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Altered axonal excitability properties in amyotrophic lateral sclerosis: impaired potassium channel function related to disease stage

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Fasciculations are a characteristic feature of amyotrophic lateral sclerosis (ALS), and can arise proximally or distally in the motor neuron, indicating a widespread disturbance in membrane excitability. Previous studies of axonal excitability properties (i.e. threshold electrotonus, strength-duration time constant) have suggested respectively that change in potassium or sodium channels may be involved. To reinvestigate these changes and explore their correlation with disease stage, multiple axonal excitability properties (threshold electrotonus, strength-duration time constant, recovery cycle and current-threshold relationship) were measured for the median nerve at the wrist in 58 ALS patients, and compared with 25 age-matched controls. In ALS, there were greater changes in depolarizing threshold electrotonus (i.e. less accommodation) ($P < 0.001$) and greater supernormality in the recovery cycles ($P < 0.001$). These abnormalities were more prominent in patients with moderately reduced CMAP (1–5 mV). Modelling the excitability changes in this group supported the hypothesis that axonal potassium conductances are reduced, resulting in increased supernormality despite membrane depolarization. The tendency for strength-duration time constant to be prolonged in ALS was only significant for patients with normal CMAP amplitude (>5 mV). Patients with severely reduced CMAP (<1 mV) alone showed reduced threshold changes to hyperpolarizing current. These results suggest a changing pattern of abnormal membrane properties with disease progression. First, persistent Na^+ conductance increases, possibly associated with collateral sprouting, and then K^+ conductances decline. Both changes cause axonal hyperexcitability, and may contribute to the generation of fasciculations. These serial changes in axonal properties could provide insights into the pathophysiology of ALS, and implications for future therapeutic options.

Keywords: amyotrophic lateral sclerosis; threshold electrotonus; strength-duration time constant; fasciculation; potassium channel; persistent sodium channel

Abbreviations: ALS = amyotrophic lateral sclerosis; CMAP = compound muscle action potential; SMA = spinal muscular atrophy; TEd = depolarizing threshold electrotonus; TEh = hyperpolarizing threshold electrotonus

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Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive fatal disorder involving both upper and lower motor neurons and is characterized by muscle weakness, atrophy, and fasciculation. Fasciculation is a prominent and diagnostic feature of ALS and is due to ectopic activity originating in the motor neuron. The fasciculations are thought to arise proximally in early stages in the disease and distally in later stages (de Carvalho and Swash, 1998). A better understanding of the membrane instability leading to fasciculations may therefore provide evidence of pathophysiological mechanisms and clues to improved treatment of the disease. Following this rationale, several studies have examined different aspects of

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axonal excitability, and have found evidence of abnormalities that could contribute to hyperexcitability (Bostock *et al.*, 1995; Mogyoros *et al.*, 1998), but no evidence relating a membrane abnormality to disease progression.

Threshold electrotonus tests excitability changes in axons induced by subthreshold conditioning currents, and can reflect accommodation due to K^+ channels (Bostock *et al.*, 1998; Burke *et al.*, 2001). Bostock *et al.* (1995), for the first time, tested threshold electrotonus in 11 patients with ALS. Motor axons of the ALS patients responded abnormally to subthreshold depolarizing currents, becoming either more (6 out of 11) or much less excitable (4 out of 11) than normal, which they referred to as Type 1 and Type 2 abnormalities respectively. They inferred that inward sodium currents were not counteracted by outward potassium currents as in normal fibres and concluded that there was a pathological imbalance in the ratio of sodium conductance to potassium conductances, most likely a reduction in potassium conductances. Modelling suggested that progressive loss of fast and slow potassium conductances could result in Type 1, then Type 2 abnormalities, fasciculations and finally cell death as the membrane potential becomes progressively unstable and finally depolarizes irreversibly.

Subsequent nerve excitability studies have provided only limited confirmation of that small initial study. Another threshold electrotonus study by Horn *et al.* (1996) of 27 ALS patients confirmed the increase in depolarizing electrotonus, but the proportion of abnormal recordings was much lower, and the increase in depolarizing electrotonus was accompanied by an increase in hyperpolarizing electrotonus, so that the 'fanning-out' of the waveforms resembled to some extent the changes seen on membrane hyperpolarization (Kiernan and Bostock, 2000). Another threshold electrotonus study of 46 ALS patients (Cikurel *et al.*, 1997) found other distinctive abnormalities in addition to the Type 1 and Type 2 (e.g. some relatively flat responses), and that the main distinguishing feature of the ALS group was abnormal variability: the mean responses were not significantly different from controls.

Meanwhile, study of another axonal excitability property, strength-duration time constant, reopened the question of which conductances are altered in ALS. Strength-duration time constant (τ_{SD}) is a measure of the rate at which stimulus current decreases as the duration of a threshold stimulus pulse increases, and equates to chronaxie. τ_{SD} partly depends on the persistent Na^+ conductance active at the resting membrane potential (Bostock and Rothwell, 1997). A study of the strength-duration properties of motor and sensory axons in 23 ALS patients found that the motor nerve τ_{SD}/sensory nerve τ_{SD} ratio was abnormally high, suggesting a greater nodal persistent Na^+ conductance in motor axons (Mogyoros *et al.*, 1998).

