

1 **Nerve excitability changes related to muscle weakness in chronic progressive external**
2 **ophthalmoplegia**

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23 Running Title: Nerve dysfunction and weakness in CPEO

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2 *Conflict of interest Statement*

3 HB receives royalties from UCL for sales of his Qtrac software used in this study.

4

5 *Funding*

6 This study received no financial support.

7

8

1 **Abstract**

2 *Objective:* To explore potential spreading to peripheral nerves of the mitochondrial
3 dysfunction in chronic progressive external ophthalmoplegia (CPEO) by assessing axonal
4 excitability.

5 *Methods:* CPEO patients (n=13) with large size deletion of mitochondrial DNA and matching
6 healthy controls (n=22) were included in a case-control study. Muscle strength was quantified
7 using MRC sum-score and used to define two groups of patients: CPEO-weak and CPEO-
8 'normal' (normal strength). Nerve excitability properties of median motor axons were assessed
9 with the TROND protocol and changes interpreted with the aid of a model.

10 *Results:* Alterations of nerve excitability strongly correlated with scores of muscle strength.
11 CPEO-weak displayed abnormal nerve excitability compared to CPEO-'normal' and healthy
12 controls, with increased superexcitability and responses to hyperpolarizing current.
13 Modelling indicated that the CPEO-weak recordings were best explained by an increase in the
14 'Barrett-Barrett' conductance across the myelin sheath.

15 *Conclusion:* CPEO patients with skeletal weakness presented sub-clinical nerve excitability
16 changes which were not consistent with axonal membrane depolarization, but suggested
17 Schwann cell involvement

18 *Significance:* This study provides new insights into the spreading of large size deletion of
19 mitochondrial DNA in CPEO patients.

1 **Highlights**

2 • Patients with chronic progressive external ophthalmoplegia due to large size deletions
3 of mtDNA were studied

4 • Nerve excitability abnormalities were found that correlated with skeletal muscle
5 weakness

6 • Abnormalities suggested a spread of mitochondrial dysfunction to Schwann cells

7

8 **Keywords:** CPEO, Mitochondrial disease, Nerve excitability, Myelin sheath, Barrett-Barrett
9 conductance.

10

11 Word Count: Abstract: 180; Text: 2425

1 **Introduction**

2 Chronic progressive external ophthalmoplegia (CPEO) is one of the most frequent
3 forms of mitochondrial disease. It may be due to diverse genetic alterations, including
4 mitochondrial DNA (mtDNA) large-size deletion, mtDNA point mutation and alteration of a
5 nuclear gene involved in the mtDNA replication (Dey et al., 2000; Hirano et al., 2001; Holt et
6 al., 1988; Kaukonen et al., 2000; Seneca et al., 2001; Spelbrink et al., 2001; Van Goethem et
7 al., 2001; Zeviani et al., 1989).

8 In the case of large-size deletions, deleted mtDNA molecules coexist with normal ones
9 (heteroplasmy) and linear correlation has been observed between cellular energy supply and
10 percentage of mutated mtDNA, without a clear threshold above which symptoms appear
11 (Gellerich et al., 2002). The proportion of large-size deletions can differ greatly between
12 different tissues (Shanske et al., 1990), directly influencing the phenotype (Berenberg et al.,
13 1977; Drachman 1968; Shy et al., 1967). The spreading of large-size deletions to the different
14 tissues is not totally understood. It might be related to the time point at which the genetic
15 alteration occurred during embryogenesis: nervous system as well as muscle and other
16 mesoderm tissues would be involved if the alteration occurred before mesoderm and ectoderm
17 differentiation. Mitotic segregation of deleted mtDNA molecules during oocyte maturation
18 could also explain restricted tissue distribution. (Chinnery et al., 2004; Wai et al 2008).
19 Peripheral neuropathy is common in mitochondrial diseases (Filosto et al., 2003; Hirano et al.,
20 2001; Peyronnard et al., 1980; Seneca et al., 2001; Yiannikas et al., 1986). In CPEO it has
21 preferentially been associated with nuclear gene mutations and less classically with large-size
22 deletions. However peripheral nerve functional abnormalities may be overlooked by standard
23 electroneuromyography (ENMG).

24 The development of computerized threshold-tracking techniques has facilitated non-
25 invasive assessment of the excitability properties of peripheral nerve fibers (Bostock et al.,

1 1998). These techniques have previously been used to explore the pathophysiology of a wide
2 variety of diseases affecting nerve excitability (Lin et al., 2006; Kiernan & Lin, 2013),
3 including the mitochondrial disease MELAS (mitochondrial myopathy, encephalopathy, lactic
4 acidosis and stroke-like episodes (Farrar et al., 2010). The aim of this study was to use the
5 high sensitivity of nerve excitability measurements to detect spread of mitochondrial
6 dysfunction to peripheral nerve in CPEO.

