Preserved slow conducting corticomotoneuronal projections in amyotrophic lateral sclerosis with autosomal recessive D90A CuZn-superoxide dismutase mutation

Markus Weber,1 Andrew Eisen,1 Heather G. Stewart1 and Peter M. Andersen2

1The Neuromuscular Diseases Unit, Vancouver Hospital and the University of British Columbia, Vancouver, Canada and 2Department of Neurology, Umeå University Hospital, Umeå, Sweden

Correspondence to: Dr A. Eisen, Neuromuscular Diseases Unit, Vancouver Hospital, 855 West 12th Avenue, Vancouver, British Columbia, Canada V5Z 1M9 E-mail: eisen@unixg.ubc.ca

Summary

Recently, a subgroup of the amyotrophic lateral sclerosis (ALS) syndrome associated with mutations in the gene encoding the free radical scavenging enzyme CuZn-superoxide dismutase (CuZn-SOD, SOD1) has been identified. Some 67 different mutations have been reported worldwide to date, comprising about one-fifth of familial ALS cases in the populations studied. The autosomal recessively inherited D90A CuZn-SOD mutation has been associated with a very slowly progressive, clinically distinct phenotype, and is neurophysiologically characterized by very slow central motor conduction. It is not known which physiological and/or biochemical mechanisms are responsible for the different clinical course. To delineate ALS associated with this particular CuZn-SOD mutation from ALS without mutations, we performed a detailed neurophysiological study of the corticomotoneuronal function using peristimulus time histograms (PSTHs) in eight ALS patients homozygous for the D90A CuZn-SOD mutation. The results were compared with those obtained in 12 non-hereditary ALS patients and 11 healthy subjects. PSTHs were constructed from three to seven different, voluntarily recruited motor units of the extensor digitorum communis muscle (EDC) in each patient. The onset latency, number of excess bins, duration and synchrony of the primary peak were analysed. All measurements differed significantly between healthy controls and the D90A patients (P < 0.0007). The mean onset latency of the primary peak in D90A patients was 35.3 ms, compared with 24.2 ms for non-hereditary ALS patients and 19.3 ms for normal subjects (P < 0.0000). Delayed primary peaks in the D90A patients were desynchronized and characteristically preceded by a marked suppression phase. This suppression phase was not seen in non-hereditary ALS patients. We conclude that the mainly slow conducting and/or polysynaptic corticomotoneuronal connections are preserved in the D90A homozygous cases, and that the cortical and possibly spinal inhibitory circuitry is preserved. These events may partially protect the motor neurons, slowing down the degenerative process.

Keywords: ALS; corticomotoneuronal system; D90A; CuZn-SOD mutation; central motor conduction time

Abbreviations: ALS = amyotrophic lateral sclerosis; CMCT = central motor conduction time; CUSUM = cumulative sum analysis; CuZn-SOD = CuZn-superoxide dismutase; EDC = extensor digitorum communis muscle; EPSP = excitatory postsynaptic potential; FALS = familial ALS; LMN = lower motor neuron; MEP = motor evoked potentials; MUP = motor unit potential; PSTH = peristimulus time histogram; UMN = upper motor neuron

Introduction

In 1993 a collaborative consortium (Rosen et al., 1993) reported the finding of missense mutations in the gene encoding the free radical scavenging enzyme CuZn-superoxide dismutase (CuZn-SOD, SOD1) in a small group of patients with familial amyotrophic lateral sclerosis (FALS). This finding sparked worldwide research interest in free radical homeostasis and motor neuron degeneration. At present, 67 CuZn-SOD mutations have been reported worldwide (Al-Chalabi et al., 1998). In most populations studied, one-fifth of FALS cases and a small percentage of sporadic ALS cases have been found to have a CuZn-SOD mutation (Andersen et al., 1997; Eisen and Krieger, 1998),

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all but one of them being inherited as a dominant trait. The exception is the D90A mutation, which was first reported as a recessive trait in Sweden and Finland (Andersen et al., 1995), and later in non-Scandinavian populations in southern France, Germany and Italy as well as Canada and the USA (through recent migration). Uniquely for a disease associated mutation, rare cases of ALS heterozygous for the D90A mutation show a definitive dominant pattern of inheritance in other ethnic populations in Belgium (Robberecht et al., 1996), England, Scotland, central France and the USA (through migration). This makes the D90A mutation the most globally widespread CuZn-SOD mutation and the single most common known cause of ALS.

