

Motor cortical excitability and plasticity in patients with neurofibromatosis type 1



Jesminne Castricum^{a,b,c}, Joke H.M. Tulen^{b,c}, Walter Taal^{c,d}, Myrthe J. Ottenhoff^c, Steven A. Kushner^{b,c}, Ype Elgersma^{a,c,*}

^a Department of Neuroscience, Erasmus Medical Center, Rotterdam, the Netherlands

^b Department of Psychiatry, Erasmus Medical Center, Rotterdam, the Netherlands

^c ENCORE Expertise Center for Neurodevelopmental Disorders, Erasmus Medical Center, Rotterdam, the Netherlands

^d Department of Neurology, Erasmus Medical Center, Rotterdam, the Netherlands

ARTICLE INFO

Article history:

Accepted 11 August 2020

Available online 11 September 2020

Keywords:

Transcranial magnetic stimulation

Neurofibromatosis type 1

Cortical plasticity

Motor evoked potentials

Intracortical inhibition

HIGHLIGHTS

- Single-pulse TMS resulted in lower motor evoked potential amplitudes in NF1 patients than controls.
- Intermittent theta burst stimulation (iTBS) increased cortical excitability in both NF1 patients and controls.
- After iTBS, NF1 patients showed an attenuation of the initial potentiated response that might be used as an outcome measure.

ABSTRACT

Objective: 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.

Methods: 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).

Results: 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 ($F(1, 41) = 7.552, p = 0.009$).

Conclusions: 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.

Significance: These findings have potential utility as neurophysiological outcome measures for intervention studies to treat cognitive deficits associated with NF1.

© 2020 International Federation of Clinical Neurophysiology. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Neurofibromatosis type 1 (NF1) is an autosomal dominant genetic disorder with a birth incidence of approximately 1:2000 (Uusitalo et al., 2015). 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 (Ferner, 2007). Many individuals with NF1 suffer from cognitive deficits which adversely impacts their quality of life (Krab et al., 2008a, 2009; Ottenhoff et al., 2020). These deficits include attention, visual-spatial abilities, motor learning, executive functioning, and intelligence (Hyman et al., 2005; Krab et al., 2008a; Ottenhoff et al., 2020). Loss-of-function of neurofibromin is well established to result in hyperactivity of the *RAS* signaling pathway.

* Corresponding author at: Department of Neuroscience, Erasmus Medical Center, Rotterdam, the Netherlands.

E-mail address: y.elgersma@erasmusmc.nl (Y. Elgersma).

However, despite several clinical trials aimed at improving cognitive deficits in NF1 through RAS reducing treatments, no effective treatment has yet been established (Krab et al., 2008b; Van der Vaart et al., 2013; Payne et al., 2016).

Studies of the cellular mechanism underlying the cognitive deficits associated with NF1 have largely focused on animal models of NF1 (Costa et al., 2002; Shilyansky et al., 2010; Omrani et al., 2015). Based on the animal studies, reduced NF1 activity has been shown to result in abnormal hyperactivation of RAS signaling in inhibitory interneurons (Costa et al., 2002; Shilyansky et al., 2010; Omrani et al., 2015). RAS hyperactivation leads to enhanced inhibition through abnormally high gamma-aminobutyric acid (GABA) neurotransmission, thereby causing a reduction of glutamatergic synaptic plasticity (Costa et al., 2002; Cui et al., 2008; Shilyansky et al., 2010; Omrani et al., 2015). Furthermore, Omrani et al. (2015) 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 (Omrani et al., 2015).

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 (Violante et al., 2013, 2016). 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 (Sheikh and Martin, 1998), 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 (Kujirai et al., 1993; Huang et al., 2005), were used in human NF1 studies (Mainberger et al., 2013; Zimmerman et al., 2015). 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 (Barker et al., 1985). 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 (Mainberger et al., 2013; Zimmerman et al., 2015). One study showed a trend towards more cortical inhibition in NF1 patients compared to neurotypical controls (Mainberger et al., 2013). Furthermore, reduced task-related intracortical inhibition was observed during motor learning in NF1 patients (Zimmerman et al., 2015). Additionally, reduced cortical plasticity was shown in the motor cortex of NF1 patients using the paired associative stimulation (PAS) repetitive TMS paradigm (Mainberger et al., 2013).

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 (Bajbouj et al., 2006; Levinson et al., 2010). 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 (Siebner et al., 1998; Di Lazzaro et al., 2005). In addition, a pharmacological study using a GABA reuptake inhibitor confirmed the role of GABA_B receptors in CSP modulation (Werhahn et al., 1999). 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 (Costa et al., 2002; Oberman et al., 2011; Omrani et al., 2015). 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 (Huang et al., 2007). 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 (Wischniewski and Schutter, 2015).

Notably, recent studies have also pointed out the high inter-subject variability in response to TMS paradigms (López-Alonso et al., 2014; Huang et al., 2017). 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 (Ridding and Ziemann, 2010; Huang et al., 2017). 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.

2. Materials and methods

2.1. Subjects

In this study, 32 NF1 patients and 32 controls between 18 and 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 (Oldfield, 1971) and met the criteria of the safety screening questionnaire for undergoing a TMS-measurement (Rossi et al., 2009, 2011). 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.

