IN VIVO ASSESSMENT OF INTERICTAL SARCOLEMMAL MEMBRANE PROPERTIES IN HYPOKALAEMIC AND HYPERKALAEMIC PERIODIC PARALYSIS

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ABSTRACT

Objective: Hypokalaemic periodic paralysis (HypoPP) is caused by mutations of Ca_v 1.1, and Na_v1.4 which result in an aberrant gating pore current. Hyperkalaemic periodic paralysis (HyperPP) is due to a gain-of-function mutation of the main alpha pore of Na_v1.4. This study used muscle velocity recovery cycles (MVRCs) to investigate changes in interictal muscle membrane properties *in vivo*.

Methods: MVRCs and responses to trains of stimuli were recorded in tibialis anterior and compared in patients with HyperPP(n=7), HypoPP (n=10), and normal controls (n=26).

Results: Muscle relative refractory period was increased, and early supernormality reduced in HypoPP, consistent with depolarisation of the interictal resting membrane potential. In HyperPP the mean supernormality and residual supernormality to multiple conditioning stimuli were increased, consistent with increased inward sodium current and delayed repolarisation, predisposing to spontaneous myotonic discharges.

Conclusions: The *in vivo* findings suggest the interictal resting membrane potential is depolarized in HypoPP, and mostly normal in HyperPP. The MVRC findings in HyperPP are consistent with presence of a window current, previously proposed on the basis of *in vitro* expression studies. Although clinically similar, HyperPP was electrophysiologically distinct from paramyotonia congenita.

Significance: MVRCs provide important *in vivo* data that complements expression studies of ion channel mutations.

HIGHLIGHTS:

- The muscle resting membrane potential is depolarised in HypoPP, but mostly normal in HyperPP
- Muscle excitability studies support the presence of a window current in HyperPP
- Excitability measures distinguish between HypoPP and HyperPP, and HyperPP and paramyotonia congenita.

KEY WORDS: Periodic paralysis, sodium channel, calcium channel, muscle excitability, membrane potential, paramyotonia congenita.

INTRODUCTION

The familial periodic paralyses are dominantly inherited disorders of skeletal muscle ion channels. Mutations in Ca_v 1.1 (*CACNA1S*), Na_v1.4 (*SCN4A*), or K_{IR} 2.1 (*KCNJ2*) result in channel dysfunction that causes destabilisation of the muscle resting membrane potential; this predisposes the muscle membrane to sustained depolarization manifesting as episodes of paralysis.

In hypokalaemic periodic paralysis (HypoPP), the susceptibility to anomalous depolarization in low external potassium is caused by a gating pore leakage current associated with mutations in the S4 voltage sensors of Ca_v1.1 (HypoPP1) and Na_v1.4 (HypoPP2).

In hyperkalaemic periodic paralysis (HyperPP), gain-of-function mutations in Na_v1.4 predispose to sustained depolarization in high external potassium. Early studies (prior to the successful expression of *human* Na_V1.4) using heterologous expression of *rat* Na_V1.4 containing mutations corresponding to the human T704M and M1592V, demonstrated a disruption in fast inactivation leading to an increase in persistent non-inactivating sodium currents which allowed sodium current to flow even after tens of milliseconds (Cannon and Strittmatter, 1993). Another early study also using the rat homologue of the T704M mutation found a shift of the voltage dependence of activation in the negative, hyperpolarised direction (Cummins et al., 1993). Subsequent studies of *human* Na_v1.4 channels with the T704M and M1592V mutations however, found no impairment of fast inactivation (Yang et al., 1994; Bendahhou et al., 1999; Rojas et al., 1999; Hayward et al., 1999), but did find a shift of activation in the hyperpolarised direction by 5-10 mV, and a shift of the midpoint of the slow inactivation curve in the depolarised direction. It was proposed that the shift in activation could lead to a lowered threshold for action potential (AP) generation, and also lead to window currents arising from overlaps between the

voltage dependence of activation and slow inactivation (Yang et al., 1994; Bendahhou et al., 1999; Bendahhou et al., 2002).

Several mouse models of periodic paralysis have been studied. Curiously, unlike the human carriers of the mutation, the mouse models of PP do not display spontaneous episodes of weakness although decreasing (HypoPP) (Wu et al., 2011; Wu et al., 2012) or increasing (HyperPP) (Hayward et al., 2008; Corrochano et al., 2014) the extracellular potassium concentration can induce muscle weakness *ex vivo*.

The results of PP mutations in expression clones of different species and lack of spontaneous episodes of weakness in animal models, highlight the need to examine the effect of ion channel mutations in humans themselves. However, because human muscle tissue is not readily available, and there is limited access to human primary muscle cultures, most studies continue to be performed using heterologous expression in non-muscle cell lines or toad oocytes.

Routine *in vivo* electrophysiological techniques are insufficiently sensitive to investigate alterations in muscle ion channel function. Even specifically designed exercise tests (Fournier et al 2004, Tan et al 2011), which may show patterns of change in patients with muscle channelopathies are limited in the information they can provide about the specific effects of a mutation on ion channel function. Muscle excitability studies, where membrane potential changes are inferred from velocity recovery cycles, provide indirect measures of muscle fibre membrane potential *in vivo*, and have proved a useful method for investigating muscle membrane properties occurring as a consequence of metabolic and electrolyte abnormalities, and in other muscle ion channelopathies (Z'Graggen and Bostock 2009; Z'Graggen et al., 2010; Z'graggen et al., 2011; Tan et al., 2012, 2014, 2016, 2018). Because the studies are performed *in vivo*, the various stimulation paradigms allow investigation, not only of changes directly related to the mutant channel, but may also detect secondary changes resulting from compensatory mechanisms or interactions with other ion channels.

When combined with results of *in vitro* expression studies, the muscle excitability data help to provide a clearer understanding of the actual consequences of a specific mutation to muscle function *in vivo*.

We have previously studied patients with periodic paralysis associated with Andersen-Tawil syndrome (*KCNJ2* mutations) (Tan et al 2012). In the current study, we used muscle excitability studies to investigate the interictal muscle membrane properties in patients with hyperkalaemic and hypokalaemic periodic paralysis.

METHODS

Patients

There were 7 patients with HyperPP aged 53.14 ± 5.14 years (range 29-68), 8 patients with HypoPP1, aged 41 ± 4.11 years (range 19-52), and two with HypoPP2 aged 17 and 18. Their gender, age, and genetic mutations are as shown in Table 1. There was no significant difference in gender or age between patients and controls. None of the patients reported any weakness typical of an acute attack at the time of the study.

Asymptomatic Controls

The MVRC studies were compared with recordings from 26 healthy volunteers, 10 men, 16 women, aged 44.2 ± 12.3 years (range 27-66) who served as normal controls (NC).

Consent

Informed written consent was obtained from all patients and controls according to the Declaration of Helsinki. This study was approved by the London-Westminster Research Ethics Committee, London, UK.

Study Protocol

Limited nerve conduction studies, muscle velocity recovery studies, electromyography (EMG) of tibialis anterior (TA), the long exercise test (Tan et al 2011), and a blood sample for electrolytes were performed on the same day. More extensive nerve conduction studies and EMG were performed prior to this study as part of the patients' neurophysiological workup.

Muscle velocity recordings

Experimental setup

The recording technique was as described previously for tibialis anterior (Tan et al., 2012; Tan et al., 2014; Tan et al., 2016). For details of the muscle velocity recovery cycle and ramp protocols, please see Tan et al., 2014, and for details of the repetitive stimulation protocol, please see Tan et al., 2012.

Data analysis

Recovery cycle data were analyzed by the QtracP program, as previously described (Z'Graggen et al., 2009; Z'Graggen et al., 2011; Tan et al., 2012; Tan et al., 2014).

