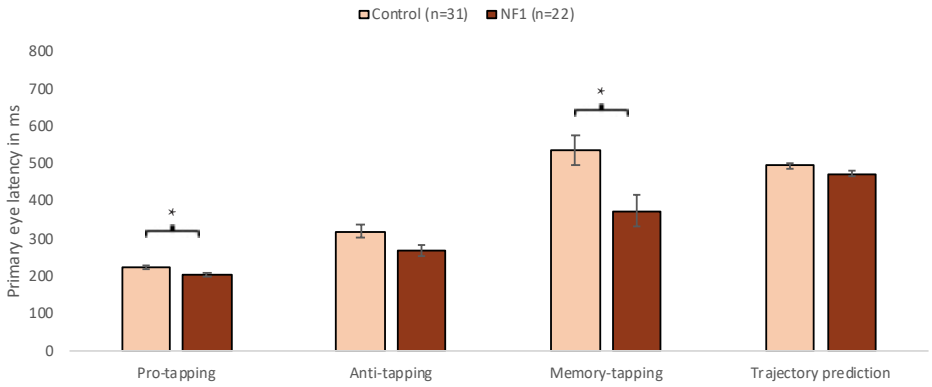
The Tapping Tasks

In the visuomotor tasks, the patient group did differ from the control group in the pro- ($F(3,43) = 8.3$, $p < 0.001$, Wilk's $\Lambda = 0.64$), the anti- ($F(3,45) = 5.4$, $p = 0.003$, Wilk's $\Lambda = 0.74$), and the memory tapping tasks ($F(3, 43) = 4.4$, $p = 0.009$, Wilk's $\Lambda = 0.77$) (Table 2; Figure 2). In the pro- and memory-tapping tasks, univariate tests showed significantly faster EL in the NF1 group than in the control group ($F_{pro}(1,45) = 8.3$, $p = 0.006$, $eta^2 = 0.2$; $F_{memory}(1,45) = 9.3$, $p = 0.004$, $eta^2 = 0.2$) (Figure 2A). Additionally, in the pro- and anti-tapping tasks, the HE was significantly higher in the NF1 group than in the control group ($F_{pro}(1,45) = 11.5$, $p = 0.001$, $eta^2 = 0.2$; $F_{anti}(1,47) = 11.4$, $p = 0.003$, $eta^2 = 0.2$; Figure 2B), indicating a reduced hand accuracy in the NF1 group. The performance on the anti-tapping did not differ between the groups ($Errorrate_{NF1} = 81.9\% \pm 20.4$; $_{control} = 69.7\% \pm 28.8$), indicating that both groups made a similar amount of reflexive eye movements towards the stimulus. In the memory-tapping task, the patient group did not differ from the control group in HL and HE (Table 2; Figure 2). Interestingly, however, the performance on the memory tapping was significantly higher in the NF1 group than in the control group, indicating that the NF1 group made more reflexive saccades prior to the disappearance of the visual stimulus ($Errorrate_{NF1} = 63.7\% \pm 29.9$; $_{control} = 43.7\% \pm 33.2$; $U = 181.5$, $p = 0.03$).

Correlations

There were no significant correlations between age and the eye and hand movement responses. However, educational attainment showed a significant correlation with HL in the memory-tapping tasks: the increase of reaction time of hand latency correlated significantly with higher education attainment ($r_{memory} = 0.4$, $p = 0.004$).

A.



B.

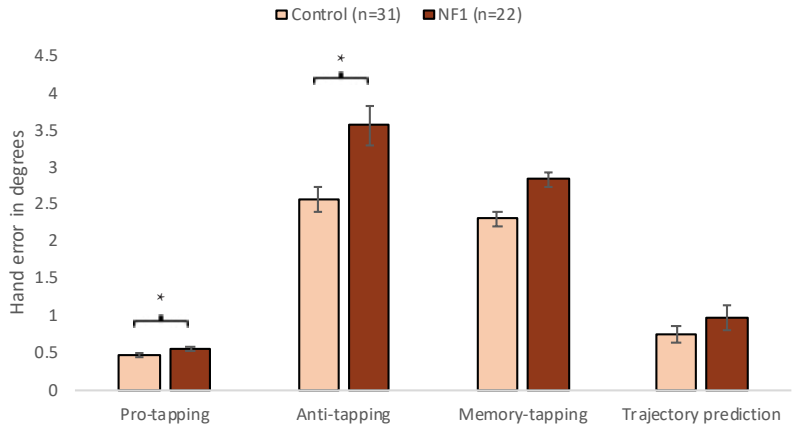


Figure 2. Eye movement responses and hand accuracy per visuomotor task per group. A. Patients showed faster eye movement responses (EL) than controls in the visuomotor tasks, these findings were significantly different between groups in the pro- and memory-tapping tasks ($F_{pro}(1,45) = 8.3$, $p = 0.006$, $eta^2 = 0.2$; $F_{memory}(1,45) = 9.3$, $p = 0.004$, $eta^2 = 0.2$). B. The hand error (HE) was significantly higher in the NF1 group than in the control group in the pro- and anti-tapping tasks ($F_{pro}(1,45) = 11.5$, $p = 0.001$, $eta^2 = 0.2$; $F_{anti}(1,47) = 11.4$, $p = 0.003$, $eta^2 = 0.2$). Significance is displayed with asterisks.

Table 2. The eye and hand movement responses per group for controls and adult patients with NF1.

	Control (n = 30)			NF1 (n = 21)		
Visual-spatial task[1] (median, IQR)	**RTF (ms)**	**FD (ms)**		**RTF (ms)**	**FD (ms)**	
VT contrast detection	253, 45	2583, 292		241, 55	2565, 565	
VT motion coherence	441, 137	2358, 581		435, 136	2271, 825	
VT form coherence	280, 57	1827, 603		280, 52	1827, 619	
VT motion detection	341, 92	2538, 373		363, 87	2475, 450	
Visuomotor tasks (mean ± SD)	**HL (ms)**	**EL (ms)**	**HE (°)**	**HL (ms)**	**EL (ms)**	**HE (°)**
Trajectory Prediction pEL | dEL	754 ± 122	495 ± 41| 598 ± 62*	0.75 ± 0.58	800 ± 184	471 ± 36 | 573 ± 67*	0.97 ± 0.79
pro-tapping	342 ± 40	224 ± 20*	0.47 ± 0.12*	342 ± 42	205 ± 22*	0.56 ± 0.12*
anti-tapping	415 ± 68	319 ± 90	2.56 ± 0.95*	426 ± 61	269 ± 66	3.56 ± 1.26*
memory-tapping	538 ± 73	537 ± 222*	2.30 ± 0.58	580 ± 75	373 ± 193*	2.84 ± 0.45

*Significantly different between patients and controls ($p < 0.05$).

[1] Only the results of the easiest option of difficulty (i.e. 100% difference) during the visual threshold task are presented for contrast detection, motion coherence, and form coherence.

NF1: neurofibromatosis type 1; IQR: interquartile range; VT: Visual Threshold Task; pEL: primary eye latency; dEL; decisive eye latency; RTF: reaction time to fixation; FD: fixation duration; HL: hand latency; HE: hand error in degrees.

DISCUSSION

Studying eye and hand movement responses using eye-tracking could be a non-invasive objective and quantitative assessment of the visual-spatial and visuomotor functioning in adults with NF1. Our findings showed no differences in primary visual-spatial information processing between the NF1 and control groups. However, the NF1 group had faster eye movement responses to visual stimuli than the control group, which was significant in the pro-and memory-tapping tasks (primary EL), and the Trajectory Prediction task (decisive EL). In the pro-and anti-tapping tasks, these faster responses occurred with significantly reduced hand accuracy. Hand latency was not significantly different between the 2 groups in the visuomotor tasks.

In contrast to our expectations, the eye movement responses during the visual-spatial assessments did not differ significantly between groups. The Visual Threshold Task reflects the primary pathway of visual-spatial information processing. In this pathway, the retina projects the visual information via the lateral geniculate nucleus to the primary visual cortex (V1). The neural pathway in the brain of visual information processing is complex, in which the cortical areas are responsible for orientation, recognition, and perception (45). V1 projects to the posterior parietal cortex (dorsal pathway) and to the inferior temporal cortex (ventral pathway), which are responsible for visual-spatial perception and visuomotor actions, and identification of visual stimuli, respectively. Clinically, visual evoked potentials (VEPs) measured with electroencephalography are used to assess the function of the visual pathway from the eye to the visual cortex. VEP studies in children and adults with NF1 have shown abnormalities in the early components of the VEPs in 26-51% of the patients, suggesting deficits to the primary visual pathway in NF1 (46–50). Moreover, previous studies showed alterations in the Visual Threshold Task in reaction time to fixation and fixation duration in children with cerebral or ocular visual impairments (28). In the present study, we did not find abnormalities in these measures of primary visual information processing in our NF1 sample. Notably, all subjects in the present study had no severe visual problems or ocular pathology to investigate cognitive deficits associated with the function of cortical networks or neural pathways in the brain. Additionally, previous studies in children with ADHD showed discriminating features

in visual-spatial function compared to controls (33,36). Specifically, they showed gaze alterations in mixed directions in detecting contrast differences in texture and color, while watching video clips. Interestingly, ADHD symptoms are common comorbid problems in children with NF1 (51). Our NF1 sample did not include clinically diagnosed ADHD patients, although symptoms could be subclinical since the existence of comorbidity of ADHD symptoms has been shown in adult patients with NF1 (51). Overall, the present study showed no significant alterations in eye movement responses during primary visual information processing in an NF1 sample without ocular pathology or ADHD comorbidity.

However, adult patients with NF1 were significantly faster in their eye latency than controls during the visuomotor pro- and memory tapping task. The tapping tasks resemble the visual-spatial function of the Visual Threshold Task: identifying a visual stimulus, its location, and visual and spatial relationships between objects, but the tapping tasks also involve visuomotor integration. Previous studies using the pro-tapping task did not find any differences in eye-latency between controls and patients with PD or AD (31,32,38,39). Hence, this feature could be specifically altered in patients with NF1. Furthermore, Kovarski et al. (52) showed significantly faster eye movements on a pro-saccade task in children with autism spectrum disorder (ASD), which is in line with present findings. Interestingly, next to ADHD, ASD symptoms are also common comorbid problems in children with NF1 (53).The eye-saccades are thought to rely on a direct connection from the incoming visual stimulus to the motor command of the eyes (35,54). Since only relevant visual stimuli need to trigger eye-saccades in everyday life, there is a decisive period between the visual input and motor processing that indicates the relevance of the stimulus.This is in accordance with hyper-reactivity to sensory input clinically observed in the NF1 comorbidities ASD and ADHD (52). Our findings suggest that hyper-reactivity to sensory input may also be present in adult patients with NF1. Although none of the subjects had a clinical diagnosis of these comorbidities, subclinical symptoms could be present. Additionally, the faster eye movement responses observed in the Trajectory Prediction task (decisive EL) in the NF1 group are in line with the hyper-reactivity hypothesis.

The faster eye movement responses occurred with significantly reduced hand accuracy in the visuomotor pro- and anti-tapping tasks. It has been suggested that the visual dorsal pathway projects further to the prefrontal, premotor, and medial temporal cortices (55). Hence, deficits in the visual dorsal pathway could lead to motor problems, which are known to be related to NF1 including deficits in fine motor skills (6,22). The faster eye movement responses and reduced hand accuracy in the NF1 group resulted in a significantly reduced performance in the memory-tapping task. In the anti-tapping task, the performance seemed to be lower in the NF1 group than the control group, although these findings were non-significant due to high variability in the error rate.

The lack of differences in the hand movement responses in the more complex visuomotor tasks is in contrast to our expectations based on previous studies. The Trajectory Prediction Task highly resembles the JLO task, which is commonly used in NF1. Impaired performance on the JLO task has been shown in children and adults with NF1 (14,18). Remarkably, the performance on the Trajectory Prediction Task did not differ between both groups in the present study. The similar performance in both groups may indicate that the Trajectory Prediction Task was not too difficult for the subjects. Notably, in the more complex visuomotor tasks, higher-order cognitive functions become involved and therefore involve other factors that influence the visuomotor function, including sensory perception, attention, or intelligence. In our study, the sensory perception was not significantly different as assessed in the before-mentioned Visual Threshold Task. Furthermore, a previous study observed no abnormalities on attention tasks in adult patients with NF1, in contrast to findings in children with NF1 (56). However, it is known that the intelligence quotient is lower than average for NF1 (3,5,57). Interestingly, a reduced intelligence quotient was associated with reduced performance on various cognitive neurophysiological tasks in controls (58). Although we have not tested intelligence quotients in the present study, the NF1 group did show a significantly lower educational attainment than the control group, therefore, this parameter was added as a covariate in all analyses. It is important to point out that the positive association between intelligence and educational attainment may be influenced by many other factors. We did observe a significant negative correlation between educational attainment and the hand latency in the memory-tapping task, indicating that

subjects with a lower educational level needed more time for visuomotor integration than subjects with a higher education level. Without including the covariate educational attainment, hand latencies were different in the NF1 and control groups in the memory-tapping task. Additional subgroup analyses based on the educational attainment were not performed due to the relative sample size and small variation in level of education (level of education: control, level 4: n = 1, 5: n = 8, 6: n = 11, 7: n = 1; NF1, level 1: n = 1, 3: n = 1, 4: n = 4, 5: n = 10, 6: n = 5). Future studies should confirm the finding of reduced hand latency in NF1 patients, and investigate whether hand latency could be a predictor of cognitive deficits.

An important issue of the study is that our NF1 sample may not be representative for the true NF1 population due to the potential overrepresentation of highly motivated or less severely affected patients. The lack of significant differences in the hand movement responses to the more complex visuomotor assessments could reflect this issue. Nevertheless, our data could also reflect a genuine presence of correct visuomotor integration in the preparation and onset of hand movements in NF1. Moreover, the faster eye movement responses and reduced hand accuracy in the NF1 group were significantly present in our sample.

To our knowledge, this is the first study that measured eye and hand movement responses to quantify visual-spatial and visuomotor functioning in NF1 adults. It has been shown that eye-tracking could be used as a potential biomarker in various disorders with a neurodevelopmental basis (36). The present study provides more information on the cognitive phenotype of adult patients with NF1. The majority of previous studies into cognitive abilities in NF1 were focused on children with NF1, while the severity of cognitive deficits might diminish from childhood to adulthood due to developmental and compensatory changes (15,59). Furthermore, the experiments lasted only ca. 20 min, thereby minimizing fatigue or diminished concentration. In addition, the tasks were simple and easy to understand, which make it useful to include these non-invasive quantitative tasks as outcome measures in clinical intervention studies.

In conclusion, we observed no alterations in primary visual-spatial information processing in adult patients with NF1. However, we did find faster eye movement responses and reduced accuracy on the visuomotor tasks, which is in line with the comorbid symptoms of NF1 such as hyper-reactivity and motor problems. Impairments in eye movement responses and hand accuracy during specific visuomotor tasks can indicate deficits in visuomotor functioning in adult patients with NF1.

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Chapter 4

Plasticity of visual evoked potentials in patients with neurofibromatosis type 1

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ABSTRACT

The inability to properly process visual information has been frequently associated with neurofibromatosis type 1 (NF1). Based on animal studies, the cause of cognitive disabilities in NF1 is hypothesized to arise from decreased synaptic plasticity. Visual cortical plasticity in humans can be investigated by studying visual evoked potentials (VEPs) in response to visual stimulation.

VEP plasticity was assessed by measuring the increase of the peak amplitudes C1, P1, and N1 induced by 10-min modulation of checkerboard reversals in 22 adult NF1 patients and 30 controls. VEP signals were recorded pre-modulation, during modulation, and at 2, 7, 12, 17, 22, 27 min post-modulation.

The C1 and P1 amplitudes increased significantly comparing post-modulation to pre-modulation in the control group. This potentiation was not observed in the NF1 group.

Visual cortical plasticity could be measured using VEPs in response to visual stimulation in the control group. Individuals with NF1 may have reduced visual cortical plasticity, as indicated by their non-potentiated response to VEP induction. These findings should be interpreted with caution due to high inter-subject variability. The present study contributes to an improved assessment of the feasibility for using neurophysiological outcome measures in intervention studies of cognitive deficits among patients with NF1.

INTRODUCTION

Neurofibromatosis type 1 (NF1) is associated with cognitive deficits and learning disabilities that can affect quality of life (1). In addition to the somatic symptoms associated with NF1, patients have a lower than average intelligence quotient score, attention deficits, impairments in motor learning, and visual information processing difficulties (2,3). Given the cognitive impairments in the visuospatial and visuoperceptual domains, the inability to properly process visual information might contribute to some of the learning disabilities in NF1 (3,4). However, it is unknown whether there is primary dysfunction of visual pathways in NF1 adults, and if there are neurophysiological deficits in the visual cortex that could contribute to the cognitive disabilities in NF1.

The underlying cause of the cognitive disabilities in NF1 might be a result of decreased synaptic plasticity, which was found in animal models of NF1 (5–8). The neurobiological process leading to enduring enhancement of strength or efficacy of synaptic transmission, i.e. long-term potentiation (LTP), is essential for learning and memory. Previous LTP studies were mostly limited to animal studies or surgically excised human cortical tissue, hampering a translation to clinical studies. Non-invasive neurophysiological methods have filled this gap and can measure changes in cognition and learning (9). These measurements include event-related potentials in response to sensory stimulation in the human brain. Ribeiro et al. (10) observed that, in response to visual stimuli, event-related potentials were already atypical at baseline for late evoked responses in 12 NF1 children, indicating alterations in high-level processing of visual stimuli in NF1. Furthermore, they found an increased amplitude of alpha brain oscillations in the visual cortex in NF1 patients. The enhancement of alpha brain oscillations is associated with decreased excitability and may be associated with attention problems in visual processing (10).

Perceptual learning involves the plasticity of responses to sensory stimulation in the primary sensory cortices. Specifically, Frenkel et al. (11) showed visual cortical plasticity of the responses to repeated visual stimulation in awake mice. They measured chronic visual evoked potentials (VEPs), which is a type of event-related potential. The measurements showed a time-dependent increase in VEP amplitude in response to a repeated visual

stimulus, which disappeared with the presentation of a novel visual stimulus. Visual cortical plasticity in the human visual cortex can be measured by changes in the amplitude of VEPs (12). In psychiatry, the study of VEPs has been used to improve the understanding of the physiology and pathology of several disorders including depression, schizophrenia and bipolar disorder (13–15).

VEPs can be elicited in the visual cortex by visual stimulation, for which a flash of light or pattern reversal of the black and white blocks in a checkerboard pattern is typically used (for an overview of studies see Table 1 of Valstad et al. (16)). Teyler et al. (12) were the first to demonstrate an increase of one of the components of the VEP in unaffected controls after repetitive visual stimulation using checkerboard reversals. Prolonged stimulation by exposure to flashing light at a high frequency or a 10-min block of checkerboard reversals at a low frequency has been shown to induce potentiation of the VEPs (14,15). Changes in VEP amplitudes seem to be more sensitive to checkerboard reversals (14,15,18,19). Potentiation of VEPs has been observed in healthy adults as indicated by a decrease in amplitude of the prominent negative component at 75ms (C1), and an increase in amplitude of the positive component at 100 ms (P1) after stimulation (18). In contrast, in 40 depressed patients, P1 did not increase after a 10-min modulation block of checkerboard reversals while it did in a group of 70 healthy controls (14). In addition, Zak et al. (19) observed a significant increase in the peak-to-peak amplitude of P1 to N1 in controls, but not in patients with bipolar disorder type II. The N1 amplitude is the negative component at 150-200 ms post-stimulus. Collectively, these studies show that VEP induction with checkerboard reversals can indicate deficits in potentiation in the visual cortex.

Clinically, VEPs are used to assess the function of the visual pathway from the eye to the occipital cortex. In NF1 patients, a few VEP studies have been performed in which VEPs were studied under baseline conditions without the induction of VEP plasticity. These studies showed abnormal VEPs at baseline in 26-51% of NF1 patients, including children (aged 6-16 years), adolescents (aged 10-18 years), and adults (aged 18-56 years), compared to controls (20–23). More specifically, NF1 patients exhibited a delayed latency of the P1. Additionally, a recent study showed a decreased amplitude of the P1 in

26 NF1 adults compared to controls (24). These findings suggest a primary abnormality of the visual pathways in NF1. Notably, optic nerve gliomas are very common in NF1 and could have influenced the VEP latencies in the previous studies. However, the number of patients in these studies with optic gliomas was low (5-15%; (22,23)) and patients with optic gliomas were often excluded (20,21,24). This indicates that delayed VEP in NF1 cannot be fully explained by the presence of gliomas.