2.2. 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 μ V \pm 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) (Mainberger et al., 2013; López-Alonso et al., 2014). RMT was defined as the stimulus intensity in percentage of MSO that elicited MEPs of >50 μ V with a 50% probability, using a maximum likelihood threshold-hunting procedure (Awiszus, 2003). The RMT measurement was repeated at 3-time points to control for changes over time (Fig. 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 (Åkerstedt and Gillberg, 1990) (Fig. 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); vocabulary, similarities, block design and matrix reasoning. Additionally, educational attainment was coded following the 7-point coding scale of Verhage (1964) (Verhage, 1964), taken from Hendriks et al. (2014).

2.3. TMS measurements

2.3.1. 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 (Kujirai et al., 1993). The standard paradigm for SICI uses a CP of 80% of RMT and an inter-stimulus interval of 3 ms. We added a 60% of RMT CP condition to avoid a potential floor effect in NF1 patients (Mainberger et al., 2013). 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.

2.3.2. 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 (Orth and Rothwell, 2004).

2.3.3. 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 (Huang et al., 2005) to avoid muscle contraction prior to iTBS. These contractions prior to iTBS might influence the direction of the TBS-aftereffects (Iezzi et al., 2008; Tse et al., 2018). The stimulus intensity seems to be similar for the two different methods (Sarfeld et al., 2012). 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 (Fig. 1) (Huang et al., 2005). Additionally, in accordance with previous studies that pointed out the high inter-

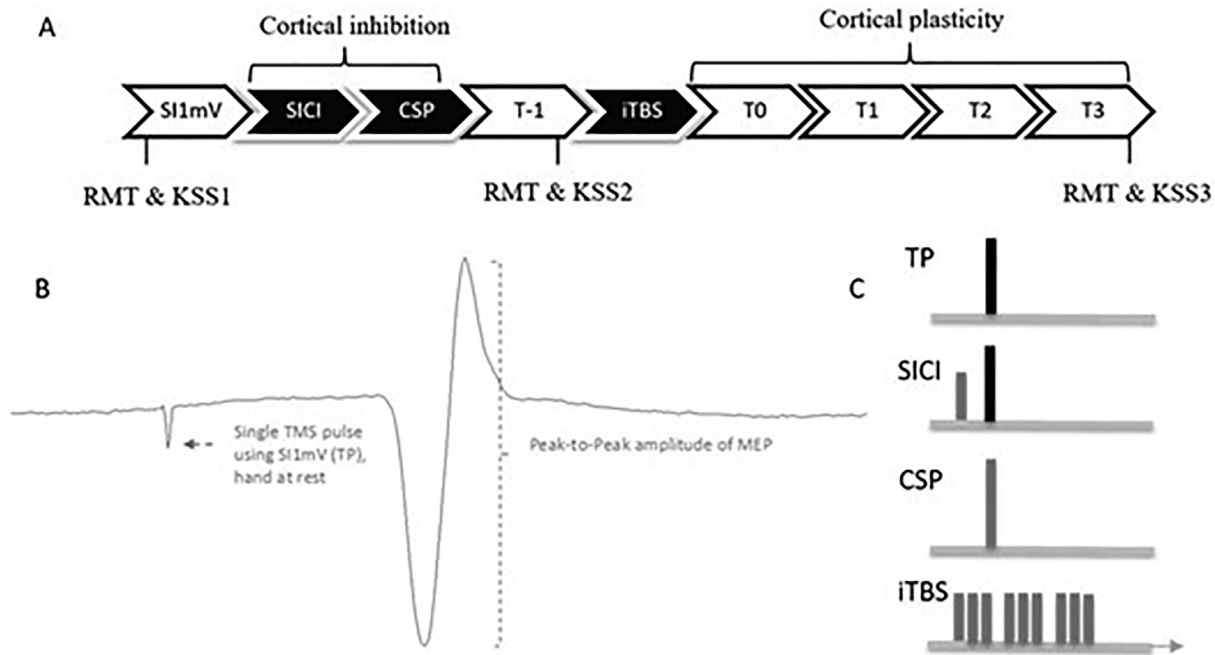


Fig. 1. Schematic overview of transcranial magnetic stimulation (TMS) measurements. A. Procedure of TMS measurements for cortical inhibition and cortical plasticity. SI_{1mV} , the procedure to establish the stimulation intensity that elicited motor evoked potentials (MEPs) with a mean between 800–1200 μ V. 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 SI_{1mV} recorded directly before iTBS. T0-T3, 20 single-pulses at SI_{1mV} 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 SI_{1mV} during hand at rest. C. Schematic presentation of the TMS pulses per paradigm. TP, single test pulse at SI_{1mV} . SICI, paired-pulse consisting of a subthreshold conditioning pulse followed by an unconditioned TP at SI_{1mV} 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 SI_{1mV} ; Grey bars represent stimulations with a stimulation intensity of a specific percentage of RMT (SICI: 60% or 80%, CSP: 120%, iTBS: 80%).

subject variability in response to iTBS independent of genotype (Hamada et al., 2013; Hinder et al., 2014; López-Alonso et al., 2014; Tse et al., 2018), 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 (Orth et al., 2003; Hinder et al., 2014; Nettekoven et al., 2014).