Statistics

We used the non-parametric unequal variance t-test (Welch rank test) for intergroup comparisons. Only $P<0.01$ was considered significant when comparing groups with multiple comparisons, but for discussion, $P<0.05$ is mentioned when relevant for individual tests.

RESULTS

Basic Neurophysiology

None of the patients had evidence of a generalised large fibre neuropathy. The EMG and long exercise test (LET) findings, and serum potassium levels are presented in Table 1. The LET was abnormal (maximum decrement >40%) in 3/6 of the HyperPP patients, and 6/8 of

the HypoPP patients in whom it was performed. The skin temperature for the groups was comparable: NC 30.38 ^{o}C ± 0.21, HyperPP 29.94 ^{o}C ± 0.43, HypoPP 30.51 ^{o}C ± 0.3.

Velocity recovery cycles

The mean MVRCs of patients with HypoPP1 and HypoPP2 are compared with the normal controls in Figure 1A. There was no significant difference between HypoPP1 and HypoPP2 patients in any of the measurements, so these two groups were combined for all subsequent figures and measurements. MVRCs of HypoPP, HyperPP and normal controls are compared in Figure 1B, and the measurements shown in Table 2 and selected ones illustrated in Figure 2.

The most striking difference is the marked increase in relative refractory period (MRRP) ($p=1.1x10^{-7}$), and the reduction in early supernormality (ESN)($p=0.00057$) seen in the HypoPP patients, which contrasted with the normal ESN and MRRP in the HyperPP group (Figure 2). Analysis of individual recordings showed that three of the HypoPP patients (patients 8, 12 and 14) had both MRRP and ESN within the normal range (mean ± 2SD: ESN = 6.99-15.39, MRRP = 2.56-4.84 ms), although in two of the three, the ESN was at the lower end of normal, and the MRRP at the upper end of normal, in line with the direction of changes seen in the other HypoPP patients. In the HyperPP group, two of the seven patients (pts 4, 6) had an increased MRRP and reduced ESN, but these parameters were normal for the rest of the group.

The time to maximal ESN was delayed in both HypoPP and HyperPP patients, but was only significant in the HypoPP group ($p=0.0015$). There was a reduction in the mean supernormality (area under the curve) after 5 conditioning stimuli (5XMSN, $p=0.0004$) and late supernormality (LSN, $p=0.0039$) in the HypoPP patients, which became highly significant after 5 conditioning stimuli (5XLSN, $p=4.5x\ 10^{-6}$).

In the HyperPP group (Table 2, Figure 2), the main difference compared with controls was an increase in the area under the curve after 2 conditioning stimuli (2XMSN, p=0.0017), and an increased extra residual supernormality after 5 conditioning stimuli (5XRSN, p=0.007). For both these parameters, and for late supernormality, the direction of change compared with controls was in the opposite direction to the HypoPP group, resulting in significant differences between HyperPP and HypoPP groups.

Frequency Ramp

The results of increasing the stimulation rate up to an average of 15.5Hz by adding intermittent 1-s trains of conditioning stimuli at frequencies from 1 to 30Hz are shown in Table 3 and illustrated in Figure 3. In this test, all groups exhibited a U-shaped latency curve, with initial increase in speed giving way to relative slowing of conduction, probably because progressive depolarization due to potassium accumulation in the t-tubules resulted in sufficient sodium channel inactivation to slow conduction.

The main difference in the ramp findings was a smaller reduction in latency during the ramp in the HypoPP group. This was significant for the first in train at 15 Hz (Lat $15Hz_{first}$, p=0.0039), and for the last in train at 30Hz (Lat $30Hz_{last}$, p=0.002) compared with controls. There also appeared to be a delay in the onset of change, with very little change in latency occurring at the lowest frequencies.

There were no significant changes between the HyperPP group and normal controls, but the latency changes at 15 Hz compared with controls were in the opposite direction to the HypoPP group, and reached significance for both the first (p=0.0053) and last (p=0.00057) in train.

There were no significant differences in the amplitude changes in either group compared with controls.

Repetitive Stimulation

The results of interspersing a 1s train of 20 stimuli between recovery cycle measurements are illustrated in Figure 4; the measurements are listed in Table 4. This relatively long test was only completed for 6 HypoPP patients, 5 HyperPP patients, and 24 normal controls, but nevertheless showed some interesting differences in the HypoPP group. The difference in MRRP from NC was abolished during the 20 Hz repetitive stimulation, but became more exaggerated in the recovery period after the 6 minutes of repetitive stimulation ($p=1.99 \times 10^{-6}$).

The latencies of the first and last in the train of 20 Hz stimuli (Cycles 1,2 first and last) were increased in the HypoPP group ($p=8.7 \times 10^{-5}$, and p=0.00035 respectfully), but this difference was abolished with continued repetitive stimulation (cycles 4,5) as conduction slowed in NC with depolarisation during the 20Hz trains.

The latencies of the last in train in the HyperPP group were less than in NCs, but this difference was not significant. However, as the latency changes in the HyperPP group were in the opposite direction from controls to the HypoPP patients, the differences between HyperPP and HypoPP were significant both in the early (cycles 1,2 first and last, both p=0.00057) and later cycles (cycles 4,5 and last, p=0.0025) during the repetitive stimulation. There were no significant differences between HyperPP and normal controls, and no significant amplitude changes in either group.

Separation of HypoPP and HyperPP on excitability measures

To explore the ability of these excitability measures to separate the different groups, we ignored the repetitive stimulation data, since it was incomplete. With the MVRC and frequency ramp data, it was not possible to separate either HypoPP or HyperPP patients completely from controls (e.g. Figures 5A, 5B). However, there were a number of combinations of two measurements that enabled the HypoPP and HyperPP patients to be

well separated. In general, the changes were in opposite directions from NC, whose measurements fell in between the HyperPP and HypoPP patients (e.g. Figures 5C, 5D).

DISCUSSION

The numbers of patients in the HyperPP and HypoPP groups were relatively small, and is a limitation of this study. However, the results were fairly consistent within the groups, and provide some insights into the underlying pathophysiological mechanisms in each group.

MVRC and interictal resting muscle membrane potential in HypoPP and HyperPP

The MVRC recordings showed an increased MRRP and reduced ESN in the HypoPP patients compared with normal controls. The ESN is thought to relate to the depolarising afterpotential (DAP) seen after a muscle action potential. In frog muscle, at a resting membrane potential of about -80mV, the DAP was of the order of 10-20mV, and was shown to decrease with depolarisation (Frank, 1957). The combination of an increased MRRP and reduced ESN is typically seen with depolarisation of the resting membrane potential (Z'Graggen and Bostock, 2009), where the MRRP is increased because of slowed sodium channel recovery from inactivation, and the DAP is reduced by a combination of increased inactivation of sodium channels, reduction in the electrochemical gradient for sodium influx, and increased outward movement of potassium, during the action potential. The MRRP is also temperature sensitive, being increased with cooling (Bostock et al 2012), but there was no difference in skin temperature over the recording site between the patient and control groups. The increased MRRP and reduced ESN in our HypoPP cohort thus suggests that the interictal resting membrane potential in this group is depolarised, even when the serum potassium is within the normal range, and when the patients are not overtly weak. This finding was similar in both HypoPP1 and HypoPP2 groups. This closely resembles the changes we found in a previous study of ATS patients (Tan et al 2012). In contrast, the

MVRC results suggest that patients in the HyperPP group mostly had normal interictal resting membrane potentials.