To investigate the plasticity of the visual cortex in NF1 patients by assessing VEP potentiation, we studied VEPs using checkerboard reversals at baseline (i.e. pre-modulation), during 10-min modulation and 30-min post-modulation. VEP plasticity was measured by change in the peak amplitude of the VEP signal compared to pre-modulation. To our knowledge, this is the first study that investigates VEP plasticity in NF1, which might be a novel neurophysiological outcome measure associated with cognitive disability.

METHODS AND MATERIALS

Subjects

In this study, 22 patients with NF1 and 31 controls between 18-55 years participated after they gave their written informed consent. According to the in- and exclusion criteria, subjects were included if they had no severe visual problems or neurological illness that involved the visual system. Furthermore, all subjects had no optic nerve gliomas and were without any other ocular pathology based on a general health questionnaire. Subjects had no history or current presence of neurological or psychiatric disorders and did not use psychoactive agents at the time of the study. Patients with NF1 were outpatients from the ENCORE NF1 expertise center for neurodevelopmental disorders at the Erasmus University Medical Center, Rotterdam. Patients with NF1 had a genetic and/or clinical diagnosis of NF1. Controls matched for age and gender were unaffected unrelated peers of the patients or recruited through online advertisements. The Medical Ethics Review Committee of the Erasmus Medical Center Rotterdam approved the study (MEC-2020-0095), which was conducted following the Declaration of Helsinki (2013).

Procedures

VEP recordings took place in the afternoon between 12 PM and 5 PM at the Department of Clinical Neurophysiology at the Erasmus University Medical Center Rotterdam. Subjects were seated in a comfortable chair during the VEP recordings while maintaining focus on a red fixation dot on a screen located 54 cm in front of the subject. We recorded from Oz, Cz, and a reference electrode on the forehead (ground) according to the 10-20 system of electrode placement (25). The impedance between electrode and scalp was minimized by injecting conductive, non-alcoholic, viscous gel (OneStep Cleargel, H + H Medizinprodukte GbR, Münster, Germany) in the electrodes. VEP signals were recorded using a NicoletTM Viking EDX system (Natus Neurology Incorporated, Middleton, Wisconsin, USA) with settings according to the ISCEV guidelines (17). We used a classical cathode ray tube (CRT) stimulator with a mean photopic luminance of 45 Cd/m2. The mean luminance of the visual stimulator was constant during the checkerboard reversals and identical to the grey screen presented during the intervals. The light in the room was dimmed. We used a custom programmed Raspberry PI to facilitate accurate timing of the protocol. In addition, before the start and at the end of the VEP recording, the level of sleepiness was reported using the Karolinska sleepiness scale (KSS), a self-report questionnaire on a nine-point Likert scale (26).

VEP measurements

VEPs were elicited by checkerboard reversals at a low frequency of 1.92 Hz (14,15,17). In each stimulation block, 40 sweeps were presented within 20 seconds to both eyes. The responses to the sweeps were averaged. An identical checkerboard reversal was presented continuously for 10 minutes during the modulation block. A grey screen was shown during the intervals between the stimuli. During the experiment, signals were analogous band-pass filtered of 0.05 to 100 Hz and amplified according to the ISCEV guidelines (17). Traces exceeding 130 μV were considered blink artifacts and were discarded.

We recorded the mean VEP signals in the stimulation blocks at pre-modulation, modulation, and post-modulation (13,14,18) (Figure 1). Pre-modulation, the stimulation blocks with a duration of 20 seconds each, started 1 and 3 min after the start of the experiment

(i.e. B1 and B2). We used two measurements at baseline for better data stability. If the two measurements showed no significant difference in amplitude or latency, the average of the two was used in further analyses as pre-modulation. Otherwise, the recordings of the second stimulation block was used as pre-modulation. The modulation with a duration of 10 min started 5 min after the start of the experiment. We recorded VEP signals 10 times for 20 seconds each (i.e. M1 to M10) to observe the data stability during modulation. Post-modulation, the stimulation blocks with a duration of 20 seconds each, started at 2 (T1), 7 (T2), 12 (T3), 17 (T4), 22 (T5), and 27 (T6) minutes after the end of the modulation (Figure 1). VEP plasticity is measured by a change in the peak amplitudes of the P1, C1, or N1 component when comparing the average of the post-modulation, or the individual time points (T1-T6) of the post-modulation, to pre-modulation.

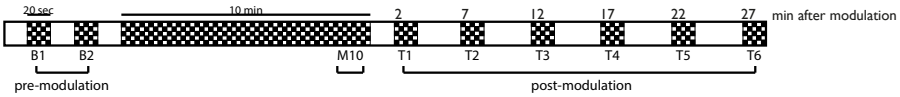


Figure 1. Schematic time course of VEP induction. B1 and B2: checkerboard reversals pre-modulation with each 20 seconds, started 1 and 3 min after the start of the experiment. M10: VEP measurement of 20 seconds during checkerboard reversals given in the 10th minute of continuous stimulation of 10 min. Modulation with a duration of 10 min started 5 min after the start of the experiment. T1-T6: checkerboard reversals post-modulation with each 20 seconds given at 2 (T1), 7 (T2), 12 (T3), 17 (T4), 22 (T5), and 27 (T6) minutes after the end of the modulation.

Statistical analysis

VEP data were analyzed using MATLAB R2019b (Mathworks). The data was baseline corrected (-50 – 0 ms prior to stimulus) and digitally low-pass filtered at 48 Hz. We performed peak detection semi-automatically using a custom-made MATLAB script. We calculated the mean of the VEPs from all recordings per subject to create a subject average VEP signal. In this subject average VEP signal, P1 was identified as the maximum amplitude between 90 and 130 ms, C1 as the last minimum preceding P1, and N1 as the first minimum following P1. Subsequently, peaks were automatically detected from each recorded time point by finding the minimum/maximum within a 20 ms window surrounding the subject average peak latencies. Peaks were manually detected if no minimum/maximum was found within this 20 ms window. Amplitudes of the C1, P1 and N1 peaks were calculated to the 50 ms baseline. Two experimenters run the analysis

independently and came to a consensus of the exclusion of VEPs or individual peaks that could not be identified.

Statistical analyses were performed using IBM Statistics SPSS (version 25). Correlations between age, educational attainment, and the main outcomes were evaluated using Pearson or the nonparametric Spearman's correlation coefficients, and p-values were corrected for multiple testing with the Holm-Bonferroni correction. Significant data had a p-value ≤ 0.05.

VEP response pre-modulation

We tested whether there were differences between groups regarding the confounding variables gender, age, and educational attainment with a Chi-squared test and non-parametrically with Mann-Whitney U test, respectively. The peak latencies and amplitudes of the stimulation blocks pre-modulation (i.e. B1 and B2) were tested with a paired t-test. The differences between the two groups in mean peak latencies and amplitudes pre-modulation were tested with independent t-tests.

VEP response post-modulation

We tested the change per peak amplitude of C1, P1, and N1 of averaged pre-and post-modulation with paired t-tests in controls and patients separately to detect the effect of the modulation (13). Averaged post-modulation is the average of all measurements after modulation (T1-T6) (13,16,18). We also tested the change in peak-to-peak amplitude of C1 to P1 with paired t-tests (17). A within-subject repeated-measures ANOVA was used to measure the effect of the factor time on mean VEP amplitudes post-modulation (T1-T6) in controls and patients separately (13). Degrees of freedom were corrected using Greenhouse-Geiser estimates of sphericity.

VEP response at M10

We tested the effect of modulation without any delay by comparing the VEP amplitudes of the last minute during continuous visual stimulation (i.e. M10, Figure 1) with pre-modulation using paired t-tests.

RESULTS

In total, 42 patients with NF1 agreed to eligibility screening, of which 22 subjects were enrolled. In addition, 35 eligible subjects without NF1 were screened for study eligibility, of which 31 subjects were enrolled (Figure 2). After the measurements, subjects were excluded if VEPs could not be identified ($n_{control}$ = 1; n_{NF1} = 2), and individual peaks were excluded if they could not be identified (15 of 558 traces in $n_{control}$ = 4; 14 of 396 traces in n_{NF1} = 5).

No significant differences were found between patients and controls in age ($M_{patient}$ = 29.2 ± 11.4, $M_{control}$ = 33.17 ± 11.2; U_{age} = 161.0, p = 0.10), gender (χ^2 = 0.35, p = 0.56) or level of sleepiness at the start and end of the experiment (U_{start} = 261, p = 0.55, U_{end} = 162, p = 0.16). We did find an expected difference in the level of education, for which patients had a significantly lower level of education than controls (U = 127.5, p < 0.001) (Table 1).

VEP response pre-modulation

Peak latencies and amplitudes of B1 and B2 were not significantly different between groups, for which the averages were used as pre-modulation in further analyses. We did not find significant differences in pre-modulation peak latencies between the NF1 and control groups. Additionally, we did not find significant differences in peak amplitudes between the groups pre-modulation (Table 1).

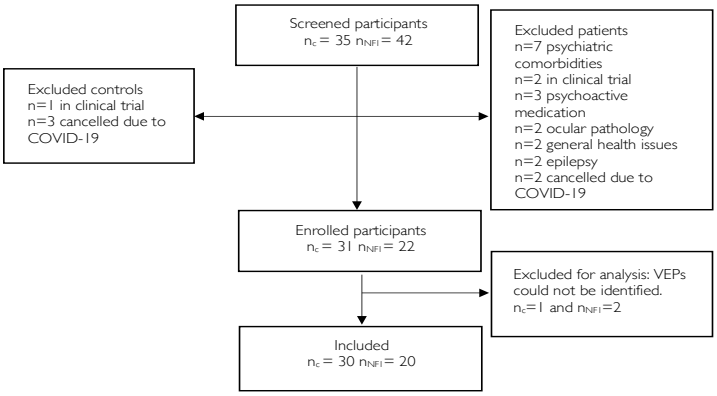


Figure 2. Flowchart. The number of included and excluded participants. c, control; NF1, Neurofibromatosis type 1; VEPs = visual evoked potentials.

Table 1. Demographics (Mean ± SD), VEP responses pre-modulation, post-modulation, and during modulation of the neurofibromatosis type 1 (NF1) group and the control group separately.

	Control (n = 30)		NF1 (n = 20)	
Demographics				
age in years	33.2 ± 11.2		29.2 ± 11.4	
gender: male in % (#)	36.7 (11)		45 (9)	
Sleepiness (median, range) [1]	2.0, 1-7 | 3.0, 1-7		2.0, 1-6 | 2.0, 1-6	
educational attainment, median (range)*	6.0, 4-7		5.0, 1-6	
VEP response pre-modulation	*latencies*	*amplitudes*	*latencies*	*amplitudes*
C1 in ms | in µV	79.9 ± 6.5	-2.6 ± 2.2	80.3 ± 9.2	-2.7 ± 3.9
P1 in ms | in µV	114.0 ± 4.6	8.6 ± 4.5	112.3 ± 3.8	8.3 ± 5.5
N1 in ms | in µV	161.0 ± 17.6	-4.0 ± 2.6	156.9 ± 16.1	-4.8 ± 4.1
C1 – P1		11.2 ± 4.9		11.0 ± 6.3
VEP response during modulation (in µV) [2]		*amplitudes*		*amplitudes*
C1		-1.2 ± 2.9**		-1.7± 3.5
P1		10.4 ± 4.9**		9.1 ± 7.8
N1		-1.6 ± 2.5**		-2.4 ± 3.4**
C1 – P1		11.4 ± 5.3		10.8 ± 6.8
VEP response post-modulation (in µV) [3]				
C1		-1.9 ± 1.9**		-2.2 ± 2.9
P1		9.8 ± 5.0**		8.3 ± 5.5
N1		-3.5 ± 2.5		-3.9 ± 4.2**
C1 – P1		11.6 ± 5.7		10.6 ± 5.7

* Significantly different between patients and controls (p-value ≤ 0.05)

** Significantly different from pre-modulation (p-value ≤ 0.05)

[1] Karolinska sleepiness scale (KSS) at the start of the VEP recordings | Karolinska sleepiness scale at the end of the VEP recordings

[2] Values represent the average amplitude during the visual stimuli given in the 10th minute of continuous visual stimulation (M10).

[3] Values represent the average amplitude during the visual stimuli given at 2 (T1), 7 (T2), 12 (T3), 17 (T4), 22 (T5), and 27 (T6) minutes post-modulation.

VEP, visual evoked potentials; NF1, neurofibromatosis type 1; C1, the prominent negative component at ca. 75 ms; P1, positive component at ca. 100 ms; N1, negative component at ca. 150-200 ms post-stimulus.

VEP response post-modulation

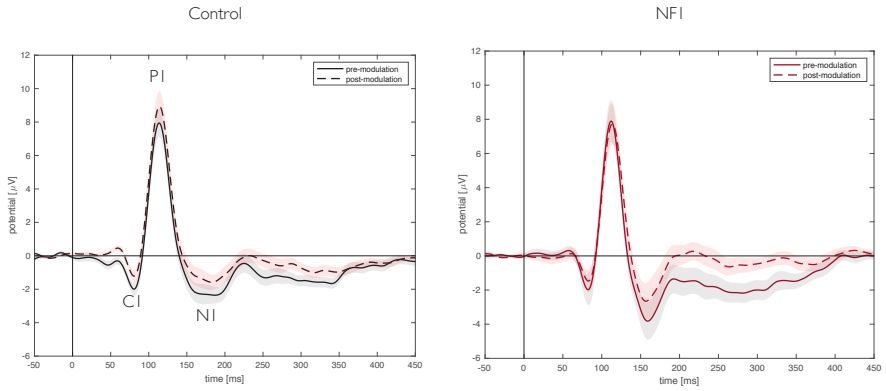
To examine the effect of modulation on VEP plasticity, we tested the change per peak amplitude (C1, P1, N1) comparing averaged post-modulation to pre-modulation in the control group. C1 was significantly decreased and P1 was significantly increased in amplitude when comparing averaged post-modulation to pre-modulation ($t_{C1}(29) = 3.6$, $p = 0.001$, $d = 0.4$; $t_{P1}(29) = 2.9$, $p = 0.008$, $d = 0.2$), indicating VEP potentiation (Figure 3; Figure 4). The N1, and C1-P1 peak-to-peak amplitude did not significantly differ from pre- to averaged post-modulation (Table 1; Figure 3). Within-subject repeated measures ANOVA showed a significant effect of the factor time (T1-T6) for C1 and trended towards significant for P1 amplitude ($F_{C1}(6, 162) = 2.1$, $p = 0.05$, $\eta^2 = 0.07$; $F_{P1}(6, 162) = 2.1$, $p = 0.055$, $\eta^2 = 0.07$) (Figure 4).

In contrast to the control group, the C1 and P1 peak amplitudes were not significantly different between pre-and averaged post-modulation in the NF1 group. The N1 significantly decreased in amplitude from pre- to averaged post-modulation ($t_{N1}(19) = 1.1$, $p = 0.02$, $d = 0.2$), which was in the opposing direction to N1 modulation in previous studies. Furthermore, a within-subject repeated measures ANOVA showed no significant effect of the factor time on VEP amplitudes post-modulation (T1-T6) (Figure 3; Figure 4).

VEP-response at M10

We investigated modulation without delay by comparing VEP-amplitude during the 10th minute of continuous stimulation (M10) to pre-modulation using paired t-tests. In the control group, P1 amplitude was significantly increased, and C1 and N1 amplitudes were significantly decreased between M10 and pre-modulation ($t_{C1}(28) = 4.0$, $p < 0.001$; $t_{P1}(29) = 3.0$, $p = 0.005$, $t_{N1}(29) = 5.4$, $p < 0.001$). In the NF1 group, the peaks C1 and P1 did not significantly change between M10 and pre-modulation, although N1 amplitude was significantly decreased at M10 compared to pre-modulation ($t_{C1}(17) = 0.5$, $p = 0.6$; $t_{P1}(17) = 1.2$, $p = 0.24$, $t_{N1}(17) = 3.3$, $p = 0.04$) (Figure 4).

A.



B.

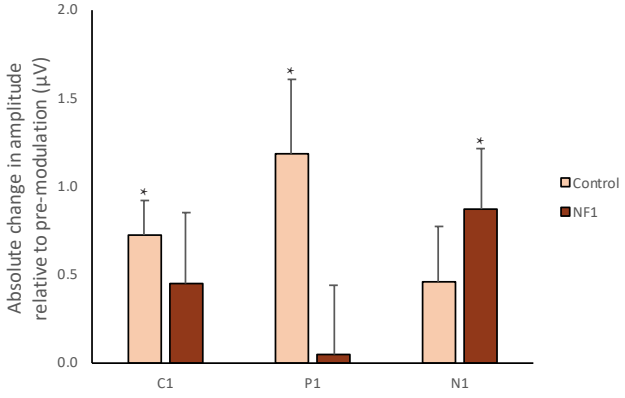


Figure 3. The VEP response in the control and the NF1 group. A. The mean VEP response pre-modulation (solid line) and post-modulation (dashed line) in the control group (left) and NF1 group (right) ± SEM. B. Absolute change in amplitude ± SEM relative to pre-modulation for C1, P1, and N1 separately. C1 was significantly decreased and P1 was significantly increased in amplitude comparing post-modulation to pre-modulation in the control group ($t_{C1}(29) = 3.6$, $p = 0.001$, $d = 0.4$; $t_{P1}(29) = 2.9$, $p = 0.008$, $d = 0.2$). The peaks C1 and P1 were not significantly different in amplitude comparing pre-and post-modulation in the NF1 group, although the N1 was significantly decreased in amplitude post-modulation compared to pre-modulation in the NF1 group ($t_{N1}(19) = 1.1$, $p = 0.02$, $d = 0.2$).

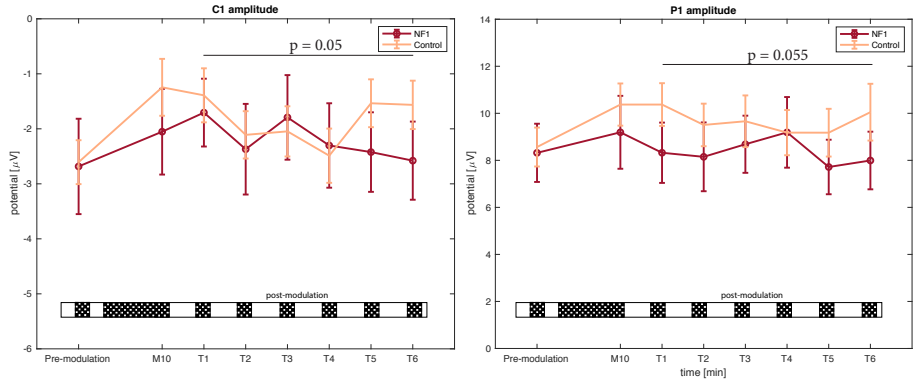


Figure 4. VEP plasticity of the C1 and P1 peak amplitude in the control and NF1 group. Mean amplitude of C1 (left) and P1 (right) ± SEM per group per VEP measurement. Pre-modulation: mean VEP amplitudes of B1 and B2. M10: mean VEP amplitudes during checkerboard reversals given in the 10th minute of continuous stimulation of 10 min. T1-T6 (post-modulation): checkerboard reversals post-modulation with each 20 seconds given at 2 (T1), 7 (T2), 12 (T3), 17 (T4), 22 (T5), and 27 (T6) minutes after the end of the modulation. A simplified schematic scheme of the VEP measurements is presented above the x-axis.

Correlations

There were no significant correlations between the change in VEP amplitude (post-minus pre-modulation) with age (r_{C1} = 0.03, p = 0.09; r_{P1} = -0.02, p = 0.9; r_{N1} = 0.2, p = 0.09) and education level (r_{C1} = -0.05, p = 0.7; r_{P1} = 0.005, p = 0.9; r_{N1} = 0.09, p = 0.5).

DISCUSSION

VEP plasticity offers a non-invasive metric to quantify cortical plasticity in the visual cortex. Our findings showed that VEPs were potentiated in control subjects in response to a 10-min block of visual stimulation. C1 and P1 amplitudes of the VEP between post-modulation and pre-modulation were significantly decreased and increased, respectively, in control subjects. In contrast, these amplitudes were not potentiated in response to modulation in the NF1 group, which might suggest deficits in visual cortical plasticity in adults with NF1.

In contrast to our expectations, the latencies and amplitudes of the VEP components pre-modulation were not significantly different between groups. Previous studies of VEP characteristics in patients with NF1 showed a delayed latency and reduced amplitude of the P1 at baseline (20–24). In the present study, we did not find abnormalities in VEP components pre-modulation, which suggests a lack of abnormalities of the visual pathways in our NF1 sample. In contrast to previous studies, all subjects were without ocular pathology, optic gliomas or severe visual problems.

VEP plasticity in the control group

Our findings support the hypothesis that VEP plasticity could be used to identify visual cortical plasticity in humans. The underlying mechanism of VEP plasticity induced by a modulation block has been shown to resemble characteristics of synaptic plasticity. More specifically, VEP plasticity in mice was inhibited by manipulation of N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors (11). The activation of these receptors is important for induction of long-term potentiation at cortical synapses (27).