2.4. 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 >70 μV peak-to-peak amplitude within 50 ms pre-trigger (Hermesen et al., 2016; Guthrie et al., 2018). 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 (Chang et al., 2016). 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 (Carson et al., 2004; Fujiyama et al., 2017). 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 (Holm, 1978).

2.5. Data availability

Data are available from the corresponding author on reasonable request.

3. Results

In total, 155 eligible subjects were invited of which 91 subjects declined participation. We measured 64 participants ($n_{\text{control}} = 32$ $n_{\text{NF1}} = 32$). After the measurement, some participants were excluded due to either no observations of MEPs above >50 μV despite the use of a high stimulus intensity ($n_{\text{control}} = 2$ $n_{\text{NF1}} = 2$); artifacts and high background EMG-activity during SICI and iTBS ($n_{\text{NF1}} = 2$); technical problems during SICI measurements ($n_{\text{control}} = 1$); or significant outliers (>3 standard deviations from the mean) in CSP measurements ($n_{\text{control}} = 1$) (Fig. 2). Age and

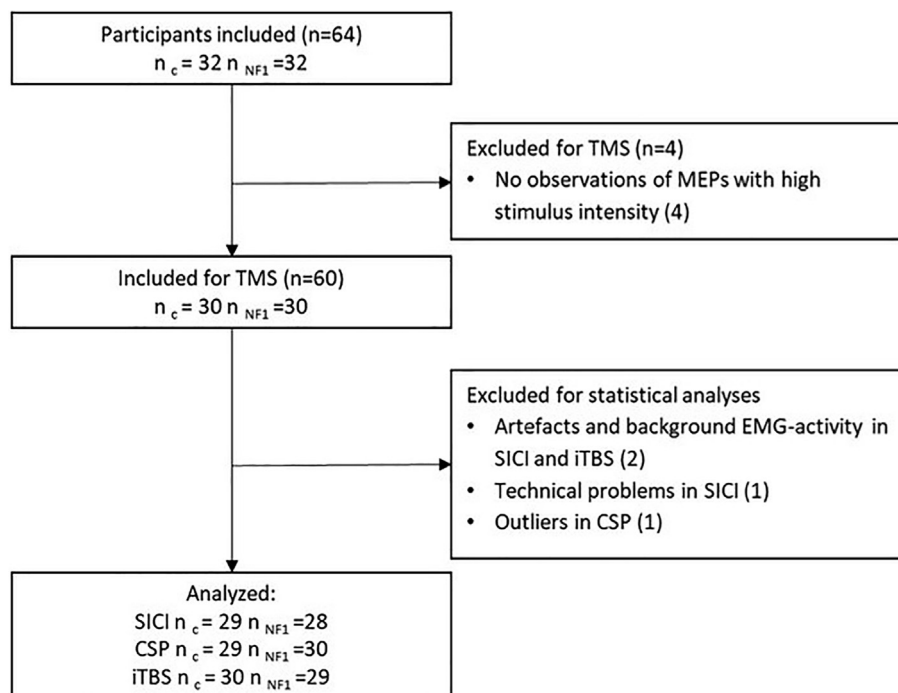


Fig. 2. Flow-chart of inclusions. _c, control; _{NF1}, Neurofibromatosis type 1; TMS, transcranial magnetic stimulation; MEP, motor evoked potential; EMG, electromyography; SICI, short interval cortical inhibition; iTBS, intermittent theta burst stimulation; CSP, cortical silent period.

gender were not different between the groups ($t_{age(57)} = 1.08$, $p = 0.28$; $\chi^2_{gender} = 0.167$, $p = 0.68$). 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} ($M_{control} = 56 \pm 10$; $M_{NF1} = 55 \pm 15$) was similar between patients and controls ($t_{SI_{1mV}(57)} = 0.417$, $p = 0.68$) (Table 1). Although the mean amplitude of MEPs at SI_{1mV} was between 800–1200 μV in both

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

	NF1 group (n = 30)	Control group (n = 30)
<i>Demographics</i>		
Age in years	31.24 \pm 12.3	34.52 \pm 10.8
Gender: Male in % (#)	41 (12)	47 (14)
<i>Educational attainment & estimated IQ</i>		
Educational attainment (median, range)*	5.0, 1–7	6.0, 4–7
Verbal IQ*	85 \pm 16.6	99 \pm 12.9
Performance IQ*	87 \pm 15.3	98 \pm 19.6
<i>Sleepiness (Median, range)</i>		
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
<i>During TMS measurements</i>		
RMT %MSO	46.0 \pm 10.9	48.4 \pm 8.6
SI_{1mV} %MSO	55.2 \pm 15.1	56.7 \pm 10.8
Mean amplitude of MEPs at SI_{1mV} *	886.7 \pm 270.2	1062.5 \pm 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; SI_{1mV} , Stimulus Intensity at 1 mV; MSO, Maximum Stimulator Output; NF1, neurofibromatosis type 1.

