Clinical Neurophysiology 142 (2022) 220–227

Contents lists available at ScienceDirect

Clinical Neurophysiology

journal homepage: www.elsevier.com/locate/clinph

Plasticity of visual evoked potentials in patients with neurofibromatosis type 1

J. Castricum [a,b,d], J.H.M. Tulen [b,d], A.M. Heuvelmans [a,b,d], G. Geleijnse [c], D.C.G. Straver [c], W. Taal [d,e], S.A. Kushner [b,d], Y. Elgersma [a,d,*]

[a] Department of Clinical Genetics, Erasmus University Medical Center, Rotterdam, the Netherlands
[b] Department of Psychiatry, Erasmus University Medical Center, Rotterdam, the Netherlands
[c] Department of Clinical Neurophysiology, Erasmus University Medical Center, Rotterdam, the Netherlands
[d] ENCORE Expertise Center for Neurodevelopmental Disorders, Erasmus Medical Center, Rotterdam, the Netherlands
[e] Department of Neurology/Neuro-oncology, Erasmus University Medical Center Cancer Institute, Rotterdam, the Netherlands

ARTICLE INFO

Article history:
Accepted 10 August 2022
Available online 24 August 2022

Keywords:
Visual evoked potentials
Neurofibromatosis type 1
Cortical plasticity

HIGHLIGHTS

- Studying VEP plasticity might be a novel neurophysiological outcome measure associated with cognitive disability in NF1.
- The NF1 group had a non-potentiated response to VEP induction.
- The control group revealed a potentiated VEP response during continuous visual stimulation without delay.

ABSTRACT

Objective: 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.
Methods: 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.
Results: The P1 amplitude increased significantly comparing post-modulation to pre-modulation in the control group. This potentiation was not observed in the NF1 group.
Conclusions: 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.
Significance: 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.

1. Introduction

Neurofibromatosis type 1 (NF1) is associated with cognitive deficits and learning disabilities that can affect quality of life (Varni et al., 2019). 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 (Hyman et al., 2005; Descheemaeker et al., 2013). 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 (Descheemaeker et al., 2013; Ribeiro et al., 2015). However, it is unknown whether there is primary dysfunction of visual pathways in NF1 adults, and if there are

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

https://doi.org/10.1016/j.clinph.2022.08.009
1388-2457/

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 (Costa et al., 2002; Cui et al., 2008; Shilyansky et al., 2010; Omrani et al., 2015). 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 (Tinga et al., 2019). These measurements include event-related potentials in response to sensory stimulation in the human brain. Ribeiro et al. (Ribeiro et al., 2014) 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 (Ribeiro et al., 2014).

Perceptual learning involves the plasticity of responses to sensory stimulation in the primary sensory cortices. Specifically, Frenkel et al. (Frenkel et al., 2006) 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 (Teyler et al., 2005). 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 (Normann et al., 2007; Çavuş et al., 2012; Elvsåshagen et al., 2012).

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., 2020)). Teyler et al. (Teyler et al., 2005) 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 (Normann et al., 2007; Çavuş et al., 2012). Changes in VEP amplitudes seem to be more sensitive to checkerboard reversals (Normann et al., 2007; Çavuş et al., 2012; Klöppel et al., 2015; Zak et al., 2018). Potentiation of VEPs has been observed in healthy adults as indicated by a decrease in amplitude of the prominent negative component at 75 ms (C1 or N75), and an increase in amplitude of the positive component at 100 ms (P1 or P100) after stimulation (Klöppel et al., 2015). 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 (Normann et al., 2007). In addition, Zak et al., (Zak et al., 2018) 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 or N135 is the negative component following P1. 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 (Jabbari et al., 1985; Iannaccone et al., 2002; Ammendola et al., 2006; Yerdelen et al., 2011). 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 (Nebbioso et al., 2020). 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 %; (Ammendola et al., 2006; Yerdelen et al., 2011)) and patients with optic gliomas were often excluded (Jabbari et al., 1985; Iannaccone et al., 2002; Nebbioso et al., 2020). 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.

2. Materials and methods

2.1. 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 visual problems or neurological illness that involved the visual system. Furthermore, all subjects had no optic nerve gliomas, no MRI (magnetic resonance imaging) lesions, 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).