While most patients with a dominantly inherited CuZn-SOD mutation show variable phenotypes with different sites of onset and survival time indistinguishable from patients without a CuZn-SOD mutation (Radunovic and Leigh, 1996), ALS patients homozygous for the D90A CuZn-SOD mutation show a very characteristic and uniform disease phenotype irrespective of ethnic background (Andersen et al., 1996).

At least two-thirds of D90A homozygous patients experience an insidious pre-paretic phase with lower extremity stiffness and cramps, unsteadiness or clumsiness, and general fatigue. Approximately half of patients complain of burning or aching pain in the lower back, buttocks, hips and/or legs. The pre-paretic phase lasts from a few months to a few years during which time the clinical and neurophysiological studies are reportedly normal. This initial phase is followed by a stereotypic paretic phase with slowly ascending usually asymmetric paresis, with a combination of lower motor neuron (LMN) and upper motor neuron (UMN) findings such as muscle wasting, fasciculations, spastic tone, absent ankle and brisk upper extremity reflexes, and bilateral Babinski signs. This is followed by a slow progression to proximal paresis, occasional clumsiness and fasciculations. In all four limbs, signs of an UMN lesion appear before signs of LMN involvement with a characteristic pathological reflex pattern (Andersen et al., 1997). Upper extremity involvement appears on average 4.1 years and bulbar symptoms on average 5.4 years after onset of the first symptoms distally in the lower extremities. Bulbar symptoms, especially progress very slowly, and only very late in the disease do D90A homozygous patients develop aphony, anarthria and aphagia (Andersen et al., 1996). Mean survival time from onset of paresis is 12.6 years (n = 16) (Andersen et al., 1997). In most of the patients, LMN features dominate over UMN features during the progression of the disease. Urgency of micturition and/or difficulty initiating urination are common in some patients even early in the disease (Andersen et al., 1996), which supports neuropathological findings in patients with different CuZn-SOD mutations that the disease process is not restricted to the motor system (Ince et al., 1998).

Marked slowing of central motor conduction has been reported earlier in D90A homozygous patients and in single FALS cases heterozygous for the D76Y and K12A mutation (Andersen et al., 1996; Andersen, 1997; Penco et al., 1999). This is unusual for ALS in which central motor conduction time (CMCT) is normal or very modestly slowed (Ingram and Swash, 1987; Thompson et al., 1987; Schriefer et al., 1989; Eisen et al., 1990; Berardelli et al., 1991). The slow progressive course and characteristic neurophysiological findings in D90A homozygous cases makes this particular mutation an ideal model to study UMN dysfunction in ALS, prompting us to perform a detailed study of central motor function in these patients.

Subjects and methods
We studied eight patients homozygous for the D90A mutation (mean age 54.1 ± 8.5 years, range 46–66 years). Twelve patients with El Escorial definite (Brooks, 1994) non-hereditary ALS (mean age 58.7 ± 13.4 years, range 39–79 years) and 11 (six male, five female) healthy control subjects (mean age 55.8 ± 16.0 years, range 33–82 years) served as controls for the peristimulus time histogram (PSTH) studies. Relevant parameters for the patients are shown in Table 1. None of the studied subjects or patients had a history of CNS or PNS trauma, unconsciousness, coma or CNS infection. Subjects voluntarily consented to participate in the experiments, which were approved by the University of British Columbia Human Ethics Committee. Before commencing the electrophysiological studies, a complete neurological examination was performed. None of the subjects was on medication that would alter cortical excitability or responses to transcranial magnetic stimulation.

With informed consent, peripheral blood samples were drawn, the DNA was extracted using standard procedures and analysed for CuZn-SOD mutations, as described earlier (Andersen et al., 1995).

Routine neurophysiological studies
Needle EMG studies were performed using a monopolar needle electrode (Dantec 13R1, Dantec Elektronik, Skovlunde, Denmark). Six upper and lower limb muscles, paraspinal muscles and the chest wall musculature, including the diaphragm, were examined. Motor and sensory (orthodromic method) nerve conduction studies including F-waves were performed on the median, ulnar, tibial and sural nerves. Recordings were made with surface electrodes (Medicotest E-10-VS, Medicotest, Ølstykke, Denmark).