Our findings in the HypoPP group is consistent with studies showing mild depolarisation in muscle fibres biopsied from human HypoPP patients (Rüdel et al., 1984; Jurkatt-Rott et al., 2009). Interestingly, slowed interictal muscle fibre velocity has also been reported in HypoPP patients (Troni et al., 1983; Zwarts et al., 1988), suggesting that this depolarisation may cause conduction slowing. This may appear to stand in contradiction to evidence that the supernormality following a spike, which we record with the MVRC, is associated with a depolarising afterpotential. However, the effects of more prolonged depolarisation on muscle excitability are exceedingly complex, with slow and ultra-slow inactivation reducing excitability on the one hand (Goldin, 2003), while on the other hand compensating shifts of the Nav1.4 activation and inactivation curves to maintain excitability have been reported (Filatov et al., 2005).

Our findings suggesting a depolarised interictal resting membrane potential is also consistent with a rightward shift of the potassium concentration at which paradoxical depolarisation occurs in HypoPP patients (Cannon 2015). With an already mildly depolarised resting V_m, a smaller reduction in $[K]_o$ would be required to cause (V_m-E_K) to exceed the level (~20 mV) at which there is cessation in the K_{IR} current. In the bi-stable range, especially if the E_{CL} is already mildly shifted in the depolarised direction after exercise (Foppen et al., 2002, Fuster et al., 2017, Mi et al., 2019), a mild reduction in serum potassium would now strongly favour the depolarised state associated with paralysis.

Explanation for the reduction in LSN in the HypoPP patients

In addition to the reduced ESN, there was also reduced mean and late supernormality after 5 conditioning stimuli in the HypoPP patients. The process underlying these later

supernormalities is thought to be the accumulation of potassium in the t-tubules, and the reduction in 5XLSN in the HypoPP patients could be explained either by a reduction of potassium entry into the t-tubules, or more efficient removal and/or return of potassium to the cytosol. A similar reduction in LSN was seen previously in our ATS cohort carrying loss of function K_{IR} mutations (Tan et al 2012), and in patients with depolarisation due to ischaemia (Humm et al 2010). It is unlikely that less potassium is extruded with each AP, since with depolarisation, the outward electrochemical driving force for potassium would be increased. The main mechanisms for returning potassium from the t-tubules to the cytosol is via the K_{IR} channels, and the activity of the Na/K ATPase alpha-2 (α2-pump) situated in the t-tubules. Both are stimulated by an increase in the potassium concentration in the t-tubules. The α2-pump is also voltage sensitive, with its activity increasing with depolarisation. One possible explanation for the reduction in LSN in the HypoPP patients may be increased α2-pump activity related to the depolarised resting membrane potential.

Simulation of the effects of exercise on membrane potential in HypoPP by repetitive stimulation

The differences between HypoPP and NC in the baseline measurements of MRRP and ESN in the repetitive stimulation protocol are similar to the changes seen with the MVRC protocol, and are consistent with depolarisation of resting membrane potential in HypoPP. During the 20 Hz trains, these differences are abolished when the trains of APs lead to mild depolarisation of the muscle membrane in the NC group, but cause only a small increase in MRRP and no fall in the ESN in the HypoPP group. Within 2-3 minutes of the start of the repetitive stimulation, there is a gradual 'correction'/return towards baseline in the MRRP and ESN changes in NC, which likely reflects the hyperpolarising influence of the sodium pump (Na/K ATPase alpha-1 pump). In the HypoPP patients, this 'correction' is delayed and

appears less effective, such that the MRRP remains increased beyond baseline values after cessation of repetitive stimulation and remains elevated above the pre-stimulation baseline value for the remainder of the 10 minute recording period (Figure 4A), suggesting that the pump may have difficulty restoring the baseline membrane potential after a period of exercise, consistent with the suggestion that exercise may exacerbate the degree of depolarisation of the muscle membrane in HypoPP.

Velocity recovery cycles and channel properties in HyperPP mutations

In the HyperPP patients, there is an increase in extra mean supernormality after multiple conditioning stimuli (significant after 2 CS [2XMSN]), and an increased extra residual supernormality after 5 conditioning stimuli (5XRSN). Four of the seven HyperPP patients in our cohort had either T704M or M1592V sodium channel mutations. These mutations are associated with a shift in the mid-point of steady state activation in the hyperpolarised direction. They also impair slow inactivation by shifting the midpoint of the inactivation curve in the depolarised direction. This combination creates a range of voltages (-65 to -30 mV) where the activation and inactivation curves overlap, and over which a steady-state sodium current will flow (Yang et al., 1994). It is possible that the mild depolarisation occurring following the 2-5 conditioning stimuli in the MVRC protocol is sufficient to push the membrane potential transiently into this 'window current' range, resulting in an increased inward sodium current, which manifests as an increased 2XMSN and 5XRSN. The resulting increased residual supernormality following a short burst of conditioning stimuli (5XRSN) explains the propensity for myotonic discharges in HyperPP.

High external potassium likely triggers a paralytic attack because the depolarising shift in E_K causes the resting membrane potential to enter the 'window current' range, resulting in an inactivating inward sodium current that then depolarises the membrane.

HyperPP, Paramyotonia congenita (PMC) and Sodium channel myotonia (SCM)

It has been suggested that HyperPP and PMC should perhaps not be regarded as distinct syndromes but as part of a spectrum of presentation of sodium channel gain-of-function mutations associated with myotonic discharges and episodes of weakness (Cannon, 2015). Although there may be an overlap in the clinical phenotype, it was possible to achieve complete separation between the muscle excitability data from our HyperPP cohort and our previously published data from PMC patients with T1313M mutations (Tan et al., 2018) (Figures 5E, 5F). This was largely because of differences in the amplitude measures during the frequency ramp and repetitive stimulation. In the PMC patients, the amplitude of the responses dropped dramatically following trains of action potentials, both during the ramp and during the 20Hz repetitive stimulation protocol (Tan et al., 2018). The T1313M mutation has been shown by *in vitro* heterologous expression experiments to generate unusually large persistent (non-inactivating) sodium currents (Hayward et al., 1996). Consistent with this, at high stimulation frequencies, the PMC muscle fibres became depolarized to inexcitability, causing the amplitude of the responses to fall and eventually become unrecordable (Tan et al., 2018). Had there been a similar non-inactivating sodium current associated with T704M and M1592V, as suggested by the early expression studies using the rat homologue of these mutations (Cannon and Strittmatter 1993), a similar phenomenon might have been expected in our HyperPP cohort. Instead, the amplitudes remained normal during both the frequency ramp and repetitive stimulation protocols.

It was not possible however, to achieve complete separation between the excitability measurements from the HyperPP and previously published SCM patients (Tan et al., 2018), and statistically, there were only marginal differences of borderline significance in the MRRP ($p=0.049$) and the timing of maximal ESN ($p=0.03$), but no other significant differences

between the HyperPP and SCM groups. This may be because in the interictal state, the resting membrane potential is normal in both, and our current stimulation paradigm is insufficient to maintain membrane potential in the 'window current' range long enough for the impaired slow inactivation to provoke the sustained depolarisation associated with an attack of paralysis.

Comparisons with other muscle ion channelopathies

When comparing our current data with previous data recorded from patients with other muscle ion channelopathies, certain combinations of excitability measures showed a clustering together of conditions associated with myotonic discharges at one end, and HypoPP and ATS at the other, the changes being in the opposite direction compared with normal controls (Figure 6 A-D).

As an overview of changes seen in different muscle ion channels disorders, the findings are of interest in illustrating how specific alterations in the function of an ion channel can affect specific excitability parameters. However, we are not proposing that any specific measurements be used as a 'diagnostic test' for a specific channel dysfunction, since similar changes in excitability properties can be caused by different factors, as is demonstrated by the overlap of the polygons in Figure 6.