2.2. 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 (Klem et al., 1999). 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

Table 1
Demographics (Mean ± SD), and 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 % (N)	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[2] (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) [3]		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) [4]		amplitudes		amplitudes
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 $\leq$ 0.05).
VEPs, 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.
[1] Karolinska sleepiness scale (KSS) at the start of the VEP recordings | Karolinska sleepiness scale at the end of the VEP recordings.
[2] The level of education was scored using the ISCED (Schneider, 2013).
[3] Values represent the average amplitude during the visual stimuli given in the 10th minute of continuous visual stimulation (M10).
[4] 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.

to the ISCEV guidelines (Odom et al., 2016). We used a classical cathode ray tube (CRT) stimulator with a mean photopic luminance of 45Cd/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 (Åkerstedt and Gillberg, 1990).

2.3. VEP measurements

VEPs were elicited by checkerboard reversals at a low frequency of 1.92 Hz (Normann et al., 2007; Çavuş et al., 2012; Odom et al., 2016). The checkerboard reversal stimuli were presented at a distance of 54 cm with a NICOLET 2015 visual stimulator connected to a ViewSonic E70fB 17-inch display. This setup met the criteria as defined by ISCEV guidelines (Odom et al., 2016). We used a field size of 33 × 25 degrees with large checks of 1x1°. The mean luminance at the center was between 40 and 60Cd/m^2 and had a Michelson contrast ratio larger than 80 %. We accepted variation up to 30 % from the center to the periphery. The Viking EDX had a sampling frequency of 48 kHz and the checkerboard pattern changed every half-second. 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 (Odom et al., 2016). Traces (epoched data with one sweep locked to the pattern reversals) 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 (Normann et al., 2007; Elvsåshagen et al., 2012; Klöppel et al., 2015) (Fig. 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 the paired t-tests as pre-modulation in comparison to the average of post-modulation (T1-T6) in accordance with previous literature (Elvsåshagen et al., 2012; Klöppel et al., 2015; Zak et al., 2018). 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 (Fig. 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.

2.4. Statistical analysis

VEP data were analyzed using MATLAB R2019b (Mathworks). A trial was the average of 40 traces with 18 trials over time per individual (i.e. B1, B2, M1-M10, T1-T6). The trials started 50 ms before the onset of each checkerboard reversal and continued for 450 ms after onset (Elvsåshagen et al., 2012). The trial data was baseline corrected (-50 – 0 ms prior to stimulus) and digitally low-pass filtered at 48 Hz (Heinrich and Bach, 2001; Di Russo et al., 2002; Elvsåshagen et al., 2012). 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 (Supplementary Fig. 1a). In this subject

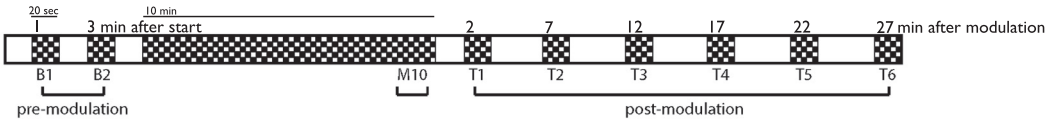

Fig. 1. Schematic time course of VEP induction. B1 and B2: checkerboard reversals pre-modulation with each 20 seconds, started 1 and 3 min after the start of the experiment. M10: VEP measurement of 20 seconds during checkerboard reversals given in the 10th minute of continuous stimulation of 10 min. 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.

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 (i.e. trial) 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 ran the analysis independently and came to a consensus of the exclusion of VEPs or individual peaks that could not be identified (see example in Supplementary Fig. 1b). If VEPs or individual peaks could not be identified within a subject, the whole trial was excluded from the analyses.

Statistical analyses were performed using IBM Statistics SPSS (version 25). 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. Correlations between age, educational attainment (following the ISCED; (Schneider, 2013)), 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.

2.4.1. The VEP response

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.

2.4.2. VEP plasticity

We tested the overall effect of the modulation in the NF1 and control group with a repeated measures ANOVA between-within subject design with factor time (pre-modulation, T1-T6) and factor group. In the ANOVA, we included the first baseline measurement to compare the VEPs acquired using the same number of traces.

Subsequently, a within-subject repeated-measures ANOVA was used to measure the effect of the factor time (pre-modulation, T1-T6) on mean VEP amplitudes in controls and patients separately if a significant interaction effect was established with the overall repeated measures ANOVA (Elvsåshagen et al., 2012). Degrees of freedom were corrected using Greenhouse-Geiser estimates of sphericity.