Taken together, the threshold electrotonus and strength-duration studies in ALS suggest that potassium conductances may be reduced and persistent Na^+ conductance increased, both of which would increase axonal excitability, but because the different types of conductance interact by changing the resting potential, identifying the primary membrane abnormality by such indirect techniques is problematic. A recent review (Kiernan and Burke, 2005) has suggested that uncertainties about the interpretation of threshold electrotonus abnormalities in ALS might be overcome by using threshold electrotonus in combination with other techniques that explore nerve excitability. A method is now available for estimating multiple measures of axonal excitability (recovery cycle and current/threshold relationship, in addition to threshold electrotonus and strength-duration time constant) in human subjects non-invasively and rapidly (Kiernan *et al.*, 2000), and the multiplicity of parameters recorded in the method has helped to reduce the ambiguities that had been inherent in previous clinical studies of a single excitability parameter (Kiernan *et al.*, 2000, 2001*a*, 2001*b*, 2002, 2005; Kuwabara *et al.*, 2000, 2002; Kanai *et al.*, 2003; Kitano *et al.*, 2004). Furthermore, fitting a computer model of nerve excitability may provide an objective aid to interpretation (Kiernan *et al.*, 2005).

The present study was undertaken to reassess the membrane abnormalities in motor axons of ALS patients, using multiple excitability measurements and computer modelling, and sufficient patients to enable the excitability changes to be related to the stage of the disease.

Methods

Subjects

Fifty-eight consecutive patients with sporadic ALS, seen at Chiba University Hospital between 2000 and 2004, were studied. There were 29 men and 29 women with ages ranging from 37 to 80 years (mean, 63 years). The patients fulfilled the El Escorial criteria for definite or probable ALS (World Federation of Neurology, 1994). We excluded coincidental carpal tunnel syndrome on the basis of clinical examination and nerve conduction studies. We also excluded ALS patients with other neurological disorders (diabetic neuropathy, cervical spondylosis, dementia, and past history of poliomyelitis). Control data for multiple excitability measurements were obtained from 25 age-matched ($P = 0.32$) normal subjects (ages 45–77 years, mean 61 years). Twenty-eight patients with spinal muscular atrophy (SMA) were also examined and the results of excitability testing were compared with those of normal controls and patients with ALS. All subjects gave informed consent, and the study was approved by the Ethics Committee of Chiba University School of Medicine.

Conventional electrophysiological studies

Nerve conduction studies in the median nerve were done by conventional procedures (Liveson and Ma, 1992). Compound muscle action potentials (CMAPs) were recorded from the abductor pollicis brevis muscle, and amplitudes of the initial negative peaks of the CMAPs were measured. We divided patients into three subgroups according to the CMAP amplitudes: (i) Group A (CMAP > 5 mV), where 5.0 mV is the lower limit of normal in our laboratory ($n = 100$), (ii) Group B (1–5 mV), and (iii) Group C (<1 mV).

Multiple excitability measurements based on threshold tracking

Multiple excitability measurements were performed by a recently reported protocol using a computerized program (QTRAC version 4.3 with multiple excitability protocol TRONDHM; copyright Institute of Neurology, London, UK) as described elsewhere (Kiernan *et al.*, 2000; Kiernan and Bostock, 2000; Kuwabara *et al.*, 2000; Kanai *et al.*, 2003). Briefly, CMAP was recorded from the abductor pollicis brevis with stimulation at the wrist. The protocols examining stimulus-response curves used durations of 0.2 and 1.0 ms. From these curves, τ_{SD} was calculated using the following formula: (Kuwabara *et al.*, 2002):

$$\tau_{SD} = 0.2(I_{0.2} - I_{1.0})/(I_{1.0} - 0.2I_{0.2})$$

where $I_{0.2}$ and $I_{1.0}$ were the respective threshold currents for test stimuli of 0.2- and 1.0-ms duration.

In the following measurements, the current required to produce a CMAP that was ~40% of the maximum was tracked (threshold tracking). In the threshold electrotonus studies, the membrane potential was altered by the use of subthreshold DC polarizing currents that were 40% of the unconditioned threshold. Depolarizing and hyperpolarizing currents were used, each lasting 100 ms, and their effects on the threshold current for the test CMAP were examined. In a further test with subthreshold conditioning, the test stimulus was delivered at the end of a polarizing current pulse lasting 200 ms. The strength of the current pulse was changed systematically from 50% depolarizing to 100% hyperpolarizing in 10% steps. This produced a current-threshold relationship, analogous to the conventional current-voltage relationship. The recovery cycle of axonal excitability after a single supramaximal stimulus was measured by delivering the test stimulus at different intervals after the conditioning stimulus. The intervals between the conditioning and test stimulation were changed systematically from 2 to 200 ms.

For each parameter of the multiple excitability measurements, differences in medians were tested with the Mann–Whitney test, and differences in proportion with the chi-square test, using SPSS software (SPSS Japan Inc., Tokyo, Japan).

Electrical model of nerve excitability

To model the excitability changes in human motor axons and the effects of altered ion conductances, we used the same model as described by Kiernan *et al.* (2005). This was based on the mathematical model used by Bostock *et al.* (1995) to simulate threshold electrotonus, which was derived in turn from an earlier model of electrotonus in human motor axons (Bostock *et al.*, 1991). Transient sodium channels were modelled using the voltage clamp data of Schwarz *et al.* (1995), persistent sodium currents were added (Bostock and Rothwell, 1997) and further empirical parameter adjustments were made to improve the fit to normal human recovery cycle, strength-duration and current-threshold data, as well as to threshold electrotonus.