Our findings are in line with previous results of VEP plasticity in humans showing that prolonged modulation by exposure to a 10-min block of checkerboard reversals at a low frequency induces VEP plasticity in unaffected controls as indicated by the changes in peak amplitudes of the VEP (13,14,16,18,19). In these studies, C1 amplitude was significantly in the control group decreased and P1 amplitude increased between

post-modulation and pre-modulation. Notably, however, Elvsåshagen et al. (13) did not observe a change in the C1 component. In the present study, C1 and P1 amplitudes were also significantly decreased and increased, respectively, between post-modulation and pre-modulation in the control group.

In contrast to previous studies, we did not find a modulation of the N1 component in the control group (13,14,19). We did observe a decreased N1 amplitude in the control group during continuous visual stimulation, but this was in the opposite direction of N1 modulation observed in previous studies (13,14,19). In these studies, a modulation effect of N1 was shown as an increase of the N1 amplitude. The absence and opposite direction of N1modulation may be due to the high variability observed in the N1 latency and amplitude. In contrast to the latencies of the C1 and P1 components, the latency of the N1 component had a wide range of 150-200 ms post-stimulus (Figure 3A). Previous studies often used distinct protocols to identify the N1 component or it remained unmentioned. The largest cohort of control subjects (n = 415) involved in measuring VEP plasticity after 10 minutes of continuous visual stimulation was reported by Valstad et al. (16). They observed a strong modulation effect with decreased C1, and increased P1, N1 and N1b amplitudes 2-6 min after modulation. In the present study, we have not focused on the N1b component, but on the early VEP components in accordance with Elvsåshagen et al. and Normann et al. (13,14). Early VEP components might be less affected by attention or complex cognitive processes (14). Attention may especially explain the variation in N1 amplitude (28). Future studies should take the subject's attention carefully into account by implementation of an additional attention test (16,18).

Our observed duration of the potentiated VEP response in controls appeared to be shorter than some of previous studies. Normann et al. (14) showed changes in VEP amplitudes after continuous visual stimulation up to 20 min, although in some individual experiments the VEP amplitudes were potentiated up to 60 min. In the present study, the strongest modulation is observed during the 10-min modulation block and 2 min post-modulation. The latter is consistent with the findings of Valstad et al. (16). The duration of the VEP response could depend on a variety of factors, including high inter-subject

variability in the VEP response, degree of neural recruitment, and level of attention to the visual stimulus. Increasing the duration of post-modulation recording could have allowed for modulation effects later in time (i.e. >30 min), which is following the definition of LTP (14,29). Notably, we choose our methodology due to its feasibility in patients, prior demonstration of a robust modulation effect in large cohorts of unaffected controls and the lack of a VEP response in psychiatric patients (14,16).

To our knowledge, the present study is the first to examine VEP potentiation during the modulation block, which revealed a potentiated VEP response during continuous visual stimulation without delay. In animal studies, it has been shown that an increased response during LTP induction enhances the response after induction (30). But although VEP plasticity shows similarities to the properties of synaptic plasticity, it is unknown whether the potentiation during prolonged visual stimulation is dependent upon synaptic plasticity (31).

VEP plasticity in NF1

In contrast to control subjects, we observed no potentiation of the VEP response of the C1 and P1 components during continuous visual stimulation and post-modulation in adults with NF1. These findings support the theory of decreased synaptic plasticity found in animal models of NF1 (5–8). These studies describe that reduced NF1 activity in animal models of NF1 leads to an increase in gamma-aminobutyric acid (GABA) neurotransmission, which causes a decrease in glutamatergic synaptic plasticity. In support of this theory, a previous study in adults with NF1 showed alterations in motor cortical excitability and plasticity upon a form of repetitive transcranial magnetic stimulation (32). This is in line with the present study, which together indicates that adults with NF1 may have reduced visual cortical plasticity, as indicated by their non-potentiated response following VEP induction.

Interestingly, the N1 amplitude in our NF1 group was significantly decreased during modulation, and between post-modulation and pre-modulation. However, the N1 modulation effect was in the opposing direction as reported in previous studies, absent in our control group, and showed high inter-subject variability in latency and amplitude,

which makes the interpretation of the difference more difficult. Increasing the number of electrodes to record the VEP could decrease variability. Future studies of NF1 patients should be performed to further characterize the modulation effect of N1.

An important limitation of the study is that our NF1 sample may not be representative. In our NF1 sample, there may have been a participation bias towards highly motivated or less cognitively affected patients. The differences in VEP potentiation between NF1 and controls may have been larger in a more severe cognitively affected NF1 sample. Furthermore, it has been mentioned that VEP components are influenced by attention, although early components might be less affected (14). Attention in NF1 patients may be reduced due to fatigue. Increased fatigue has been associated with NF1 and has been shown to affect the daily life of adults with NF1 (33). However, we did not find any difference in fatigue based on a sleepiness scale at the start or end of the VEP recordings. Nevertheless, although we did not find a significant modulation effect in C1 or P1 amplitudes of the VEP in the NF1 group, the results should be considered cautiously due to the relatively small sample size of the NF1 group, and the small effect sizes. The strengths of the study were that the NF1 patients were not receiving mental health care and were not using psychoactive medication. Additionally, the control and NF1 groups were similar in age and sex, and the experiment was standardized to the time of day. Hence, these factors could not explain the differences in potentiation between the NF1 and control groups.

In conclusion, we showed that VEP plasticity can be measured in response to prolonged stimulation of low frequency checkerboard reversals. The non-potentiated response upon VEP modulation in patients with NF1 may indicate deficits in visual cortical plasticity. Due to the small NF1 sample, small effect sizes, and transient potentiation in controls, the results should be considered with caution. Future studies should investigate VEP plasticity more extensively by including a longer period of post-modulation and studying late VEP components in a larger group of patients in which attentional measures are considered. The present study contributes to an improved assessment of the feasibility for using neurophysiological outcome measures in intervention studies of cognitive deficits among patients with NF1.

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Chapter 5.1

Motor cortical plasticity in adults with neurofibromatosis type 1

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Clinical Neurophysiology, 2020

ABSTRACT

Neurofibromatosis type 1 (NF1) is an autosomal dominant genetic disorder that is associated with cognitive disabilities. Based on studies involving animals, the hypothesized cause of these disabilities results from increased activity of inhibitory interneurons that decreases synaptic plasticity. We obtained transcranial magnetic stimulation (TMS)-based measures of cortical inhibition, excitability and plasticity in individuals with NF1.

We included 32 NF1 adults and 32 neurotypical controls. Cortical inhibition was measured with short-interval intracortical inhibition (SICI) and cortical silent period (CSP). Excitability and plasticity were studied with intermittent theta burst stimulation (iTBS).

The SICI and CSP response did not differ between NF1 adults and controls. The response upon iTBS induction was significantly increased in controls (70%) and in NF1 adults (83%). This potentiation lasted longer in controls than in individuals with NF1. Overall, the TMS response was significantly lower in NF1 patients ($F(1,41) = 7.552$, $p = 0.009$).

Individuals with NF1 may have reduced excitability and plasticity, as indicated by their lower TMS response and attenuation of the initial potentiated response upon iTBS induction. However, our findings did not provide evidence for increased inhibition in NF1 patients. These findings have potential utility as neurophysiological outcome measures for intervention studies to treat cognitive deficits associated with NF1.

INTRODUCTION

Neurofibromatosis type 1 (NF1) is an autosomal dominant genetic disorder with a birth incidence of approximately 1:2000 (1). It is caused by a loss-of-function mutation of the NF1 gene, which encodes the protein neurofibromin. NF1 is clinically characterized by a diversity of brain and somatic symptoms (2). Many individuals with NF1 suffer from cognitive deficits which adversely impacts their quality of life (3–5). These deficits include attention, visual-spatial abilities, motor learning, executive functioning, and intelligence (4–6). Loss-of-function of neurofibromin is well established to result in hyperactivity of the RAS signaling pathway. However, despite several clinical trials aimed at improving cognitive deficits in NF1 through RAS reducing treatments, no effective treatment has yet been established (7–9).

Studies of the cellular mechanism underlying the cognitive deficits associated with NF1 have largely focused on animal models of NF1 (10–12). Based on the animal studies, reduced NF1 activity has been shown to result in abnormal hyperactivation of RAS signaling in inhibitory interneurons (10–12). RAS hyperactivation leads to enhanced inhibition through abnormally high gamma-aminobutyric acid (GABA) neurotransmission, thereby causing a reduction of glutamatergic synaptic plasticity (10–13). Furthermore, Omrani et al. (11) identified a neurofibromin-interacting protein, hyperpolarization-activated cyclic nucleotide-gated channel (HCN1), that underlies the enhanced inhibitory neurotransmission. An agonist of the HCN1 channel, lamotrigine, could rescue deficits in inhibition and plasticity in animal models of NF1 (11).

For implementation of human NF1 translational studies investigating the mechanistic findings from animals, several approaches have been used. Studies using magnetic resonance spectroscopy showed that the visual cortex of NF1 patients had reduced GABA levels (14,15). The cause of the reduced GABA levels in the cortex may be a compensatory mechanism for the increased inhibitory function of interneurons. This increase could limit GABA neurotransmission by downregulating GABA synthesizing enzymes (16), but further studies are required to investigate this potential mechanism in humans. More recently, transcranial magnetic stimulation (TMS) paradigms, that were

developed to perform non-invasive measurements of cortical inhibition and plasticity (17,18), were used in human NF1 studies (19,20). TMS is a tool to assess cortical excitability in the motor cortex via single pulse stimulations as well as the modulation of cortical excitability via TMS paradigms (21). The evaluation of cortical excitability in response to single pulse stimulations has not yet been described in NF1 patients. In two human NF1 studies, the TMS paradigm short-interval intracortical inhibition (SICI) was used in a small group of 9-11 NF1 patients (19,20). One study showed a trend towards more cortical inhibition in NF1 patients compared to neurotypical controls (19). Furthermore, reduced task-related intracortical inhibition was observed during motor learning in NF1 patients (20). Additionally, reduced cortical plasticity was shown in the motor cortex of NF1 patients using the paired associative stimulation (PAS) repetitive TMS paradigm (19).

To investigate cortical inhibition and plasticity in NF1 patients, we made use of 3 TMS paradigms: the aforementioned SICI, the cortical silent period (CSP) and the intermittent theta burst stimulation (iTBS) paradigms. The first two paradigms, SICI and CSP, are robust for investigation of motor cortical inhibition and have frequently been used in studying the pathophysiology of various psychiatric disorders (22,23). They are also sensitive to changes in GABA-mediated inhibition, as $GABA_A$ and $GABA_B$ receptor agonists increase the response on the SICI and the CSP paradigms, respectively (24,25). In addition, a pharmacological study using a GABA reuptake inhibitor confirmed the role of $GABA_B$ receptors in CSP modulation (26). The third paradigm, iTBS, is a TMS paradigm that makes use of high-frequency stimulation of the motor cortex to induce cortical plasticity, which can be measured as an increased excitability of the motor cortex. Interestingly, the iTBS stimulation paradigm highly resembles the long-term potentiation (LTP) plasticity protocols that have been used to study ex vivo plasticity in Nf1 mouse models (11,12,27). Additionally, similar to mouse studies, the after-effects of iTBS in the human motor cortex seem to depend on N-methyl-D-aspartate (NMDA) receptors (28). Moreover, iTBS is reported to have robust efficacy with advantages over the aforementioned PAS paradigm as it requires a lower stimulation intensity and has a shorter time of stimulation (29).

Notably, recent studies have also pointed out the high inter-subject variability in response to TMS paradigms (30,31). According to these studies, the response to TMS seems to depend on a variety of confounding factors including age, sex, time of day, and sleepiness (30,32). Hence, for this study, we carefully took these potential confounders into account. Additionally, we assessed motor cortical excitability prior and during the TMS paradigms in response to single pulse stimulations. We hypothesized to observe a more pronounced inhibition and reduced cortical plasticity in NF1 adults compared to neurotypical controls.

METHODS & MATERIALS

Subjects

In this study, 32 NF1 patients and 32 controls between 18-56 years participated. According to the inclusion and exclusion criteria, the subjects had no current or history of medical, psychiatric, or neurological disorders and were medication-free (excluding contraceptives) at the time of the study. Subjects were right-handed according to the Edinburgh Handedness Inventory (33) and met the criteria of the safety screening questionnaire for undergoing a TMS-measurement (34,35). NF1 patients had a genetic or clinical diagnosis and were recruited from the ENCORE-NF1 expertise center for Neurodevelopmental Disorders at the Erasmus MC or through the Dutch NF patient association (NFVN). Controls matched for age and gender were unaffected unrelated peers of the patients or recruited through online advertisements. The Dutch Central Medical Ethics Committee of the Erasmus Medical Center Rotterdam approved the study, which was conducted in accordance with the Declaration of Helsinki (2013). All subjects gave their written informed consent.

Procedures

All subjects visited the lab at noon and were asked to abstain from alcohol and caffeinated beverages 24 hours before the start of the measurements. Before and during the measurements, subjects were seated in a comfortable chair with their eyes open and arms at rest. Motor evoked potentials (MEPs) were recorded from the left First Dorsal Interosseous (FDI) muscle at rest by surface electromyography (EMG), using

silver/silver chloride electrodes in belly-tendon recording technique. Data was amplified using a universal amplifier (ANT Neuro, Enschede, The Netherlands) and filtered with a band-pass (20-2000 Hz) and a 50 Hz notch filter. The TMS set up consisted of an eight-shaped stimulation coil (MC-B70, MagVenture, Denmark) connected to a MagPro TMS stimulator (MagPro X100 with MagOption; MagVenture, Denmark). The MagPro TMS stimulator delivers pulses in a monophasic current waveform with a posterior-anterior current direction. The coil was placed on the scalp over the right primary motor cortex with its handle in a posterolateral direction at an angle of 45° from the midline. Optimal positioning of the coil (the hotspot) was established by randomly placing TMS stimulations around the reference point of the FDI. This reference point was 10% lateral to Cz over the right hemisphere at the level of the ears. The coil was held at the hotspot using a 3D neuronavigation (Visor2XT) to elicit MEPs of maximum amplitude in the FDI. The stimulation intensity that elicited MEPs with a mean and median between 800-1200 μV ±SD<1/2 of the mean (SI_{1mV}) was determined by increasing stimulus intensity with 1% of maximum stimulator output (MSO) per 10 consecutive trials starting from the resting motor threshold (RMT) (19,31). RMT was defined as the stimulus intensity in percentage of MSO that elicited MEPs of > 50 μV with a 50% probability, using a maximum likelihood threshold-hunting procedure (36). The RMT measurement was repeated at 3-time points to control for changes over time (Figure 1). Sleepiness was also measured at these time points with the Karolinska sleepiness scale (KSS), a self-report questionnaire on a nine-point Likert scale (37) (Figure 1). We studied the MEP modulation as result of the TMS paradigms SICI, CSP, and iTBS. After the measurements, the verbal and performance IQ of the subjects was estimated using four subtests of the Wechsler Adult Intelligence Scale (WAIS-IV-NL; Wechsler, 2012); vocabulary, similarities, block design and matrix reasoning. Additionally, educational attainment was coded following the 7-point coding scale of Verhage (1964) (38), taken from Hendriks et al. (39).

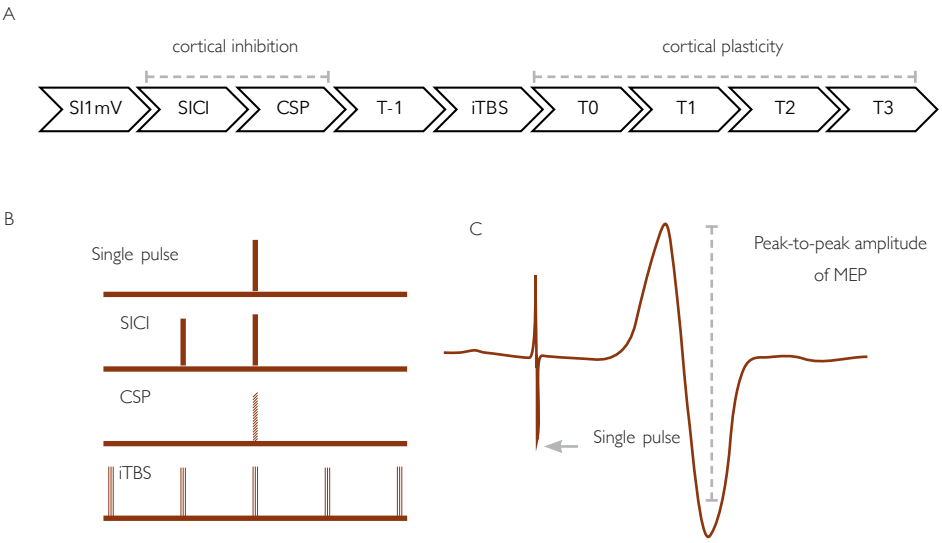

Figure 1. Schematic overview of transcranial magnetic stimulation (TMS) measurements. A. Procedure of TMS measurements for cortical inhibition and cortical plasticity. SI1mV, the procedure to establish the stimulation intensity that elicited motor evoked potentials (MEPs) with a mean between 800–1200 μV. SICI, short interval cortical inhibition, 30 pulses; iTBS, intermittent theta burst stimulation, 600 pulses; CSP, cortical silent period, 10 pulses; T-1, 20 single-pulses at SI1mV recorded directly before iTBS. T0-T3, 20 single-pulses at SI1mV recorded four times within 30 minutes after stimulation at T0, T1, T2, and T3: 0, 10, 20 and 30 minutes after stimulation. RMT, resting motor threshold. KSS1-3, Karolinska sleepiness scale. B. Example trace of the data of a single-pulse at SI1mV during hand at rest. C. Schematic presentation of the TMS pulses per paradigm. TP, single test pulse at SI1mV. SICI, paired-pulse consisting of a subthreshold conditioning pulse followed by an unconditioned TP at SI1mV after an interstimulus interval of 3 ms; CSP, a single pulse at 120% of RMT; TBS consists of bursts of 3 stimuli at 50 Hz, which are repeated at 5 Hz (shown here). The iTBS paradigm repeats a 2-sec train of TBS every 10 sec for a total of 190 sec (i.e. 600 pulses) with a stimulus intensity of 70% of resting motor threshold (RMT). Black bars represent single stimulations at SI1mV; Grey bars represent stimulations with a stimulation intensity of a specific percentage of RMT (SICI: 60% or 80%, CSP: 120%, iTBS: 80%).

TMS measurements

Short interval cortical inhibition

SICI is a paired-pulse TMS paradigm in which a subthreshold conditioning pulse (CP) is followed by a test pulse (TP) at SI_{1mV} after an interstimulus interval of <6 ms (17). The standard paradigm for SICI uses a CP of 80% of RMT and an interstimulus interval of 3ms. We added a 60% of RMT CP condition to avoid a potential floor effect in NF1 patients (19). We performed 10 paired stimulations in both the 60% CP and the 80% CP condition, as well as 10 single stimulations at the SI_{1mV} in random order. Cortical inhibition was estimated as the difference in amplitude between paired and single MEPs.

Cortical silent period

CSP is the duration of interruption of EMG activity following a single suprathreshold TMS pulse. The FDI was tonically contracted with 20% of maximum voluntary strength using a hand-held pinch gauge (B&L Engineering; Santa Ana, CA, USA). We recorded 10 single pulses at 120% of RMT with an inter-stimulus interval of 6 seconds (40).

Intermittent theta burst stimulation

TBS consists of bursts of 3 stimuli at 50 Hz, which are repeated at 5 Hz. The iTBS paradigm repeats a 2-sec train of TBS every 10 sec for a total of 190 sec (i.e. 600 pulses). We used a stimulus intensity of 70% RMT instead of the 80% active motor threshold (AMT) described in the original iTBS protocol (18) to avoid muscle contraction prior to iTBS. These contractions prior to iTBS might influence the direction of the TBS-aftereffects (41,42). The stimulus intensity seems to be similar for the two different methods (43). Changes in cortical plasticity are assumed to be reflected in a change in MEP size after iTBS induction. We recorded 20 single pulses at SI_{1mV} directly before iTBS and four times within 30 minutes after stimulation at a 10 minute interval (Figure 1) (18). Additionally, in accordance with previous studies that pointed out the high inter-subject variability in response to iTBS independent of genotype (31,42,44,45), we classified responders to iTBS using a cut-off of a minimal increase of 10% in MEP amplitude after stimulation at T0, T1, T2 or T3 (44,46,47).