In addition, we tested the change per peak amplitude of averaged pre-and post-modulation with paired t-tests in controls and patients separately if a significant interaction effect was established with the overall repeated measures ANOVA (Elvsåshagen et al., 2012). Averaged post-modulation is the average of all measurements after modulation (T1-T6) (Elvsåshagen et al., 2012; Klöppel et al., 2015; Valstad et al., 2020). We also tested the change in peak-to-peak amplitude of C1 to P1 with paired t-tests (Odom et al., 2016).

2.4.3. 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, Fig. 1) with the first baseline measurement using overall repeated measures ANOVA with a between-within subject design. Subsequently, a within-subject repeated-measures ANOVA was used to measure the effect of the factor time in controls and patients separately if a significant interaction effect was established with the overall repeated measures ANOVA. The data stability during modulation was tested with a repeated measures ANOVA to find any significant differences in VEP amplitude during modulation.

2.5. Data availability

Data are available from the corresponding author on reasonable request.

3. Results

In total, 42 patients with NF1 agreed to eligibility screening, of which 22 subjects were enrolled. Subjects were excluded due to psychiatric comorbidities (n = 7), participation in other clinical trials (n = 2), use of psychoactive medication (n = 3), ocular pathology (n = 2), general health issues (n = 2), epilepsy (n = 2), or cancelled due to COVID-19 (n = 2) (Fig. 2). In addition, 35 eligible subjects without NF1 were screened for study eligibility, of which 31 subjects were enrolled. During the measurements, the number of excluded traces exceeding 130 microV was not significantly different between group (U = 337, p = 0.9, excluded traces: control = 0.21 %; NF1 = 0.15 %). After the measurements, subjects were excluded if VEPs could not be identified ($n_{control}$ = 1; n_{NF1} = 2), and trials (18 trials per individual; B1, B2, M1-M10, T1-T6) were excluded if they could not be identified (2.7 % of total trials in controls; 3.5 % of total trials in patients with NF1).

No significant differences were found between patients and controls in age (*Mean* ± *SD*: $M_{patient}$ = 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).

3.1. The VEP response

Peak latencies and amplitudes of B1 and B2 were not significantly different between groups; only the statistics of P1 are presented here ($t_{latency}(49)$ = 0.7, p = 0.5; $t_{amplitude}$ (49) = 1.1, p = 0.3) (Table 1). We did not find significant differences in pre-modulation peak latencies between the NF1 and control groups ($t(48)$ = 1.3, p = 0.2). Additionally, we did not find significant differences in peak amplitudes between the groups pre-modulation ($t(48)$ = -0.2, p = 0.9) (Table 1).

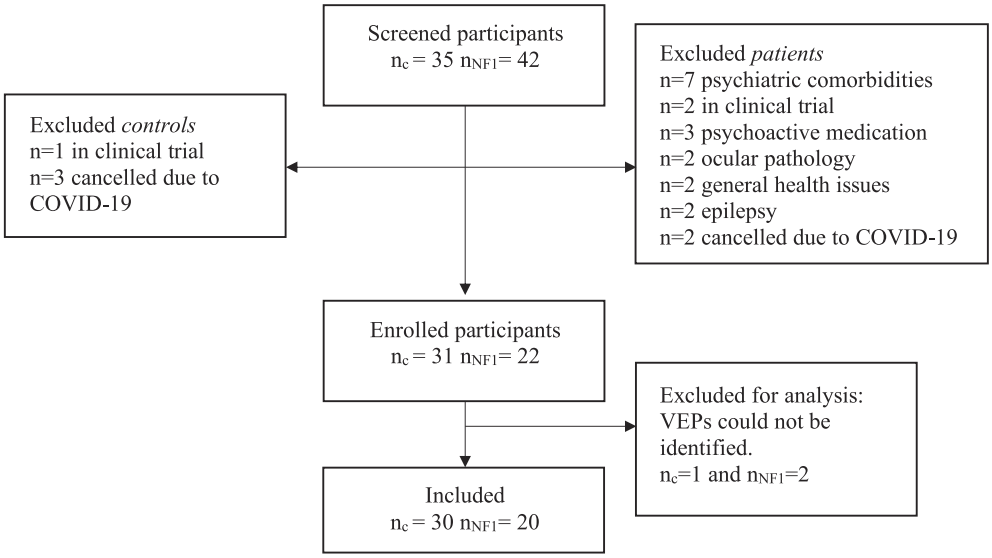

Fig. 2. Flowchart. The number of included and excluded participants. $_c$ = controls; $_{NF1}$ = neurofibromatosis type 1; VEPs = visual evoked potentials.