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

the control group and the NF1 group ($M_{control} = 1062 \pm 304$; $M_{NF1} = 886 \pm 270$), 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).

3.1. Cortical inhibition

During the SICI paradigm, the mean MEP size of single pulse stimulations ($M_{control} = 798 \pm 425$; $M_{NF1} = 625 \pm 315$) was not different between groups ($t(55) = 1.59$, $p = 0.12$) (Fig. 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 ($F(2, 110) = 49.72$, $p < 0.001$, $\eta^2 = 0.47$) (Fig. 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.

The mean CSP duration, i.e. the time from the single TMS pulse onset to the time of reappearance of voluntary EMG activity (Fig. 4A), was not significantly different between NF1 patients and controls ($M_{control} = 131 \pm 29$; $M_{NF1} = 124 \pm 31$) ($t(57) = 0.87$, $p = 0.39$, $d = -0.23$) (Fig. 4B). 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 ($r = -0.054$, $p = 0.69$).

3.2. Cortical plasticity

3.2.1. 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.

3.2.2. Responder group analysis

We performed an explorative subgroup-analysis on the responders to assess whether there were differences in excitability and

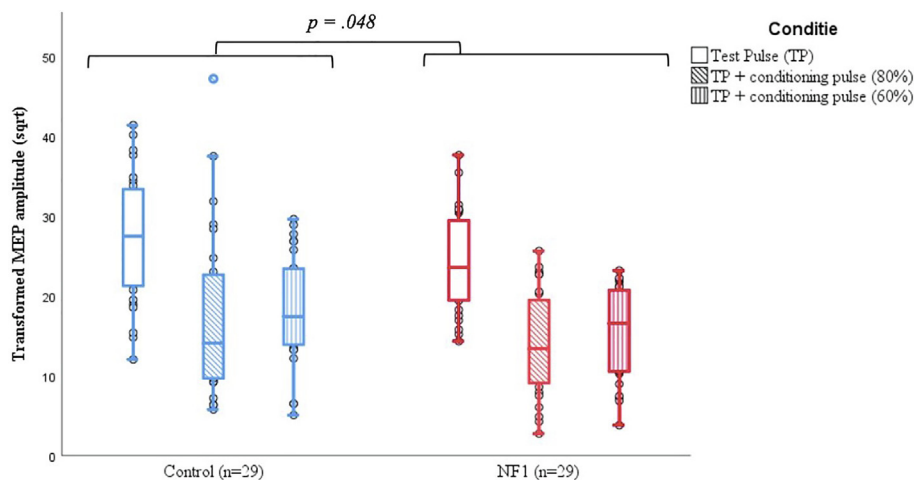


Fig. 3. Response to the short interval cortical inhibition (SICI) paradigm. Boxplots of square-root (sqrt) transformed mean motor evoked potential (MEP) amplitudes per subject in response to the SICI, for both groups separately. Mean MEP amplitudes in response to the test pulse (TP) + conditioning pulse 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 ($F(1, 55) = 4.075$, $p = 0.048$).

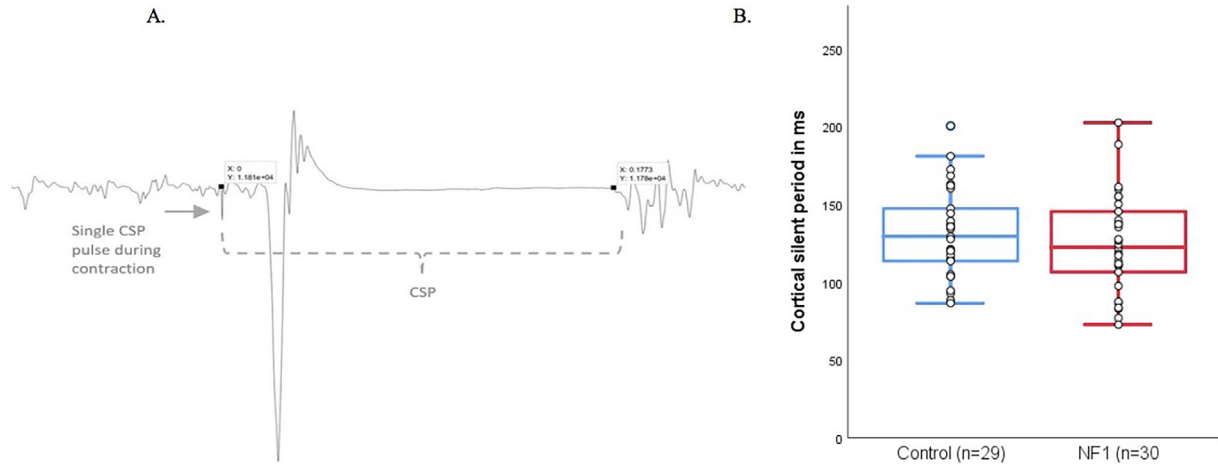


Fig. 4. Response to the cortical silent period (CSP) paradigm. A. Example trace of the data of a single CSP pulse with visual computation of the CSP. B. Boxplot of individual means of CSP duration for the control group and the neurofibromatosis type 1 (NF1) group. There were no significant differences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

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 \pm SD), for all subjects of both groups.