Statistical analysis

EMG epochs were cut offline from the continuously recorded EMG data of 100 ms before and after the TMS pulse. These epochs were analyzed with Signal version 5.08 (CED Ltd., UK) and screened automatically and visually for technical artifacts and excessive background EMG activity and were discarded if there was activity with a >70μV peak-to-peak amplitude within 50 ms pre-trigger (48,49). If more than 50% of the responses at one-time point within an individual needed to be discarded, all the data at that time point were excluded from the analysis to avoid unreliable measurements (50). Peak-to-peak MEP amplitude following the TMS-trigger was measured within each trial and subjected to a square-root transformation due to the positive skewness of the raw MEPs (51,52). The duration of the CSPs was analyzed using MATLAB (2019), (version 9.6.0 (R2019a), Natick, Massachusetts: The MathWorks Inc.). CSP duration was defined as the time from the single TMS pulse onset to the time of reappearance of voluntary sustained EMG activity. Statistical analyses were performed using the transformed MEPs in IBM Statistics SPSS (version 25).

Similarity of patient and control groups regarding the confounding variables age, gender and sleepiness was established with a Chi-squared test, independent t-test or non-parametrically with Mann-Whitney U test. Relationships between confounding factors that differed between groups and the main outcomes (absolute MEP size during iTBS and SICI, and CSP duration) were evaluated using Pearson correlation coefficients, and p-values were corrected for multiple testing with the Bonferroni correction. The difference in CSP durations between groups was evaluated with an independent t-test. A repeated measures ANOVA was used to compare mean MEP amplitudes during SICI between groups, between the different conditions of single and paired stimulations (60% and 80% of RMT), and the interaction effect of group and condition. In addition, a repeated measures ANOVA was performed to compare MEP amplitudes between NF1 patients and controls, time points before and after iTBS (T0, T1, T2, T3), and the interaction between group and time. Degrees of freedom were corrected using Greenhouse-Geiser estimates of sphericity. The difference in the number of responders and non-responders upon iTBS was tested with a Chi-squared test. If there was no difference between groups in the number of responders, we performed a subgroup-

analysis using a similar repeated measures ANOVA as for the whole group analyses. Furthermore, a secondary analysis in the subgroup included within-group analyses to clarify the effect of iTBS over time within each responder subgroup by means of t-tests using the uniformly powerful Holm-Bonferroni correction (53).

RESULTS

In total, 155 eligible subjects were invited of which 91 subjects declined participation. We measured 64 participants ($n_{control}$ = 32 n_{NF1} = 32). After the measurement, some participants were excluded due to either no observations of MEPs above >50μV despite the use of a high stimulus intensity ($n_{control}$ = 2 n_{NF1} = 2); artifacts and high background EMG-activity during SICI and iTBS (n_{NF1} = 2); technical problems during SICI measurements ($n_{control}$ = 1); or significant outliers (>3 standard deviations from the mean) in CSP measurements ($n_{control}$ = 1) (Figure 2). Age and gender were not different between the groups ($t_{age}(57)$ = 1.08, p = 0.28; χ^2_{gender} = 0.167, p = 0.68). However, as expected, educational attainment and IQ scores were significantly lower in the NF1 group than in the control group ($U_{Verhage}$ = 237, p = 0.001; $t_{VIQ}(59)$ = 3.66, p = 0.001, $t_{PIQ}(60)$ = 2.42, p = 0.018) (Table 1).

During the measurements, the overall sleepiness score was low (i.e. subjects were alert) and did not differ between the groups ($median_{control}$ = 3.5, IQR = 1.4, $median_{NF1}$ = 3.7, IQR = 2.0, U = 367.5, p = 0.55). The RMT was not different between the groups ($t_{RMT}(57)$ = 0.927, p = 0.36) and did not change over time (F(2) = 0.236, p = 0.79). Also, the SI_{1mV} ($M_{control}$ = 56±10; M_{NF1} = 55±15) was similar between patients and controls ($t_{SI1mV}(57)$ = 0.417, p = 0.68) (Table 1). Although the mean amplitude of MEPs at SI_{1mV} was between 800-1200 μV in both the control group and the NF1 group ($M_{control}$ = 1062±304; M_{NF1} = 886±270), it was significantly smaller in the NF1 group than in the control group prior to the start of the paradigms (t(57) = 2.32, p = 0.024) (Table 1).

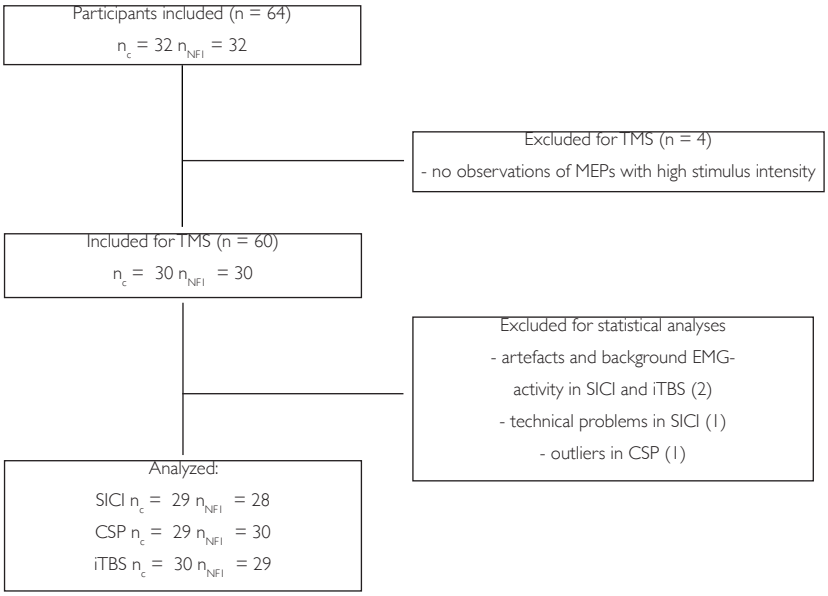

Figure 2. Flow-chart of inclusions. c, control; NF1, Neurofibromatosis type 1; TMS, transcranial magnetic stimulation; MEP, motor evoked potential; EMG, electromyography; SICI, short interval cortical inhibition; iTBS, intermittent theta burst stimulation; CSP, cortical silent period.

Table 1. Demographics, estimated intelligence quotient (IQ) and variables during transcranial magnetic stimulation (TMS) (Mean ± SD) of the neurofibromatosis type 1 (NF1) group and the control group separately.

	NF1 group (n = 30)	Control group (n = 30)
Demographics		
age in years	31.24 ± 12.3	34.52 ± 10.8
gender: male in % (#)	41 (12)	47 (14)
Educational attainment & estimated IQ		
Educational attainment (median, range)*	5.0, 1-7	6.0, 4-7
Verbal IQ*	85 ± 16.6	99 ± 12.9
Performance IQ*	87 ± 15.3	98 ± 19.6
Sleepiness (Median, range)		
Total KSS	3.7, 1-6	3.5, 1-7
KSS1	3.0, 1-6	3.0, 1-7
KSS2	4.0, 1-8	4.0, 1-7
KSS3	4.0, 1-7	3.0, 1-7
During TMS measurements		
RMT %MSO	46.0 ± 10.9	48.4 ± 8.6
SI1mV %MSO	55.2 ± 15.1	56.7 ± 10.8
Mean amplitude of MEPs at SI1mV *	886.7 ± 270.2	1062.5 ± 304.4
Maximal force (Median, range)*	4.0, 2-9	5.0, 3-9

#, number of subjects; IQ, intelligence quotient; KSS1-3, Karolinska sleepiness scale at time points 1-3; TMS, transcranial magnetic stimulation; RMT, Resting Motor Threshold; SI1mV, Stimulus Intensity at 1 mV; MSO, Maximum Stimulator Output; NF1, neurofibromatosis type 1.
* Significantly different between patients and controls (p-value <.05)

Cortical Inhibition

During the SICI paradigm, the mean MEP size of single pulse stimulations ($M_{control}$ = 798±425; M_{NF1} = 625±315) was not different between groups ($t(55) = 1.59$, $p = 0.12$) (Figure 3). There was a significant main effect of SICI condition, indicating that the paired stimulations (60% and 80% of RMT) sufficiently inhibited the MEPs in both groups ($F(2, 110) = 49.72$, $p<0.001$, $\eta^2 = 0.47$) (Figure 3), although there was no significant difference between the paired stimulations of 60% and 80% of RMT. A significant overall group

difference was found in mean MEP amplitudes ($F(1, 55) = 4.075$, $p = 0.048$, $\eta^2 = 0.07$): NF1 patients showed overall lower mean MEP amplitudes than controls, but there was no significant interaction effect between group and the conditions.

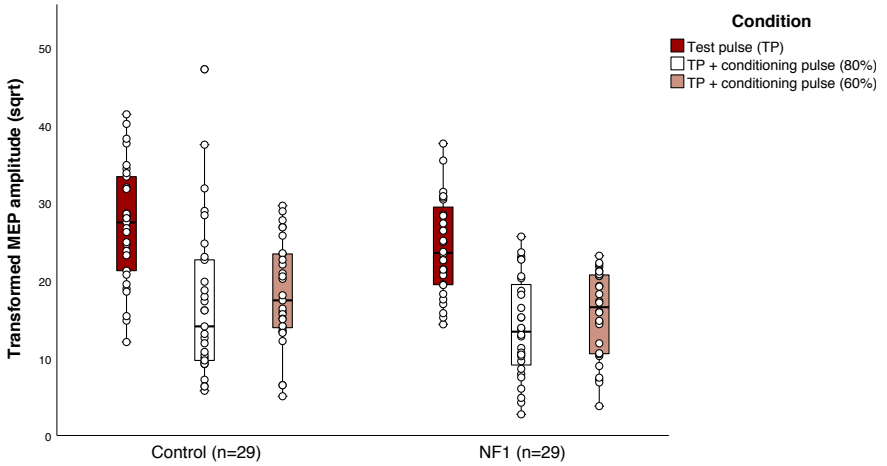

Figure 3. Response to the short interval cortical inhibition (SICI) paradigm. Boxplots of square-root (sqrt) transformed mean motor evoked potential (MEP) amplitudes per subject in response to the SICI, for both groups separately. Mean MEP amplitudes in response to the test pulse (TP) + conditioning pulse with a stimulus intensity of 60% or 80% of resting motor threshold (RMT) did not differ between the neurofibromatosis type 1 (NF1) group and the control group. Overall, a significant group difference was found in mean MEP amplitudes ($F(1, 55) = 4.075$, $p = 0.048$).

The mean CSP duration, i.e. the time from the single TMS pulse onset to the time of reappearance of voluntary EMG activity (Figure 4), was not significantly different between NF1 patients and controls ($M_{control} = 131 \pm 29$; $M_{NF1} = 124 \pm 31$) ($t(57) = 0.87$, $p = 0.39$, $d = -0.23$) (Figure 4). There was a significant difference in maximal force ($median_{control} = 5.0$, IQR = 2.0, $median_{NF1} = 4.0$, IQR = 2.0, $U = 262$, $p = 0.008$) (Table 1), but there was no significant correlation between CSP duration and maximal force ($r = -0.054$, $p = 0.69$).

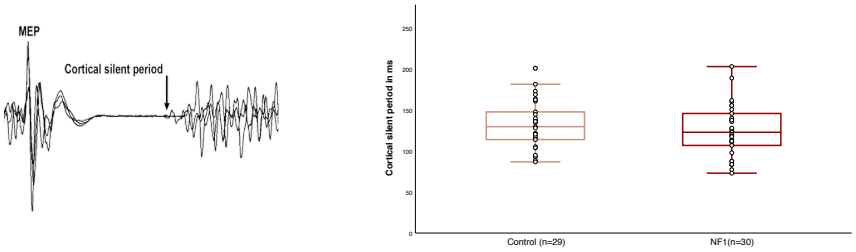

Figure 4. Response to the cortical silent period (CSP) paradigm. Left. Example trace of the data of a single CSP pulse with visual computation of the CSP. Right. Boxplot of individual means of CSP duration for the control group and the neurofibromatosis type 1 (NF1) group. There were no significant differences in mean CSP duration between the groups ($t(57) = 0.87$, $p = 0.39$).

Cortical plasticity

Whole group analysis

At baseline, MEPs in response to single pulse TMS before iTBS induction were not different between the groups (Table 2). There was a significant main effect of group: overall, MEPs were significantly lower in NF1 patients than in controls ($F(1,54) = 9.68$, $p = 0.003$, $\eta^2 = 0.15$). There was no significant main effect of time ($F(3.49, 188.77) = 1.75$, $p = 0.19$, $\eta^2 = 0.03$) and no significant interaction effect between group and time.

Table 2. Whole group analysis of cortical plasticity. Square-root transformed mean motor evoked potentials (MEPs) in response to single pulses directly before intermittent theta burst stimulation (iTBS) (T-1) and four times (T0-T3) within 30 minutes after stimulation (Mean ± SD), for all subjects of both groups.

	T-1[2]	T0	T1	T2	T3
NF1[1] (n = 30)	23.8 ± 7.4	25.9 ± 6.7	23.6 ± 7.5	22.9 ± 7.5	22.1 ± 8.6
Control[1] (n = 30)	27.9 ± 8.4	29.2 ± 7.3	28.6 ± 7.7	27.6 ± 8.1	27.9 ± 7.5

MEP, motor evoked potential; iTBS, intermittent theta burst stimulation; NF1, neurofibromatosis type 1; T-1, 20 single-pulses at stimulus intensity of 1 mV (SI1mV) recorded directly before iTBS; T0-T3, 20 single-pulses at SI1mV recorded four times within 30 minutes after stimulation at T0, T1, T2, and T3: 0, 10, 20 and 30 minutes after stimulation.

[1] Significant main effect of group $F(1,54) = 9.68$, $p = 0.003$.

[2] No significant main effect of time $F(3.49, 188.77) = 1.75$, $p = 0.19$.

Responder group analysis

We performed an explorative subgroup-analysis on the responders to assess whether there were differences in excitability and plasticity between responder NF1 patients and responder controls. Therefore, participants were classified as responders if an increase of 10% in MEP size at any given time point after iTBS was observed. Importantly, there was no difference in the number of responders who showed a significant increase in motor cortical excitability at T0, T1, T2 or T3 after iTBS, being 21 out of 30 controls (70%) and 24 out of 29 NF1 patients (83%) ($\chi^2(1) = 1.326$, $p = 0.25$). There was a significant main effect of group ($F(1,41) = 7.552$, $p = 0.009$, $\eta^2 = 0.16$): MEPs were significantly lower in the responder NF1 patients than in the responder controls. There was also a significant main effect of time ($F(3,123.1) = 3.73$, $p = 0.013$, $\eta^2 = 0.08$). There was no significant interaction effect between time and group ($F(3, 123.1) = 0.91$, $p = 0.43$) (Figure 5).

Within-group analyses in controls showed that the increased MEP amplitude following iTBS was significantly higher than baseline for all time points ($p_{T0} = 0.001$, $p_{T1} = 0.025$, $p_{T2} = 0.012$, $p_{T3} = 0.049$) (Table 2). In contrast, within-group analysis in NF1 patients showed that the increased MEP amplitude following iTBS was only significantly higher than baseline for T0 ($p_{T0} = 0.003$, $p_{T1} = 0.217$, $p_{T2} = 0.695$, $p_{T3} = 0.942$), suggesting that the increased MEP amplitude lasted longer in the responder controls than in the responder NF1 patients (Figure 5).

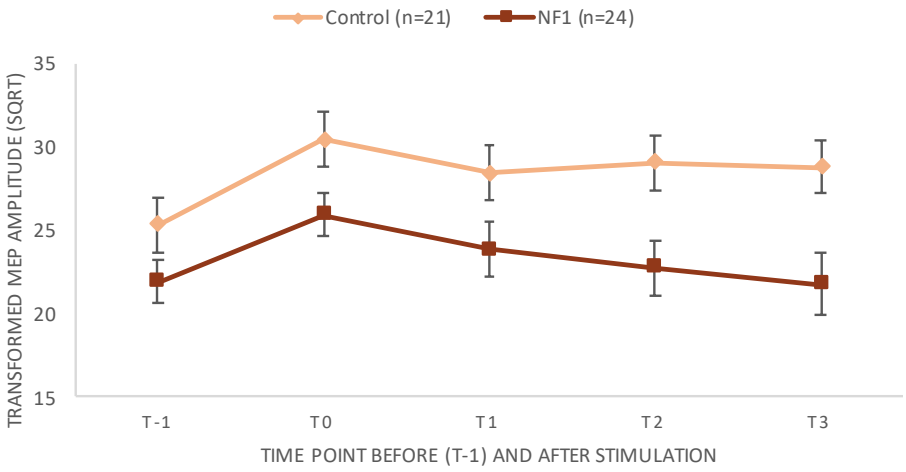

Figure 5. Responder group analysis of cortical plasticity. Transformed (sqrt, square root) mean motor evoked potentials (MEP) amplitudes ± SEM of the responders to intermittent theta burst stimulation (iTBS). T-1: mean MEP in response to single pulses directly before iTBS. T0-T3: mean MEP in response to single pulses four times within 30 minutes after stimulation: 0, 10, 20 and 30 minutes after stimulation. There was a significant main effect of group ($F(1,41) = 7.552$, $p = 0.009$) and a significant main effect of time ($F(3,123.1) = 3.73$, $p = 0.013$). Controls showed significantly increased MEP amplitude following iTBS for all time points ($p_{T0} = 0.001$, $p_{T1} = 0.025$, $p_{T2} = 0.012$, $p_{T3} = 0.049$); neurofibromatosis type 1 (NF1) patients only showed a significantly increased MEP amplitude following iTBS at T0 ($pT0 = 0.003$, $p_{T1} = 0.217$, $p_{T2} = 0.695$, $p_{T3} = 0.942$).

Correlations

There were no significant correlations between any variables of the main outcomes, and between confounders and the main outcomes. Only the statistics of the most relevant correlations are presented here. There were no significant correlations between the absolute MEPs size of inhibited MEPs measured with $SICI_{80\%}$ and the MEP size post-iTBS ($r_{T0} = 0.21$, $p = 0.12$). There were also no significant correlations between the duration of CSP and the MEPs inhibited by SICI ($r = -0.07$, $p = 0.61$), or the MEPs induced by iTBS ($r_{T0} = 0.13$, $p = 0.35$). We also did not find significant correlations between IQ and the MEP amplitudes during the $SICI_{80\%}$ ($r_{SICI\text{-}VIQ} = 0.03$, $p = 0.81$, $r_{SICI\text{-}PIQ} = -0.17$, $p = 0.20$), during iTBS time points ($r_{T0\text{-}VIQ} = 0.03$, $p = 0.82$, $r_{T0\text{-}PIQ} = -0.11$, $p = 0.42$), or the CSP duration ($r_{CSP\text{-}VIQ} = 0.11$, $p = 0.43$, $r_{CSP\text{-}PIQ} = 0.11$, $p = 0.41$).

DISCUSSION

Using mouse models of NF1, it has been shown that decreased NF1 function causes increased inhibition and consequently decreased synaptic plasticity (11,12). Whether changes in neuronal plasticity are also underlying the cognitive deficits in NF1 patients is unknown. We obtained TMS-based measures of inhibition, excitability and plasticity in the human primary motor cortex in controls and NF1 patients. We hypothesized that we would observe reduced plasticity using iTBS, as well as changes in the inhibitory measures SICI and CSP. Although we indeed observed an attenuation of the initial potentiated MEPs upon iTBS induction in the subgroup-analysis, the SICI and CSP paradigms did not provide evidence for increased inhibition. Moreover, individuals with NF1 may have reduced excitability, as indicated by their overall lower MEP amplitudes.

The lack of an effect in the SICI paradigm is in contrast to previous small studies, measuring 9-11 individuals with NF1, which demonstrated a stronger inhibitory response to SICI in the motor cortex and reduced task-related inhibition in patients compared to controls (19,20). Although magnetic resonance spectroscopy studies showed evidence for increased inhibitory function of interneurons in the visual cortex (14,15), less is known about cortical inhibition in the primary motor cortex. We did find a significant overall group difference in mean MEP amplitudes during the SICI procedure, which could be explained by an overall reduction of MEP amplitudes in NF1 individuals compared to neurotypical controls.

Although the test pulses during the SICI were not significantly different between the groups, the mean amplitude of MEPs at SI_{1mV} prior to the start of the paradigms were lower in NF1 patients than in controls. We used a margin of 800-1200 μV for the mean amplitude of MEPs at SI_{1mV} consistent with previous research (19,31). However, we observed in some NF1 patients no increase in the mean MEP-size after repeated attempts with increasing stimulus intensity, which was less frequently observed in controls. Interestingly, lower MEP sizes in NF1 patients could also reflect reduced neuronal excitation and/or deficits in the balance of excitation and inhibition in the primary motor cortex (54,55). Future TMS research should investigate more extensively

whether NF1 patients indeed respond less to single-pulse TMS, which could indicate deficits in the balance of excitation and inhibition.