Conclusions

In summary, this study provides *in vivo* evidence of interictal membrane depolarisation in HypoPP and, to our knowledge, provides the first *in vivo* assessment of interictal sarcolemmal membrane properties in HyperPP patients. In the HyperPP patients, our findings are consistent with the proposed presence of a 'window current'. These muscle excitability studies provide complementary *in vivo* information of membrane potential and

sarcolemmal excitability for comparison with *in vitro* expression studies of ion channel mutations, and may prove useful in helping to interpret the likely pathogenicity of novel ion channel variants identified by next generation sequencing in patients with features of a muscle ion channelopathy. This method is minimally invasive, and can be performed repeatedly without causing undue trauma, similar to the use of needle EMG, and could potentially be used to provide physiological outcome measures in future treatment trials.

Abbreviations

AP: action potential

ATS: Andersen-Tawil Syndrome

CMAP: compound muscle action potential

ESN: early supernormality (largest percentage decrease in latency for ISIs below 15 ms)

ESN@: interstimulus interval for maximum ESN

5ESN: early supernormality after 5 conditioning stimuli

HypoPP: Hypokalaemic periodic paralysis

HyperPP: Hyperkalaemic periodic paralysis

ISI: inter-stimulus interval

Lat(15 Hz)First: % change in latency for first muscle action potential of 15-Hz train

Lat(15 Hz)Last: % change in latency for last muscle action potential of 15-Hz train

Lat(30 Hz)First: % change in latency for first muscle action potential of 30Hz train

Lat(30 Hz)Last: % change in latency for last muscle action potential of 30-Hz train

LET: long exercise test

LSN: late supernormality (mean percentage decrease in latency for ISIs between 50 and 150 ms)

2XLSN: extra supernormality after 2 conditioning stimuli compared with 1 conditioning stimulus

5XLSN: extra supernormality after 5 conditioning stimuli compared with 1 conditioning stimulus

MC: Myotonia Congenita

MRRP: muscle relative refractory period

MSN: mean supernormality (average latency reduction between MRRP and 1 sec, corresponding to area under curve when plotted with linear ISI axis)

2XMSN: extra mean supernormality after 2 conditioning stimuli

5XMSN: extra mean supernormality after 5 conditioning stimuli

MPkf(20Hz C4+5): % change in amplitude for the first in train during the 4^{th} and 5^{th} cycle of 20Hz repetitive stimulation.

MSuperN(20Hz Bline): baseline early supernomality before the start of the 20Hz trains.

MVRC: muscle velocity recovery cycle

NC: normal controls

Pk(30Hz)Last: peak amplitude for last action potential in 30Hz train as percentage of baseline

Pk(30 Hz+30 s): peak amplitude 30s after end of 30-Hz train as percentage of baseline

PMC: paramyotonia congenita

RSN: residual supernormality (mean percentage decrease in latency at the end of the sweep, averaged for ISIs of 900 and 1000 ms)

5XRSN: extra residual supernormality after 5 conditioning stimuli

SCM: sodium channel myotonia

TA: tibialis anterior

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Figure Legends

Figure 1. Mean muscle velocity recovery cycle waveforms. Top panels: MVRCs after single impulse, middle panels: MVRCs after 5 impulses, bottom panels: differences between 1 and 5 impulses. A. HypoPP1 (black squares, n=8) compared with hypoPP2 (black diamonds, n=2) and normal controls (grey open circles, n=26). B. HypoPP combined (black circles, n=10), compared with HyperPP (black triangles, n=7) and normal controls (grey open circles, n=26).

Figure 2. MVRC measurements compared between 26 normal controls (NC), 10 HypoPP and 7 HyperPP patients. A: MRRP = muscle relative refractory period in ms. B: ESN (%) = early supernormality, measured as maximum percentage reduction in latency. C: 5XLSN(%) = extra late supernormality for 5 as compared with 1 impulse, measured as mean percentage reduction in latency between 50 and 150 ms. D: 5XRSN(%) = extra residual supernormality for 5 as compared with 1 impulse, measured as mean reduction in latency between 900 and 1000 ms. Measurements are plotted as medians, interquartile ranges, and extreme values. Asterisks indicate P values according to Welch rank test comparison with normal controls: NS = $P>0.05$, * = $P<0.05$, ** = $P<0.01$, *** = $P<0.001$, ***** = $P<0.00001$.

Figure 3. A: Frequency ramp recordings comparing HypoPP patients (black, n=10) and normal controls (grey, n=26). B: Frequency ramps for HyperPP patients (black, n=7) and controls (grey). Lines plotted are means +/- standard errors, plotted separately for first impulse in train and last impulse in train, with train frequency increasing linearly to 30 Hz over 1 min.

Figure 4. A: Repetitive stimulation recordings, comparing HypoPP patients (black, n=6) and normal controls (grey, n=24). B: Repetitive stimulation for HyperPP patients (black, n=5) and controls (grey). Lines plotted are means +/- standard errors. During the repetitive stimulation, as for the frequency ramp, latencies are plotted separatedly for the first and last impulses in each train.

Figure 5. Separation of groups by combinations of two MVRC and frequency ramp measurements. A & B Show only incomplete separation of HypoPP (n=10) and HyperPP (n=7) patient groups respectively from normal controls (n=26). C & D show complete separation of

HypoPP from HyperPP patient groups. E & F show complete separation of HyperPP (n=7) and paramyotonia congenita (PMC, n=8) groups. (PMC data from Tan et al., 2018).

Figure 6.

Separation of groups by combinations of MVRC and repetitive stimulation measurements. A: Showing increased extra mean supernormality to 5 conditioning stimuli compared with NC (n=24) in the conditions associated with myotonia, with a slight differential increase in the baseline supernormality before the start of the 20Hz trains partially separating the PMC (n=8), HyperPP (n=5), and myotonia congenita (MC) (n=10, MC patients not on sodium channel blockers) groups. The changes are in the opposite direction from NC for HypoPP (n=6). B: The groups can also be partially separated plotting extra mean supernormality to 5 conditioning stimuli against changes in the amplitude of the first in train for the fourth and fifth cycles during 20 Hz repetitive stimulation. C: As in A, but with NC removed and SCM (n=10) and ATS (n=10) data included to show the overlap between SCM and HyperPP, and between ATS and HypoPP. D: as in B, but with NC removed and again showing the overlap between SCM, HyperPP, and MC, and between ATS and HypoPP. (MC data from Tan et al., 2014. ATS data from Tan et al., 2012).