7

8 **Materials and Methods**

9 Thirteen CPEO patients with large-size deletions and 22 healthy controls, matching in
10 sex and age, were enrolled in a prospective case-control study. Inclusion criteria were
11 progressive ptosis and ophthalmoplegia, orbital muscle atrophy on MRI or Scan and muscle
12 mitochondrial histologic abnormalities with presence of large-size deletion of mtDNA. The
13 patients were part of the cohort of 69 CPEO patients followed in the neurological department
14 of “Fondation Ophtalmologique A. de Rothschild”. The study was approved by the ethical
15 committee of Ile de France VI and conducted in accordance with the provisions of the
16 Declaration of Helsinki. Patients and controls were fully informed of the study modalities and
17 gave their written consent. Medical history was collected in the patients’ chart.

18 Large-size deletion was shown in total DNA extracted from muscle biopsy, using
19 Southern blot and long-range PCR to determine the presence, size and proportion of the
20 deletion. When muscle sample was unavailable for molecular studies, large-size deletion was
21 also looked for in urinary sediment sample using long-range PCR.

22 MRC sum-score evaluation was performed on eleven groups of muscles (shoulder
23 abductors, elbow flexors and extensors, wrist flexors and extensors, first dorsal interosseous,
24 hip flexors, knee flexors and extensors, foot flexors and extensors) (Kleyweg et al., 1991;
25 Merckies et al., 2003). The MRC sum-score was used to define two groups of CPEO patients:

1 with normal strength (CPEO-'normal') or with weakness (CPEO-weak). The patients of the
2 latter group presented with skeletal muscle weakness in the four limbs and MRC sum-score
3 <108/110. Slight deltoid weakness was frequent and did not suffice to include in the CPEO-
4 weak patients in the absence of additional muscle weakness.

5 The multiple measures of nerve excitability were performed on the median motor
6 nerve, once an ENMG procedure had been achieved. The cutaneous temperature was
7 monitored before and after excitability recordings at the stimulation site, to ensure a
8 temperature around 32°C. The stimulating current was applied over the median nerve at the
9 wrist. The anode was placed 10 cm proximally to the cathode over muscle on the lateral part
10 of the forearm. Stimuli were delivered using bipolar constant current stimulator (DS5
11 stimulator; Digitimer, U.K.). The current intensity was adjusted to reach a CMAP of target
12 amplitude by means of the Qtrac program (written by H. Bostock, copyright Institute of
13 Neurology, UCL, London, UK). CMAP was recorded from the abductor pollicis brevis using
14 a belly-tendon configuration. The signal had 50 Hz noise eliminated with a Humbug (Quest
15 Scientific, Canada) before digitization with a data acquisition card (PCI-6221 National
16 Instruments, U.S.A.) and recording in a computer. Assessment of nerve excitability was
17 performed using the TRONDNF Qtrac recording protocol.

18 The stimulus-response relationship was established by manually increasing the intensity
19 of stimulation until a CMAP of maximum amplitude was obtained. The program then
20 generated a descending stimulus-response relationship and fitted a curve, which was
21 afterwards used to optimally track a target response set to 40% of the maximal CMAP.
22 Threshold tracking was used to determine the following excitability properties: (i) strength-
23 duration relationship (evaluated as the 'threshold current' to reach the target response when
24 the duration of the stimulation decreased from 1 ms to 0.2 ms in 0.2 ms steps); (ii) threshold
25 electrotonus (assessed as the threshold changes during and 100 ms after 100 ms polarizing

1 currents set to $\pm 20\%$ and $\pm 40\%$ of the control threshold); (iii) current/threshold relationship
2 (evaluated as the threshold changes at the end of 200 ms polarizing currents varied from 50%
3 to -100% of the control current threshold in 10% steps); and (iv) recovery cycle (determined
4 as the threshold changes following a supra maximal conditioning pulse at inter-pulse intervals
5 that were gradually decreased from 200 ms to 2 ms). Analysis of the excitability recordings
6 was carried out automatically by the Qtrac software, which generated multiple excitability
7 measurements as described previously (e.g. Table 2 in Tomlinson et al., 2010).

8

9 *Statistical analysis*

10 Clinical characteristics and excitability parameters were analysed with Fischer test
11 (discrete variables) and Mann-Whitney U-test (continuous variables). Wilcoxon-test was used
12 to analyse repetitive assessments of excitability parameters. Since MRC sum-scores were not
13 normally distributed, they were compared with nerve excitability measurements by
14 Spearman's rank correlation coefficient rho.