Additionally, reduced MEP sizes in individuals with NF1 could potentially mask a SICI inhibitory effect. It has been shown that the SICI effect can be smaller at a lower stimulus intensity of the conditioning pulse (19,46). Therefore, we expected reduced inhibition by reducing the stimulus intensity of the conditioning pulse from 80% to 60% of RMT in order to detect differences between NF1 patients and controls. However, in both groups, this reduction in stimulus intensity did not affect the level of SICI inhibition in contrast to a previous study (46). It could be that the stimulus intensity of the conditioning pulse should be reduced even more to avoid a potential floor effect. However, previous studies did not find a significant difference in the SICI effect using a stimulus intensity lower than 60% of RMT between NF1 patients and controls (19,46). Furthermore, the control group showed also no differences in inhibition between the 80% and 60% of RMT conditions, while they showed a trend towards higher MEP amplitudes than NF1 patients. This suggests that reduced MEPs sizes in individuals with NF1 could not fully explain the lack of a SICI inhibitory effect. Furthermore, repeated attempts to achieve SI_{1mV} could have been tiresome, which could have affected the MEP-size in NF1 patients (56). However, sleepiness measured with the KSS was not different in both groups during the experiment. Additionally, stimulus intensities were similar in both groups and a significant difference in mean MEP amplitudes at SI_{1mV} was not present at the baseline-values during the paradigms.

To our knowledge, this is the first study that used the CSP and iTBS paradigms to quantify plasticity and inhibition in NF1 adults. Contrary to our expectations based on animal findings and findings in other cortical areas, we found no evidence for a change in CSP duration in patients with NF1 in the motor cortex. The CSP paradigm has been proposed as a suitable paradigm to study the pathophysiology of various psychiatric disorders related to inhibitory GABAergic dysfunction. Previous magnetic resonance spectroscopy studies in NF1 patients indicated changes in the functioning of $GABA_A$ and $GABA_B$ receptors in the visual cortex (14,15) to compensate for the presumed increase of inhibitory function of interneurons as observed in NF1 mice. This theory

of increased cortical inhibition is not strongly supported for the primary motor cortex by the present study. Consistent with previous findings (57), maximal voluntary muscle force was reduced in NF1 patients. However, this does not appear to have affected our results, as there was no significant correlation between CSP and muscle force. A recent study observed a significant decrease in CSP duration with a high tonic contraction of more than 60% of maximal force (58). However, in the present study we used a tonic contraction of 20% of maximal force to avoid fatigue of the muscle, and we consider it unlikely that reduced muscle force explains the lack of a CSP phenotype.

The induction of plasticity with iTBS is analogous to ex vivo LTP protocols used to demonstrate deficits in synaptic plasticity in mouse models of NF1 (11,12). Additionally, iTBS has advantages over the PAS paradigm as it requires lower stimulation intensity and less time to stimulate. Hence, we considered the iTBS paradigm to be superior as a potential neurophysiological outcome measure for NF1 patients. However, we did not observe an overall effect of time with iTBS in the whole group analysis. Therefore, the findings in the subgroup analysis should be interpreted with caution. When only including the data of responders, we observed a normal response at T0, but a marked effect in the ability to maintain this potentiation, as the MEP size decreased with 10 minutes to baseline values in NF1 patients. Importantly, the number of responders at T0, T1, T2 or T3 after iTBS was not significantly different between groups ($70\%_{Control}$, $83\%_{NF1}$). A previous study measured plasticity in 11 NF1 patients using the TMS PAS paradigm (19). That study indicated a relative inability to induce MEP potentiation in NF1 patients, which was already evident immediately after stimulation. This difference was not observed in our study using iTBS, as the number of NF1-responders to iTBS was similar to controls. Non-responsiveness to iTBS might be explained by high inter-individual variability (31,44). Recent studies suggest that high inter-individual variability could be due to genetics or the current state of neuronal activity of neuronal networks recruited by each TMS pulse (45,59), which would be interesting to take into consideration in future studies. It could be argued that it would have been more accurate to use the optimal individual stimulus intensity based upon an input-output curve for each participant (60). This could reduce variability between subjects and decrease stimulus intensity. The rationale for using SI_{1mV} was to avoid ceiling and floor effects, and to create

a baseline measure of excitability that is approximately in the middle of the smallest and largest response to the TMS pulse. The SI_{1mV} method is in line with the majority of the TBS-studies, which makes it easier to interpret the results of NF1 patients. Future studies should aim to combine these approaches that may improve the method.

Interestingly, responses to single pulse stimulations showed a trend to lower MEP amplitudes in NF1 patients throughout the whole experiment. This finding was observed despite the use of similar stimulus intensities and RMT values, and a mean amplitude of MEPs at SI_{1mV} between 800-1200 μV. Cortical excitability in response to single pulse stimulations has not been explored previously in NF1 patients. TMS is used to estimate the corticospinal state by measuring MEPs to single pulse stimulations (54,61). However, the interpretation of the underlying physiology of observed lower MEP amplitudes in NF1 patients in response to single stimulations is difficult due to multiple circuits contributing to MEPs (54).

This study has three key strengths: the rather large sample size for TMS studies, the inclusion of measurements of parameters that could affect the outcome if they differed, and the absence of any psychoactive medication in the subjects. A large sample size is needed, as an elaborated meta-analysis showed publication bias specific for iTBS studies with small sample sizes (62). Although our sample size is already quite high for a rare disease patient study, we recommend including an even higher number of patients in the future, due to the high inter-individual variability after iTBS (62). This limitation of high inter-individual variability can potentially be reduced by further optimizing iTBS protocols. A previous study on the optimization of the iTBS protocol showed that increasing the stimulation dose did not improve the responder-rate to iTBS (47). Additionally, it has been suggested that priming neural networks with other TMS paradigms might standardize the history of neural activity, and consequently reduce the variability in response to iTBS (63). Furthermore, Hamada et al. (45) state that the current state of neuronal activity and recruitment of early or late indirect waves (I-waves) are probably of high influence on the after-effects of iTBS, which should be addressed in future research. It has been shown that iTBS aftereffects are correlated with I-wave recruitment indicating differential recruitment of cortical pathways (45,64). Interestingly, previous studies have

shown that iTBS can increase excitability of the cortical pathways reflected in the generated later I-waves (65,66). Future research should address later I-waves after iTBS in adult NF1 patients to clarify further cortical excitability and plasticity in NF1. In the present study, we matched for age and sex, and standardized the time of day. We also measured whether sleepiness was different to avoid its effect on the outcome. Moreover, in contrast to previous studies (31,44), all MEPs were recorded from the non-dominant hand due to the more pronounced cortical inhibition in the non-dominant hemisphere than in the dominant hemisphere (67). Although the severity of behavioral problems of the participating NF1 patients in daily life was not known, none of the patients were receiving mental health care or using psychoactive medications. Additionally, the average estimated IQ of the NF1 patients that participated in our study closely resembled previously reported IQ scores (4–6), which is a good predictor of neuropsychological functioning in other cognitive domains (68). This suggests that there was not a strong participation bias towards patients with less severe cognitive dysfunction. Patients had either a clinical (40%) or genetic diagnosis (60%) of NF1. Those patients with a genetic diagnosis included both intragenic mutations (61%, n = 11) or deletions (22%, n = 4), as well as a chromosomal microdeletion of the NF1 gene (17%, n = 3). The latter genotype is associated with a more severe cognitive phenotype (5). The estimated-IQ was not significantly correlated with any of the TMS outcomes, which indirectly suggests the absence of a meaningful relationship between plasticity and inhibition, with IQ. Hence, the TMS findings of this study need to be further substantiated before they can be used as reliable neurophysiological outcome measures in treatment intervention studies and in relation to the cognitive deficits in NF1 patients. It would be of interest to validate the findings of optimized TMS protocols with combinations of neuroimaging methods to control for the high inter-individual variability of TMS-responses.

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Chapter 5.2

Motor cortical plasticity in adults with major depressive disorder

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ABSTRACT

Major depressive disorder (MDD) is a severe psychiatric disorder that is associated with various cognitive impairments, including learning and memory deficits. As synaptic plasticity is considered an important mechanism underlying learning and memory, deficits in cortical plasticity might play a role in the pathophysiology of patients with MDD. We used Transcranial Magnetic Stimulation (TMS) to assess inhibitory neurotransmission and cortical plasticity in the motor cortex of MDD patients and controls.

We measured the cortical silent period (CSP) and short interval cortical inhibition (SICI), as well as intermittent theta-burst stimulation (iTBS), in 9 drug-free MDD inpatients and 18 controls.

The overall response to the CSP, SICI, and iTBS paradigms was not significantly different between the patient and control groups. iTBS induction resulted in significant potentiation after 20 minutes in the control group ($t(17) = -2.8$, $p = 0.01$), whereas no potentiation was observed in patients.

We did observe plasticity deficits, but found no evidence for medium-to-large effect size differences in CSP and SICI measures in severely depressed drug-free patients, suggesting that reduced cortical inhibition is unlikely to be a robust correlate of the pathophysiological mechanism in MDD. However, these findings should be interpreted with caution due to the high inter-subject variability and the small sample size. These findings advance our understanding of neurophysiological functioning in drug-free severely depressed inpatients.

INTRODUCTION

Major depressive disorder (MDD) is a severe psychiatric disorder with a prevalence of 4.7% worldwide (1). MDD comprises a depressed mood and loss of interest or pleasure in life activities. The majority of MDD patients also suffer from cognitive dysfunction (2,3). Previous research showed various cognitive impairments in MDD patients, including deficits in memory, attention, language, and visual-motor speed (4,5).

The mechanism underlying the cognitive deficits associated with MDD remains poorly understood. The cellular mechanism of learning and memory is believed to depend on the ability to induce long-lasting changes in synaptic efficacy. The ability of synapses to enhance their strength or efficacy of synaptic transmission over time, i.e. long-term potentiation (LTP), has been well studied in animals. Although findings of cortical plasticity in humans show important parallels with LTP, there is a lack of evidence that the cortical potentiation is due to synaptic changes. Therefore, in the literature, the term LTP-like plasticity is often used when referring to lasting cortical plasticity. A previous study reported significant performance impairment in three learning tasks in MDD patients (5). Based on neurophysiological findings, previous studies hypothesized that cortical plasticity is impaired in patients with MDD (6–9). These studies made use of Transcranial Magnetic Stimulation (TMS), a neurophysiologic technique to assess inhibitory and excitatory neurotransmission in the motor cortex via single-pulse stimulations, as well as the modulation of cortical excitability via TMS paradigms (10). Reduced cortical plasticity was shown in 23 and 27 MDD patients, taking psychotropic drugs at the time of measurement, in response to the paradigm of paired associative stimulation (PAS) (6,7). Recently, one study investigated cortical plasticity in 11 drug-free MDD patients with the intermittent theta-burst stimulation (iTBS) paradigm (8). The iTBS paradigm bears a strong resemblance with the methodology used in ex vivo preclinical studies to measure LTP (11). In addition, due to its shorter duration and low stimulus intensity, the iTBS paradigm is less demanding than PAS and therefore more suitable to use in severely depressed patients. Vignaud et al. (8) showed impaired cortical plasticity upon iTBS in MDD treatment-resistant patients, although they observed high variability in the response to iTBS. There are only a few studies of cortical plasticity in MDD patients, in

which the effect of psychoactive drug use and the variation in depression severity has remained unclear.

Cortical plasticity in patients with MDD seems to be modulated by gamma-aminobutyric acid (GABA), the main inhibitory neurotransmitter in the central nervous system (12). GABAergic interneurons inhibit other neurons in the cortex to coordinate cortical activity and modulate synaptic plasticity. Several preclinical studies have shown that GABAergic deficits play a role in cognitive dysfunction associated with MDD traits such as anxiety and distortion of attention to threat cues (13,14). In addition, Stockmeier et al. (15) observed a reduction in GABAergic connections postmortem in the hippocampus of 19 MDD patients. Following the theory that synaptic plasticity is essential for learning and memory, cognitive dysfunction in MDD could be caused by deficits in the GABAergic neurotransmitter system.

GABA deficits in MDD have been extensively studied in preclinical and treatment studies, including TMS (12). The TMS paradigms short-interval intracortical inhibition (SICI) and cortical silent period (CSP) are sensitive to changes in GABA-mediated inhibition. Previous studies have shown that the response to the SICI or CSP paradigms can be increased by $GABA_A$ or $GABA_B$ receptor agonists (16,17). However, TMS studies of GABA-mediated cortical inhibition in MDD patients have yielded contradictory findings. The response to the SICI and CSP was significantly reduced in 20 drug-free patients with treatment-resistant MDD, indicating deficits in cortical inhibition in MDD (18). Conversely, the response to the SICI was not significantly different in 16 MDD patients (19). Moreover, 16 depressed patients had significantly higher CSP, suggesting an increase of cortical inhibition in MDD (20). However, most of these patients either received psychoactive drugs at the time of the study (19,20) or had a nonresponse to treatment with antidepressants (18). Notably, drugs that act on the central nervous system can strongly influence the response to TMS paradigms (21).

Considering the prevalence of cognitive dysfunction clinically reported in MDD patients, and the inconsistent TMS findings in MDD patients, further clarification of the presence of underlying neurophysiological deficits in drug-free severely depressed patients is

relevant. Interestingly, it has been shown that age similarly affects GABAergic cortical inhibition as late-life depression (22). Therefore, it is highly important to age-match patients and controls. Additionally, the effect of confounders as experimental factors, sleepiness, time of day, and gender should be considered due to high variability in TMS measures observed in healthy individuals (9,23). Hence, we examined both cortical inhibition and cortical plasticity in drug-free severely depressed inpatients compared to age-matched neurotypical controls using the TMS paradigms SICI, CSP, and iTBS, in which potential confounders were systematically considered.

METHODS & MATERIALS

Subjects

In this study, we included drug-free MDD patients and controls matched for age and gender. Participants were included with an age between 18-85 years. Patients were included if they had a confirmed diagnosis of major depression according to the criteria of the DSM V (24) and were being free of psychoactive drugs (see Table 1 for 'days without medication'). Patients were excluded if they had other somatic or psychiatric comorbidities as bipolar disorder or psychotic symptoms. Additionally, patients had no neurological diseases as Parkinson's disease or Alzheimer's disease, or any brain pathology as a cerebrovascular accident. Lastly, patients with an indication for acute electroconvulsive therapy were excluded. Controls were included if they had a score on the Beck Depression Inventory (BDI) (25,26) below 9 and were being medication free (excluding contraceptives). Controls had no current or history of medical, psychiatric, or neurological disorders. Furthermore, subjects had no neurological illness that could affect the motor system and used no psychoactive drugs. Subjects met the criteria for undergoing a TMS measurement (27,28). Inpatients with MDD were recruited by a senior psychiatrist from the depression unit of the Department of Psychiatry at the Erasmus University Medical Center. Recruitment of unaffected controls took place through online advertisements.

Table 1. Characteristics of MDD patients. Age, gender, education, Hamilton score (HAM-D), and medication specifications per patient with MDD.

MDD patients	Age	Gender	Education	HAM-D	medication	Dose in mg (times per day)	Days without medication	Mean half-life (hrs)
p1	58	F	-	17	NA	NA	NA	NA
p2	59	M	-	17	Clomipramine	75 (2)	20	21
						25 (1)	16	
p3	46	F	6	20	Olanzapine	5 (1)	11	30
					Lamotrigine	50 (2)	4	33
p4	44	F	-	24	Lorazepam	1 (2)	2	12-16
p5	70	F	2	20	Venlafaxine	375(1)	13	5
						75 (1)	6	
						37.5(1)	1	
p6	47	M	7	16	NA	NA	NA	NA
p7	47	M	3	18	Lorazepam	0.5 (1)	2	12-16
					Venlafaxine	37.5 (1)	2	5
p8	56	F	3	14	Pregabaline	75 (1)	0	6
					Lithium	200 (1)	13	12-48
						600 (1)	11	
					Nortriptyline	25 (1)	13	26
p9	66	F	3	29	Propranolol	10 (2)	1	3-6
					Haloperidol	0.5 (1)	7	12-38
					Trazodon	50 (1)	9	8
					Temazepam	20 (1)	13	7-11
						10 (1)	10	
					Lorazepam	1 (1)	10	12-16
					Lithium	400 (1)	13	12-48
						200 (1)	10	
					Escitalopram	10 (1)	13	30

* Education, level of education using the International Standard Classification of Education (ISCED) (30). -, level of education unknown.

MDD, major depressive disorder; Edu, education level; HAM-D, Hamilton score; F, female; M, male; NA, not applicable.

We achieved our a priori sample size estimations (41) based on data from previous studies (7,18,19). To detect a medium to large-sized effect for cortical plasticity (η^2 = 0.12) with a power of 80% and a significance level of 0.025 (Bonferroni corrected), we needed a sample size of minimal 7 subjects per group (patient and control groups). To detect a large-sized effect for cortical inhibition (η^2_{SICI} = 0.22; d_{CSP} = 1.02) with a power of 80% and a significance level of 0.025, we needed a sample size of minimal 7 and 17 subjects per group, respectively.

This study was conducted following the Declaration of Helsinki (2013) and was approved by the Dutch Central Medical Ethics Committee of the Erasmus Medical Center Rotterdam.

Procedures

Participants were screened before the start of the TMS measurements using the questionnaires Transcranial magnetic stimulation Adult Safety Screen (TASS) (28), Beck Depression Inventory (BDI) (controls) (25), and Hamilton Rating Scale for Depression (HAM-D) (patients) (29) (see 2.2.1 Questionnaires). We classified the level of education using the International Standard Classification of Education (30). We started the TMS measurements at noon for all subjects after they had a light lunch. Subjects had their eyes open and arms at rest while sitting in a comfortable chair. We recorded motor evoked potentials (MEPs) from the left first dorsal interosseous (FDI) muscle using electromyography (EMG) with silver/silver chloride electrodes in belly-tendon recording technique. We used a universal amplifier (ANT Neuro, Enschede, The Netherlands). Data was filtered online with a 20-2000 Hz band-pass filter and a 50 Hz notch filter, and raw data was stored for offline analysis. TMS stimulations were given by a TMS stimulator (MagPro X100 with MagOption; MagVenture, Denmark) via an eight-shaped stimulation coil (MC-B70, MagVenture, Denmark) placed on the scalp. The handle of the coil was held in a posterolateral direction at an angle of 45° from the midline. First, we determined the optimal positioning of the coil on the primary motor cortex in the right hemisphere (i.e. the hotspot) in accordance with the reference point of the FDI. The reference point was defined on the right hemisphere as the place at 10% of the ear-to-ear span lateral to Cz. We placed randomly around this reference point TMS

stimulations to define the hotspot with the highest peak-to-peak amplitude of the MEP in the FDI muscle. Throughout the experiment, the coil was held at the hotspot using a 3D neuronavigation (Visor2XT). The resting motor threshold (RMT) was defined with a maximum likelihood threshold-hunting procedure (31). RMT is the stimulus intensity that elicited MEPs of > 50 μV with a 50% probability. The RMT measurement was repeated at 3-time points to control for changes over time. Sleepiness was also measured at these time points with the Karolinska sleepiness scale (KSS), a self-report questionnaire on a nine-point Likert scale (32). Furthermore, throughout the experiment, single-pulse stimulations were given with a stimulus intensity that elicited a mean and median between 800-1200 μV ± SD <1/2 of the mean (SI1mV). The SI1mV was determined by the mean of 10 stimulations with increasing stimulus intensity starting from the RMT (33,34). The differences in MEP size as a response to the TMS paradigms SICI, CSP and iTBS were studied.

Questionnaires

The TASS is a validated questionnaire to screen TMS candidates consisting of 15 questions (28). Positive answers to one or more questions do not represent absolute contraindications to TMS. The BDI is a 21-question multiple-choice self-report inventory to measure the severity of depression in controls (25). The HAM-D is a 17-item questionnaire (29), commonly used to rate the severity of depression.

TMS measurements

SICI: Short interval cortical inhibition (SICI) is a paired-pulse TMS paradigm that measures cortical inhibition. In this paradigm, a subthreshold pulse of 80% of RMT is followed by a pulse at SI1mV after an interstimulus interval of <6 ms. The SICI has been reliable and reproducible within individuals (35). We performed in random order 17 paired stimulations with the conditioning pulse at 80% of RMT, and 13 single stimulations at the SI1mV. The difference in MEP amplitude between the response to paired and single pulses was used to estimate cortical inhibition.

CSP: During the cortical silent period (CSP) paradigm, the FDI was tonically contracted with 20% of maximum voluntary strength using a hand-held pinch gauge (B&L Engineering;

Santa Ana, CA, USA). The CSP is determined from the time the single suprathreshold TMS pulse is given until EMG activity reappears after the MEP. Single pulses consisted of 10 pulses at 120% of RMT with an inter-stimulus interval of 6 seconds (36). The CSP has been shown to have good test-retest reliability (37).