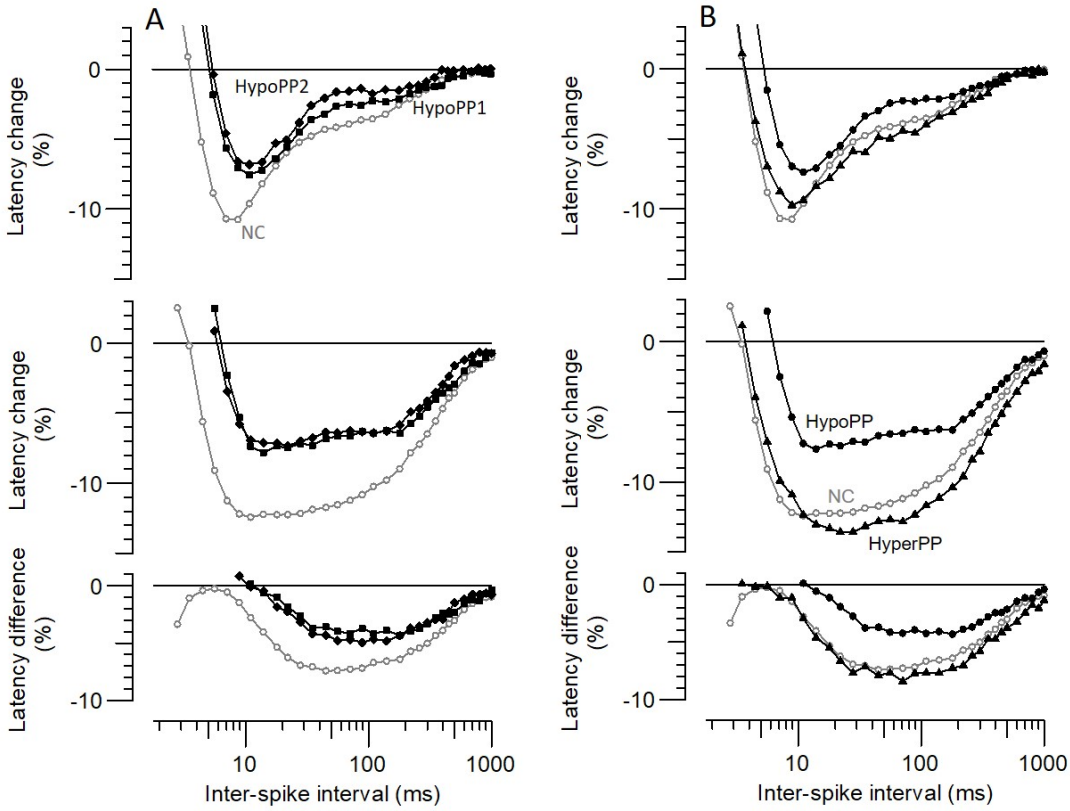
A
B
Latency change (%)
0
-10
HypoPP2
HypoPP1
NC
HypoPP
HyperPP
Latency difference (%)
10
100
1000
Inter-spike interval (ms)

Figure 1

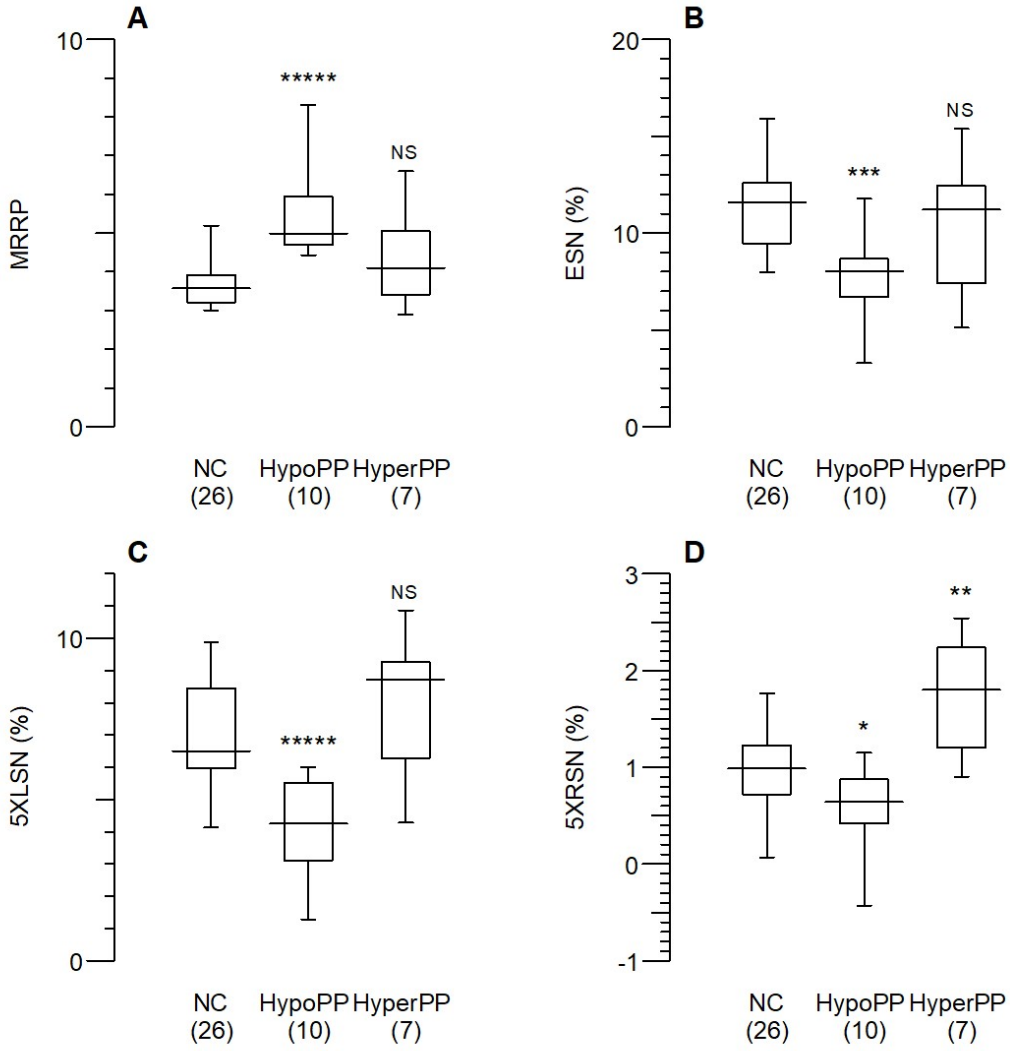
A
MRRP
10
0
*****
NS
NC (26)
HypoPP (10)
HyperPP (7)
B
ESN (%)
20
10
0
***
NS
NC (26)
HypoPP (10)
HyperPP (7)
C
5XLSN (%)
10
0
*****
NS
NC (26)
HypoPP (10)
HyperPP (7)
D
5XRSN (%)
3
2
1
0
-1
*
**
NC (26)
HypoPP (10)
HyperPP (7)