Transformed mean MEP	T-1 ²	T0	T1	T2	T3
NF1 ¹ (n = 30)	23.8 \pm 7.4	25.9 \pm 6.7	23.6 \pm 7.5	22.9 \pm 7.5	22.1 \pm 8.6
Control ¹ (n = 30)	27.9 \pm 8.4	29.2 \pm 7.3	28.6 \pm 7.7	27.6 \pm 8.1	27.9 \pm 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 (S1_{1mV}) recorded directly before iTBS; T0-T3, 20 single-pulses at S1_{1mV} 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 $F(3,49, 188.77) = 1.75, p = 0.19$.

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 ($F(3, 123.1) = 0.91, p = 0.43$) (Fig. 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 (Fig. 5).

3.2.3. 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

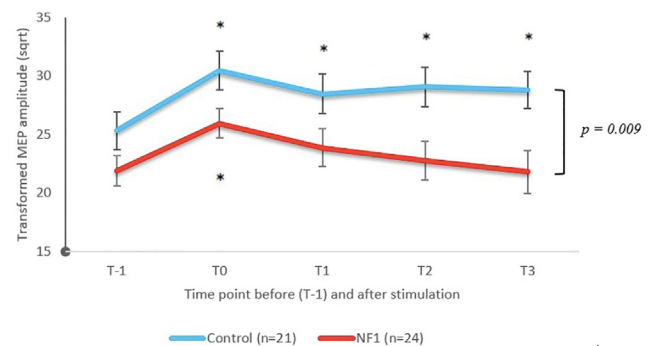


Fig. 5. Responder group analysis of cortical plasticity. Transformed (sqrt, square root) mean motor evoked potentials (MEP) amplitudes \pm SEM of the responders to intermittent theta burst stimulation (iTBS). T-1: mean MEP in response to single pulses directly before iTBS. T0-T3: mean MEP in response 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 ($F(3,123.1) = 3.73, p = 0.013$). Asterisks show the results of the within-group analyses: 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 ($p_{T0} = 0.003, p_{T1} = 0.217, p_{T2} = 0.695, p_{T3} = 0.942$).

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%} ($r_{SICI-VIQ} = 0.03, p = 0.81, r_{SICI-PIQ} = -0.17, p = 0.20$), during iTBS time points ($r_{T0-VIQ} = 0.03, p = 0.82, r_{T0-PIQ} = -0.11, p = 0.42$), or the CSP duration ($r_{CSP-VIQ} = 0.11, p = 0.43, r_{CSP-PIQ} = 0.11, p = 0.41$).

4. Discussion

Using mouse models of NF1, it has been shown that decreased NF1 function causes increased inhibition and consequently decreased synaptic plasticity (Costa et al., 2002; Omrani et al., 2015). 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 (Mainberger et al., 2013; Zimmerman et al., 2015). Although magnetic resonance spectroscopy studies showed evidence for increased inhibitory function of interneurons in the visual cortex (Violante et al., 2013, 2016), 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 (Mainberger et al., 2013; López-Alonso et al., 2014). 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 (Di Lazzaro et al., 2004; Bestmann and Krakauer, 2015). 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 (Orth et al., 2003; Mainberger et al., 2013). 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 (Orth et al., 2003). 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 (Orth et al., 2003; Mainberger et al., 2013). 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 MEP 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 (De Gennaro et al., 2007). 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 (Violante et al., 2013, 2016) 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 (Souza et al., 2009), 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 (Matsugi, 2019). 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 (Costa et al., 2002; Omrani et al., 2015). 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 (Mainberger et al., 2013). 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 (Hinder et al., 2014; López-Alonso et al., 2014). 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 (Suppa and Berardelli, 2012; Hamada et al., 2013), 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 (Pitcher et al., 2015). 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 and 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 (Cuypers et al., 2014; Bestmann and Krakauer, 2015). 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 (Bestmann and Krakauer, 2015).

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 (Chung et al., 2016). 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 (Chung et al., 2016). 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 (Nettekoven et al., 2014). 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 (Opie et al., 2017). Furthermore, Hamada et al. (2013) 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 (Hamada et al., 2013; Volz et al., 2019). Interestingly, previous studies have shown that iTBS can increase excitability of the cortical pathways reflected in the generated later I-waves (Di Lazzaro et al., 2008; Cárdenas-Morales et al., 2010). 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 (Hinder et al., 2014; López-Alonso et al., 2014), 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 (Ridding and Flavel, 2006).

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 (Hyman et al., 2005; Krab et al., 2008a; Ottenhoff et al., 2020), which is a good predictor of neuropsychological functioning in other cognitive domains (Diaz-asper et al., 2004). 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 chromoso-

mal microdeletion of the NF1 gene (17%, $n = 3$). The latter genotype is associated with a more severe cognitive phenotype (Ottenhoff et al., 2020). 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.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank all the subjects who participated in this study. We also thank Tomas van der Velde for his contribution to the data-analysis of this study.