A Dantec Magpro II magnetic stimulator (Dantec MMC 140) was used to deliver stimuli to the contralateral motor cortex and the cervical roots through a large, cup-shaped, round coil. Surface recordings were made from the extensor digitorum communis muscle (EDC). For cortical stimulation the same preactivation was used as for single motor unit studies. CMCT was calculated by subtracting the latency after cervical stimulation from the latency after cortical stimulation.
### Table 1 Clinical data

(A) D90A patients

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age of onset (years)</th>
<th>Duration (years)</th>
<th>First symptom</th>
<th>Spasticity</th>
<th>Weakness/wasting</th>
<th>Fascics</th>
<th>Reflexes</th>
<th>Babinski</th>
<th>Bulbar signs</th>
<th>Bladder</th>
<th>Remarks</th>
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<tr>
<td>1</td>
<td>F</td>
<td>53</td>
<td>4</td>
<td>Back pain, lack of control of left leg</td>
<td>Legs, L &gt; R</td>
<td>–</td>
<td>–</td>
<td>***</td>
<td>–/±</td>
<td>–</td>
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<td>Depressed ankle jerks</td>
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<tr>
<td>2</td>
<td>F</td>
<td>58</td>
<td>8</td>
<td>Weakness of legs, loss of balance</td>
<td>Legs &gt; arms</td>
<td>+</td>
<td>+</td>
<td>***</td>
<td>+/-</td>
<td>+</td>
<td>–</td>
<td>Absent ankle jerks</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>44</td>
<td>4</td>
<td>Several falls, twitching left thigh</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>***</td>
<td>+/-</td>
<td>+/±</td>
<td>+</td>
<td>Absent ankle jerks</td>
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<tr>
<td>4</td>
<td>M</td>
<td>56</td>
<td>4</td>
<td>Several falls, difficulties climbing stairs</td>
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<td>++</td>
<td>+</td>
<td>***</td>
<td>+/-</td>
<td>+/±</td>
<td>+</td>
<td>Absent ankle jerks</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>37</td>
<td>9</td>
<td>Weakness of legs, loss of balance</td>
<td>Legs &gt; arms</td>
<td>+</td>
<td>–</td>
<td>***</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
<td>Absent ankle jerks</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>39</td>
<td>13</td>
<td>Several falls, stiffness of legs</td>
<td>Legs</td>
<td>++</td>
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<td>Absent jerks in legs</td>
</tr>
<tr>
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<td>F</td>
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<td>2</td>
<td>Limping left leg</td>
<td>Arms</td>
<td>++</td>
<td>+</td>
<td>***</td>
<td>+/-</td>
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<td>+</td>
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(B) Non-hereditary ALS patients

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<tr>
<th>Case</th>
<th>Sex</th>
<th>Age of onset (years)</th>
<th>Duration (months)</th>
<th>Onset symptom</th>
<th>Spasticity</th>
<th>Weakness/wasting</th>
<th>Fascics</th>
<th>Reflexes</th>
<th>Babinski</th>
<th>Bulbar signs</th>
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<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>64</td>
<td>30</td>
<td>Unilateral, upper limb</td>
<td>+</td>
<td>+++</td>
<td>+</td>
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<td>+/-</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>39</td>
<td>15</td>
<td>Bulbar signs</td>
<td>Legs</td>
<td>++</td>
<td>+</td>
<td>***</td>
<td>+/-</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>60</td>
<td>60</td>
<td>Bulbar signs</td>
<td>–</td>
<td>++</td>
<td>+</td>
<td>***</td>
<td>+/-</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>46</td>
<td>21</td>
<td>Bulbar signs</td>
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<td>++</td>
<td>+</td>
<td>*</td>
<td>–/–</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>77</td>
<td>11</td>
<td>Bulbar signs</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>*</td>
<td>–/–</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>55</td>
<td>13</td>
<td>Unilateral, upper limb</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>***</td>
<td>+/-</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>61</td>
<td>35</td>
<td>Bulbar signs, unilateral upper limb</td>
<td>Legs</td>
<td>+</td>
<td>+</td>
<td>**</td>
<td>+/-</td>
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</tr>
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<td>F</td>
<td>61</td>
<td>34</td>
<td>Unilateral, lower limb</td>
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<td>+</td>
<td>+</td>
<td>*</td>
<td>–/–</td>
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<tr>
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<td>M</td>
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<td>45</td>
<td>Unilateral, upper limb</td>
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<td>++</td>
<td>++</td>
<td>**</td>
<td>–/–</td>
<td>–</td>
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<tr>
<td>10</td>
<td>M</td>
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<td>23</td>
<td>Unilateral, upper limb</td>
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<td>++</td>
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<td>***</td>
<td>+/-</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>44</td>
<td>18</td>
<td>Unilateral, upper limb</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>*</td>
<td>–/–</td>
<td>–</td>
</tr>
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<td>12</td>
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<td>20</td>
<td>Bulbar</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>*</td>
<td>–/–</td>
<td>–</td>
</tr>
</tbody>
</table>