ITBS: Theta burst stimulations are repetitive bursts of 3 stimuli at a frequency of 50Hz repeated at 5Hz. In the intermittent TBS (iTBS) paradigm, a train of TBS of 2 seconds was repeated every 10 seconds for a total of 190 seconds (11). We used a stimulus intensity of 70% of RMT for the iTBS and recorded 20 single pulses at SI1mV before iTBS and at 0, 10, 20, 30 minutes after iTBS modulation (11,38–40). Changes in mean MEP size after iTBS induction compared to the mean MEP size before iTBS induction are assumed to reflect changes in cortical plasticity.

Data analyses

EMG data was online continuously recorded with Visor software (Visor2XT). The raw data from the Visor program were analyzed using Matlab (Matlab, version 2019b). First, all the traces were detrended if a linear trend was present. Secondly, a bandpass filter between 20 and 2000 Hz and a notch filter at 50 Hz with an elliptic design was applied to the raw EMG data. Thereafter, traces were discarded if the peak-to-peak amplitude of the EMG activity in rest was higher than 70 μV and a standard deviation higher than 25 μV within a 50 ms pre-trigger interval (42,43). We used a range of 10 μV lower than the cut-off values to visually detect technical artifacts or excessive background EMG activity during rest. TMS responses of one-time point within a participant were discarded if more than 50% of the epochs were discarded at that time point (40). Lastly, MEP size and peak latencies were calculated within a time window of 0.2-48 ms. We defined the MEP onset automatically and visually within 20-35 ms after the TMS trigger. If the data was not normally distributed, MEP-sizes were transformed with a square root transformation to reduce right skewness (44,45). Statistical analyses were performed using the (transformed) MEPs in IBM Statistics SPSS (version 25).

We tested for differences in age, gender, educational attainment, and sleepiness between groups with an independent t-test, a Chi-square test, and non-parametrically with a

Mann-Whitney U test, respectively. The change over time in RMT during the experiment was tested with a repeated-measures ANOVA. The difference in CSP durations between groups was evaluated with an independent t-test. We performed a repeated-measures ANOVA to compare mean MEP amplitudes between the patient group and the control group during SICI and their interaction with the SICI condition (paired or single pulses). We performed a repeated-measures ANOVA to compare mean MEP amplitudes between the patient group and the control group during iTBS and their interaction with time (T0, T1, T2, T3). In addition, we tested separately the responders to iTBS in both groups, classified as a minimal increase of 10% in MEP amplitude after iTBS induction at T0, T1, T2, or T3 (38,46). Relationships between confounding factors such as age and the HAM-D score and the main outcomes were evaluated using Pearson or Spearman's rho (r_s) correlation coefficients, respectively, and p-values were corrected for multiple comparisons with the Bonferroni correction.

RESULTS

In total, 35 eligible drug-free patients with MDD were invited of which 11 subjects declined participation, 13 subjects were excluded due to other (psychiatric) comorbidities, and 2 subjects had no diagnosis of severe depression. In total, we included 9 patients. In addition, 49 eligible control subjects were invited of which 18 subjects were included (n_c = 18, n_{MDD} = 9). Two patients discontinued participation during the iTBS paradigm due to fatigue and were consequently excluded from further analysis of cortical plasticity.

Age was not significantly different between the patient group (M = 54.8 ± 9.4) and the control group (M = 51.1 ± 10.6) (t_{age} (25) = -0.9, p = 0.4). Gender was not significantly different between the groups (χ^2_{gender} = 1.2, p = 0.3). The level of education also was not significantly different between the groups (U = 37.0, p = 0.3). The mean HAM-D score for patients was 19.4 ± 1.5. The mean drug-free period was 10.5 ± 8.1 days at the time of testing. Before the drug-free period, patient psychotropic usage in the month prior included: tricyclic antidepressants (n = 2; clomipramine, nortriptyline), selective serotonin reuptake inhibitors (n = 3; trazodone, escitalopram), selective serotonin norepinephrine inhibitors (n = 1, venlafaxine), antipsychotics (n = 2; olanzapine,

haloperidol), anti-epileptics (n = 2; lamotrigine, pregabalin), benzodiazepines (n = 4; lorazepam, temazepam), lithium (n = 2) and beta-blockers (n = 1; propranolol) (Table 1). The mean sleepiness score during the measurements was significantly higher (i.e. less alert) in the patient group than in the control group (U = 31.5, p = 0.02).

RMT was not different between the groups ($t_{RMT}(25) = -0.8, p = 0.4$) and did not change over time ($F(2, 38) = 0.12, p = 0.9$). The mean amplitude of MEPs at SI1mV was similar for the patient group and the control group ($M_c = 967 \pm 297$; $M_{MDD} = 837 \pm 340$, $t(25) = 1.0, p = 0.3$). Stimulus intensities were similar for both groups ($t_{SI1mV}(25) = -0.4, p = 0.7$) (Table 2).

Cortical inhibition

The mean MEP size of singe pulse stimulations was not different between groups ($t(25) = -1.0, p = 0.4$). Mean MEP size differed significantly between conditions, indicating that the SICI paradigm sufficiently inhibited the MEPs in both groups ($F(1, 25) = 44.0, p < 0.001, \eta^2 = 0.6$) (Figure 1). We did not find a significant group effect ($F(1, 25) = 0.8, p = 0.4$) or interaction effect between group and conditions ($F(1, 25) = 0.3, p = 0.6$). The SICI paradigm inhibited the MEPs in both groups equally. Additionally, there was no significant difference in mean CSP duration between the MDD group and the control group ($M_c = 132.0 \pm 30.0$; $M_{MDD} = 117.7 \pm 38.8$) ($t(25) = 1.1, p = 0.3$) (Figure 2).

Table 2. Demographics and variables during transcranial magnetic stimulation (TMS) measurements (Mean ± SD) of the MDD group and the control group separately.

	MDD group (n=9)	Control group (n=18)
Demographics		
age in years	54.8 ± 9.4	51.1 ± 10.6
gender: male in % (#)	33 (3)	56 (9)
educational attainment, range	3.0, 2-7	6.0, 2-7
Sleepiness, median KSS, range*	7.0, 1-9	3.0, 1-5
During TMS measurements		
RMT %MSO	50.4 ± 9.9	47.3 ± 9.4
SI_{1mV} %MSO	59.2 ± 13.6	57.4 ± 12.0
Mean amplitude of MEPs at SI_{1mV}	837.5 ± 340.6	966.5 ± 297.8

#, number of subjects; KSS, Karolinska sleepiness scale; TMS, transcranial magnetic stimulation; RMT, Resting Motor Threshold; SI_{1mV}, Stimulus Intensity at 1 mV; MSO, Maximum Stimulator Output; MEP; motor evoked potential; MDD, major depressive disorder.
* Significantly different between the patient and control group (p-value <0.05)

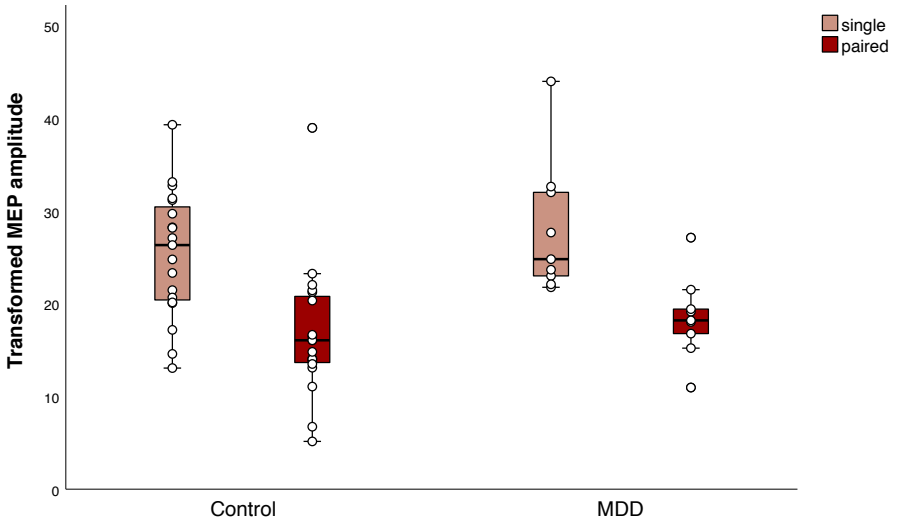

Figure 1. Response to the short interval cortical inhibition (SICI) paradigm. Boxplots of square-root (sqrt) transformed mean motor evoked potential (MEP) amplitudes per subject in response to the SICI, for both groups separately. Mean MEP amplitudes in response to the single pulses or paired pulses did not differ between the major depressive disorder (MDD) group and the control group. Mean MEP size differed significantly between conditions, indicating that the SICI paradigm sufficiently inhibited the MEPs in both groups ($F(1, 25) = 44.0$, $p<0.001$, $\eta^2 = 0.6$).

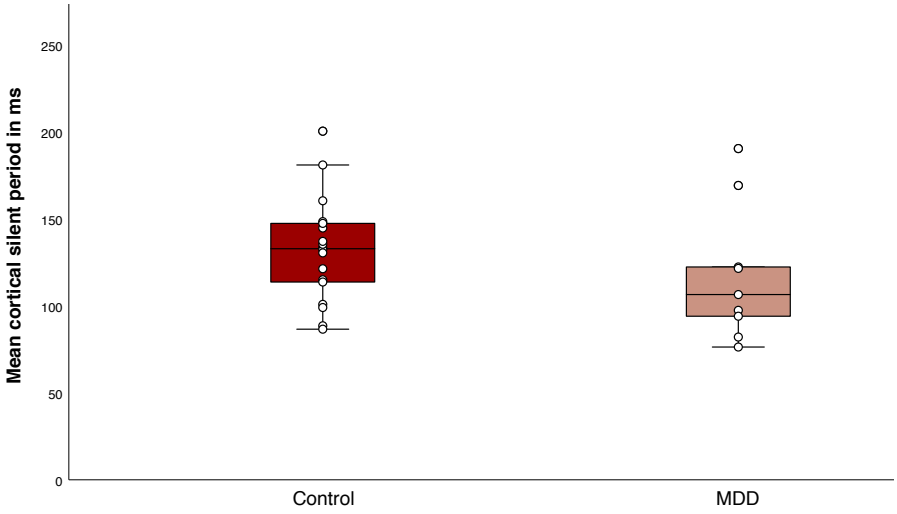

Figure 2. Response to the cortical silent period (CSP) paradigm. Boxplot of individual means of CSP duration for the control group and the major depressive disorder (MDD) group. There were no significant differences in mean CSP duration between the groups ($t(25) = 1.1$, $p = 0.3$).

Cortical plasticity

At baseline, MEPs in response to single-pulse TMS before iTBS induction were not different between the groups (t(24) = -0.5, p = 0.6). Repeated measures ANOVA revealed that MEPs measured after stimulation were significantly higher in both groups ($F_{time}(4, 88) = 3.4$, $p = 0.01$, $\eta^2 = 0.13$). We did not find a significant group effect ($F_{group}(1, 22) = 0.09$, $p = 0.8$), and an interaction effect on trend level between group and time ($F(4, 88) = 2.5$, $p = 0.05$). In the control group, within-group analyses by means of t-tests showed that the MEP-size following iTBS was significantly higher 20 minutes after stimulation (i.e. T3) (t(17) = -2.8, p = 0.01). In the patient group, within-group analyses showed that the MEP-size following iTBS was not significantly higher for any time point compared to baseline (Figure 3). The standard errors of the mean were large in both groups indicating a high inter-subject variability.

Additionally, the number of responders to iTBS, classified as a minimal increase of 10% in MEP amplitude after iTBS induction at T0, T1, T2 or T3, was not significantly different between groups (control = 83%; MDD = 67%, $\chi^2(1) = 0.96$, $p = 0.3$). Repeated measures ANOVA of the responder group revealed similar results as the whole group analysis ($F_{time}(4, 76) = 4.6$, $p = 0.002$, $\eta^2 = 0.2$; $F_{group}(1, 19) = 0.4$, $p = 0.5$; $F_{interaction}(4, 76) = 2.4$, $p = 0.05$). In the control group of responders, within-group analyses showed a significant potentiation of the MEP-size following iTBS at 0-20 minutes after stimulation ($t_{T1}(14) = -3.7$, $p = 0.002$; $t_{T2}(14) = -3.3$, $p = 0.005$; $t_{T3}(14) = -4.4$, $p = 0.001$). In the patient group of responders, within-group analyses showed that the MEP-size following iTBS was not significantly higher for any time point compared to baseline.

Correlations

There were no significant correlations between the severity of the depression of inpatients measured with HAM-D and the main outcomes nor between the potential confounders age, gender, educational attainment, or sleepiness and the main outcomes. There were no significant correlations between the HAM-D score of inpatients and the duration of the CSP ($r_s = -0.2$, $p = 0.6$), the MEPs inhibited by SICI ($r_s = -0.7$, $p = 0.07$), nor the increase of the MEPs induced by iTBS ($r_{s\_T0} = 0.8$, $p = 0.08$). We also did not find significant correlations in either group between age and the duration of the CSP (r =

-0.1, p = 0.5), the MEPs inhibited by SICI (r = 0.05, p = 0.8), or the increase of the MEPs induced by iTBS (r_{T0} = -0,08, p = 0.7). There were no significant correlations between sleepiness and the increase of the MEPs induced by iTBS (r_{s_T0} = -0.2, p = 0.4).

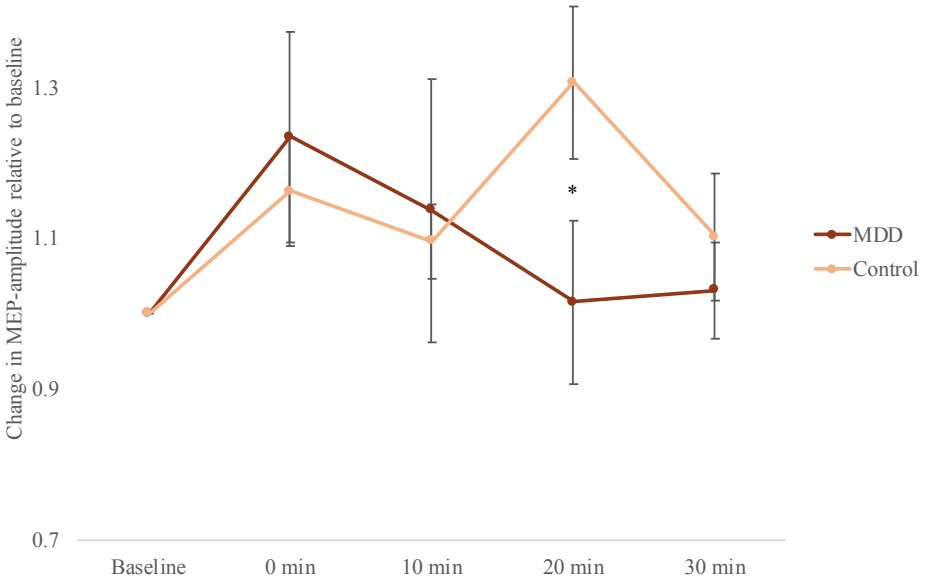

Figure 3. Whole group analysis of cortical plasticity. The change in MEP-amplitude (motor evoked potential) amplitudes ±SEM of the response upon induction of intermittent theta-burst stimulation (iTBS). Baseline: mean MEP in response to single pulses directly before iTBS. 0-30 min: mean MEP in response to single pulses four times within 30 minutes after stimulation: 0, 10, 20 and 30 minutes after stimulation. MEPs measured after stimulation were significantly higher in both groups ($F_{time}(4, 88) = 3.4$, p = 0.01, $\eta^2 = 0.13$). We did not find a significant group effect ($F_{group}(1, 22) = 0.09$, p = 0.8). However, there was an interaction effect between group and time ($F(4, 88) = 2.5$, p = 0.05, $\eta^2 = 0.10$). In the control group, within-group analyses by means of t-tests showed that the MEP-size following iTBS was significantly higher 20 minutes after stimulation (i.e. T3) ($t(17) = -2.8$, p = 0.01). In the patient group, within-group analyses showed that the MEP-size following iTBS was not significantly higher for any time point compared to baseline

DISCUSSION

Whether changes in neurophysiological measures are present in drug-free severely depressed patients causing the cognitive deficits remains poorly understood. We examined TMS-based measures of cortical inhibition and plasticity in drug-free severely depressed inpatients and controls. Previous studies showed inhibitory GABAergic dysfunction in MDD patients, which might consequently affect cortical plasticity. Based on these previous findings, we expected alterations in the response to the inhibitory TMS measures CSP and SICI, as well as a reduced response to the plasticity measure iTBS in MDD patients. We did observe plasticity deficits, but found no evidence for medium-to-large effect size differences in CSP and SICI measures in severely depressed drug-free patients, although a high inter-subject variability was noted in both groups.

We found no evidence for differences in CSP and SICI measures reflecting GABA-mediated inhibition in MDD patients. In both the MDD and control groups, the MEP amplitudes were similarly inhibited in response to the paradigms. Although previous studies were inconsistent, several studies showed a trend towards decreased inhibition in MDD patients as measured with the CSP and SICI (18,19,47). The CSP paradigm is considered to be a very robust paradigm used in the study of the pathophysiology of several psychiatric disorders such as obsessive-compulsive disorder and schizophrenia (48,49). The CSP is associated with deficits in $GABA_B$ receptor-mediated inhibitory neurotransmission (17,50). It was shown in a meta-analysis that the CSP duration was shortened in MDD patients compared to controls (47). Contradictory, in the present study, we did not find a significant difference in CSP duration between the patient and control group, although our MDD group was quite small according to a priori sample size estimations. However, previous studies did not use a standardized protocol to measure the CSP, causing difficulties in comparing the findings. Previous studies used different stimulus intensities (range 110%-200% of RMT), different strengths of muscle contraction and measured from different hemispheres (i.e. dominant vs non-dominant). The increasing stimulus intensity is known to increase the CSP duration (51). Nevertheless, the stimulus intensity of 120% of RMT in the present study provides a reliable and informative CSP (52). In addition, this stimulus intensity was used in the present study, because MDD

patients can be more sensitive to potential discomfort as induced by increasing stimulus intensities of the TMS. The strength of muscle concentration was relatively low to avoid fatigue of the muscle, although CSP duration seems not to be affected by the strength of muscle contraction (52). Lastly, we stimulated the non-dominant hemisphere due to less cortical inhibition in the dominant hemisphere than in the non-dominant hemisphere (53).

In the present study, the SICI paradigm sufficiently inhibited the MEPs in both groups, but the amount of inhibition was not different between the groups in contrast to previous studies. Although Levinson et al. (19) showed no difference in the SICI response between unmedicated MDD patients (i.e. without medication for at least 1 month) and controls, previous studies did find a difference between treatment-resistant MDD patients and controls (18,19). Possibly, these patients had a more severe illness causing more inhibitory deficits, although the HAM-D score was not different between the unmedicated and treatment-resistant depressed patients. In the present study, all patients were inpatients admitted to the hospital for a longer period, nevertheless, some patients suffered from moderate depression (Table 1). It is important to note that the treatment-resistant patients measured in Levison et al. (19) used medication during the study which could have influenced the results (21), whereas in our study, the patients were drug-free. The SICI and CSP might not only measure inhibitory processes mediated by GABA, but also measure processes that interact with the GABAergic neurotransmitter system or processes from independent inhibitory pathways in the primary motor cortex (19). Despite evidence of reduced GABA levels in depressed patients by studying the treatment of selective serotonin reuptake inhibitor and electroconvulsive therapy (ECT) (54,55), the present findings are in line with some previous studies that looked into GABA-related deficits (56,57). More specifically, Knudsen et al. (57) found no differences in GABA levels between depressed and healthy participants before or after ECT treatment. Bhagwagar et al. (58) suggested that reduced GABA levels might be associated with a trait of vulnerability to mood disorder based on findings in recovered patients, instead of a direct neurochemical correlate of MDD.

The second main finding of our study is the difference in cortical plasticity upon iTBS induction between the patient and control group. We found that the MEP size had a marked effect in potentiation following iTBS induction at 20 minutes in the control group, as the MEP size decreased quickly after induction to baseline values in the MDD group. This effect was even stronger when only including the data of the responders: we observed a significant increase in MEP-size immediately after iTBS-induction up to 20 minutes in the control group, but there was no potentiation in the patient group. It is important to note that the number of responders was not significantly different between the groups, nevertheless, the group sizes were quite small. To our knowledge, only one study investigated cortical plasticity in 11 drug-free treatment-resistant depressive patients with the iTBS paradigm (8). Interestingly, our findings are consistent with Vignaud et al. (8) who showed a significant potentiation following iTBS induction at 20 minutes in controls and no potentiation in MDD patients. However, in the present study, we did not find a significant overall group effect. Furthermore, reduced cortical plasticity was also shown in MDD patients taking psychoactive drugs at time of measurement, in response to the paradigm paired associative stimulation (PAS) (6,7). The present study showed comparable TMS results in drug-free MDD patients using a more favorable method, iTBS, due to its lower stimulation intensity and duration. The effect of iTBS induction seems to depend on N-methyl-D-aspartate (NMDA) receptors (59), of which alterations in the levels have been shown in the brain of depressive patients (60).