3.2. VEP plasticity

The overall repeated measures ANOVA with a between-within subject design showed no overall effects of time (pre-modulation, T1-T6) or group for C1 (F_{time}(6,270) = 1.6, p = 0.2; F_{group}(1,45) = 0.2, p = 0.6). However, there was a significant interaction effect for P1, but no time or group effect ($F_{interaction}$(6,270) = 2.5, p = 0.023; F_{time}(6,270) = 1.5, p = 0.2; F_{group}(1,45) = 0.9, p = 0.3). Furthermore, there was a significant main effect for time for N1 (F_{N1}(6,270) = 2.3, p = 0.034), but no group or interaction effect (F_{group}(1,45) = 0.3, p = 0.6; $F_{interaction}$(6,270) = 0.5, p = 0.8) (Fig. 3).

Subsequently, in the control group, the within-subject repeated measures ANOVA showed a significant effect of the factor time (pre-modulation, T1-T6) for P1 (F_{P1}(6, 162) = 3.1, p = 0.007, η^2 = 0.1) (Holm-Bonferroni correction: p_{P1} < 0.02;) (Fig. 4; Supplementary Fig. 2). In the NF1 group, the within-subject repeated measures ANOVA showed no significant effect of the factor time for P1 (F_{P1}(6, 108) = 1.3, p = 0.3) (Fig. 3).

In addition, to examine the effect of modulation on VEP plasticity, we tested the change per peak amplitude comparing averaged post-modulation to pre-modulation in the control group. P1 was significantly increased in amplitude when comparing averaged post-modulation to pre-modulation (t_{P1}(29) = 2.9, p = 0.008, d = 0.2), indicating VEP potentiation (Holm-Bonferroni correction: p_{P1} < 0.02) (Fig. 3). The C1-P1 peak-to-peak amplitude did not significantly differ from pre- to averaged post-modulation (t(29) = -1.1, p = 0.1) (Table 1; Fig. 3).