Weakness and wasting refers to LMN type. Reflexes: * = slightly, ** = moderately brisk, *** = exaggerated. Bulbar signs = dysarthria and/or dysphagia; bladder = urgency of micturition; – = absent, + = modest, ++ = moderate, +++ = severe. †F = female; M = male.
Fig. 1 (A) PSTH recorded for a normal subject. The x-axis indicates the time scale (ms). It shows events occurring for 50 ms before and 200 ms after the cortical stimulus, which was applied at time 0 ms. The apparent inhibition of activity immediately following the stimulus is due to stimulus artefact suppression built into the program. Following the primary peak there is a variable period of inhibition. Events were collected in 1 ms bins. Values are: onset latency, 17 ms; amplitude, 6.3 mV; excess bins, 3; duration, 3 ms. Note that the scales for the y-axes (bin count) are different in each histogram. (B) PSTH recorded for a D90A patient. The major abnormality is the delayed, desynchronized primary peak preceded by a marked suppression period. The activated threshold was 65%. Onset of the suppression phase is at 24 ms and it has a duration of 14 ms. The suppression phase is also seen in the CUSUM. Values of the primary peak are: onset latency, 45 ms; amplitude, 5.9 mV; excess bins, 11; duration, 14 ms. Desynchronized primary peaks with normal (C) and delayed (D) onset recorded from the same non-hereditary ALS patient (nHALS) on two separate occasions are indicated. In C, the primary peak has a normal number of excess bins (6) but an abnormal duration (11 ms) which is mainly due to the appearance of a 'double peak'. In D, CUSUM and the histogram show a reduced firing probability preceding the delayed primary peak, which is not as marked as in B. Values (C and D, respectively) are: onset latency, 19 ms, 31 ms; amplitude, 3.45 mV, 2.02 mV; excess bins, 6, 6; duration, 11 ms, 12 ms.
patients there is a bimodal distribution with onset latencies either in D90A patients and also in the non-hereditary ALS (nHALS) histogram of the primary peak onset latencies. Note that in the hereditary ALS have a normal or only slightly prolonged onset latency.

The scalp site at which the lowest intensity stimulus stand, delivered stimuli to the contralateral motor cortex. The technique used has been described previously.

Subjects were instructed to maintain steady recruitment of a pronated on a pillow. A monopolar needle electrode was located. While the subject or patient maintained a steady recruitment of the indexed motor unit the stimulus output was then adjusted to an intensity just below that which evoked a complex response consisting of two or more motor units. This intensity is referred to as the activated threshold, which was then reduced by 2.5% of the stimulator output and a series of 100–200 stimuli were randomly delivered at intervals of 1 to 5 s. In each individual, three to seven different motor units were examined sequentially. It was assumed that during repositioning of the needle electrode, there would be movement of the subject’s head relative to the coil and that levels of activation would vary with the newly recruited indexed motor unit. This necessitated re-adjusting the stimulus intensity between each MUP studied by increasing it in 2.5% increments until the compound motor evoked potential (MEP) was again evoked and then reducing it by 2.5%. Identification of the indexed motor unit was verified off-line by visual inspection of individual sweeps. If the post-stimulus indexed motor unit was contaminated with other MUPs the sweep was discarded. The average number of sweeps included in each histogram was 140.9 ± 40.1 for the D90A patients, 95.9 ± 7.1 for normal subjects and 97.1 ± 24.0 for the non-hereditary ALS patients. Discharges of an indexed motor unit were collected into 1-ms bins of stimulus-triggered sweeps and changes in the firing probability were expressed as a PSTH (Fig. 1). Each stimulus-triggered sweep had a total analysis time of 250 ms (50 ms before the stimulus and 200 ms after the stimulus). An initial period of increased firing probability (the primary peak) was readily discernible in the PSTHs occurring at about 20–50 ms after the stimulus. An excess bin was defined as a bin with a count exceeding the mean pre-stimulus background by >2 SD.