Figure 2

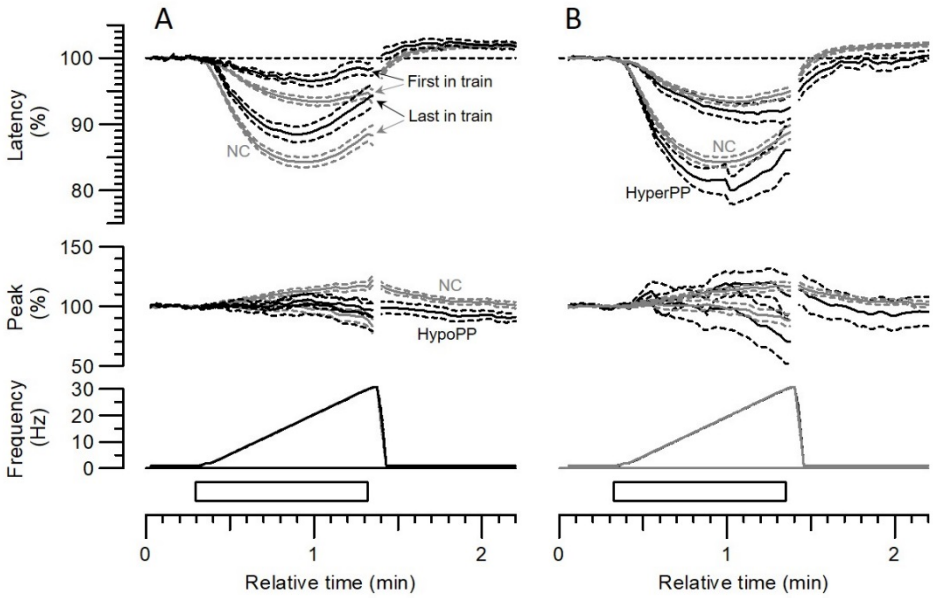

Figure 3

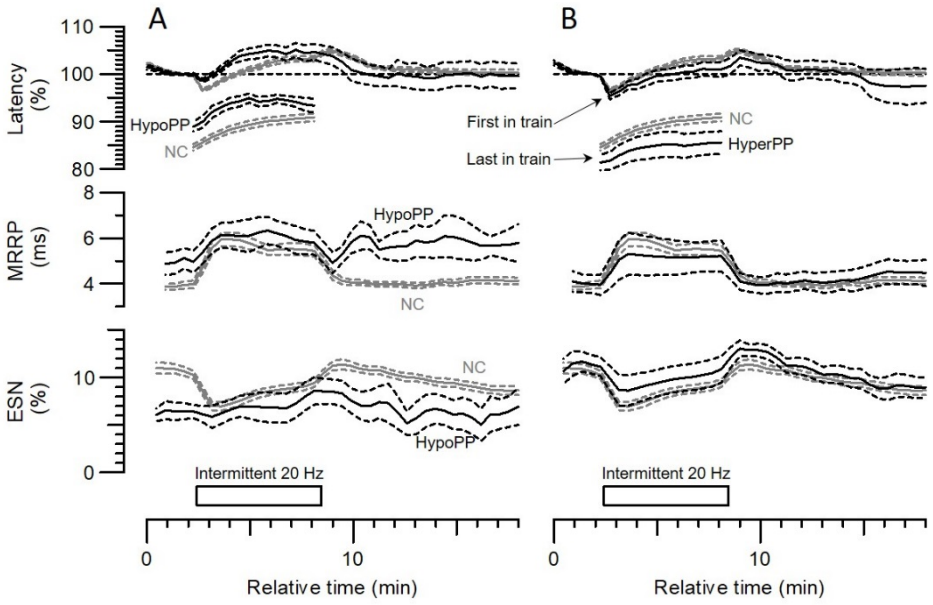

Figure 4

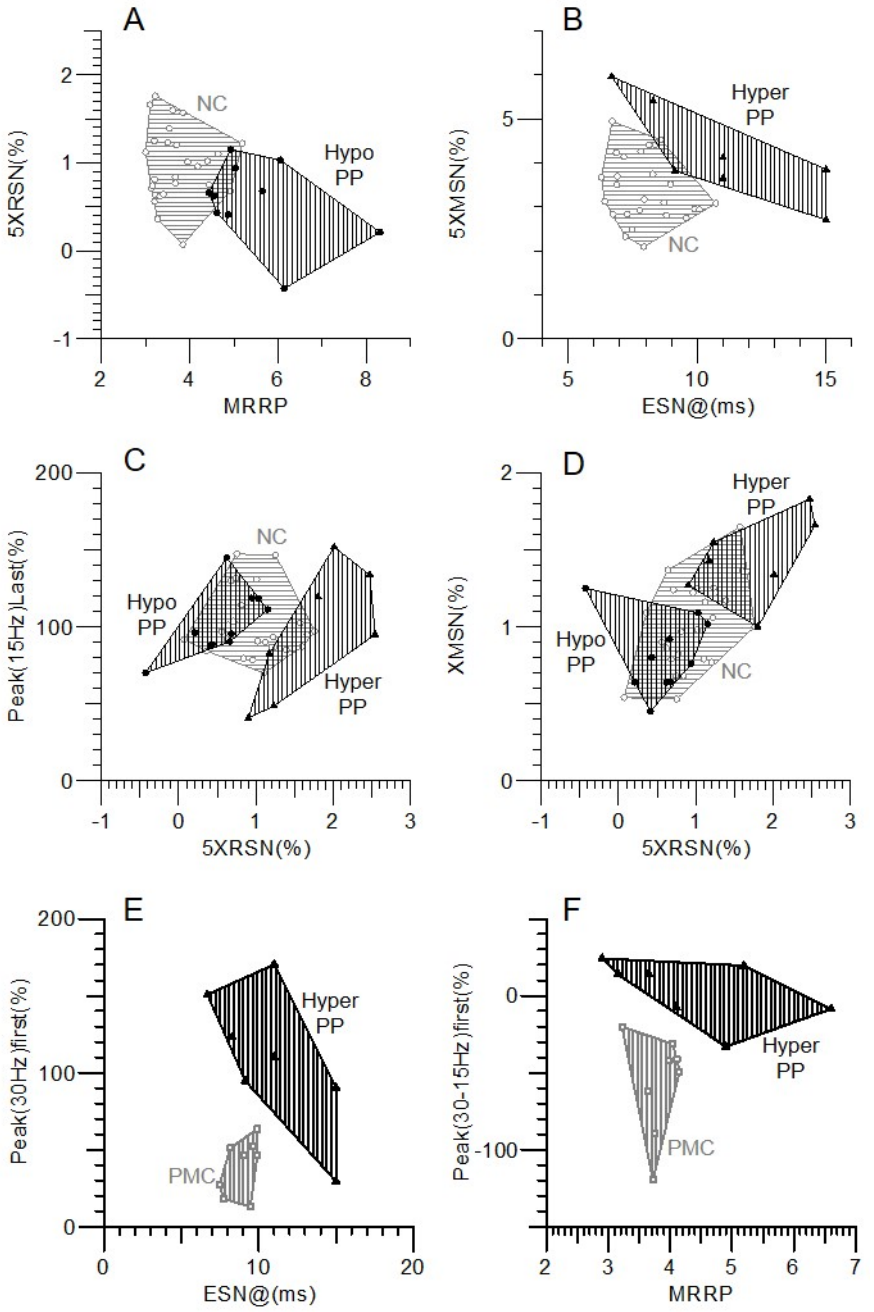
A
NC
Hypo
PP
5XRSN(%)
MRRP
B
Hyper
PP
NC
5XMSN(%)
ESN@(ms)
C
NC
Hypo
PP
Hyper
PP
Peak(15Hz)Last(%)
5XRSN(%)
D
Hyper
PP
Hypo
PP
NC
XMSN(%)
5XRSN(%)
E
Hyper
PP
PMC
Peak(30Hz)first(%)
ESN@(ms)
F
Hyper
PP
PMC
Peak(30-15Hz)first(%)
MRRP