The equations for a single node and internode, representing a spatially uniform axon, were evaluated by integration over successive small time steps (Euler's method, Press *et al.*, 1992), using a maximal integration interval of 3 μs. At times corresponding to those in the human nerve excitability recordings, the excitability of the model nerve was tested repeatedly to determine threshold with an accuracy of 0.5%. The discrepancy between the thresholds determined for the model and those determined from a sample of real nerves was scored as the weighted sum of the error terms: $[(x_m - x_n)/s_n]^2$, where x_m is the threshold of the model, x_n the mean and s_n the standard deviation of the thresholds for the real nerves. The weights were the same for all thresholds of the same type (e.g. recovery cycle), and chosen to give an equal total weight to the four different types of threshold measurement: threshold electrotonus, current/threshold relationship, recovery cycle and strength-duration properties. The standard model was obtained by minimizing the discrepancy between the model and the normal control data with an iterative least squares procedure, so that alteration of any of the above parameters would make the discrepancy worse.

Results

CMAP amplitudes

In the 58 ALS patients, the mean CMAP amplitude after median nerve stimulation at the wrist was 4.5 mV (range, 0.2–10.6 mV; normal >5.0 mV). We divided patients into three subgroups as described in Methods: 21 patients were classified in group A (CMAP > 5 mV), 30 patients in group B (1–5 mV), and 7 patients in group C (<1 mV).

Multiple excitability measurements in the total ALS group

In the stimulus-response curves, threshold currents were slightly higher, but not significantly, in ALS patients than in normal subjects (Fig. 1A and Table 1). Patients with ALS tended to have longer τ_{SD} than normal subjects (Fig. 1B and Table 1), but the difference did not reach statistical significance ($P = 0.09$). There was a reciprocal relationship between τ_{SD} and rheobase in both the ALS and normal groups. In threshold electrotonus studies, ALS patients had greater threshold changes to depolarizing conditioning currents, resembling the Type 1 abnormality described by Bostock *et al.* (1995). The threshold changes in depolarizing threshold electrotonus at 10–30 ms (TEd [10–30 ms]) and at 90–100 ms (TEd [90–100 ms]) of ALS patients were significantly greater than those of normal controls ($P < 0.05$ and $P < 0.001$, respectively). However, the grand average of hyperpolarizing threshold electrotonus at 90–100 ms (TEh [90–100 ms]) was similar for the ALS and normal groups (Fig. 1C and Table 1). The Type 2 abnormality (Bostock *et al.*, 1995), in which the early part of TEd shows an abrupt threshold increase or 'dip', accompanied by a reduction in latency, was only observed in one patient out of 58, in contrast to the 4 patients out of 11 in the previous study. The reason for this discrepancy is unclear, but the new results are consistent with those of Cikurel *et al.* (1997) in which the frequency was 2 in 46.

The findings for the recovery cycle in ALS patients are shown in Fig. 1E and Table 1. The mean supernormality was significantly greater for ALS patients than for normal subjects ($P < 0.001$), but refractoriness and late supernormality were not significantly different for the two groups. The current/threshold relationships in ALS patients are shown in