Funding

This work was supported by resources of the Department of Neuroscience and the Department of Psychiatry of the Erasmus MC, Rotterdam, The Netherlands.

References

- Akerstedt T, Gillberg M. Subjective and objective sleepiness in the active individual. *Int J Neurosci* 1990;52(1-2):29–37.
- Awiszus F. TMS and threshold hunting. *Suppl Clin Neurophysiol* 2003;56:13–23.
- Bajbouj M, Lisanby SH, Lang UE, Danker-Hopfe H, Heuser I, Neu P. Evidence for impaired cortical inhibition in patients with unipolar major depression. *Biol Psychiatry* 2006;59(5):395–400.
- Barker AT, Jalinous R, Freeston IL. Non-invasive magnetic stimulation of human motor cortex. *The Lancet* 1985;325(8437):1106–7.
- Bestmann S, Krakauer JW. The uses and interpretations of the motor-evoked potential for understanding behaviour. *Exp Brain Res* 2015;233(3):679–89.
- Cárdenas-Morales L, Nowak DA, Kammer T, Wolf RC, Schönfeldt-Lecuona C. Mechanisms and applications of theta-burst rTMS on the human motor cortex. *Brain Topogr* 2010;22(4):294–306.
- Carson RG, Riek S, Mackey DC, Meichenbaum DP, Willms K, Forner M, Byblow WD. Excitability changes in human forearm corticospinal projections and spinal reflex pathways during rhythmic voluntary movement of the opposite limb. *J Physiol* 2004;560(3):929–40.
- Chang WH, Fried PJ, Saxena S, Jannati A, Gomes-Osman J, Kim Y-H, et al. Optimal number of pulses as outcome measures of neuronavigated transcranial magnetic stimulation. *Clin Neurophysiol* 2016;127(8):2892–7.
- Chung SW, Hill AT, Rogasch NC, Hoy KE, Fitzgerald PB. Use of theta-burst stimulation in changing excitability of motor cortex: A systematic review and meta-analysis. *Neurosci Biobehav Rev* 2016;63:43–64.
- Costa RM, Federov NB, Kogan JH, Murphy GG, Stern J, Ohno M, et al. Mechanism for the learning deficits in a mouse model of neurofibromatosis type 1. *Nature* 2002;415(6871):526–30.
- Cui Y, Costa RM, Murphy GG, Elgersma Y, Zhu Y, Gutmann DH, et al. Neurofibromin regulation of ERK signaling modulates GABA release and learning. *Cell* 2008;135(3):549–60.
- Cuypers K, Thijs H, Meesen RLJ, Theoret H. Optimization of the transcranial magnetic stimulation protocol by defining a reliable estimate for corticospinal excitability. *PLoS ONE* 2014;9.
- De Gennaro L, Marzano C, Veniero D, Moroni F, Fratello F, Curcio G, et al. Neurophysiological correlates of sleepiness: A combined TMS and EEG study. *NeuroImage* 2007;36(4):1277–87.
- Diaz-asper CM, Schretlen DJ, Pearlson GD. How well does IQ predict neuropsychological test performance in normal adults? *J. Inter. Neuropsych. Soc.* 2004;10(01).
- Di Lazzaro V, Oliviero A, Pilato F, Saturno E, Dileone M, Mazzone P, et al. The physiological basis of transcranial motor cortex stimulation in conscious humans. *Clin Neurophysiol* 2004;115:255–66.
- Di Lazzaro V, Oliviero A, Saturno E, Dileone M, Pilato F, Nardone R, et al. Effects of lorazepam on short latency afferent inhibition and short latency intracortical