Our findings of reduced cortical plasticity should be interpreted with caution due to the high inter-subject variability. To reduce the variability, we increased the single pulse stimulations per timepoint (8,40). Moreover, we standardized experimental settings such as time of day, and we matched for sex and age (61). It has been shown that age similarly affects GABAergic cortical inhibition as late-life depression (22). Additionally, sleepiness was measured to consider its potential confounding on the outcome. Patients were less alert than the control group, although this could be associated with their MDD symptoms. Nevertheless, there were no significant correlations between the main outcomes and the sleepiness score. We also used the personalized SI_{1mV} to avoid ceiling and floor effects within subjects instead of a standard percentage of the RMT. This could

be optimized further, however, by using an input-output curve of the individual stimulus intensity (62). Nevertheless, the high inter-individual variability in TMS responses could be the result of genetics, the current state of neuronal activity, or the recruitment of early or late indirect waves (I-waves) (63–65). Future research should address the high inter-subject variability in the response to iTBS to clarify further cortical excitability and plasticity in drug-free MDD patients.

The key strength of the present study is the relative homogeneity of the patient group. All patients had severe symptoms and were inpatients at the time of the study, admitted to the hospital for extended stays for treatment of a major depressive disorder. Patients had no psychiatric comorbidities or discernible brain pathology. Additionally, patients were free of psychoactive medication for an average of 10 days prior to the study. Although the severity of the depression in patients was slightly lower ($M = 19.4 \pm 1.5$) than previous studies that found large effects on inhibitory measures ($M = 21.2 \pm 6.0$; 21.1 ± 1.1) (18,66), the scores were still within the range of moderate to severe depression. Nevertheless, Lewis et al. (67) showed that the severity of the depressive symptoms might correlate with the degree of neurophysiological dysfunction in a pediatric sample. In the present study, we did not find such a correlation, in line with studies of an adult sample with MDD (19,20).

Our study is limited by its small sample size, the location of stimulation on the human cortex, the variability in the duration of the drug-free period, and the variability in the use of psychoactive drugs before the drug-free period. The number of inpatients was low due to the known inherent lack of motivation among patients with severe MDD, the low number of admissions to the hospital, and the short amount of time to test the patients between admission and the start of treatment with antidepressants. We achieved our a priori sample size estimations (41) for the iTBS and SICI outcomes based on data from previous studies (7,18,19), but we were unable to reproduce the large differences in TMS measures reported in previous studies. Nevertheless, a larger sample size might reduce the observed high inter-subject variability (68), although the effect of age on the outcome measures should be still taken into account. Furthermore, our measures are limited to the primary motor cortex, while we are interested in neurophysiological

processes that are not involved in motor function. The dorsolateral prefrontal cortex might be more interesting to stimulate with TMS combined with electroencephalography to study the pathophysiology of major depressive disorder (69). Potentially, the findings of neurophysiological processes in the primary motor cortex could be translated to other cortices. Lastly, the duration of the drug-free period was rather short in some of the patients. Medication could affect the outcome measures in patients that had a short wash-out period of the psychoactive drugs. We carefully acknowledged medication half-life in the study design. Furthermore, the patients that had a short wash-out period of the medication before the measurement used medication with no known effect on the inhibitory TMS measures (21). Only one patient with a short wash-out period used a benzodiazepine agonist that increases GABA-mediated inhibition, but no clear deviations were found in this patient in relation to the other study subjects.

Future research should investigate further whether deficits in cortical inhibition is a robust pathophysiological mechanism in MDD, and if the observed plasticity deficits are still present with a larger sample size in drug-free patients. Perhaps, in the future, treatment could be optimized by making use of these TMS measurements to indicate neurophysiological deficits in MDD patients.

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Chapter 6

General discussion and conclusions

GENERAL DISCUSSION AND CONCLUSIONS

We investigated the use of various outcome measures for cognitive deficits in adult individuals with NF1 to aid future clinical trials to identify treatments for cognitive disorders. The aims defined in the general introduction were addressed in this thesis by administering neuropsychological tasks and non-invasive neurophysiological paradigms to unaffected controls, adult patients diagnosed with NF1, and adult patients diagnosed with MDD.

The main findings of this thesis are:
1) In contrast to previous findings in children with NF1, there were no significant differences in attention and motor learning tasks between the adult NF1 and control groups measured with neuropsychological tasks. Notably, the visuomotor integration task did show deficits in NF1: the NF1 group showed faster eye movement responses and reduced hand accuracy than the control group as measured with eye-hand coordination tasks. Overall, performance on the visual-spatial information processing task was not different between the NF1 and control groups as measured with the eye-tracking Visual Threshold task.
2) The NF1 group showed reduced motor cortical plasticity and reduced visual cortical plasticity versus controls, as indicated by their attenuation of the initial potentiated response after iTBS induction and by their non-potentiated response after VEP induction.
3) The neurophysiological outcome measure TMS showed similar results in motor cortical plasticity in severely depressed unmedicated patients compared to NF1 patients. Furthermore, the use of the neurophysiological outcome measures TMS and VEP are sensitive to many parameters causing high variability in our results. Nevertheless, in the absence of neuropsychological deviations on the attention tasks and performance on the motor skill learning task, we still found small but significant neurophysiological changes in NF1 as measured with the eye-hand coordination tasks, VEP paradigm, and TMS paradigms.

1) Cognitive functions in adult patients with NF1

The neurodevelopmental disorder NF1 is associated with cognitive deficits that have been extensively studied in children with NF1, but limited studies have focused on adults with NF1. Notably, cognitive deficits in adult patients with NF1 have been associated with reduced quality of life (1). The findings of this thesis indicate that, in an experimental setting, the performance on attention tasks as tested with neuropsychological tests is not different between the NF1 and control groups. These observations are unexpected given the attention deficits observed in children with NF1 (2). However, the findings are in agreement with previous studies in adult patients with NF1 (3,4). This could imply that the severity of attention deficits might diminish from childhood to adulthood due to developmental changes (3,5). In addition, the performance in motor learning tested with the neuropsychological motor skill learning task was not different between the NF1 and control groups, despite previous observations of disabilities in motor performance in children with NF1 (6–9). However, the motor skill learning task did show delayed reaction times, despite similar performance and accuracy in both groups. These slower reaction times could suggest a slower information processing overall in NF1 when learning a new skill (5,10,11). The neuropsychological motor learning task assesses multiple domains of how the nervous system manifests in actions such as skill acquisition and decision-making. However, the effect of motivation on our results could not be ruled out, which may be related to the increased fatigue or other associated complaints in NF1 patients in their daily life. In addition, it is possible that our studies suffer from ascertainment bias and that our NF1 participants do not reflect the entire adult NF1 population (discussed in the section 'Limitations and strengths of the studies' below).

The use of a neurophysiological approach may be a more sensitive measure of brain functioning compared to neuropsychological testing. By objectively recording eye movements in combination with hand movements to assess visuomotor integration, we showed small differences in the hand accuracy and eye movement responses between the NF1 and control groups. We observed faster eye movement responses in the NF1 group than in the control group to visual stimuli, which may be associated with hyperreactivity to sensory input (12). It can, therefore, be hypothesized that neurophysiological changes can be identified in the absence of overt changes on neuropsychological tests. In

contrast to our finding of delayed reaction times on the motor learning task, the eye-hand coordination tasks showed no deficits in hand latencies in both groups indicating correct preparation and onset of the hand movements in NF1. Basic functions of the visual and motor system were measured in the eye-hand coordination tasks, while the motor skill learning task measured the ability to acquire new motor skills.

NF1 adults showed similar performance on attention and motor learning in a neuropsychological experimental design, despite potential problems in these cognitive domains seen in the overall NF1 population. Small neurophysiological deficits in the hand accuracy and eye-movement responses were present in the eye-hand coordination tasks, which suggests that deficits in visuomotor integration may be of significance in the pathophysiology of NF1.

2) Cortical plasticity in adult patients with NF1

Plasticity changes in the functional strength of the synaptic connections are hypothesized to represent learning and memory processes (13). In this thesis, adult patients with NF1 showed reduced cortical plasticity in the motor and visual cortex using TMS and VEP paradigms compared to the control group. These findings support our hypotheses based on preclinical findings, in which increased GABA neurotransmission and decreased glutamatergic synaptic plasticity have been described in animal models of NF1 (14–17). In addition, cortical plasticity as assessed with TMS and VEP paradigms may be associated with the enhancement of attention, or improved executive, planning, and motor functions. It has to be stressed, however, that high variability was noted in our studies. Nevertheless, these findings underline the use of neurophysiological outcome measures to study the pathophysiology of cognitive deficits in adult patients with NF1. Interestingly, our findings of reduced VEP plasticity are similar to previous studies in other clinical populations, including depression, schizophrenia, and bipolar disorder (18–20). This may suggest that cortical plasticity deficits can be observed in many brain disorders and that these outcome measures are widely applicable.

Our findings provided no evidence for changes in motor cortical inhibition in NF1, as the TMS measures SICI and CSP did not differ between the NF1 group and the control group. This was surprising, based on the inhibitory deficits observed in preclinical and

clinical studies in NF1 (16,21–23). The paradigms SICI and CSP are robust measures for determining cortical inhibition and are suggested to be sensitive to changes in GABA-mediated inhibition (24,25). More specifically, $GABA_A$ and $GABA_B$ receptor agonists can increase the response on the SICI and the CSP paradigms, respectively (26,27). Hence, we expected deficits in the response to SICI and CSP in the NF1 group (28,29). Small deficits in the balance of excitation and inhibition in the primary motor cortex of NF1 patients may not have been detected, although Mainberger et al. (21) did show a trend towards more cortical inhibition in 10 NF1 patients compared to neurotypical controls as tested with the SICI. The SICI and CSP might also measure processes interacting with the inhibitory system or processes from independent inhibitory pathways that could compensate or mask potential GABAergic inhibitory deficits.

Motor and visual cortical plasticity were reduced in patients with NF1, which could underlie the deficits in cognitive functioning observed in the adult NF1 population. No evidence was found for altered motor cortical inhibition in patients with NF1, which could suggest there are compensatory mechanisms to balance excitation and inhibition changes in the primary motor cortex.

3) The use of neurophysiological outcome measures

Non-invasive neurophysiological measures can assess the functioning of the central and peripheral nervous systems through the recording of spontaneous or stimulated bioelectrical activity, and document changes in cognition and learning (30). The faster eye movement responses measured with the eye-tracking task could be a specific feature of NF1, as eye movement responses were not altered in patients with Parkinson's Disease or Alzheimer's Disease (31–34). Notably, faster eye movement responses have been reported in patients with autism, which is a common comorbidity of NF1 (12). As mentioned before, the lack of effects in NF1 patients on the TMS paradigms SICI and CSP were not in line with our hypotheses based on animal studies (15–17,35). We did show similar results on these TMS paradigms in severely depressed unmedicated patients, although we expected reduced cortical inhibition based on the underlying mechanism of MDD (24). In our MDD study, patients showed reduced motor cortical plasticity upon iTBS induction and no alterations in cortical inhibition. These findings partly support

our hypotheses based on the preclinical studies in MDD such as Crestani et al. (36). They studied $GABA_A$ dysfunction related to MDD traits in γ2+/– mice and observed inhibitory deficits that could consequently affect synaptic strengthening. Although MDD differs from NF1 in many ways, including the disease severity and genetic background, both patient groups frequently show complex interactions between cognitive deficits and psychiatric symptoms. Therefore, reduced motor cortical plasticity measured with TMS paradigms appears to be an outcome measure that can potentially be used for assessing multiple neurocognitive disorders.

Importantly, to understand the meaning of the neurophysiological changes observed in patients, it is essential to translate the preclinical neurophysiological findings to the human brain. The neurophysiological paradigms iTBS and VEP used in this thesis show a high resemblance to the long-term potentiation plasticity protocols that have been used to study ex vivo plasticity in Nf1 mouse models (14,17,37), and the measurement of visual cortical plasticity of the responses to repeated visual stimulation in awake mice (38), respectively. The underlying cause of cognitive deficits may be due to deficits in the long-lasting strengthening ('potentiation') of synapses, which is the most studied form of synaptic plasticity. The potentiation of cortical responses following high-frequency stimulation resembles the properties of synaptic long-term potentiation including the dependency of the functioning of the NMDA receptor (39,40). Although findings of impaired cortical plasticity in the human brain as measured with the iTBS and VEP paradigm are consistent with findings of reduced long-term potentiation in animals, there is a lack of evidence that the cortical potentiation is due to synaptic changes. Furthermore, it should be mentioned that the between subject variability of the TMS and VEP findings was high. Previous studies using the TMS and VEP paradigms have suggested several factors for within and between subject variability of the motor and visual evoked potentials (41). In our studies, we carefully considered these factors, such as the study design. The study design of the TMS studies included the use of a neuronavigation system to ensure that the 'hotspot' was targeted during the whole experiment (42,43). In addition, we aimed at a number between 20-30 trials per time point during pre-and post-induction to establish reliable measurements (44). In all studies, the distribution of the data was carefully taken into account in parametric or

non-parametric statistical analyses (41). We accounted for potential confounders such as hormonal circadian cycles (i.e. consistent time of day) and sleepiness. Nevertheless, between subject variability could be further reduced by optimizing the study design, and the consensus for solutions to the high variability in response to non-invasive brain stimulation is still in development (41). Previous studies have shown that a combination of neuroimaging and non-invasive brain stimulation techniques could control for the within and between subject variability or could increase the response upon induction. This includes a combination of TMS and transcranial electrical stimulation (45,46) or a combination of TMS and EEG (47). The combination of techniques could control for the fluctuations in neuronal excitability dependent on brain oscillations. Real-time information of the brain excitability by EEG could be used to enhance plasticity induction with TMS by stimulating always the low-excitability state of brain oscillations (48).

Despite the lack of neuropsychological changes, we still found small significant neurophysiological differences between the NF1 and control groups measured with the eye-hand coordination tasks, VEP paradigm, and TMS paradigms. As mentioned before, these observations lead to the hypothesis that small neurophysiological changes could be detected in the absence of overt neuropsychological dysfunction. Additionally, we could speculate that neurophysiological deficits may even predict future neuropsychological deficits, but future studies should investigate this further in a follow-up study by using a combination of neurophysiological and neuropsychological assessments.

The use of the neurophysiological measure TMS provided similar results in NF1 and MDD, indicating that the use of neurophysiological outcome measures can improve the understanding of the pathology and physiology of cognitive deficits in various disorders. A combination of outcomes measures should be considered to account for the within and between subject variability.

Limitations and strengths of the studies

Our findings have to be interpreted with caution due to several limitations. There is a complex interaction of many factors that can affect the performance on neuropsychological and neurophysiological tasks. These factors include the higher-order cognitive functions as attention, sensory perception, or intelligence, but also factors such as fatigue, motivation, or anxiety (30). In addition, potential cognitive deficits in daily life in the NF1 and MDD population were not assessed in this thesis due to the experimental settings, although education level of the participants was obtained. in the NF1-studies.

We acknowledge a potential overrepresentation of highly motivated or less severe patients in our NF1 patient groups. Interestingly, the variability in cognitive ability in NF1 children is similar to the healthy general population, although the overall IQ distribution is shifted leftward (49). There is an exception for NF1 patients with a chromosomal microdeletion who showed a more severe cognitive phenotype (49). In our studies, additional genetic testing was not performed to confirm the clinical diagnosis of NF1. Additional subgroup analyses based on the severity of the cognitive abilities were also not performed due to the relatively small sample sizes. It has to be stressed that educational attainment was significantly lower in NF1 patients in our studies, although no significant correlations were found with the outcome measures. For the MDD group, the sample size was limited mainly due to the patient's motivation and admission rate. These relatively small sample sizes could have affected the statistical power. Nevertheless, the MDD patient group was a relatively homogeneous group of only inpatients with severe symptoms, who were admitted to the hospital for an extended stay for treatment of a major depressive disorder.

The main limitations of the TMS technique were the high variability of the MEP amplitude and finding the optimal individual stimulus intensity. The average of the MEP amplitudes over trials was calculated to obtain a reliable outcome. The optimal individual stimulus intensity was not reached for all participants, resulting in overall lower MEP amplitude. These lower MEP sizes may also reflect reduced excitability in the motor cortex (50,51). Another issue is the level of activity of a brain region before induction. Silvanto et al. (52) showed that the initial activity of a neural population can affect TMS aftereffects.

However, the strengths of the studies in this thesis should also be emphasized. All studies followed a case-control study design. The potential confounders as age (in the range of 18-55 years), gender, and sleepiness did not influence our outcome measures. Furthermore, the patient groups were homogenous, free of psychoactive drugs (medical and non-medical), and without clinically diagnosed comorbidities. Despite the rare nature of the NF1 disease, we included a sample size of 20-30 subjects per group, which previous studies have suggested to be able to observe a reliable ±20% difference between groups in TMS responses (53,54). Moreover, the procedures were standardized and feasible, all subjects were able to complete the procedures. Finally, the use of multiple approaches to investigate cognitive deficits in the human brain provide us with more knowledge of the physiology of cognitive deficits in NF1 and MDD to improve future study designs.

Future perspectives

This thesis demonstrated useful indications of the use of outcome measures to detect cognitive deficits in adult patients with NF1, but future research is suggested to draw stronger conclusions. Based on our findings, we formulate recommendations to investigate outcome measures related to cognitive functions below.

As mentioned before, our outcome measures are highly dependent on and influenced by many variables, which should be carefully taken into account. Fatigue is an important issue in neurocognitive disorders and should be addressed before and during experiments. In NF1 adults, fatigue affects the daily life and coping skills of patients (55). In addition, motivation could be an important issue in the performance of the tasks. Motivation is a subject's willingness to make an effort to receive a reward (56). Future studies may benefit from the inclusion of a motivational feature, such as the implementation of a reward during the experiment. Furthermore, the implementation of an additional test during the non-invasive stimulation paradigms, such as reading out numbers or pressing a key with changing colors (57,58), could improve the subject's attention. To account for the experimental setting, measurements should be performed repeatedly over multiple days, or outcome measures should be implemented in daily life, such as eye-tracking

in everyday environment. Medication use is also a factor that could influence the outcome measures, and future studies on the underlying neurophysiology of cognitive functions should include only medication-free patients. To account for statistical power issues, the sample size could be increased by collaboration with other (international) institutes. Consequently, a larger sample size makes it possible to perform additional subgroup analyses. These subgroup analyses of neurophysiological outcomes could be performed based on level of education, intelligence score, or the severity of cognitive deficits assessed with the neuropsychological tasks. In addition, cognitive functioning in daily life could be assessed before inclusion with self-ratings of cognitive deficits (59), or a more general questionnaire to assess adaptive functioning and emotional, social, and behavioral problems in the general population, e.g. the Adult Self-Report (ASR) (60). Furthermore, additional subgroup analyses could be performed based on age, as previous studies have shown that cortical inhibition is similarly affected in older subjects as in subjects with late-life depression (61). In addition, it would be interesting to consider the maturation process in future prospective longitudinal studies to NF1. It has been argued that treatment should occur at an early age due to developmental processes, but a previous study on neurodevelopmental disorders suggested potential reversal of the physiological and behavioral deficits into adulthood by pharmacological treatments or genetic modifications (62).

It could be argued to improve several methodical issues of the TMS and VEP paradigms in future studies. Using an optimal individual TMS stimulus intensity based upon an input-output curve may be more accurate (63). Moreover, to validate our findings, the optimized TMS protocols should be combined with neuroimaging methods to control for high variability. To decrease this variability in the VEP paradigm, the number of electrodes could be increased to confirm visual cortical plasticity deficits. In addition, since all the outcome measures were non-invasive, future studies should further investigate the test-retest reliability of the outcome measures (see also Table 1).