15 The Memfit facility within the QtracP program was used to assess the likely
16 biophysical basis for the abnormal excitability data, by comparison with the 'Bostock' model
17 of nerve excitability (Howells et al., 2012). To fit the model to the real nerve excitability
18 measurements a least squares method was used. For each measurement in the Trond protocol,
19 the discrepancy between the model and the recordings was calculated as: $[(x_m - x_n)/s_n]^2$,
20 where x_m is the threshold of the model, x_n the mean and s_n the standard deviation of the
21 thresholds for the real nerves. The weights for the measurements within each test were the
22 same but the weights for each test were different (strength-duration relationship = 0.5,
23 threshold electrotonus = 2, current/threshold relationship = 1, recovery cycle = 1).

1

2 **Results**

3 The characteristics of the two patient groups are compared in Table 1. Nerve
4 excitability did not differ in any of its parameters between the CPEO and control group
5 (Figure 1A-D). Within the CPEO group however threshold changes during polarizing currents
6 (i.e. ‘fanning-out’ of threshold electrotonus) strongly correlated with muscular weakness, as
7 measured by the MRC sum-score (Figure 2). The greatest correlations were with the
8 responses to hyperpolarizing currents, TEh(10-20 ms) ($\rho = 0.805$), TEh(20-40 ms) ($\rho =$
9 0.832) and TEh(90-100 ms) ($\rho = 0.635$).

10 CPEO-weak patients were thus compared as a group to the CPEO-'normal' and control
11 groups and found to differ with respect to both threshold electrotonus and recovery cycle
12 (Figure 1E-H, Table 2). In response to depolarizing current, reduced threshold was observed
13 during the early stage, ranging from 10 – 20 ms after the beginning of the applied current.
14 From early to late stage, important increase of the threshold was observed during the
15 hyperpolarized current, associated with an abnormal restoration of the threshold after the end
16 of the current. Superexcitability was increased. In contrast, strength duration and current
17 threshold relationship did not differ in any group. CPEO-'normal' and control groups mainly
18 overlapped.

19 A second evaluation could be performed few months after the initial one in two
20 CPEO-weak patients. In both cases the results of the two evaluations were superimposable,
21 showing very little within-patient variability.

22

23 *Modelling*

24 To address the biophysical basis of these changes, we used the MEMFIT facility in the
25 QtracP program. Adjustments were made until the discrepancy to the standard model of

1 human motor axons with the CPEO-'normal' group recordings could not be further reduced.
2 All the model parameters were tested to find which change in the CPEO-'normal' model could
3 best minimize the discrepancy with the CPEO-weak recordings. An increase by 17 % of the
4 conductance of the Barrett and Barrett conductance (GBB) from 36.4 nS (model value) to
5 42.6 nS reduced the discrepancy between CPEO-weak and CPEO-'normal' by 58.7 % (Figure
6 3). The GBB parameter represents conduction through the parallel paths across the myelin
7 sheath and around it at the paranode (Barrett et al., 1982). The next best discrepancy reduction
8 was obtained by reducing the nodal and internodal leak conductances (54.5 % and 54.4 %
9 respectively). These changes are physiologically implausible, however, since they involved
10 reducing the conductance by 96.9 % and 99.7 % respectively. We also optimized the fit of the
11 model first to the control group. The best parameter alteration to fit the CPEO-weak patient
12 was an increasing of GBB from 39.3 to 45.3 nS (15.5% increase), which reduced the
13 discrepancy by 65.5%. Modelling of the recording in the most abnormal individual CPEO-
14 weak patient was also best achieved by an increase of GBB, in this case from 36.4 nS to 52.5
15 nS, which reduced the discrepancy with the CPEO-'normal' patients by 61.5 % (Figure 3).

16

17 **Discussion**

18 We have found a correlation between muscle strength and nerve excitability in CPEO
19 patients. Abnormal nerve excitability was observed in CPEO patients with skeletal muscle
20 weakness. These changes involved mostly the changes in excitability occurring during and
21 after hyperpolarising currents (i.e. hyperpolarizing threshold electrotonus) as well as the
22 superexcitable period of the recovery cycle. Here we discuss first the likely biophysical basis
23 of the nerve excitability changes, and secondly why they should correlate with weakness.