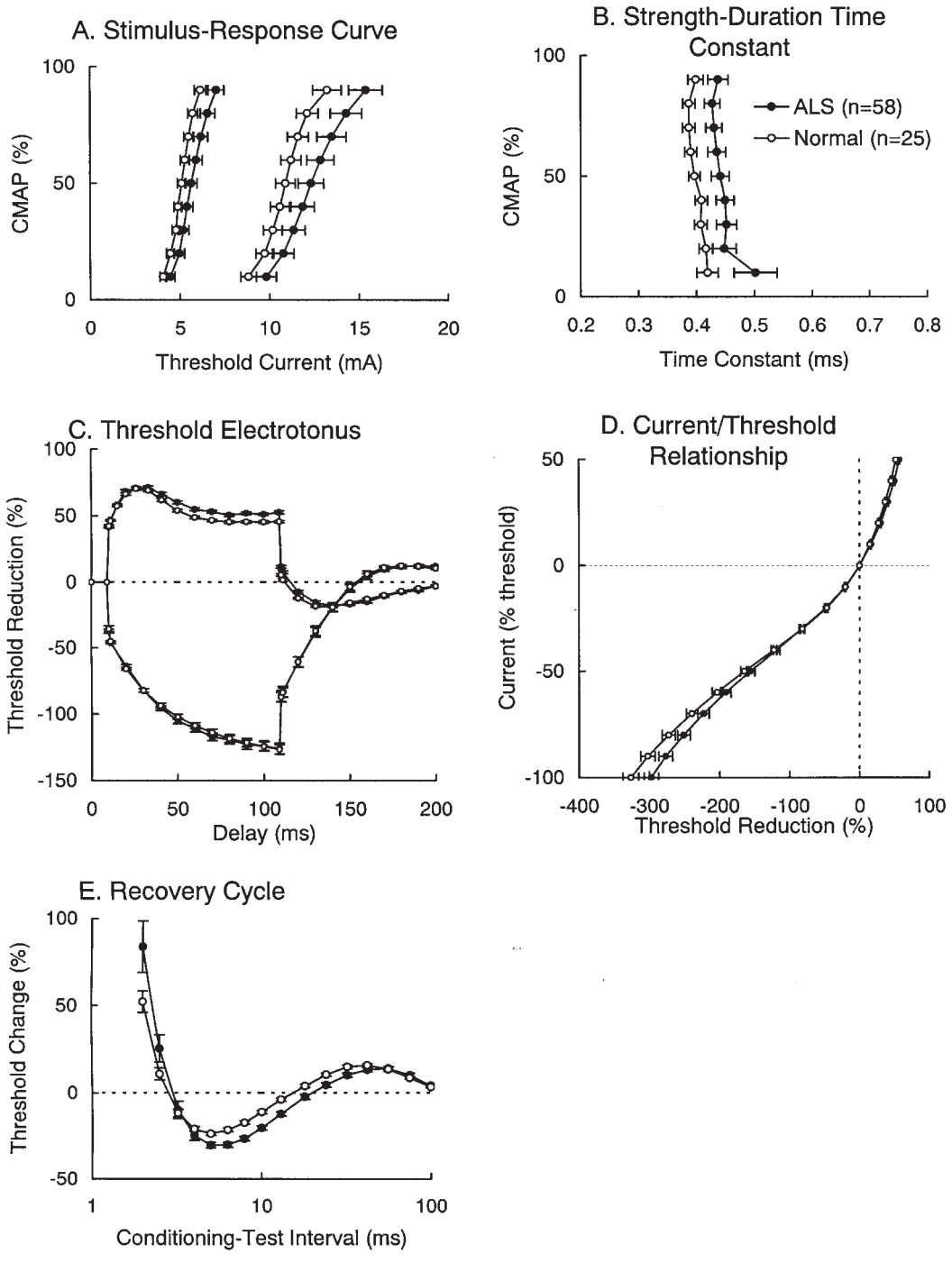

Fig. 1 Grand-averaged data of multiple excitability measurements in patients with ALS (n = 58; filled circle) and in age-matched normal subjects (n = 25; open circle). In Stimulus-Response Curve, left curves were obtained by stimulus duration = 1.0 ms; right curves were obtained by stimulus duration = 0.2 ms. Error bars indicate SEM.

Fig. 1D and Table 1. Threshold reduction by a 50% depolarizing current lasting 200 ms is significantly greater in ALS patients than in normal subjects ($P < 0.005$). The threshold increase induced by a 100% hyperpolarizing current of 200 ms duration in ALS patients is smaller than that in normal subjects, but the difference is not significant ($P = 0.10$). The results both in the hyperpolarizing current/threshold relationships and the TEh of ALS patients are inconsistent with a previous report (Horn *et al.*, 1996) showing that the mean value of the TEh in ALS patients was significantly larger than in normal subjects. The reason for this discrepancy is unclear, but may be associated with the fact that the mean value of the TEh of the normal subjects in the previous study was much smaller than

Table 1 Indices in multiple excitability measurements: mean (SEM)

	Normal (n = 25)	ALS All (n = 58)	Group A (CMAP > 5 mV) (n = 21)	Group B (CMAP, 1–5 mV) (n = 30)	Group C (CMAP < 1 mV) (n = 7)	SMA (n = 28)
Threshold current[a] (mA)	5.1 (0.2)	5.3 (0.2)	5.4 (0.4)	5.1 (0.3)	5.8 (0.8)	5.6 (0.4)
Rheobase[a] (mA)	3.7 (0.2)	3.7 (0.2)	3.8 (0.3)	3.6 (0.2)	4.0 (0.4)	3.4 (0.2)
τ_{SD} (ms)	0.40 (0.01)	0.44 (0.02)†	0.46 (0.02)*	0.43 (0.02)	0.44 (0.06)	0.48 (0.02)****
TEd (10–30 ms) (%)	66.8 (0.9)	69.2 (0.92)*	68.4 (1.0)	70.2 (1.4)*	67.8 (2.4)	69.3 (0.8)
TEd (90–100 ms) (%)	45.3 (0.9)	51.8 (1.1)****	49.1 (1.1)*	53.5 (1.4)****	52.7 (2.7)*	48.1 (1.1)
TEh (90–100 ms) (%)	−127.0 (3.8)	−124.9 (3.9)	−125.2 (6.2)	−128.7 (5.4)	−107.9 (10.8)*	−113.0 (3.8)†
Refractoriness (%)	60.7 (7.5)	86.1 (12.3)	65.4 (7.1)	90.9 (22.1)	129.3 (43.8)	83.1 (20.0)
Supernormality (%)	−23.7 (1.1)	−30.6 (1.5)****	−28.6 (1.8)*	−32.9 (2.3)****	−26.0 (4.5)	−24.2 (1.4)
Late subnormality (%)	15.6 (1.0)	13.5 (1.0)	17.3 (1.6)	11.7 (1.2)	9.8 (3.5)	14.0 (0.7)
50% depolarizing current in CTR (%)	51.9 (1.4)	57.4 (1.0)**	55.9 (1.6)	58.4 (1.4)***	57.8 (5.1)	54.0 (1.1)
100% hyperpolarizing current in CTR (%)	−326.3 (10.9)	−298.8 (10.2)	−283.1 (13.9)*	−317.5 (14.5)	−246.8 (40.2)†	−276.2 (8.3)****

ALS, amyotrophic lateral screlosis; SMA, spinal muscular atrophy; τ_{SD}, strength duration time constant; TEd, depolarizing threshold electrotonus; TEh, hyperpolarizing threshold electrotonus; CTR, current/threshold relationship. [a]for 50% CMAP (1.0ms) †$P < 0.10$; *$P<0.05$; **$P < 0.01$; ***$P < 0.005$; ****$P < 0.001$, compared with normal values

those in this study or in the other studies (Kiernan *et al.*, 2000, 2002)

Patients with SMA had significantly longer τ_{SD} than normal subjects, but parameters of threshold electrotonus, recovery cycle, and current/threshold relationships were not significantly different from those of normal controls (Table 1).