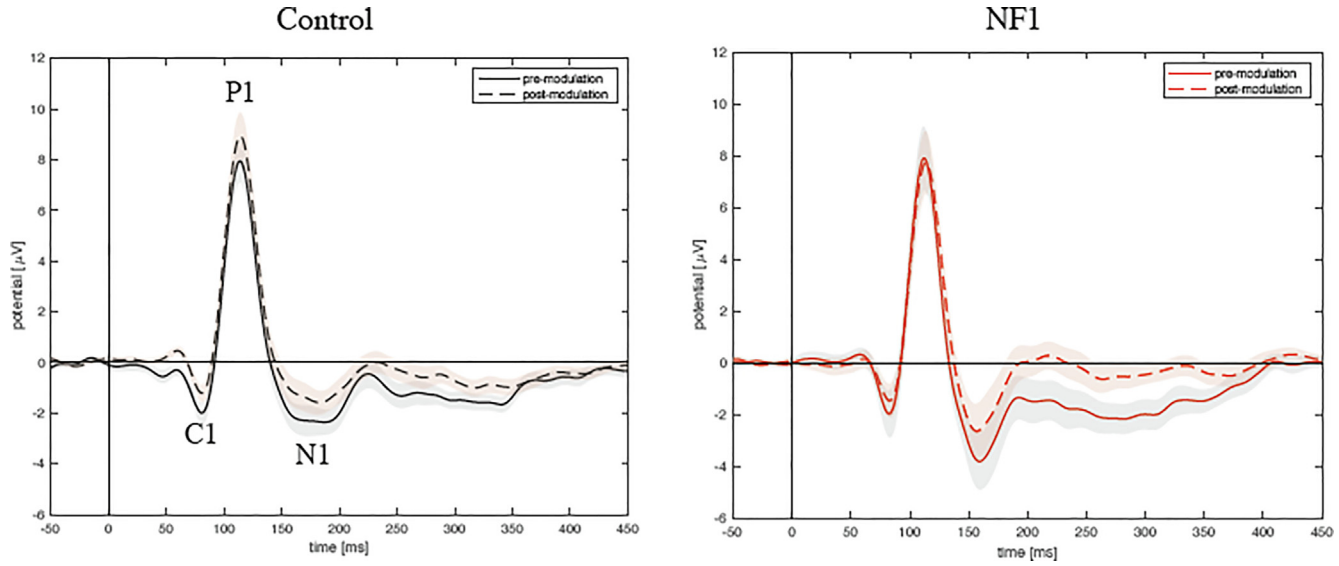

Fig. 3. The VEP response in the control and the NF1 group. The mean VEP response pre-modulation (solid line) and post-modulation (dashed line) in the control group (left) and NF1 group (right) ± SEM. There was a significant interaction effect for P1 (F_{P1}(6,270) = 2.5, p = 0.023) and a main effect for time for N1 (F_{N1}(6,270) = 2.3, p = 0.034). P1 was significantly increased in amplitude comparing post-modulation to pre-modulation in the control group, but not in the NF1 group. NF1 = neurofibromatosis type 1; VEPs = visual evoked potentials; 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.

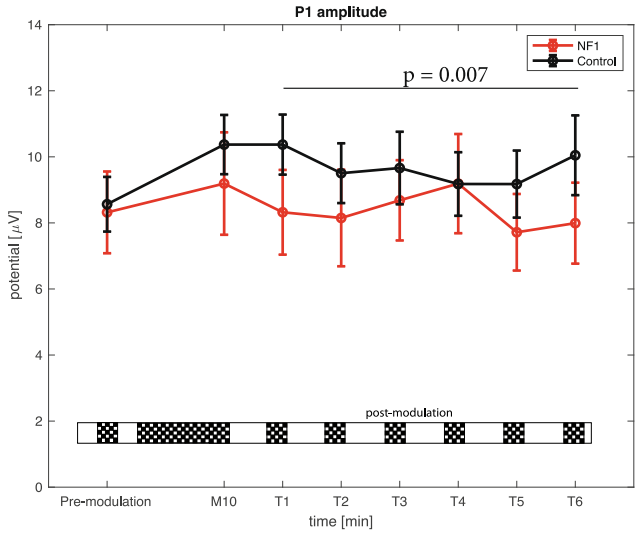

Fig. 4. VEP plasticity of the P1 peak amplitude in the control and NF1 group. Mean amplitude of P1 ± SEM per group per VEP measurement. Within-subject repeated measures ANOVA showed a significant effect of the factor time (pre-modulation, T1-T6) for P1 in the control group ($F_{P1}(6, 162) = 3.1$, $p = 0.007$, $\eta^2 = 0.1$). 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 scheme of the VEP measurements is presented above the x-axis. NF1 = neurofibromatosis type 1; VEPs = visual evoked potentials; P1 = positive component at ca. 100 ms.

In contrast to the control group, the P1 peak amplitudes were not significantly different between pre-and averaged post-modulation in the NF1 group ($t(19) = -0.3$, $p = 0.4$).

3.3. VEP-response at M10

We investigated modulation without delay by comparing VEP-amplitude during the 10th minute of continuous stimulation (M10) to pre-modulation. The overall repeated measures ANOVA showed a significant time effect for C1 ($F_{C1}(1,45) = 4.2$, $p = 0.047$), P1 ($F_{P1}(1,46) = 8.0$, $p = 0.007$) and N1 ($F_{N1}(1,46) = 35.8$, $p < 0.001$), indicating a decreased C1 and N1 amplitude and an increased P1 amplitude, which was similar to the comparison of averaged post-modulation to pre-modulation (Table 1). There were no significant group and interaction effects ($F_{C1\text{-group}}(1,45) = 0.01$, $p = 0.9$; $F_{C1\text{-interaction}}(1,45) = 0.7$, $p = 0.4$; $F_{P1\text{-group}}(1,46) = 0.3$, $p = 0.6$; $F_{P1\text{-interaction}}(1,46) = 0.8$, $p = 0.4$; $F_{N1\text{-group}}(1,46) = 1.2$, $p = 0.3$; $F_{N1\text{-interaction}}(1,46) = 0.06$, $p = 0.8$). We did not find any significant differences in VEP amplitude during the modulation (M1-M10) for all the peaks, only the statistics of P1 are presented here ($F_{time}(6.9,311) = 1.9$, $p = 0.07$; $F_{group}(1,45) = 0.7$, $p = 0.3$; $F_{interaction}(6.9,311) = 3.8$, $p = 0.8$).

3.4. 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 ($r_{C1} = -0.05$, $p = 0.7$; $r_{P1} = 0.005$, $p = 0.9$; $r_{N1} = 0.09$, $p = 0.5$).