24

25 *Biophysical basis of nerve excitability changes*

1 In mitochondrial disease an impaired function of energy-dependent sodium pump
2 (Na⁺/K⁺-ATPase) might be expected, which would result in membrane depolarization.
3 Evidence for such an axonal depolarization has been found during stroke like episode in
4 MELAS (Farrar et al., 2010). The effects of ischemic depolarization on nerve excitability are:
5 (i) Threshold Electrotonus 'fanning in' (i.e. smaller threshold changes); (ii) Current Threshold
6 relationship (IV) similarly shows smaller threshold changes; (iii) In the recovery cycle, the
7 relative refractory period is prolonged, superexcitability is reduced, and subexcitability is
8 reduced to a lesser extent (Kiernan & Bostock, 2000). The pattern of abnormal excitability in
9 CPEO-weak patients was quite different from membrane depolarization, since it included
10 increased superexcitability and increased threshold ('fanning-out') during hyperpolarizing
11 electrotonus

12
13 The nerve excitability modelling indicates that GBB increase was the most plausible
14 explanation of the observed abnormalities in the CPEO-weak patients. The GBB was first
15 described by Barrett and Barrett (1982) and is the conductive path between the internodal
16 axolemma and the external medium, which is responsible for the depolarizing afterpotential
17 (DAP), and the resulting superexcitability. GBB comprises conduction across the myelin
18 sheath itself, and also through the imperfect electrical seal between the paranodal loops of
19 myelin and the axolemma. An increase in GBB, whether due to increased leakiness of the
20 myelin or a more leaky paranodal seal, leads to an increase of the DAP and superexcitability,
21 together with an early fanning out of the threshold electrotonus waveforms, as shown in
22 figures 1 and 3. If the increase in GBB were due to a more leaky paranodal seal, one might
23 have expected an increase in nodal fast K conductance due to exposure of some
24 juxtapanodal K⁺ channels. Although a slightly better fit could be obtained by a small

1 increase in this model parameter, the modelling with two parameter changes is subject to too
2 much uncertainty to be able to draw any conclusions on this question.

3 The hypothesis of a change in the myelin sheath or paranodal sealing of the myelin is
4 supported by data available from nerve biopsies in mitochondrial myopathies reporting
5 disproportionately thin myelin (Peyronnard et al., 1980) and paracrystalline inclusions in
6 mitochondria of Schwann cells (Yiannikas et al., 1986). Reviewing neuropathies associated
7 with mitochondrial disorders, Schröder (1993) found that the majority of mitochondrial
8 abnormalities were found in Schwann cells rather than axons.

9

10 *Why does abnormal nerve excitability correlate with weakness?*

11 The correlation between nerve excitability and MRC score is unlikely to be due to any
12 effect of the subclinical nerve dysfunction on muscle, or of muscle weakness on nerve, since
13 the nerve dysfunction was recorded in APB, but weakness was not observed in this muscle in
14 any of the CPEO patients. Moreover, although abnormal nerve excitability may have been
15 present in other nerves, there is no precedent for weakness being caused by such a subclinical
16 nerve abnormality, without evidence of neuropathy on ENMG. In episodic ataxia type 2
17 (EA2), a very similar subclinical nerve excitability abnormality has recently been reported
18 (Tomlinson et al., 2016), in which an increase in Barrett-Barrett conductance was attributed to
19 nodal malformation in the early neonatal period due to the lack of P/Q calcium channels.
20 Weakness was not evident in those patients (Jen et al., 2001).

21 Direct involvement of muscle mitochondria is a much more plausible explanation of
22 skeletal muscle weakness in CPEO. We therefore regard it as most likely that the correlation
23 between weakness and nerve excitability changes indicates that the spreading of large-size
24 deletions to ectodermal tissues is associated with heteroplasmy in nerve as well as muscle.
25 The variable spreading of large-size deletion mtDNA would explain the absence of

1 abnormality in an earlier study with only 4 CPEO patients (Ng et al., 2010). The excitability
2 changes observed in weak patients provide evidence for a spreading of large-size deletions to
3 ectodermal tissue, and consistent with this, CNS involvement with cerebellar ataxia was only
4 seen in patients with nerve excitability abnormalities.

5

6 *Conclusions*

7 Nerve excitability studies have revealed subclinical axonal changes in a subgroup of large-
8 size deletion CPEO patients with weakness, which suggest a spreading of large-size deletion
9 to Schwann cells, as well as to skeletal muscle, in this more severe phenotype