Figure 5

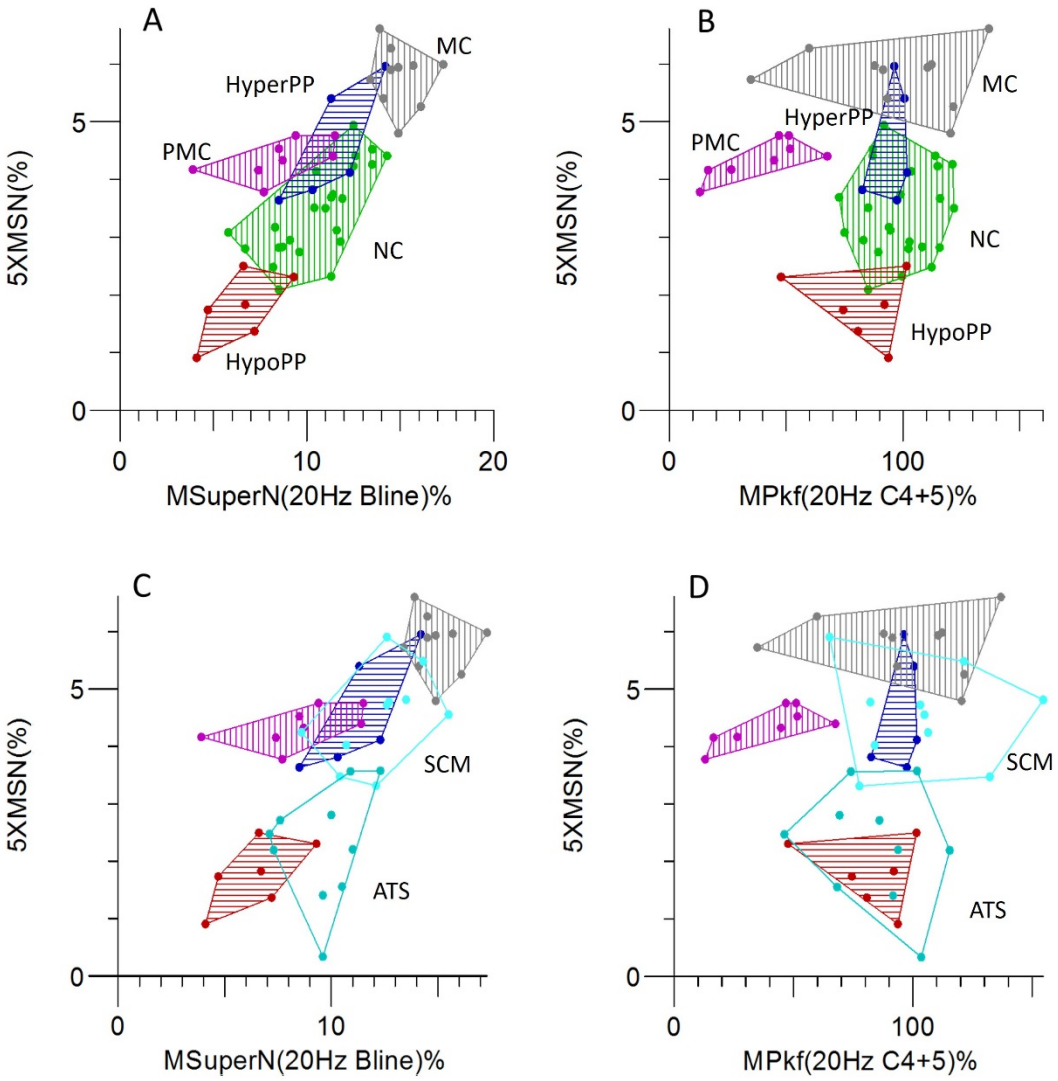
A
MC
HyperPP
PMC
NC
HypoPP
5XMSN(%)
5
0
0
10
20
MSuperN(20Hz Bline)%
B
MC
HyperPP
PMC
NC
HypoPP
5XMSN(%)
5
0
0
100
MPkf(20Hz C4+5)%
C
SCM
ATS
5XMSN(%)
5
0
0
10
MSuperN(20Hz Bline)%
D
SCM
ATS
5XMSN(%)
5
0
0
100
MPkf(20Hz C4+5)%

Figure 6

Table 1. Periodic paralysis patients.

Pt	Gender	Age	Diagnosis	Gene	Amino acid change	EMG findings	LET dec	K
1	F	49	HyperPP	SCN4A	Thr704Met	Normal units.	ND	4.4
2	M	62	HyperPP	SCN4A	Met1592Val	Myopathic. Myotonic discharges	30	3.8
3	F	29	HyperPP	SCN4A	Met1592Val	Normal units. Myotonic discharges	17	3.6
4	F	68	HyperPP	SCN4A	Met1592Val	Normal units. Myotonic discharges	ND	4.8
5	F	67	HyperPP	SCN4A	Ser653Gly	Normal units. Myotonic discharges	53	4.1
6	M	49	HyperPP	SCN4A	Ser653Gly	Normal units. Myotonic discharges	44	3.8
7	M	49	HyperPP	SCN4A	Met403Leu	Normal units. Myotonic discharges	26	4.1
8	M	52	HypoPP1	CACNA1S	Arg528His	Normal units in TA. Myopathic in proximal muscles.	ND	3.7
9	F	43	HypoPP1	CACNA1S	Arg498His	Normal units	50	4.5
10	M	48	HypoPP1	CACNA1S	Arg1239His	Mild myopathic changes and occasional fibrillations in proximal muscles	65	4
11	M	46	HypoPP1	CACNA1S	Arg1239His	Occasional fibrillations in proximal muscles, units within normal limits	ND	4
12	M	52	HypoPP1	CACNA1S	Arg900Ser	Normal units	46	3.7
13	M	19	HypoPP1	CACNA1S	Arg900Ser	Normal units	63	4.8
14	M	28	HypoPP1	CACNA1S	Arg528His	Normal units	36	3.7
15	F	37	HypoPP1	CACNA1S	Arg1239His	Normal units	52	4.6
16	M	17	HypoPP2	SCN4A	Arg1135His	Normal units	40	4
17	M	18	HypoPP2	SCN4A	Arg669His	Normal units	71	4.5

Pt, patient; HyperPP, hyperkalaemic periodic paralysis; HypoPP, hypokalaemic periodic paralysis; LET dec, maximum decrement as a percentage of the maximum CMAP during the long exercise test; ND, not done; K, serum potassium; TA, tibialis anterior

Table 2. Muscle Velocity Recovery Cycles

Velocity Recovery Cycle Measurements compared between Groups.

Excitability Measures	Mean ± SE			P for Welch rank test		
	NC (n=26)	HypoPP (n=10)	HyperPP (n=7)	NC v HypoPP	NC v HyperPP	HypoPP v HyperPP
MRRP (ms)	3.70 ± 0.12	5.46 ± 0.37	4.36 ± 0.49	1.1×10^{-7}*	0.45185	0.14997
ESN (%)	11.19 ± 0.44	7.92 ± 0.75	10.2 ± 1.41	0.00057*	0.64613	0.26006
ESN@(ms)	7.98 ± 0.23	10.46 ± 0.83	10.87 ± 1.21	0.00152*	0.0565	0.65965
5ESN (%)	13.01 ± 0.54	7.86 ± 1.09	13.32 ± 1.87	0.00031*	0.78667	0.018
MSN (%)	1.28± 0.05	0.94 ± 0.13	1.54 ± 0.12	0.02108	0.06868	0.00455*
2XMSN (%)	1.03 ± 0.06	0.82 ± 0.08	1.44 ± 0.10	0.05433	0.00174*	0.00046*
5XMSN (%)	3.40 ± 0.15	2.23 ± 0.24	4.21 ± 0.42	0.00043*	0.1327	0.00227*
LSN (%)	3.68 ± 0.17	2.28 ± 0.38	4.29 ± 0.36	0.0039*	0.17589	0.00112*
2XLSN (%)	2.25 ± 0.15	1.45 ± 0.26	2.92 ± 0.46	0.0156	0.20845	0.00935*
5XLSN (%)	7.03 ± 0.31	4.14 ± 0.49	7.85 ± 0.90	4.5×10^{-6}*	0.43503	0.00497*
RSN (%)	0.13 ± 0.04	0.26 ± 0.07	0.14 ± 0.14	0.08682	0.62096	0.25128
5XRSN(%)	0.99 ± 0.08	0.57 ± 0.15	1.73 ± 0.25	0.01481	0.00734*	0.00023*

MRRP, muscle relative refractory period; ESN, early supernormality (up to 15 ms); ESN@, interstimulus interval for maximum ESN; 5ESN, early supernormality after 5 conditioning stimuli; MSN, mean supernormality (up to 1 s); 2XMSN, extra mean supernormality after 2 conditioning stimuli; 5XMSN, extra mean supernormality after 5 conditioning stimuli; LSN, late supernormality (50–150 ms); 2XLSN extra supernormality after 2 conditioning stimuli compared with 1 conditioning stimulus; 5XLSN, extra supernormality after 5 conditioning stimuli compared with 1 conditioning stimulus; RSN, residual supernormality (900–1,000 ms); 5XRSN, extra residual supernormality after 5 conditioning stimuli; NC, normal controls; HyperPP, hyperkalaemic periodic paralysis; HypoPP, hypokalaemic periodic paralysis.