We made a simplified overview of highlighted findings of this thesis to discuss the considerations for implementation of an outcome measure in future clinical trials (Table 1). All studies compared the patient with the control group and used a repeated

measures design with a within-between interaction effect. We calculated the effect sizes and p-values. In addition, the test-retest reliability based on literature and estimated practical feasibility were taken into account (Table 1). Practical feasibility describes how easy these tests are implemented in a clinical setting, for instance as a clinical outcome measure. This includes time duration, completion rates of the experiments, and the sensitiveness of outcome measures to the effects of the intervention. Based on previous studies, the test-retest reliability of the sustained attention task used in chapter 2 varies between 0.70 and 0.85 (64). We estimated a moderate feasibility due to the time duration and effect of motivation on the outcome measure. The outcome measure eye latency during the visuomotor tasks pro- and memory-tapping used in chapter 3 has proven to have test-retest reliability of 0.64 and 0.95, respectively (65). Additionally, the estimated large feasibility is due to the short time duration of the measurements of only 5 minutes, and it is easy to perform. Although the reliability of the VEP paradigm used in chapter 4 is unknown, findings have been replicated in previous studies (57,58). The VEP paradigm has large feasibility due to the low burden of the VEP recording and the visual presentation of checkerboard reversals without side effects, which positively effect recruitment and completion rate. The effect of attention is, however, high on cortical plasticity and affects the feasibility of the paradigms used in chapters 4 and 5. Moreover, the burden of the TMS measures is higher than the VEP paradigm due to possible side effects. Additionally, TMS measures show a high intra- and inter variability, and the test-retest reliability is between 0.19-0.56 of the iTBS paradigm in controls (66,67) (see Table 1 for an overview).

Overall, the recommended outcome measure highly depends on the specifics of the research question in future research. Importantly, knowledge of the underlying mechanism of cognitive deficits in cognitive disorders can improve future study designs by including both neuropsychological and neurophysiological outcome measures, which may benefit the treatment development in cognitive disorders. The implementation of these outcome measures could show the effect of treatment on the human brain and provide more information about the optimal duration of treatment. Notably, to compare the neurophysiological outcomes across different neurocognitive disorders or brain regions, consensus should be reached concerning the protocols and statistics, which should be similar as much as possible across studies.

Table 1. Considerations for implementation of an outcome measure in future clinical trials, including our calculated effect sizes, p-values of the within-between interaction effect, test-retest reliability, and estimated feasibility in a clinical setting.

Difference between patient and control groups	Effect size η^2 [a]	p-value [b]	Test-retest reliability based on literature [c]	Estimated practical feasibility [d]	Factors affecting practical feasibility [d]
Ch2: No difference in reaction time on the sustained attention task over five periods	0.01	0.34	0.70-0.85	**	- motivation - long time duration + low burden
Ch3: Difference in eye-latency on the pro-tapping task	0.36	<0.001	0.64	***	+ easy to perform + short time duration
Ch4: Difference in peak amplitude P1 of VEP after visual stimulation	0.04	0.07	-	**	- attention + no side effects
Ch5.1: Difference in peak-to-peak amplitude of MEP after transcranial magnetic stimulation (i.e. iTBS) – NF1 study	0.16 [e]	0.003 [e]	0.19-0.56	*	- attention - side effects
Ch5.2: Difference in peak-to-peak amplitude of MEP after transcranial magnetic stimulation (i.e. iTBS) – MDD study	0.1	0.05	0.19-0.56	*	- attention - side effects

[a] value between 0 and 1: small effect = 0.01, medium effect = 0.06, large effect = 0.14 (68)

[b] repeated measures design with a within-between interaction; significantly different between patients and controls: p-value <.05

[c] value between 0 and 1: fair = 0.4 to 0.59, good = 0.60 to 0.74, excellent > 0.75 (69)

[d] estimated feasibility: * = small, ** = moderate, *** = large. Practical feasibility includes recruitment, time duration, completion rates of the experiments, and the sensitiveness of outcome measures to the effects of the intervention.

[e] no significant interaction effect, group effect is presented.

Clinical relevance

As demonstrated in this thesis, the non-invasive neurophysiological measures as eye-and hand responses, VEP, and TMS, can be used to study the central and peripheral nervous system underlying cognitive functioning. These measures contribute to showing subtle features of the human sensory cortex, which potentially could be used to monitor or treat clinically relevant deficits in functioning related to the sensory cortex. In the future, the identification of the neurophysiological characteristics of NF1 may be used as predictors for treatment outcomes. In a previous study, this approach has been shown in schizophrenia (70). In addition, in a clinical trial within the ENCORE expertise center in the Erasmus University Medical Center with NF1 adolescents, the TMS measures have been added in a study design as secondary outcome measures to assess the effect of lamotrigine on cortical plasticity; results are not available yet.

A combination of the neurophysiological measures TMS and EEG could provide optimization in the methodology to implement in research, and in the future in clinical diagnosis (71). In addition, a combination of these neurophysiological measures with various neuroimaging techniques is a potential research and diagnostic tool to study the cortical processes underlying deficits in cognitive functioning. Overall, the (combination of) non-invasive neurophysiological measures has clinical potential to monitor and track changes in inhibition and plasticity in different cortical areas by TMS treatment, pharmacotherapy, and (neuro)rehabilitation. Interestingly, Gevins et al. (72) showed that sensitivity and specificity of measuring drug effects on cognitive functions were enhanced when including both neurophysiological and neuropsychological outcomes.

Our findings are specific for adult NF1 patients, while the majority of studies to cognitive functioning have focused on children (73). A natural history should provide more insight into the development of cognitive deficits in the transition from childhood to adulthood in NF1 patients.

CONCLUSION

Cognitive deficits can strongly affect the quality of life in NF1 (74). This thesis provides more knowledge of cognitive deficits of adult NF1 patients in an experimental setting. Since no treatment has yet been identified to improve the cognitive deficits in NF1, neurophysiological measures could provide additional information and more robust characteristics than the use of neuropsychological measures alone.

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Chapter 7

Appendices

SUMMARY

Cognitive functions include attention, learning and memory, perceptual-motor function, executive function, language, and social cognition. Deficits in cognitive functioning can strongly influence the quality of life by affecting socioeconomic status, self-esteem, or mental health. The neurodevelopmental disorder neurofibromatosis type 1 (NF1) affects one in every 2000 persons and is characterized by several cognitive deficits, apart from somatic symptoms. These cognitive deficits in NF1 have been studied extensively in children with NF1, but there are limited studies on cognitive deficits in adults with NF1. The aims of this thesis were:

1) to determine the cognitive deficits in adult patients with NF1 using neuropsychological and neurophysiological outcomes, including the cognitive functions attention, motor learning, and visuomotor integration,

2) to investigate potential deficits in motor and visual cortical plasticity related to the cognitive deficits, as it is commonly accepted that synaptic plasticity is essential for learning and memory, and

3) to extend our knowledge of the use of the neurophysiological measures as outcome measures by examining their variability and specificity by including another distinct clinical population with cognitive deficits: major depressive disorder (MDD).

All subjects were in the range of 18-55 years old, free of any psychoactive drugs, and without clinically diagnosed comorbidities. All subjects completed questionnaires to general health before inclusion.

In chapter 2, the cognitive abilities attention, i.e. alertness and sustained attention, and motor skill learning were assessed in adult patients with NF1 and neurotypical controls with neuropsychological tasks. It is important to stress that our measurements were performed in an experimental setting, which does not provide information concerning these potential problems in the daily life of adults with NF1. We observed similar performance in alertness, sustained attention, and motor skill learning tasks in the NF1 group and the control group, although the control group had a faster reaction time during the motor learning task.

Neurophysiological measures may add additional information to the neuropsychological outcomes to indicate the cognitive deficits in NF1. In chapter 3, we objectively recorded eye and hand movement responses to assess deficits in visuomotor integration. We observed similar performance on the visual-spatial information processing task in the NF1 group versus the control group. However, visuomotor functioning was affected in the NF1 group by significantly faster eye movement responses and reduced hand accuracy as measured with eye-hand coordination tasks. To study the functioning of the visual system further, we investigated the cortical plasticity of the visual cortex in chapter 4. We compared the potentiation of visual evoked potentials (VEPs) between the NF1 and control group by studying VEPs induced by checkerboard reversals. Interestingly, both groups showed similar VEP latencies, which suggests intact primary visual processing. In contrast to the control group, the VEP amplitudes were not potentiated after modulation in the NF1 group.

In chapter 5, we elaborate on our knowledge of plasticity deficits in NF1 by studying a different brain region, the primary motor cortex, with the use of transcranial magnetic stimulation (TMS) (Chapter 5.1). Furthermore, the TMS outcome measures were used in a distinct clinical population with cognitive deficits: in patients with MDD (Chapter 5.2). We measured the inhibition and potentiation of the amplitude of motor evoked potentials (MEPs). The inhibitory measures short-interval intracortical inhibition (SICI) and cortical silent period (CSP) showed no alterations in cortical inhibition in the primary motor cortex between the NF1, the MDD, and the control groups. We did not observe potentiation of the MEPs upon intermittent theta-burst stimulation (iTBS) in the NF1 and the MDD groups, in contrast to the control groups.

In chapter 6, the aims of this thesis were discussed based on our main findings.
1) Despite potential problems in the cognitive functions attention and motor learning seen in the NF1 population, our findings overall showed similar performance in NF1 adults versus controls in an experimental design. Small neurophysiological deficits in the hand accuracy and eye-movement responses in NF1 adults suggested that deficits in visuomotor integration may be of significance in the pathophysiology of cognitive dysfunction in adult patients with NF1. These findings may be clinically relevant to

improve therapeutic treatment for adults with NF1.

2) We concluded that reduced visual and motor cortical plasticity could underlie deficits in cognitive functioning as measured with VEP and TMS in patients with NF1. The inhibitory measures SICI and CSP showed no alterations in patients with NF1, suggesting other compensatory mechanisms to balance excitation and inhibition.

3) We observed considerable variability in the outcome measures TMS and VEP, which are known to be sensitive to many parameters. Nevertheless, we concluded that the (combination of) non-invasive neurophysiological measures may have clinical potential to monitor and track changes in inhibition and plasticity in different cortical areas by pharmacotherapy or neurorehabilitation, especially in patients with cognitive deficits.

SAMENVATTING

Cognitieve functies zijn functies zoals aandacht, leren en geheugen, taalgebruik, sociaal-cognitief vermogen, en perceptuele en motorische vaardigheden. Cognitieve problemen kunnen van grote invloed zijn op de kwaliteit van leven. Neurofibromatose type 1 (NF1) is een ontwikkelingsstoornis met een geboorte-incidentie van ongeveer 1:2000 personen en wordt geassocieerd met lichamelijke problemen. Daarnaast komen cognitieve problemen vaak voor. Het meeste onderzoek naar cognitieve problemen in NF1 is gedaan in kinderen, maar hierover is minder bekend bij volwassenen met NF1. De doelen van dit proefschrift waren:
1) Het vaststellen van de cognitieve problemen bij volwassenen met NF1 door gebruik te maken van neuropsychologische en neurofysiologische uitkomstmaten. Wij onderzochten de cognitieve functies aandacht, het leren van een motorische vaardigheid en visuele motorische integratie.
2) Het onderzoeken van corticale plasticiteit in de motorische en visuele cortex welke mogelijk gerelateerd is aan cognitieve functies, aangezien synaptische plasticiteit theoretisch essentieel lijkt te zijn voor leren en geheugen.
3) Bepalen welke rol variabiliteit en specificiteit spelen in het gebruik van neurofysiologische uitkomstmaten voor klinische (behandel)studies. Hierbij hebben we, naast NF1, een andere klinische groep met cognitieve problemen onderzocht, namelijk patiënten met een depressie (major depressive disorder, MDD).
Alle proefpersonen waren tussen de 18 en 55 jaar oud, gebruikten geen psychofarmaca en hadden geen klinisch gediagnosticeerde comorbiditeiten. Alle proefpersonen hadden voorafgaand aan het onderzoek een vragenlijst ingevuld over de algemene gezondheid.

In hoofdstuk 2 zijn de cognitieve functies aandacht (in het bijzonder alertheid en volgehouden aandacht) en het aanleren van een motorische vaardigheid onderzocht in volwassenen met of zonder NF1 met behulp van neuropsychologische taken. Het is belangrijk om te vermelden dat onze observaties gedaan zijn in een experimentele omgeving. Daarom kunnen wij geen informatie geven met betrekking tot mogelijke cognitieve problemen in het dagelijkse leven van volwassenen met NF1. We observeerden geen verschil tussen de groepen in de uitvoering van de taken betreffende

alertheid, volgehouden aandacht en het aanleren van een motorische vaardigheid. Echter, de controle groep vertoonde een snellere reactietijd tijdens het aanleren van de motorische vaardigheid.

Neurofysiologische maten kunnen mogelijk voor extra informatie zorgen bij het indiceren van cognitieve problemen in NF1. In hoofdstuk 3 zijn oog- en handbewegingen objectief gemeten om mogelijke problemen in visuele motorische integratie te onderzoeken. We vonden geen verschil tussen de groepen in de uitvoering van de taken betreffende het verwerken van visuele ruimtelijke informatie. Echter, het verwerken van visuele motorische informatie was aangedaan in de NF1 groep. Dit werd gekenmerkt door snellere oogbewegingen en verminderde accuraatheid in de handbewegingen. We onderzochten het visuele systeem uitgebreider in hoofdstuk 4, waarin we plasticiteit in de visuele cortex onderzochten. We hebben in deze studie de toename van amplitudes van visuele opgewekte potentialen (VEPs) bestudeerd en vergeleken tussen de groepen. De VEPs werden opgewekt door een wisselend schaakbordpatroon. Beide groepen toonden geen verschil in de tijd tussen stimulus en de visueel opgewekte potentiaal (VEP). Dit suggereert een intacte visuele informatie verwerking. Maar in tegenstelling tot de controle groep, waren de VEP amplitudes in de NF1 groep niet toegenomen na herhaalde visuele stimulatie met dit schaakbordpatroon.

In hoofdstuk 5 verdiepten wij ons in de mogelijke plasticiteitsproblemen in NF1 door een ander hersengebied te bestuderen, namelijk de motorische cortex. Hiervoor maakten wij gebruik van transcraniële magnetische stimulatie (TMS) (Hoofdstuk 5.1). Bovendien werden TMS uitkomstmaten toegepast in een andere klinische groep met cognitieve problemen: patiënten met MDD (Hoofstuk 5.2). We maten de af- en toename van amplitudes van de motorische opgewekte potentialen (MEPs) in de hand. De paradigma's die de afname van MEP amplitudes meten, de short-interval intracortical inhibition (SICI) en de cortical silent period (CSP), toonden geen verschillen tussen de NF1, de MDD, en de controle groepen. In de TMS studies zagen we geen toename van de MEP amplitudes na intermitterende thetaburst stimulatie (iTBS) in de NF1 groep en in de MDD groep in tegenstelling tot de controle groepen.

In hoofdstuk 6 zijn de doelen van dit proefschrift bediscussieerd gebaseerd op onze voornaamste bevindingen:

1) Ondanks mogelijke cognitieve problemen in aandacht en het aanleren van een motorische vaardigheid in het dagelijkse leven van volwassenen met NF1, observeerden wij geen verschillen tussen de groepen in een experimentele omgeving. Kleine neurofysiologische afwijkingen in oog- en handbewegingen in volwassenen met NF1 suggereerden dat problemen in de visuele motorische integratie van belang zouden kunnen zijn in de pathofysiologie van cognitieve problemen in volwassenen met NF1. Deze bevindingen zijn klinisch relevant om behandeling voor volwassenen met NF1 te verbeteren.

2) We concludeerden dat verminderde plasticiteit in de cortex, zoals gemeten met de VEP en TMS paradigma's, ten grondslag zou kunnen liggen aan cognitieve problemen in volwassenen met NF1. De uitkomsten van de SICI en CSP paradigma's vertoonden daarentegen geen afwijkingen in volwassenen met NF1. Dit zou kunnen suggereren dat er andere compenserende mechanismen aanwezig zijn om de balans van corticale excitatie en inhibitie te behouden.

3) De uitkomstmaten van TMS en VEP paradigma's waren gevoelig voor veel parameters, wat resulteerde in variabiliteit van de metingen. Desalniettemin, de combinatie van niet-invasieve neurofysiologische metingen kan mogelijk klinische potentie hebben om veranderingen in inhibitie en plasticiteit te monitoren in verschillende corticale hersengebieden tijdens behandeling met farmacotherapie of (neuro)revalidatie, in het bijzonder in patiënten met cognitieve problemen.

LIST OF PUBLICATIONS

Castricum, J., Tulen, J.H.M., Taal, W., Ottenhoff, M. J., Kushner, S. A., & Elgersma, Y. (2020). Motor cortical excitability and plasticity in patients with neurofibromatosis type 1. Clinical Neurophysiology, 131(11): 2673-2681.

Castricum, J., Tulen, J.H.M., Taal, W., Rietman, A.B., Elgersma, Y. (2022). Attention and motor learning in adult patients with neurofibromatosis type 1. Journal of Attention Disorders, 26(4): 563–572.

Castricum. J. Birkenhager, T.K., Kushner, S.A., Elgersma, Y., Tulen, J.H.M. (2022). Cortical inhibition and plasticity in patients with major depressive disorder. Frontiers in Psychiatry, 13: 777422.

Castricum. J., Tulen, J.H.M., Heuvelmans, A.M., Geleijnse, G., Straver, D.C.G., Taal, W., Kushner, S.A., Elgersma, Y. (2022). Plasticity of visual evoked potentials in patients with neurofibromatosis type 1. Clinical Neurophysiology, under review.

CURRICULUM VITAE

Jesminne Castricum was born on February 12th, 1992 in Heemskerk, the Netherlands. In 2010, after graduating from high school at Bonhoeffer College in Castricum, she decided to follow a bachelor in Psychobiology at the University of Amsterdam. She developed her interest in clinical neurophysiology and continued studying in the master Cognitive Neurobiology and Clinical Neurophysiology. During her bachelor, she was an intern at the department of neuropsychiatry at the Vrije Universiteit Amsterdam. She worked on the study of functional correlates of response inhibition using functional MRI in patients with obsessive-compulsive disorder and Parkinson's disease. During the 2 years of master, she studied alpha laterization in children with ADHD using EEG at the Donders Institute for Brain, Cognition and Behavior in Nijmegen. Her graduation thesis was on the effect of light color on sleep onset latency while working at the Sleep & Cognition group at Netherlands Institute for Neuroscience in Amsterdam. In the fall of 2015, she obtained her master's degree and in the beginning of 2016 she started working as a research assistant at the department of Neuroscience at the Erasmus Medical Center in Rotterdam. This eventually led to the start of her PhD in May 2017 at the department of Neuroscience (she continued in the department of Clinical Genetics from January 2021) and the department of Psychiatry, under the supervision of professor dr. Y. Elgersma and dr. J.H.M. Tulen. Her main research activity is presented in this thesis.

PH.D. PORTFOLIO

Name Ph.D. student	Jesminne Castricum
Ph.D. period	May 2017 – Okt 2021
Department	Clinical Genetics, Psychiatry – Erasmus Medical Center (EMC)
Promotor	Prof. dr. Y. Elgersma
Co-Promotor	Dr. J.H.M. Tulen
Research School	Research School of Neuroscience, ONWAR

General academic skills	Year	ECTS
Basis cursus regelgeving en organisatie voor klinische onderzoekers (BROK)	2016	1.5
Literature course, EMC	2016	0.6
Integrity in Science, EMC	2017	0.3
Basistraining OpenClinica, EMC	2018	0.3
Introductory course for PhD-students, ONWAR	2018	0.9
PhD day, EMC	2017-2019	0.6
Re-registration BROK	2020	0.3
Research Skills and Courses		
Quantitative Methods, ONWAR	2017	3.6
Donders Brain Stimulation Tool-kit, Donders Institute	2017	1.0
Programming in Matlab, ONWAR	2018	1.4
Biomedical English Writing and Communication, EMC	2019	3.0
Psychopharmacology, NIHES	2021	1.5
Neural interface technology and human application, ONWAR (CEN-Utrecht)	2021	1.1

Didactic skills	Year	ECTS
Basis Kwalificatie Onderwijs, Teach the Teacher	2017	0.5
Omgaan met groepen	2018	0.2
Conferences and seminars		
12th and 13th NF1 meeting Leuven	2017/2019	2.0
ONWAR annual meeting	2017-2019	2.0
Third Dutch Neurodevelopmental Disorders Day	2018	1.0
TN2 Conference Amsterdam Innovation in Psychiatry, Neurostimulation & -inflammation	2018	1.0
Symposium NFVN, Neurofibromatose Verening Nederland	2018/2019	1.0
3rd International Brain Stimulation conference in Vancouver	2019	1.0
Neuropsychophysiology Webinar Series	2020	1.0
European Neurofibromatosis Meeting 2020 Rotterdam	2020	1.0
Sophia Research Days	2021	1.0
Bio-Medical Engineering Conference 2021	2021	1.0
Teaching activities		
Workshop Master of Neuroscience	2017-2021	2.0
Workshop Bachelor Geneeskunde	2017-2020	2.0
Supervising students (master/bachelor)	2017-2021	6.0
Workshop Master of Technical Medicine, TU Delft	2020	1.0

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