4. 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. The P1 amplitude of the VEP between post-modulation and pre-modulation was significantly increased in control subjects. In contrast, this amplitude was 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 (Jabbari et al., 1985; Iannaccone et al., 2002; Ammendola et al., 2006; Yerdelen et al., 2011; Nebbioso et al., 2020). 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 visual problems.

4.1. 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 (Frenkel et al., 2006). The activation of these receptors is important for induction of long-term potentiation at cortical synapses (Malinow et al., 2000).

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 (Normann et al., 2007; Elvsåshagen et al., 2012; Klöppel et al., 2015; Zak et al., 2018; Valstad et al., 2020). In these studies, C1 amplitude was significantly decreased, or trended towards significant, in the control group and P1 amplitude increased between post-modulation and pre-modulation. Notably, however, Elvsåshagen et al. (Elvsåshagen et al., 2012) did not observe a change in the C1 component. In the present study, P1 was also significantly increased between post-modulation and pre-modulation in the control group, although there were no significant overall group of time effects or interactions effects for C1.

We did not find a significant difference between groups in modulation of the N1 component. In contrast to previous studies, we did observe a decreased N1 amplitude in both groups during continuous visual stimulation, but this was in the opposite direction of N1 modulation observed in previous studies (Normann et al., 2007; Elvsåshagen et al., 2012; Zak et al., 2018). In these studies, a modulation effect of N1 was shown as an increase of the N1 amplitude. The opposite direction of N1 modulation 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 (Fig. 3). 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. (Valstad et al., 2020). 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. (Normann et al., 2007; Elvsåshagen et al., 2012). Early VEP compo-

nents might be less affected by attention or complex cognitive processes (Normann et al., 2007). Attention may especially explain the variation in N1 amplitude (Luck et al., 2000). Future studies should take the subject's attention carefully into account by implementation of an additional attention test (Klöppel et al., 2015; Valstad et al., 2020).

Our observed duration of the potentiated VEP response in controls appeared to be shorter than some of previous studies. Normann et al. (Normann et al., 2007) 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. (Valstad et al., 2020). 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 (Normann et al., 2007; Lisman, 2017). 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 (Normann et al., 2007; Valstad et al., 2020).

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 both groups. In animal studies, it has been shown that an increased response during LTP induction enhances the response after induction (Kushner et al., 2005). 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 (Cooke and Bliss, 2006).

4.2. VEP plasticity in NF1

In contrast to control subjects, we observed no potentiation of the VEP response of the P1 component 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 (Costa et al., 2002; Cui et al., 2008; Shilyansky et al., 2010; Omrani et al., 2015). 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, previous studies in adults with NF1 showed alterations in motor cortical excitability and plasticity upon a form of repetitive transcranial magnetic stimulation (Mainberger et al., 2013; Castricum et al., 2020). Furthermore, magnetic resonance spectroscopy showed reduced GABA levels in the visual cortex of NF1 patients (Violante et al., 2013, 2016) that could affect cortical excitability and plasticity. Interestingly, there was a potentiated VEP response during continuous visual stimulation without delay in the NF1 group and control group. This could indicate that a normal response in VEP potentiation was observed during continuous visual stimulation in the NF1 group, but the ability to maintain this potentiation was affected post-modulation in the NF1 group. A similar effect has been observed in a previous study on motor cortical plasticity using transcranial magnetic stimulation in NF1 patients (Castricum et al., 2020). These previous studies are 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, as mentioned before, the N1 modulation effect in both groups was in the opposing direction as reported in previous studies, 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. In addition, the strength of the cortical visual plasticity could depend on the history of neural activity, i.e. metaplasticity in the human visual cortex (Abraham and Bear, 1996; Bocci et al., 2014). Metaplasticity affects the ability to induce subsequent long-lasting synaptic plasticity (Abraham and Bear, 1996). In future research, metaplasticity should be taken into account by, for example, standardizing the history of neural activity using a combination of neurophysiological techniques such as TMS or transcranial direct current stimulation, and EEG (Bocci et al., 2014; Opie et al., 2017). Furthermore, future studies should investigate whether metaplasticity is aberant in patients with NF1 as has been found in several psychiatric and neurological disorders (Müller-Dahlhaus and Ziemann, 2015).