10

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12

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17

1 Figures

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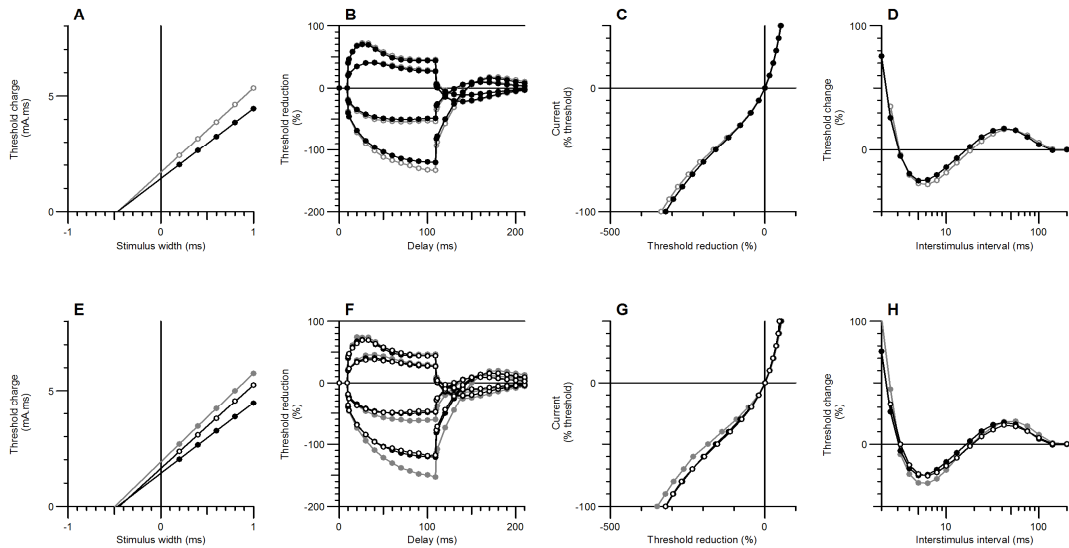


Figure 1.

3

4 **Figure 1.** Comparison of mean nerve excitability waveforms.

5 **Top row (A-D):** all CPEO patients (grey empty circles) compared to controls (black filled

6 circles). **Second row (E-H):** CPEO-weak patients (grey filled circles) compared to CPEO-

7 'normal' (black empty square) and controls (black filled circles). Excitability tests: charge

8 duration relationship (A,E), threshold electrotonus (B,F), current threshold relationship (C,G)

9 and recovery cycle (D,H).

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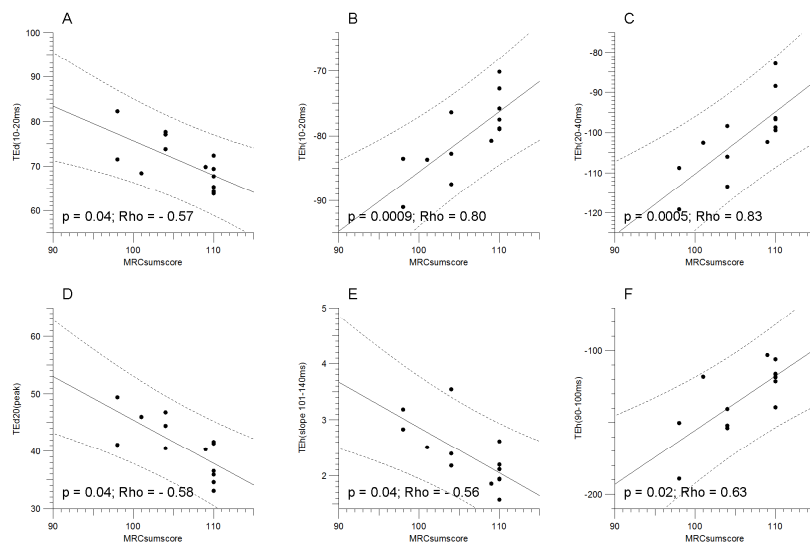


Figure 2.

- 1
- 2 **Figure 2.** Correlations between strength (MRC sum-score) and nerve excitability
- 3 measurements of CPEO patients.
- 4 Scatter plots with regression line and 95% confidence limits; **A** – TE_d (10-20ms); **B** – TE_h
- 5 (10-20ms); **C** – TE_h (20-40ms); **D** – TE_d 20 (Peak); **E** – TE_h (90-100ms); **F** – TE_h (slope 101
- 6 – 140ms). Figures show results of Spearman's rank correlation test: p = probability of
- 7 correlation occurring by chance, Rho = correlation coefficient.
- 8