Multiple excitability measurements in the subgroups of ALS patients divided according to CMAP amplitudes

To clarify the relationship between the findings of multiple excitability measurements and the disease stage, we compared the results for the three subgroups of ALS patients on the basis of CMAP amplitudes (Table 1). The mean skin temperature at the tested site (median nerve at the wrist) of each group was identical, 32.3°C.

In group A (normal CMAP), the fundamental findings of multiple excitability measurements were similar to those of the ALS grand average, but a significant increase in τ_{SD} ($P < 0.05$) was observed only in this group (Fig. 2D and Table 1). The significant change in the threshold increase to 100% hyperpolarizing current in the current/threshold relationship was also observed in this group ($P < 0.05$), but TEh was almost the same as in normal subjects (Table 1). The pattern of findings of multiple excitability measurements in group B (moderately decreased CMAP) was similar to those of all ALS patients, but abnormalities in threshold electrotonus, supernormality, and current/threshold relationships were most prominent in this group (Fig. 2 and Table 1). In group C (severely decreased CMAP), TEh [90–100 ms] was reduced ($P < 0.05$), as was the threshold increase to 100% hyperpolarizing current in the current/threshold relationships (Fig. 3 and Table 1).

Use of the model to investigate the cause of the abnormal excitability properties in ALS

To help interpret these changes in nerve excitability, the mathematical model of a human motor axon was first adjusted to provide a close match to the recordings from the control group and then the discrepancy (as defined in Methods) between the model and the group B (CMAP 1–5 mV) patient data was compared before and after adjusting the parameters of the model in turn to minimize the discrepancy. For example, the best fit that could be obtained by changing the nodal sodium conductances was a reduction in discrepancy of 17%, obtained by increasing sodium conductances by 24%. An increase in the percentage of sodium current that was persistent from 0.84 to 1.04% produced a slightly better reduction in discrepancy of 25%. A rather better fit could be obtained by a membrane hyperpolarization of 1.4 mV, which reduced the discrepancy by 48%. However, it is not clear how membrane hyperpolarization could be sustained in the dying motoneurons, and the best fits were obtained by reducing K^+ conductances: reducing the nodal slow K^+ conductance (G_{Ks}) by 38% reduced the discrepancy by 60%, and reducing the internodal fast K^+ conductance (G_{Kf}) by 60% reduced the discrepancy by 71%. This fitting procedure, which takes into account the effects of conductances on τ_{SD}, recovery cycle, current-threshold relationship and threshold electrotonus, therefore supports the interpretation by Bostock *et al.* (1995) that axonal potassium conductances are reduced in ALS.