The following measurements were made from the PSTHs. (i) Onset latency of the primary peak was defined as the first excess bin after the stimulus. (ii) The duration of the primary peak was measured from the time interval between the first and the last excess bin terminated by a period of clear suppression (inhibition phase). CUSUM (cumulative sum analysis) was used to determine the beginning of the primary peak and any intervening inhibitory phases in the PSTHs (Fig. 1B–D) (Ellaway, 1978). (iii) Synchrony index of the primary peak was defined as the number of excess bins within the primary peak multiplied by the bin width (1 ms) and divided by its duration. This index relates the number of excess bins to the duration and reflects the temporal dispersion of the primary peak. For example, in Fig. 1C the number of excess bins was six, the duration measured 11 ms and the synchrony index was therefore 0.55. (iv) The amplitude of the compound excitatory postsynaptic potential (EPSP) was estimated from the number of events occurring within the primary peak (bin count in excess bins), the number of stimuli delivered and the inter-spike interval using the following equation:

**Single unit recordings**

We studied 36 different motor units in the D90A patients, 49 motor units in the control subjects and 58 motor units in the patients with non-hereditary ALS. The EDC was used for PSTH studies. The strength of this muscle in the patients was MRC (Medical Research Council) grade 4–5 or greater.

Post-synaptic events occurring in EDC spinal motor neurons were derived from changes in the firing probability of single, voluntarily activated motor units induced by transcranial magnetic stimulation to the contralateral motor cortex. The technique used has been described previously (Eisen et al., 1996). Subjects sat comfortably with their arms pronated on a pillow. A monopolar needle electrode was used to record motor unit potentials (MUPs) from the EDC. Subjects were instructed to maintain steady recruitment of a single motor unit. This was aided by auditory and visual feedback of the spike discharges. A window discriminator was used to separate the indexed motor unit from other, contaminating motor units. Only units whose amplitudes exceeded 150 V and whose rise times were <50 s were accepted.

The magnetic stimulator, which was supported on a stand, delivered stimuli to the contralateral motor cortex. The scalp site at which the lowest intensity stimulus capable of inducing a visible muscle contraction of the EDC was located. While the subject or patient maintained a steady recruitment of the indexed motor unit the stimulus output was then adjusted to an intensity just below that which evoked a complex response consisting of two or more motor units. This intensity is referred to as the activated threshold, which was then reduced by 2.5% of the stimulator output and a series of 100–200 stimuli were randomly delivered at intervals of 1 to 5 s. In each individual, three to seven different motor units were examined sequentially. It was assumed that during repositioning of the needle electrode, there would be movement of the subject’s head relative to the coil and that levels of activation would vary with the newly recruited indexed motor unit. This necessitated re-adjusting the stimulus intensity between each MUP studied by increasing it in 2.5% increments until the compound motor evoked potential (MEP) was again evoked and then reducing it by 2.5%. Identification of the indexed motor unit was verified off-line by visual inspection of individual sweeps. If the post-stimulus indexed motor unit was contaminated with other MUPs the sweep was discarded. The average number of sweeps included in each histogram was 140.9 ± 40.1 for the D90A patients, 95.9 ± 7.1 for normal subjects and 97.1 ± 24.0 for the non-hereditary ALS patients. Discharges of an indexed motor unit were collected into 1-ms bins of stimulus-triggered sweeps and changes in the firing probability were expressed as a PSTH (Fig. 1). Each stimulus-triggered sweep had a total analysis time of 250 ms (50 ms before the stimulus and 200 ms after the stimulus). An initial period of increased firing probability (the primary peak) was readily discernible in the PSTHs occurring at about 20–50 ms after the stimulus. An excess bin was defined as a bin with a count exceeding the mean pre-stimulus background by >2 SD.