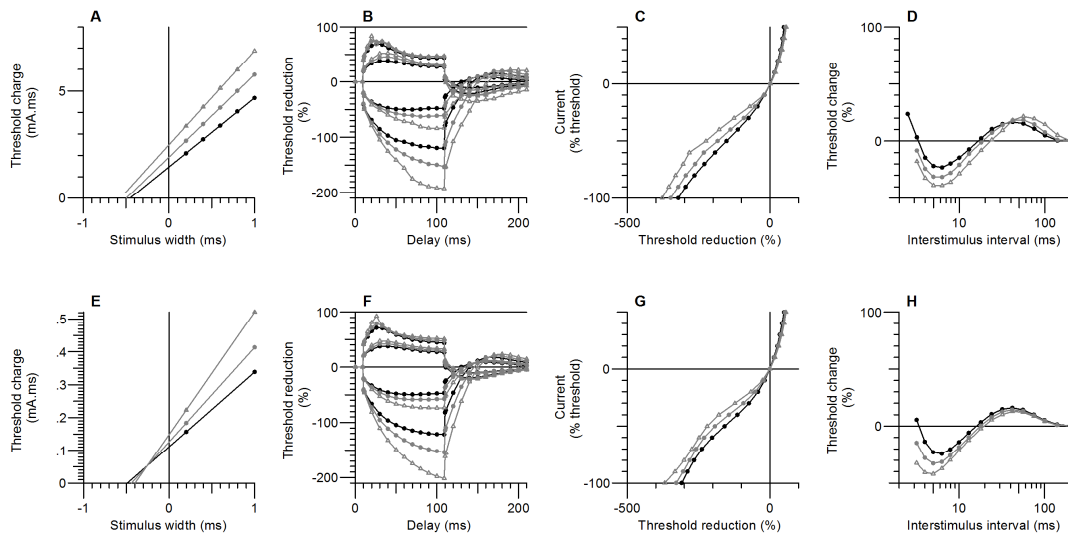


Figure 3.

1

2 **Figure 3.** Modelling of individual and mean nerve excitability waveforms.

3 **Top row (A-D):** recorded waveforms: CPEO-'normal' (black filled circles), CPEO-weak
 4 (grey filled circles) and the most abnormal CPEO-weak patient (grey filled triangle).

5 **Second row (E-H):** modelled waveforms: CPEO-'normal' (GBB=36.4 nS) (black filled
 6 circles), CPEO-'normal' modified (GBB=42.6 nS) to fit CPEO-weak (grey filled circles) and
 7 CPEO-'normal' modified (GBB=52.5 nS) to fit the most abnormal CPEO-weak patient (grey
 8 filled triangle). Excitability tests: charge duration relationship (A,E), threshold electrotonus
 9 (B,F), current threshold relationship (C,G) and recovery cycle (D,H).

1

Table 1. CPEO characteristics

	CPEO-'normal' n = 7	CPEO-weak n = 6	P
Age at onset (years)	19.3 [6– 20]	11.6 [7 – 14]	ns
Sex F/M	3/4	4/2	ns
Ptosis	7	6	ns
Ophthalmoplegia	7	6	ns
Bulbar	1 (14)	4 (66)	0.006
Skeletal UL/LL	1 / 0	6 / 6	-
MRC sum-score	110 [109 – 110]	101 [98 – 104]	-
Heart	2 (28)	1 (16)	ns
Ataxia	0	4 (67)	< 0.0001
Retinopathy	0	0	-
Large size deletion mt DNA	7	6	
Southern blot muscle	7	5	
Long range PCR urine	-	1	-
Size of LSD (Kb)	7 [3 – 8]	6 [4 – 8]	-
mtDNA with LSD (%)	20 [10 – 40]	45 [10 – 50]	-

2

3 Data: median [range] or number of patient (percentage); Medical Research Council : MRC;

4 Mitochondrial DNA : mtDNA; skeletal weakness in the upper / lower limbs : skeletal UL/LL; PCR :

5 polymerase chain reaction; LSD : large-size deletion; Percentage of mitochondrial DNA with large

6 size deletion : mtDNA with LSD.