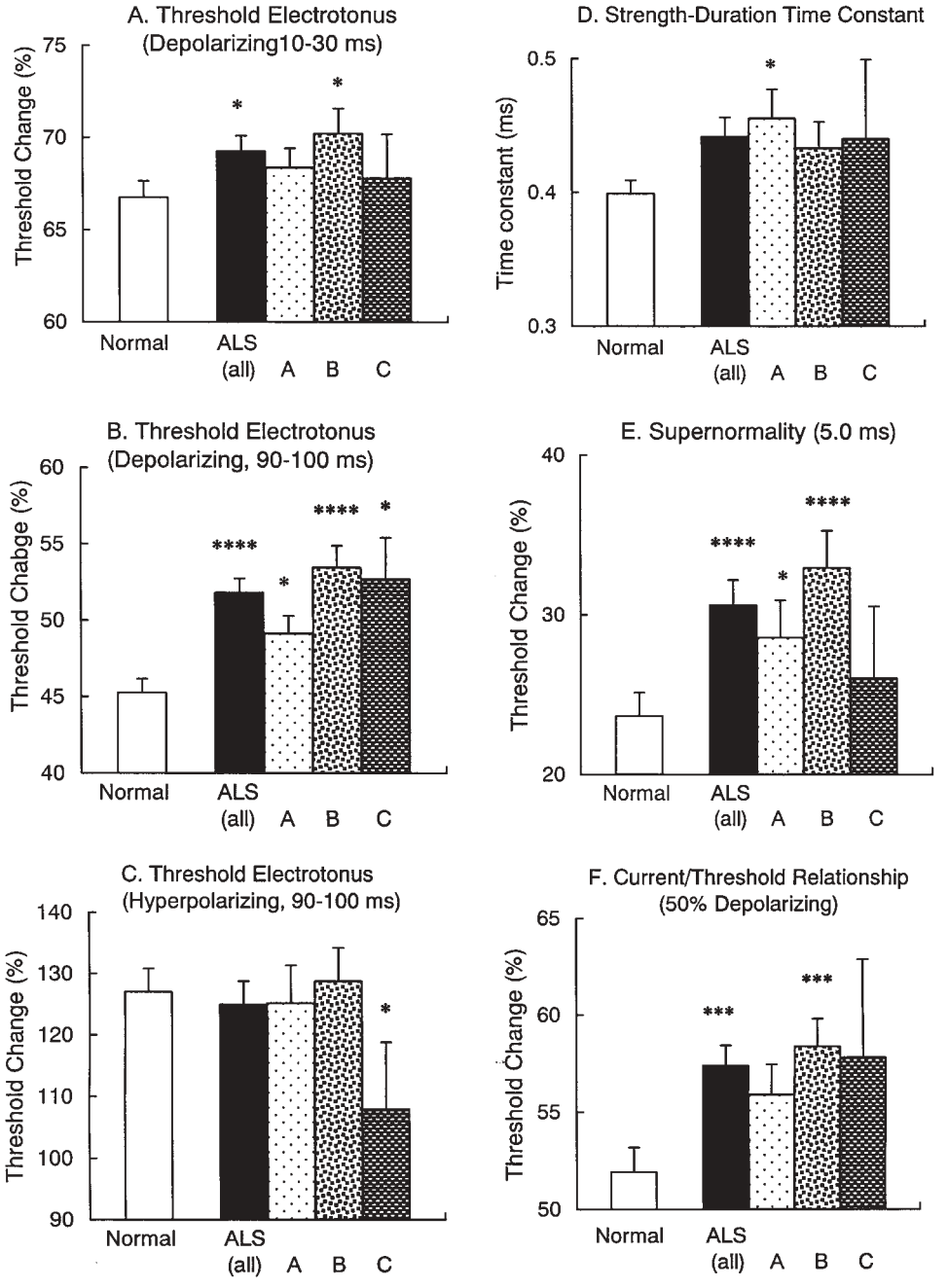

Fig. 2 Comparison of multiple excitability indices in the subgroups of ALS patients divided by CMAP size. Group A: normal CMAP (>5 mV) (n = 20). Group B: moderately decreased CMAP (CMAP, 1–5 mV) (n = 31). Group C: severely decreased CMAP (<1 mV) (n = 7).

Both fast and slow K^+ conductances are probably involved, since although a reduction in G_{Kf} gave the better overall fit, a reduction in G_{Ks} was required to produce the observed increase in TEd [90–100 ms]. Because blocking K^+ conductances results in membrane depolarization, it indirectly leads to an increase in τ_{SD}. Thus the block in G_{Kf} caused a 1.2 mV depolarization, and an increase in τ_{SD} from 0.41 to 0.44 ms, similar to that observed in the patients.

The model was further used to help understand the later changes in axonal membrane properties, by testing what changes in the model that best fitted the group B (CMAP 1–5 mV) data were required to match the group C (CMAP < 1 mV) data. We found that 87% of the discrepancy between the group B model and group C data could be accounted for by increasing the nodal leak conductance, and the next best changes were increases in internodal leak conductance (82% reduction) or inward rectification (79% reduction). The best fit obtainable by changing two parameters was a 90% reduction in discrepancy, when an increase in nodal leak conductance was combined with a further 27% decrease in the internodal fast K^+ conductance. As a check, we also tested what changes in the original model that best fitted the control

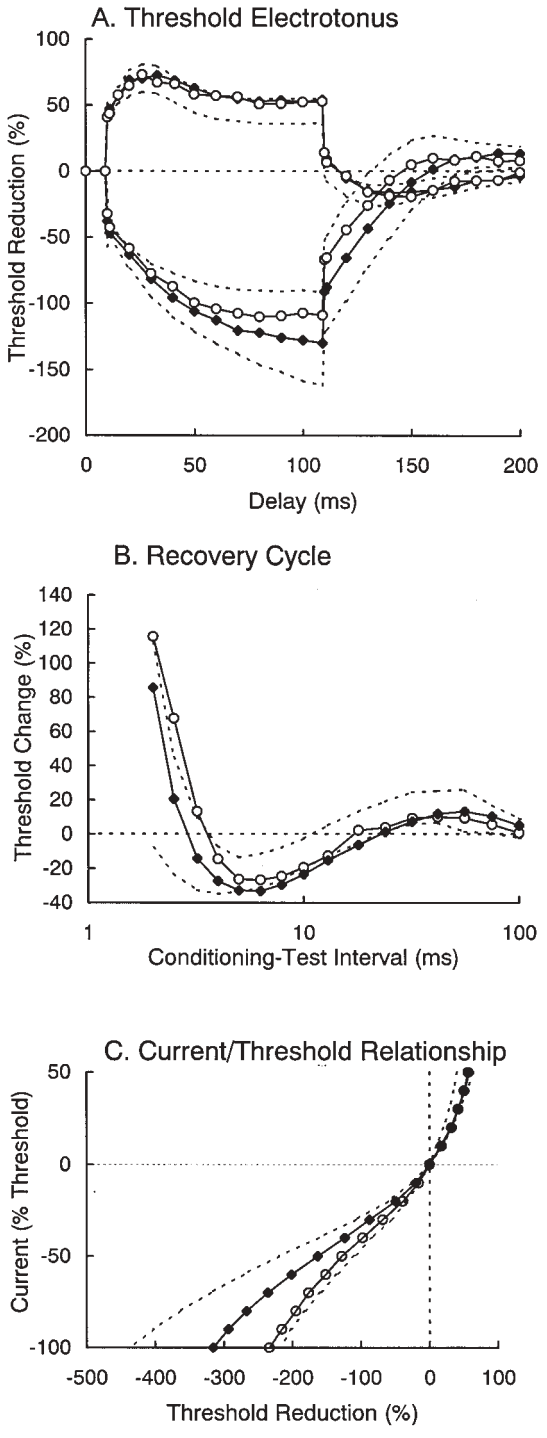

Fig. 3 Multiple excitability measurements in the subgroups of ALS patients divided by CMAP amplitudes of abductor pollicis brevis. Filled circles indicate the mean values of patients with moderately decreased CMAP (group B; $n = 31$). Open circles indicate the mean values of patients with severely decreased CMAP (group C; $n = 7$).