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 (Normann et al., 2007). 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 (Rietman et al., 2018). However, we did not find any difference in fatigue based on a sleepiness scale at the start or end of the VEP recordings. Nevertheless, we acknowledge the lack of control for eye movements during stimulation. We did instruct and motivate the subjects to keep their focus during the experiment. Furthermore, the used binocular pattern stimulation facilitates fixation and attention (Odom et al., 2010). In addition, the VEP amplitude of P1 usually varies minimally with repeated recordings (Odom et al., 2004). Nevertheless, future studies may benefit from the inclusion of a motivational feature to keep focus during the experiment, such as the implementation of a reward during the experiment. The implementation of an additional test during the non-invasive stimulation paradigms, such as reading out numbers or pressing a key with changing colors (Klöppel et al., 2015; Valstad et al., 2020), could improve the subject's attention. Overall, we did not observe any deviations in the VEP recordings that could indicate visual deprivation.

Although we did not find a significant modulation effect in the P1 amplitude 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.

Conflict of Interest Statement

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.

Acknowledgments

We thank all the subjects who participated in this study.

Funding

This work was supported by resources of the Department of Neuroscience, the Department of Clinical Genetics, the Department of Clinical Neurophysiology, and the Department of Psychiatry of the Erasmus Medical Center, Rotterdam, The Netherlands.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clinph.2022.08.009.