Table2. Excitability measurements

	Controls	CPEO		CPEO-'normal'		CPEO- weak		
	n=22	n=13	P	n=7	P	n=6	P	P†
Matching parameters								
Age (years)	57.4 ± 1.4	54.3 ± 2.7	ns	49.4 ± 3.4	0.03	59.8 ± 2.9	ns	ns
Sex (M = 1, F = 2)	1.7 ± 0.1	1.5 ± 0.1	ns	1.3 ± 0.2	ns	1.7 ± 0.2	ns	ns
Temperature (C°)	33.1 ± 0.2	32.8 ± 0.4	ns	33.30 ± 0.6	ns	32.4 ± 0.4	ns	ns
Stimulus response relationship								
Peak response\ (mv)	7.9 ± 1.1	7.6 ± 1.1	ns	8.4 ± 1.1	ns	7.2 ± 1.1	ns	ns
Stimulus-response\ slope	4.7 ± 1.1	5.1 ± 1.1	ns	5.1 ± 1.1	ns	4.9 ± 1.1	ns	ns
Stimulus (mA) for 50% max response	4.4 ± 1.1	5.0 ± 1.1	ns	4.9 ± 1.2	ns	5.5 ± 1.2	ns	ns
Strength-duration relationship								
Strength-duration time constant (ms)	0.48 ± 0.0	0.49 ± 0.0	ns	0.48 ± 0.0	ns	0.52 ± 0.0	ns	ns
Rheobase (mA)	2.9 ± 1.1	3.4 ± 1.1	ns	3.4 ± 1.7	ns	3.5 ± 1.2	ns	ns
Threshold Electrotonus								
TEd40(10-20ms)	69.9 ± 0.9	71.0 ± 1.5	ns	67.5 ± 1.2	ns	73.9 ± 1.4	0.03	0.01
TEd40(peak)	68.3 ± 0.9	70.6 ± 1.4	ns	67.6 ± 1.2	ns	73.2 ± 1.5	0.02	0.02
TEd20(peak)	40.0 ± 0.9	40.9 ± 1.4	ns	37.6 ± 1.3	ns	45.1 ± 1.6	0.02	0.01
TEd40(Accom)	25.1 ± 1.0	25.6 ± 1.1	ns	24.0 ± 1.3	ns	27.07 ± 1.4	ns	ns
TEd(90-100ms)	44.0 ± 1.0	45.4 ± 0.9	ns	43.8 ± 0.7	ns	46.6 ± 1.4	ns	ns
Accommodation half-time (ms)	38.9 ± 0.9	41.9 ± 1.8	ns	45.4 ± 2.1	0.01	38.45 ± 1.8	ns	0.02
TEh40(10-20ms)	-76.7 ± 1.2	-79.9 ± 1.6	ns	-76.4 ± 1.4	ns	-84.2 ± 2.1	0.007	0.01
TEh40(20-40ms)	-95.0 ± 2.0	-101.0 ± 2.7	ns	-95.0 ± 2.6	ns	-108.8 ± 3.7	0.004	0.008
TEh40(90-100ms)	-120.5 ± 4.3	-132.7 ± 6.7	ns	-117.3 ± 4.4	ns	-151.1 ± 9.8	0.004	0.008
TEh40(slope 101-140ms)	2.1 ± 0.1	2.4 ± 0.1	ns	2.0 ± 0.1	ns	2.7 ± 0.2	0.01	0.01
Current/threshold Relationship								
Resting I/V slope	0.59 ± 0.0	0.56 ± 0.0	ns	0.58 ± 0.0	ns	0.53 ± 0.0	ns	ns
Minimum I/V slope	0.24 ± 0.0	0.23 ± 0.0	ns	0.24 ± 0.0	ns	0.21 ± 0.0	ns	ns
Hyperpolarisation I/V slope	0.38 ± 0.0	0.38 ± 0.0	ns	0.40 ± 0.0	ns	0.38 ± 0.0	ns	ns
Recovery cycle								
RRP (ms)	3.0 ± 1.0	3.1 ± 1.0	ns	3.2 ± 1.1	ns	3.0 ± 1.0	ns	ns
Superexcitability (%)	-24.3 ± 1.2	-27.4 ± 1.4	ns	-24.6 ± 1.4	ns	-30.5 ± 2.2	0.02	0.05
Subexcitability (%)	16.6 ± 0.8	16.2 ± 1.7	ns	14.5 ± 2.7	ns	18.25 ± 1.8	ns	Ns

- 1 CPEO-'normal' = CPEO patients with normal strength, CPEO-weak = patients with muscular weakness. Data
- 2 expressed as mean \pm SE; Mann-Whitney U-test are used to compare (i) CPEO, CPEO-'normal' and CPEO-weak
- 3 to controls : P (ii) CPEO-weak to CPEO;'normal' : P†.
- 4