data were required to match the group C data. The best reduction in discrepancy that could be achieved by changing a single parameter was an unimpressive 56% reduction by increasing the nodal leak conductance, but a much better fit, and the best achieved by changing two parameters, was an 81% reduction in discrepancy obtained by both reducing the internodal fast K^+ conductance and increasing the nodal leak conductance. Taken together, these results strongly suggest that the reason that supernormality was maximal in the group B patients was not because the underlying decline in voltage-dependent K^+ conductances stopped, but rather that the further disease progression was accompanied by an increase in another conductance, with relatively weak voltage dependence, that is adequately modelled as a leak conductance.

Discussion

In the present study, measurements of multiple excitability indices confirmed altered ion channel function in ALS. The main finding was that supernormality and the threshold changes to long depolarizing currents were greater in ALS patients than in normal subjects. This finding is consistent with reduced K^+ conductances as previously proposed. Moreover, our findings showed that patterns of altered ion channel function are related to the size of CMAP, presumably reflecting progression of the motoneuronal degeneration.

Altered fast and slow K^+ conductance in ALS

The present study confirmed abnormally increased threshold changes in TEd (10–30 ms) and TEd (90–100 ms) in ALS (Bostock *et al.*, 1995). This abnormality could in principle result from either K^+ channel dysfunction or membrane hyperpolarization. However, additional measurements provided good evidence that axonal hyperpolarization is unlikely to explain the abnormality. Changes in other excitability indices sensitive to membrane potential, such as refractoriness, TEh, and τ_{SD}, were not consistent with membrane hyperpolarization. For these reasons, and the objective evidence provided by modelling that a reduction in K^+ conductances best explains the abnormalities in ALS group B patients, we conclude that impaired K^+ channel function is most likely responsible for the abnormal TEd and supernormality.

These electrophysiological findings are consistent with a recent gene expression study in spinal motor neurons of ALS patients with microarray analysis (Jiang *et al.*, 2005), which found moderately reduced mRNA expression of several potassium channels (paranodal fast K^+ channel gene *KCNA1* and *KCNA2*, and nodal M-current associated K^+ channel gene *KCNQ2*) in the appendix data of the article. In addition, recent studies revealed that axonal transport deficiencies could play important roles in the pathophysiology of ALS (LaMonte *et al.*, 2002), and deficiency of transport of axonal membrane proteins including several K^+ channels or proteins required for maintaining K^+ channels might also contribute to a reduction in fast and slow K^+ conductance. Although the microarray study did not show whether the

reduced expression of potassium channels was significant or not, the results of this study indicate that a physiologically significant effect of reduced potassium currents probably does occur.

Probable relation between fasciculations and reduced K^+ conductance in ALS

A previous study suggested the possibility that reduced K^+ conductance was associated with the fasciculations frequently observed in ALS patients (Bostock *et al.*, 1995). The further evidence of impaired fast and slow K^+ conductances in this study supports this hypothesis, since blocking of presynaptic K^+ channels has been shown to lead to spontaneous firing at the neuromuscular junction (Anderson and Harvey, 1988; Dadson *et al.*, 2003). As described below, increased persistent Na^+ conductance can also enhance axonal hyperexcitability. Therefore, we propose that decreased K^+ conductance and increased persistent Na^+ currents (whether caused simply by the depolarization resulting from reduced K^+ conductance, or by another mechanism) may cause hyperexcitability of peripheral motor axons in ALS patients, resulting in ectopic impulse generation and consequent fasciculation and/or muscle cramping. However, the relevance of our observations to the origin of fasciculations is limited by the fact that the ectopic impulses arise more often from the nerve terminals than from the nerve trunk. Studies of excitability changes in motor nerve terminals in ALS should clarify this question.

A peculiar feature of the ectopic discharges causing fasciculations, as in ALS, is that they occur at long and irregular intervals (Hjorth *et al.*, 1973; Layzer, 1994), unlike the regular high-frequency bursts of action potentials caused by the 'flip-flop' mechanism in post-ischaemic axons or the regular ectopic discharges from demyelinated axons associated with 'pacemaker' oscillations in membrane potential (Baker, 2000). It was recently shown that intrinsically aperiodic ectopic impulses could be generated in human motor axons by a third mechanism: amplification by membrane depolarization of the normal spontaneous variations in threshold caused by the random openings of sodium channels (Hales *et al.*, 2004). The axons would have to be depolarized sufficiently to eliminate supernormality for one impulse not to trigger a high-frequency burst at the end of the refractory period. The circumstances in which such a level of depolarization could occur spontaneously (i.e. without external application of a depolarizing current) have not been explored, but presumably must include either high extracellular potassium or loss of potassium conductances that would otherwise maintain the resting potential near the potassium equilibrium potential.