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ALS was 51.3 (eight patients). Urgency of micturition was a common complaint (six of signi... weakness and/or wasting in at least three limbs. Patient 1 D90A patients had predominant lower limb spasticity, LMN time of examination was 7.0/H11006/H11006 9.7 ms, respectively). It was not possible to determine a clear

**Statistical analysis**

The UNISTAT version 4.0 for Windows was used for data analysis. Differences among the three groups (D90A, controls and non-hereditary ALS) were first analysed by one-way ANOVA (analysis of variance). In instances where significant differences were found, analysis was continued with multiple comparisons (F-test, t-test) to determine differences among groups. If the F-test revealed significantly different variances the separate variance t-test was applied, otherwise the pooled variance t-test was used.

**Results**

The clinical data of the patients are summarized in Table 1A and B. The mean age of symptom onset in the D90A patients was 47.1 ± 8.2 years and the mean disease duration to the time of examination was 7.0 ± 4.1 years. All but one of the D90A patients had predominant lower limb spasticity, LMN weakness and/or wasting in at least three limbs. Patient 1 was in the early stages of the disease and symptoms were confined to the lower limbs. All the patients reported significant improvement of spasticity in warm weather. Urgency of micturition was a common complaint (six of eight patients).

The mean age of onset of the patients with non-hereditary ALS was 51.3 ± 19.2 years; the disease duration, defined as the time after onset of obvious neurological deficit, was 27.1 ± 14.5 months.

**Routine neurophysiological studies**

There was evidence for active denervation with fibrillation and/or positive sharp waves in two or more limbs in six of eight D90A patients. Chronic neurogenic changes consisting of complex, unstable motor unit potentials in three or more muscles were present in all the patients except in patient 1 who had sparse evidence of LMN disease limited to the EDC. Motor and sensory nerve conduction studies including F-waves were normal.

The mean MEP onset latency to the EDC muscle after cortical stimulation in the D90A patients measured 30.7/H11006/H11006 6.3 ms (normal 17.9 ± 1.9 ms) and the mean CMCT measured 21.4 ± 6.3 ms (normal 8.5 ± 1.3 ms). Figure 2A depicts the individual values for the MEP latencies and CMCTs of the D90A patients, and the upper normal limits (22.6 ms and 9.7 ms, respectively). It was not possible to determine a clear

**Single unit recordings**

A primary peak in the PSTH was identified in all the indexed motor units studied except for one in a patient with sporadic ALS. In normal subjects the primary peak of the PSTH was well synchronized with a mean onset latency of 19.3 ± 2.8 ms (Figs 1A and 2B).

**D90A patients**

In the D90A patients, 31 out of 36 (86%) of the primary peaks were profoundly delayed in onset (>31 ms) and desynchronized (Figs 1B and 2B). If the primary peaks of patient 1 (Table 1), who also had a normal CMCT, are excluded then there is only one other primary peak with a normal onset latency. The delayed primary peaks were always preceded by a prominent suppression phase. This commenced 22.1 ± 4.2 ms after the stimulus and its duration ranged from 3 to 19 ms (mean duration 13.1 ± 4.3 ms). No firing of the indexed motor unit was observed during this suppression phase (Fig. 1B).

**Non-hereditary ALS patients**

In non-hereditary ALS patients, the primary peak was also very desynchronized. This occurred with and without delay in the onset of the primary peak (Fig. 1C and D). Primary peaks with a normal onset latency consisted occasionally of a double peak (Fig. 1C). A delay of the primary peak onset of greater than 31 ms occurred in 15 motor units, but was seen in five out of 12 patients (Fig. 1D). CUSUM indicated that there was a slightly reduced firing probability preceding the delayed primary peaks in four out of 15 delayed primary peaks. This suppression never exceeded 3 ms in duration (Fig. 1D).

**Statistical analysis**

In the D90A patients, all PSTH measurements were significantly different from normal subjects. Significant differences for the onset latency, number of excess bins, duration and estimated amplitude were found in patients with non-hereditary ALS when compared with normal subjects (Table 2). We further analysed the delayed primary peaks in D90A patients and non-hereditary ALS after excluding all primary peaks with a normal onset latency (Table 3). The activated thresholds of single motor units in the D90A and non-hereditary ALS patients were significantly higher than in normal subjects, but there was no difference between D90A and non-hereditary ALS patients. The means of onset latency, number of excess bins, duration and estimated amplitude
Table 2 PSTHs: statistical data