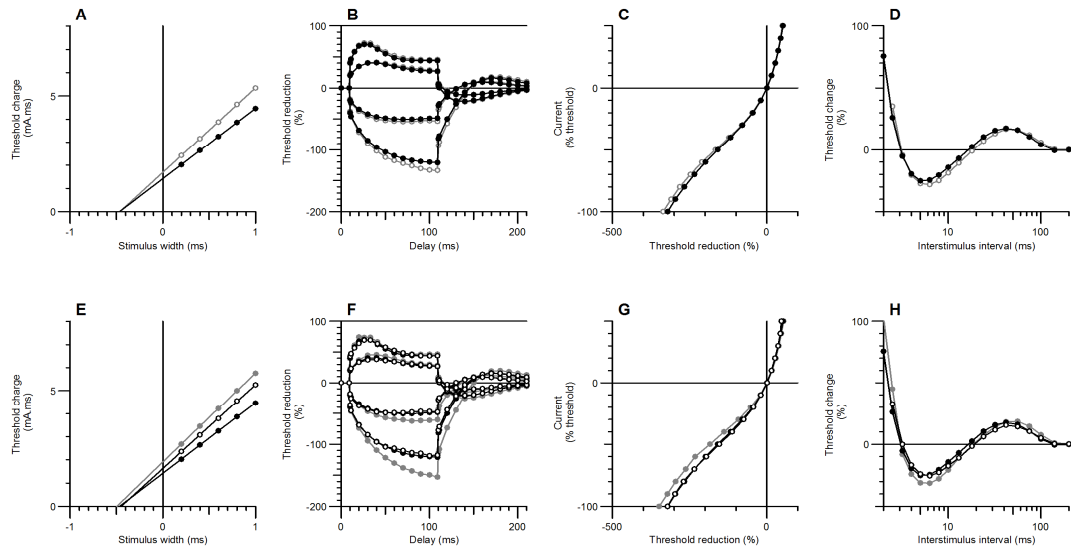


Figure 1.

5

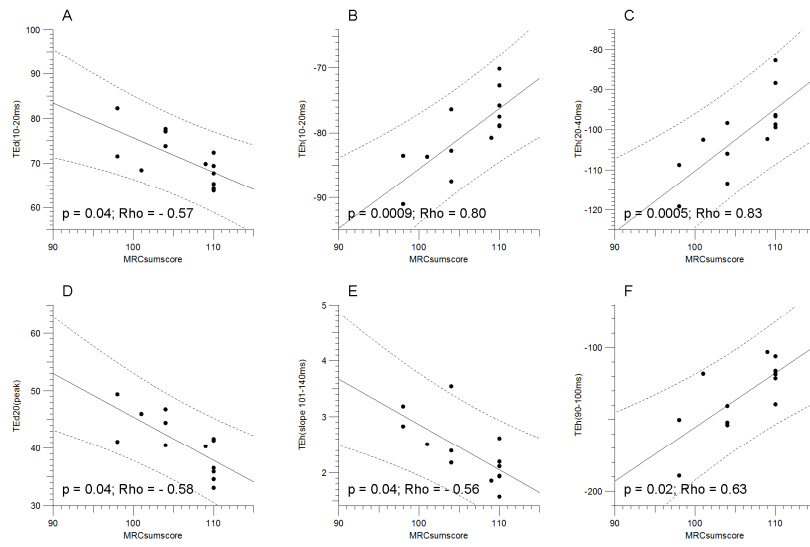


Figure 2.

1

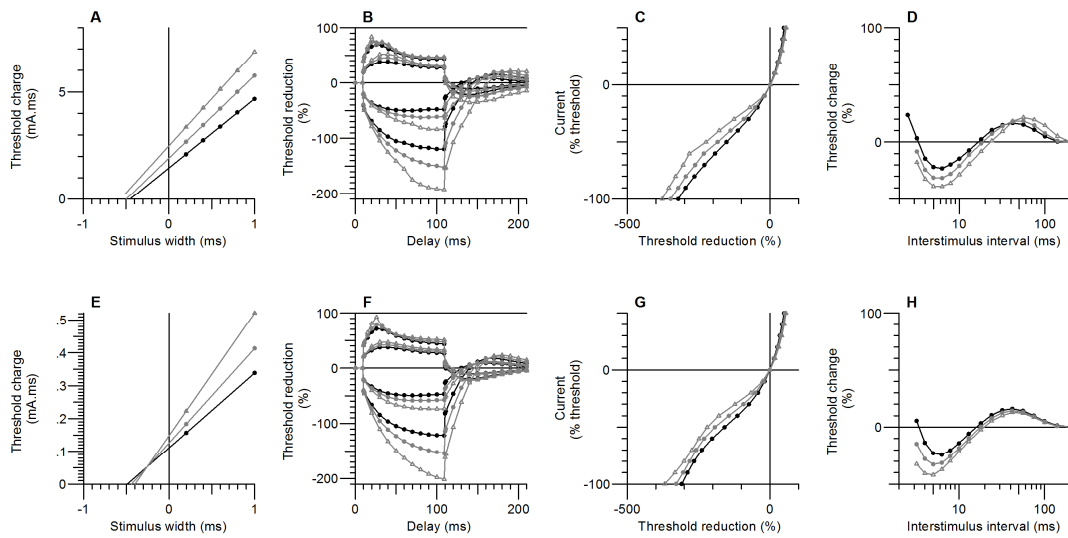


Figure 3.

2