Changes in τ_{SD} in ALS with progression of motoneuronal degeneration

Strength-duration time constant partly depends on the persistent Na^+ conductance active at the resting potential, and therefore it increases with membrane depolarization (Bostock and Rothwell, 1997). According to our modelling, the increased τ_{SD} in the patients with moderately reduced CMAP (group B) was fully accounted for by the membrane depolarization resulting from the reduced K^+ conductance. However, the increase in τ_{SD} was present in the patients with normal CMAP (group A), who had less evidence of reduced K^+ conductances, suggesting that membrane depolarization was not the only factor affecting τ_{SD}. We have reported previously (Kanai *et al.*, 2003) that τ_{SD} is commonly increased in the motor axons of patients with SMA or peripheral neuropathy. The cause of prolonged τ_{SD} is still unclear. As well as membrane depolarization, for example, metabolic abnormalities in degenerative motor neurons could affect sodium channel gating, resulting in the prolonged τ_{SD}. Interestingly, increased persistent Na^+ current has recently been found in motoneurons cultured from transgenic SOD1 mice, an animal model of the motoneuron degeneration in ALS (Kuo *et al.*, 2005). This model may lead to a better understanding of the mechanism of altered Na^+ channel gating and the increase in axonal $\tau_{SD.}$ However, an increase in τ_{SD} is by no means specific to ALS: it occurs in peripheral neuropathies (Kanai *et al.*, 2003) and was more pronounced in the patients with SMA (Table 1), and we have suggested previously that it may be related to regeneration and sprouting (Kanai *et al.*, 2003).

Changes in ion conductances in ALS with progression of motoneuronal degeneration

Our results suggest that changes in K^+ conductances in ALS depend on the size of CMAP and therefore on the stage of progression of the disease. An unexpected feature is that the abnormalities in TEd and superexcitabity do not increase progressively but are most prominent in patients with moderately decreased CMAP (group B). The computer modelling suggested that this was not due to a reversal of the reduction in voltage-dependent K^+ conductances in the advanced stages of the disease, but to the development of an increased 'leakiness' of the axonal membrane, presumably due to the activation of a non-voltage-dependent or weakly voltage-dependent conductance. Weakly voltage-dependent channels described in vertebrate myelinated axons include the 'flicker' K^+ channel (Koh *et al.*, 1992), the sodium-activated K^+ channel (Koh *et al.*, 1994) and the ATP-sensitive K^+ (K_{ATP}) channel (Jonas *et al.*, 1991). Activation of any such K^+-selective channels would offset the depolarization caused by the reduction in voltage-dependent K^+ conductances, and account for the 'flat' type of threshold electrotonus recordings previously described and their increased variability when recordings were not separated according to the size of the CMAP (Cikurel *et al.*, 1997).

Relevance to therapy

Regarding treatment of ALS, riluzole is the only therapeutic agent for which effectiveness in human ALS patients has been

confirmed by a large scale, randomized, placebo-controlled trial (Lacomblez *et al.*, 1996). It is clear, however, that riluzole does not represent a cure in ALS. Further therapeutic agent and strategies are needed (Morrison, 2002). Up to now many potential therapeutic targets have been evaluated in ALS (Rowland and Shneider, 2001; Morrison, 2002), and rational combined therapy has also been discussed (Eisen and Weber, 1999; Kriz *et al.*, 2000).

Riluzole is thought to act on the glutamate system, by activating a G-protein-dependent process that inhibits glutamate release and by blockade of postsynaptic NMDA glutamate receptors. The drug has many effects, however, and the precise action responsible for its therapeutic effect in ALS is unknown (Morrison, 2002). It blocks persistent Na^+ channels (Urbani and Belluzzi, 2005), and removes the excess persistent Na^+ current expressed in motoneurons cultured from mutant SOD1 mice (Kuo *et al.*, 2005). Riluzole also activates multiple types of K^+ channel (Grunnet *et al.*, 2001) and inhibits slow inactivation of voltage-dependent K^+ channels (Xu *et al.*, 2001). Both of these effects result in the increase of K^+ currents in neurons, which may be associated with the inhibition of glutamate release and with neuroprotection (Beltran-Parrazal and Charles, 2003).

The activation of K^+ channels recently has been considered as a potential target in many neuronal diseases including neurodegenerative diseases (Wickenden, 2002). Potassium channel activators have therefore been considered as candidate neuroprotective drugs for the treatment of neurological disorders. One example is retigabine (Rekling, 2003), an antiepileptic drug which activates *KCNQ2* channels, recently demonstrated at nodes of Ranvier (Devaux *et al.*, 2004) and thought to be responsible for the slow K^+ current. Recently an opener of K_{ATP} channels has been proposed as a candidate neuroprotective agent for ischaemic stroke (Wang *et al.*, 2004).

Since our results implicate a reduction of fast and slow K^+ conductances and an increase in persistent Na^+ current in the pathophysiology of human sporadic ALS, and since the only approved therapeutic agent has the effects of inhibiting persistent Na^+ current and of activating multiple types of potassium channels, we endorse an earlier suggestion (Bostock *et al.*, 1995) that other K^+ channel activators and inhibitors of persistent Na^+ current be considered as candidate neuroprotective targets for the treatment of ALS. This approach could be tested in transgenic mouse models of ALS (Gurney, 1997; Kriz *et al.*, 2003).

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