*$p<0.01$;

Table 3. Frequency Ramp

Frequency ramp measurements compared between Groups.

Excitability Measures	Mean ± SE			P for Welch rank test		
	NC (n=26)	HypoPP (n=10)	HyperPP (n=7)	NC v HypoPP	NC v HyperPP	HypoPP v HyperPP
$Lat(15Hz)_{First}$%	94.04 ± 0.591	96.95 ± 0.585	92.8 ± 1.1	0.00396*	0.34444	0.00533*
$Lat(15Hz)_{Last}$%	84.75 ± 0.72	88.68 ± 1.13	81.56 ± 1.78	0.01871	0.08901	0.00057*
$Lat(30Hz)_{First}$%	94.86 ± 0.699	98.23 ± 0.95	96.49 ± 3.78	0.01135	0.758	0.15496
$Lat(30Hz)_{Last}$ %	88.75 ± 1.05	94.39 ± 1.7	85.98 ± 4.37	0.00215*	0.67872	0.12854
Lat(30Hz+30s)%	101.7 ± 0.312	102.2 ± 0.537	100.8 ± 1.09	0.5605	0.34039	0.35889
$Pk(15Hz)_{First}$%	111.5 ± 2.44	101.6 ± 3.54	106.6 ± 10.3	0.02742	0.45665	0.72302
$Pk(15Hz)_{Last}$%	100.5 ± 4.21	102.1 ± 6.75	95.94 ± 15.9	0.69057	0.88263	0.84178
$Pk(30Hz)_{First}$%	115 ± 2.9	97.28 ± 5.24	109.8 ± 17.3	0.01038	0.78894	0.33511
$Pk(30Hz)_{Last}$%	87.47 ± 5.09	91.18 ± 11.1	70.63 ± 18.7	0.79742	0.55862	0.43835
$Pk(30\text{-}15Hz)_{First}$%	3.44 ± 1.77	-4.34 ± 4.2	3.2 ± 7.74	0.04211	0.60666	0.3999
Pk(30Hz+30s)%	104.5 ± 2.2	93.08 ± 4.67	94.86 ± 12.1	0.08113	0.65028	0.53352

Lat(15HZ)First%, percentage change in latency for first muscle action potential of 15Hz train; Lat(15HZ)Last%, percentage change in latency for last muscle action potential of 15-Hz train; Lat(30HZ)First%, percentage change in latency for first muscle action potential of 30Hz train; Lat(30HZ)Last%, percentage change in latency for last muscle action potential of 30Hz train; Lat (30Hz+30s)%, percentage change in latency 30s after end of 30Hz train; Pk(30Hz)Last%, peak amplitude for last action potential in 30Hz train as percentage of baseline; Pk(30–15HZ)First%, difference in peak amplitude of first action potentials at 15 and 30HZ; Pk(30Hz+30 s)%, peak amplitude 30s after end of 30 Hz train as percentage of baseline; NC, normal controls; HyperPP, hyperkalaemic periodic paralysis; HypoPP, hypokalaemic periodic paralysis.
*$p<0.01$;

Table 4. Repetitive Stimulation

Repetitive Stimulation measurements compared between Groups.

Excitability Measures	Mean ± SE			P for Welch rank test		
	NC (n=26)	HypoPP (n=6)	HyperPP (n=5)	NC v HypoPP	NC v HyperPP	HypoPP v HyperPP
MRRP (ms)						
Baseline	3.94 ± 0.16	5.05 ± 0.46	3.96 ± 0.41	0.00474*	0.90588	0.10379
Cycles 1,2	5.91 ± 0.29	6.233 ± 0.47	5.32 ± 0.87	0.4031	0.57115	0.32535
Cycles 4,5	5.48 ± 0.26	6.3 ± 0.81	5.2 ± 0.65	0.50255	0.70155	0.21783
Recovery	4.04 ± 0.12	5.83 ± 0.39	3.98 ± 0.37	1.99×10^{-6}*	1	0.00449*
ESN (%)						
Baseline	10.56 ± 0.45	6.43 ± 0.76	11.32 ± 0.96	0.00028*	0.66817	0.00254*
Cycles 1,2	7.13 ± 0.46	6.45 ± 1.02	8.8 ± 1.56	0.34114	0.45397	0.32535
Cycles 4,5	9.03 ± 0.42	7.68 ± 1.47	10.4 ± 1.45	0.54445	0.38182	0.14567
Recovery	10.91 ± 0.41	7.42 ± 1.47	12.26 ± 0.61	0.08595	0.16088	0.03185
LSN (%)						
Baseline	3.82 ± 0.25	2.37 ± 0.37	4.8 ± 0.37	0.00712*	0.14779	0.00057*
Cycles 1,2	3.13 ± 0.21	2.43 ± 0.35	3.98 ± 0.5	0.14391	0.26644	0.02414
Cycles 4,5	3.41 ± 0.23	2.93 ± 0.61	4 ± 0.37	0.44727	0.10148	0.19745
Recovery	3.24 ± 0.20	2.4 ± 0.80	3.88 ± 0.61	0.29221	0.27969	0.19851
Latency (% baseline, 20Hz)						
Cycles 1,2 first	98.6 ± 0.33	101 ± 0.49	97.42 ± 0.75	8.7×10^{-5}*	0.30777	0.00057*
Cycles 1,2 last	87.46 ± 0.71	92.67 ± 1.13	83.5 ± 2.24	0.00035*	0.2301	0.00057*
Cycles 4, 5 first	103.1 ± 0.57	104.6 ± 1.48	100.7 ± 1.42	0.39103	0.26621	0.15494
Cycles 4,5, last	90.6 ± 0.80	94.07 ± 1.12	85.32 ± 2.43	0.02175	0.05758	0.00254*
Recovery	102.6 ± 0.43	100.4 ± 2.19	102 ± 1.75	0.27022	0.46504	0.54368
Peak (% baseline, 20Hz)						
Cycles 1,2 first	106.3 ± 1.57	96.75 ± 6.56	104.9 ± 2.82	0.26632	0.65556	0.27435
Cycles 1,2 last	95.3 ± 4.43	92.97 ± 10.6	95.12 ± 14.3	0.74089	0.79961	0.84301
Cycles 4,5 first	99.05 ± 2.93	81.77 ± 7.86	95.78 ± 3.45	0.04392	0.48092	0.0922
Cycles 4,5 last	80.22 ± 3.8	74.72 ± 10.3	89.3 ± 13.7	0.70599	0.82068	0.38223
Recovery	86.8 ± 3.57	70.87 ± 11.1	78.72 ± 2.73	0.36564	0.01316	0.83716

ESN, early supernormality; MRRP, muscle relative refractory period; LSN, late supernormality; Latency Cycles 1,2 first = latency of the first response in the 20 Hz train (averaged for cycles 1 and 2) as percentage of baseline; cycles 1,2 last = latency of the last response in the 20 Hz train (averaged for cycles 1 and 2) as percentage of baseline; Cycles 4, 5 first= latency of the first response in the 20 Hz train (averaged for cycles 4 and 5) as percentage of baseline; Cycles 4,5 last = latency of the last response in the 20 Hz train (averaged for cycles 4 and 5) as percentage of baseline; Peak Cycles 1,2 first = amplitude of the first response in the 20 Hz train (averaged for cycles 1 and 2) as percentage of baseline; Peak cycles 1,2 last = amplitude of the last response in the 20 Hz train (averaged for cycles 1

and 2) as percentage of baseline; similarly for cycles 4, 5; NC, normal controls; HyperPP, hyperkalaemic periodic paralysis; HypoPP, hypokalaemic periodic paralysis.
*$p<0.01$