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Normal (n = 49)</th>
<th>D90A (n = 36)</th>
<th>nHALS (n = 58)</th>
<th>P-values, two-tail probabilities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Normal versus D90A</td>
</tr>
<tr>
<td>Stimulus threshold (%)</td>
<td>51.4 ± 9.5 (35–70)</td>
<td>71.9 ± 18.3 (40–100)</td>
<td>59.6 ± 14.0 (40–80)</td>
<td>0.016</td>
</tr>
<tr>
<td>Primary peak onset latency (ms)</td>
<td>19.3 ± 2.8 (16–27)</td>
<td>35.3 ± 8.4 (17–48)</td>
<td>24.2 ± 6.5 (16–39)</td>
<td>0.0000</td>
</tr>
<tr>
<td>Primary peak duration (ms)</td>
<td>3.5 ± 1.4 (1–6)</td>
<td>12.2 ± 5.4 (4–33)</td>
<td>8.2 ± 5.7 (1–21)</td>
<td>0.0000</td>
</tr>
<tr>
<td>Excess bins (ms)</td>
<td>3.2 ± 1.1 (1–5)</td>
<td>7.9 ± 2.5 (2–15)</td>
<td>4.8 ± 2.9 (1–14)</td>
<td>0.0000</td>
</tr>
<tr>
<td>Synchrony</td>
<td>0.93 ± 0.12 (0.5–1)</td>
<td>0.69 ± 0.17 (0.44–1)</td>
<td>0.71 ± 0.26 (0.15–1)</td>
<td>0.0000</td>
</tr>
<tr>
<td>Amplitude (mV)</td>
<td>2.9 ± 1.6 (0.8–7.5)</td>
<td>4.2 ± 1.6 (2.0–8.7)</td>
<td>2.8 ± 1.8 (0.0–7.3)</td>
<td>0.0007</td>
</tr>
</tbody>
</table>

Values are mean ± 1 SD, and the range is given. A P-value of <0.05 was considered significant. The pooled variance t-test was used except in cases where the F-test showed significantly different variances; the separate variance t-test was then used. nHALS = non-hereditary ALS; NS = not significant.

Table 3 PSTHs: statistical data (as Table 2 but excluding all primary peaks with a normal onset latency)

<table>
<thead>
<tr>
<th>Measurement</th>
<th>D90A (n = 31)</th>
<th>nHALS (n = 15)</th>
<th>P-values, two-tail probabilities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>D90A versus nHALS</td>
</tr>
<tr>
<td>Stimulus threshold (%)#</td>
<td>75.3 ± 12.6 (60–100)</td>
<td>73.6 ± 6.1 (60–80)</td>
<td>NS</td>
</tr>
<tr>
<td>Primary peak onset latency (ms)</td>
<td>37.9 ± 5.6 (29–48)</td>
<td>34.9 ± 2.7 (31–39)</td>
<td>0.0000</td>
</tr>
<tr>
<td>Primary peak duration (ms)</td>
<td>12.5 ± 3.5 (7–21)</td>
<td>7.6 ± 2.5 (3–12)</td>
<td>0.0000</td>
</tr>
<tr>
<td>Excess bins (ms)</td>
<td>8.3 ± 1.7 (5–11)</td>
<td>5.2 ± 2.1 (2–9)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Synchrony</td>
<td>0.69 ± 0.16 (0.44–1)</td>
<td>0.70 ± 0.20 (0.22–1)</td>
<td>NS</td>
</tr>
<tr>
<td>Amplitude (mV)</td>
<td>4.4 ± 1.6 (2.2–8.7)</td>
<td>2.7 ± 1.9 (0.7–7.3)</td>
<td>0.0028</td>
</tr>
</tbody>
</table>

Values are mean ± 1 SD, and the range is given. A P-value of <0.05 was considered significant. The pooled variance t-test was used except in cases where the F-test showed significantly different variances; the separate variance t-test was then used. Only PSTHs with onset latencies greater than 31 ms are included. #Activated thresholds of single motor units. nHALS = non-hereditary ALS; NS = not significant.