References

Abraham WC, Bear MF. Metaplasticity: the plasticity of synaptic plasticity. Trends Neurosci 1996;19(4):126–30.
Åkerstedt T, Gillberg M. Subjective and objective sleepiness in the active individual. Int J Neurosci 1990;52(1-2):29–37.
Ammendola A, Ciccone G, Ammendola E. Utility of multimodal evoked potentials study in neurofibromatosis type 1 of childhood. Pediatr Neurol 2006;34(4):276–80.
Bocci T, Caleo M, Tognazzi S, Francini N, Briscese L, Maffei L, et al. Evidence for metaplasticity in the human visual cortex. J Neural Transm 2014;121(3):221–31.
Castricum J, Tulen JHM, Taal W, Ottenhoff MJ, Kushner SA, Elgersma Y. Motor cortical excitability and plasticity in patients with neurofibromatosis type 1. Clin Neurophysiol 2020;131(11):2673–81.
Çavuş I, Reinhart RMG, Roach BJ, Gueorguieva R, Teyler TJ, Clapp WC, et al. Impaired visual cortical plasticity in schizophrenia. Biol Psychiatry 2012;71(6):512–20.
Cooke SF, Bliss TVP. Plasticity in the human central nervous system. Brain 2006;129:1659–73.
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.
Descheemaeker M-J, Plasschaert E, Frijns J-P, Legius E. Neuropsychological profile in adults with neurofibromatosis type 1 compared to a control group. J Intellect Disabil Res 2013;57(9):874–86.
Elvsåshagen T, Moberget T, Bøen E, Boye B, Englin NOA, Pedersen PØ, et al. Evidence for impaired neocortical synaptic plasticity in bipolar II disorder. Biol Psychiatry 2012;71(1):68–74.
Frenkel MY, Sawtell NB, Diogo ACM, Yoon B, Neve RL, Bear MF. Instructive effect of visual experience in mouse visual cortex. Neuron 2006;51(3):339–49.
Heinrich SP, Bach M. Adaptation dynamics in pattern-reversal visual evoked potentials. Doc Ophthalmol 2001;102:141–56.
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.
Iannaccone A, McCluney RA, Brewer VR, Spiegel PH, Taylor JS, Kerr NC, et al. Visual evoked potentials in children with neurofibromatosis type 1. Doc Ophthalmol 2002;105:63–81.
Jabbari B, Maitland CG, Morris LM, Morales J, Gunderson CH. The value of visual evoked potential as a screening test in neurofibromatosis. Arch Neurol 1985;42(11):1072–4.
Klem GH, Lüders HO, Jasper HH, Elger C. The ten-twenty electrode system of the International Federation. The International Federation of Clinical Neurophysiology. Electroencephalogr Clin Neurophysiol Suppl 1999;52:3–6.
Klöppel S, Lauer E, Peter J, Minkova L, Nissen C, Normann C, et al. LTP-like plasticity in the visual system and in the motor system appear related in young and healthy subjects. Front Hum Neurosci 2015;9.
Kushner SA, Elgersma Y, Murphy GG, Jaarsma D, van Woerden GM, Hojjati MR, et al. Modulation of presynaptic plasticity and learning by the H-ras/extracellular signal-regulated kinase/synapsin I signaling pathway. J Neurosci 2005;25:9721–34.
Lisman J. Glutamatergic synapses are structurally and biochemically complex because of multiple plasticity processes: long-term potentiation, long-term depression, short-term potentiation and scaling. Phil Trans R Soc B 2017;372(1715):20160260.
Luck SJ, Woodman GF, Vogel EK. Event-related potential studies of attention. Trends Cogn Sci 2000;4(11):432–40.
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).
Malinow R, Mainen ZF, Hayashi Y. LTP mechanisms: from silence to four-lane traffic. Curr Opin Neurobiol 2000;10(3):352–7.
Müller-Dahlhaus F, Ziemann U. Metaplasticity in human cortex. Neuroscientist 2015;21(2):185–202.
Nebbioso M, Moramarco A, Lambiase A, Giustini S, Marenco M, Miraglia E, et al. Neurofibromatosis Type 1: Ocular Electrophysiological and Perimetric Anomalies. Eye Brain 2020;12:119–27.
Normann C, Schmitz D, Fürmaier A, Döing C, Bach M. Long-term plasticity of visually evoked potentials in humans is altered in major depression. Biol Psychiatry 2007;62(5):373–80.
Odom JV, Bach M, Brigell M, Holder GE, McCulloch DL, Mizota A, et al. ISCEV standard for clinical visual evoked potentials: (2016 update). Doc Ophthalmol 2016;133(1):1–9.
Odom JV, Bach M, Barber C, Brigell M, Marmor MF, Tormene AP, et al. Visual evoked potentials standard (2004). Doc Ophthalmol 2004;108(2):115–23.
Odom JV, Bach M, Brigell M, Holder GE, McCulloch DL, Tormene AP, Vaegan. ISCEV standard for clinical visual evoked potentials (2009 update). Doc Ophthalmol 2010;120(1):111–9.
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.
Ribeiro MJ, d'Almeida OC, Ramos F, Saraiva J, Silva ED, Castelo-Branco M. Abnormal late visual responses and alpha oscillations in neurofibromatosis type 1: a link to visual and attention deficits. J Neurodev Disord 2014;6:4.
Ribeiro MJ, Violante IR, Bernardino I, Edden RAE, Castelo-Branco M. Abnormal relationship between GABA, neurophysiology and impulsive behavior in neurofibromatosis type 1. Cortex 2015;64:194–208.
Rietman AB, van Helden H, Both PH, Taal W, Legerstee JS, van Staa AnneLoes, et al. Worries and needs of adults and parents of adults with neurofibromatosis type 1. Am J Med Genet A 2018;176(5):1150–60.
Di Russo F, Martínez A, Sereno MI, Pitzalis S, Hillyard SA. Cortical sources of the early components of the visual evoked potential. Hum Brain Mapp 2002;15(2):95–111.
Schneider SL. The international standard classification of education 2011. In: Elisabeth Birkelund G, editor. Class and Stratification Analysis. Emerald Group Publishing Limited; 2013.
Shilyansky C, Lee YS, Silva AJ. Molecular and cellular mechanisms of learning disabilities: a focus on NF1. Annu Rev Neurosci 2010;33(1):221–43.
Teyler TJ, Hamm JP, Clapp WC, Johnson BW, Corballis MC, Kirk IJ. Long-term potentiation of human visual evoked responses. Eur J Neurosci 2005;21:2045–50.
Tinga AM, de Back TT, Louwerse MM. Non-invasive neurophysiological measures of learning: A meta-analysis. Neurosci Biobehav Rev 2019;99:59–89.
Valstad M, Moberget T, Roelfs D, Slapø NB, Timpe CMF, Beck D, et al. Experience-dependent modulation of the visual evoked potential: Testing effect sizes, retention over time, and associations with age in 415 healthy individuals. Neuroimage 2020;223:117302.
Varni JW, Nutakki K, Swigonski NL. Pain, skin sensations symptoms, and cognitive functioning predictors of health-related quality of life in pediatric patients with Neurofibromatosis Type 1. Qual Life Res 2019;28(4):1047–52.
Violante IR, Patricio M, Bernardino I, Rebola J, Abrunhosa AJ, Ferreira N, Castelo-Branco M. 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.
Yerdelen D, Koc F, Durdu M, Karakas M. Electrophysiological findings in neurofibromatosis type 1. J Neurol Sci 2011;306(1-2):42–8.
Zak N, Moberget T, Bøen E, Boye B, Waage TR, Dietrichs E, et al. Longitudinal and cross-sectional investigations of long-term potentiation-like cortical plasticity in bipolar disorder type II and healthy individuals. Transl Psychiatry 2018;8(1).