- inhibition in humans: GABA modulation of cholinergic motor cortex inhibition. *J Physiol* 2005;564(2):661–8.
- Di Lazzaro V, Pilato F, Dileone M, Profice P, Oliviero A, Mazzone P, et al. The physiological basis of the effects of intermittent theta burst stimulation of the human motor cortex: Theta-burst rTMS of the human motor cortex. *J Physiol* 2008;586(16):3871–9.
- Ferner RE. Neurofibromatosis 1 and neurofibromatosis 2: a twenty first century perspective. *Lancet Neurol* 2007;6(4):340–51.
- Fujiyama H, Hinder MR, Barzideh A, Van de Vijver C, Badache AC, Manrique-C MN, et al. Preconditioning tDCS facilitates subsequent tDCS effect on skill acquisition in older adults. *Neurobiol Aging* 2017;51:31–42.
- Guthrie MD, Gilbert DL, Huddleston DA, Pedapati EV, Horn PS, Mostofsky SH, et al. Online transcranial magnetic stimulation protocol for measuring cortical physiology associated with response inhibition. *J Vis Exp* 2018.
- Hamada M, Murase N, Hasan A, Balaratnam M, Rothwell JC. The role of interneuron networks in driving human motor cortical plasticity. *Cereb Cortex* 2013;23(7):1593–605.
- Hendriks MPH, Kessels RPC, Gorissen MEE, Schmand BA, Duits AA. Neuropsychologische diagnostiek: De klinische praktijk. Amsterdam: Boom; .
- Hermesen AM, Haag A, Duddek C, Balkenhol K, Bugiel H, Bauer S, et al. Test–retest reliability of single and paired pulse transcranial magnetic stimulation parameters in healthy subjects. *J Neurol Sci* 2016;362:209–16.
- Hinder MR, Goss EL, Fujiyama H, Carty AJ, Garry MI, Rodger J, et al. Inter- and intra-individual variability following intermittent theta burst stimulation: Implications for rehabilitation and recovery. *Brain Stimul* 2014;7(3):365–71.
- Holm S. A simple sequentially rejective multiple test procedure. *Scand J Stat* 1978;6:65–70.
- Huang Y-Z, Chen R-S, Rothwell JC, Wen H-Y. The after-effect of human theta burst stimulation is NMDA receptor dependent. *Clin Neurophysiol* 2007;118(5):1028–32.
- Huang Y-Z, Edwards MJ, Rounis E, Bhatia KP, Rothwell JC. Theta burst stimulation of the human motor cortex. *Neuron* 2005;45(2):201–6.
- Huang Y-Z, Lu M-K, Antal A, Classen J, Nitsche M, Ziemann U, et al. Plasticity induced by non-invasive transcranial brain stimulation: A position paper. *Clin Neurophysiol* 2017;128(11):2318–29.
- Hyman SL, Shores A, North KN. The nature and frequency of cognitive deficits in children with neurofibromatosis type 1. *Neurology* 2005;65(7):1037–44.
- Iezzi E, Conte A, Suppa A, Agostino R, Dinapoli L, Scotrini A, et al. Phasic voluntary movements reverse the aftereffects of subsequent theta-burst stimulation in humans. *J Neurophysiol* 2008;100(4):2070–6.
- Krab LC, Aarsen FK, de Goede-Bolder A, Catsman-Berrevoets CE, Arts WF, Moll HA, et al. Impact of neurofibromatosis type 1 on school performance. *J Child Neurol* 2008a;23(9):1002–10.
- Krab LC, de Goede-Bolder A, Aarsen FK, Pluijm SM, Bouman MJ, van der Geest JN, et al. Effect of simvastatin on cognitive functioning in children with neurofibromatosis type 1: a randomized controlled trial. *JAMA* 2008b;300:287–94.
- Krab LC, Oostenbrink R, de Goede-Bolder A, Aarsen FK, Elgersma Y, Moll HA. Health-related quality of life in children with neurofibromatosis type 1: Contribution of demographic factors, disease-related factors, and behavior. *J Pediatr* 2009;154(3):420–5.
- Kujirai T, Caramia MD, Rothwell JC, Day BL, Thompson PD, Ferbert A, et al. Corticocortical inhibition in human motor cortex. *J Physiol* 1993;471(1):501–19.
- Levinson AJ, Fitzgerald PB, Favalli G, Blumberger DM, Daigle M, Daskalakis ZJ. Evidence of cortical inhibitory deficits in major depressive disorder. *Biol Psychiatry* 2010;67(5):458–64.
- López-Alonso V, Cheeran B, Río-Rodríguez D, Fernández-del-Olmo M. Inter-individual variability in response to non-invasive brain stimulation paradigms. *Brain Stimul* 2014;7(3):372–80.
- Mainberger F, Jung NH, Zenker M, Wahlländer U, Freudenberg L, Langer S, et al. Lovastatin improves impaired synaptic plasticity and phasic alertness in patients with neurofibromatosis type 1. *BMC Neurol* 2013;13(1).
- Matsugi A. Changes in the cortical silent period during force control. *Somatosens Mot Res* 2019;36(1):8–13.
- Nettekoven C, Volz LJ, Kutscha M, Pool E-M, Rehme AK, Eickhoff SB, et al. Dose-dependent effects of theta burst rTMS on cortical excitability and resting-state connectivity of the human motor system. *J Neurosci* 2014;34(20):6849–59.
- Oberman L, Edwards D, Eldaief M, Pascual-Leone A. Safety of theta burst transcranial magnetic stimulation: a systematic review of the literature. *J Clin Neurophysiol* 2011;28(1):67–74.
- Oldfield RC. The assessment and analysis of handedness: The Edinburgh inventory. *Neuropsychologia* 1971;9(1):97–113.
- Omrani A, van der Vaart T, Mientjes E, van Woerden GM, Hojjati MR, Li KW, et al. HCN channels are a novel therapeutic target for cognitive dysfunction in neurofibromatosis type 1. *Mol Psychiatry* 2015;20(11):1311–21.
- Opie GM, Vosnakis E, Ridding MC, Ziemann U, Semmler JG. Priming theta burst stimulation enhances motor cortex plasticity in young but not old adults. *Brain Stimul* 2017;10(2):298–304.
- Orth M, Rothwell JC. The cortical silent period: intrinsic variability and relation to the waveform of the transcranial magnetic stimulation pulse. *Clin Neurophysiol* 2004;115(5):1076–82.
- Orth M, Snijders AH, Rothwell JC. The variability of intracortical inhibition and facilitation. *Clin Neurophysiol* 2003;114(12):2362–9.
- Ottenhoff MJ, Rietman AB, Mous SE, Plasschaert E, Gawehns D, Brems H, et al. Examination of the genetic factors underlying the cognitive variability associated with neurofibromatosis type 1. *Genet Med* 2020;22(5):889–97.
- Payne JM, Barton B, Ullrich NJ, Cantor A, Hears SJ, Cutter G, et al. Randomized placebo-controlled study of lovastatin in children with neurofibromatosis type 1. *Neurology* 2016;87(24):2575–84.
- Pitcher JB, Doeltgen SH, Goldsworthy MR, Schneider LA, Vallence A-M, Smith AE, et al. A comparison of two methods for estimating 50% of the maximal motor evoked potential. *Clin Neurophysiol* 2015;126(12):2337–41.
- Ridding MC, Flavel SC. Induction of plasticity in the dominant and non-dominant motor cortices of humans. *Exp Brain Res* 2006;171(4):551–7.
- Ridding MC, Ziemann U. Determinants of the induction of cortical plasticity by non-invasive brain stimulation in healthy subjects: Induction of cortical plasticity by non-invasive brain stimulation. *J Physiol* 2010;588(13):2291–304.
- Rossi S, Hallett M, Rossini PM, Pascual-Leone A. Screening questionnaire before TMS: An update. *Clin Neurophysiol* 2011;122(8):1686.
- Rossi S, Hallett M, Rossini PM, Pascual-Leone A. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clin Neurophysiol* 2009;120(12):2008–39.
- Sarfeld A-S, Diekhoff S, Wang LE, Liuzzi G, Uludağ K, Eickhoff SB, et al. Convergence of human brain mapping tools: Neuronavigated TMS parameters and fMRI activity in the hand motor area. *Hum Brain Mapp* 2012;33(5):1107–23.
- Sheikh SN, Martin DL. Elevation of brain GABA levels with vigabatrin (gamma-vinylGABA) differentially affects GAD65 and GAD67 expression in various regions of rat brain. *J Neurosci Res* 1998;52:736–41.
- Sihlyansky C, Lee YS, Silva AJ. Molecular and cellular mechanisms of learning disabilities: A focus on NF1. *Annu Rev Neurosci* 2010;33(1):221–43.
- Siebner HR, Dressnandt J, Auer C, Conrad B. Continuous intrathecal baclofen infusions induced a marked increase of the transcranially evoked silent period in a patient with generalized dystonia. *Muscle Nerve* 1998;21(9):1209–12.
- Souza JF, Passos RL, Guedes AC, Rezende NA, Rodrigues LO. Muscular force is reduced in neurofibromatosis type 1. *J Musculoskelet Neuronal Interact* 2009;9:15–7.
- Suppa A, Berardelli A. Horizons in LTP-like plasticity in human primary motor cortex. *Clin Neurophysiol* 2012;123(11):2111–3.
- Tse NY, Goldsworthy MR, Ridding MC, Coxon JP, Fitzgerald PB, Fornito A, et al. The effect of stimulation interval on plasticity following repeated blocks of intermittent theta burst stimulation. *Sci Rep* 2018;8(1):1–10.
- Uusitalo E, Leppävirta J, Koffert A, Suominen S, Vahtera J, Vahlberg T, et al. Incidence and mortality of neurofibromatosis: A total population study in Finland. *J Invest Dermatol* 2015;135(3):904–6.
- van der Vaart T, Plasschaert E, Rietman AB, Renard M, Oostenbrink R, Vogels A, et al. Simvastatin for cognitive deficits and behavioural problems in patients with neurofibromatosis type 1 (NF1-SIMCODA): a randomised, placebo-controlled trial. *Lancet Neurol* 2013;12(11):1076–83.
- Verhage F. Intelligence and age: study with Dutch people aged 12–77. Assen Van Gorcum 1964.
- Violante IR, Patricio M, Bernardino I, Rebola J, Abrunhosa AJ, Ferreira N, et al. GABA deficiency in NF1: A multimodal [11C]-flumazenil and spectroscopy study. *Neurology* 2016;87(9):897–904.
- Violante IR, Ribeiro MJ, Edden RAE, Guimarães P, Bernardino I, Rebola J, et al. GABA deficit in the visual cortex of patients with neurofibromatosis type 1: genotype–phenotype correlations and functional impact. *Brain* 2013;136(3):918–25.
- Volz LJ, Hamada M, Michely J, Pool E-M, Nettekoven C, Rothwell JC, et al. Modulation of I-wave generating pathways by theta-burst stimulation: a model of plasticity induction. *J Physiol* 2019;597(24):5963–71.
- Werhahn KJ, Kunesch E, Noachtar S, Benecke R, Classen J. Differential effects on motor cortical inhibition induced by blockade of GABA uptake in humans. *J Physiol* 1999;517(2):591–7.
- Wischniewski M, Schutter DJLG. Efficacy and time course of theta burst stimulation in healthy humans. *Brain Stimul* 2015;8(4):685–92.
- Zimmerman M, Wessel MJ, Timmermann JE, Granström S, Gerloff C, Mautner VF, et al. Impairment of procedural learning and motor intracortical inhibition in neurofibromatosis type 1 patients. *EBioMedicine* 2015;2(10):1430–7.