Discussion

All the D90A patients we studied demonstrated phenotypic clinical characteristics with a slowly progressive paraparesis, and all but one of the patients had the characteristic prolonged CMCT. The patient with normal central motor conduction had no clinical signs of UMN involvement in the upper limbs, which may explain this finding. Prolongation of central motor conduction, as seen in the D90A mutation patients, is atypical in non-hereditary ALS in which central motor conduction is usually reported as normal or marginally prolonged. (Ingram and Swash, 1987; Thompson et al., 1987; Schriefe et al., 1989; Eisen et al., 1990; Berardelli et al., 1991). The characteristic abnormality in ALS is a reduced MEP amplitude (Eisen et al., 1990, 1993), especially in cases where UMN findings predominate, as is the case in the D90A phenotype. This is particularly true when spastic bulbar palsy is marked (Eisen et al., 1993). Slowing of central motor conduction to the extent seen in the D90A patients is typical of demyelination and characteristic of multiple sclerosis (Hess et al., 1987; Ingram et al., 1988; Rossini et al., 1989; Mayr et al., 1991; Ravnborg et al., 1992) and primary lateral sclerosis (Pringle et al., 1992; Salerno et al., 1996). To date there have been no reported autopsy studies in D90A patients; however, it is reasonable to assume that the pathology of these patients is no different from that of patients with or without CuZn-SOD mutations, which is not associated with demyelination (Pringle et al., 1992; Nihei et al., 1993; Ince et al., 1998).

There are other explanations for slowing of central conduction. They include (i) conduction through a slowly conducting monosynaptic (corticospinal) or polysynaptic (corticospinal) projection (Porter and Lemon, 1993; Mills, 1995; Kohara et al., 1996); (ii) delayed initiation of corticomotoneuronal volleys resulting in late occurring I waves (Mills, 1995); and (iii) failure of the anterior horn cell to respond to the descending volley. The results of the single unit recordings and PSTHs were helpful in distinguishing these possibilities.
**Physiology**

Modulations in the firing of a single motor unit in response to an imposed transcranial stimulus (or other stimulus) can be studied using a PSTH (Ashby and Zilim, 1982; Fetz and Gustafsson, 1983; Day et al., 1987, 1989; Brouwer and Ashby, 1990; Palmer and Ashby, 1992; Mills, 1999). Changes in the firing probability of the motor unit after the stimulus are seen as peaks and troughs in the PSTH. More subtle deviations from the baseline can be detected using the CUSUM (Ellaway, 1978). A single threshold magnetic stimulus to the motor cortex excites many corticomotorneurons. However, a subset of these, the cortical colony, converge on to a single spinal motor neuron. The compound EPSP generated at the anterior horn cell is a summated composite of the unitary EPSPs evoked at each synapse.

Single cortical stimuli evoke up to six descending volleys termed D and I waves (Kernell and Wu, 1967; Day et al., 1987; Nakamura et al., 1997; Di Lazzaro et al., 1998). In normal subjects, arrival of these volleys is seen in the PSTH as a well synchronized peak (the primary peak), occurring at ~20 ms after the stimulus. This has been ascribed to impulse traffic through fast conducting, monosynaptic corticomotorneuronal projections (Day et al., 1987, 1989; Porter and Lemon, 1993; de Noordhout et al., 1999). The primary peak may have several subcomponents reflecting the sequential arrival of subsequent descending volleys (Kernell and Wu, 1967; Day et al., 1987, 1989; Mills, 1995). In normal subjects there are rarely more than three subcomponents to the primary peak and the total duration of the primary peak does not exceed 6 ms so that the interval between subcomponents is ~2 ms (Day et al., 1989; Mills, 1995; Eisen et al., 1996; Kohara et al., 1996). The onset of the primary peak usually corresponds closely to the onset of the surface evoked MEP (Day et al., 1989; Kohara et al., 1999).

**Slow conduction in non-hereditary ALS**

In sporadic ALS several different abnormalities of the primary peak have been reported (Awiszus and Feistner, 1993, 1995; Mills, 1995; Eisen et al., 1996; Kohara et al., 1996; Nakajima et al., 1997; Weber et al., 2000). The most frequent is desynchronization of the primary peak, characterized by an increased number of excess bins and prolonged duration. Frequently, as found in our non-hereditary ALS patients, two distinct peaks (‘a double peak’) are recognizable. The interval between the two peaks can be up to 17 ms (Kohara et al., 1996, 1999; Weber et al., 2000). The later peak often has a longer duration than the earlier one. Two peaks within the primary peak separated by such a long interval suggests that two subpopulations of corticomotorneuronal connections are stimulated: a fast conducting monosynaptic pathway originating from large Betz cells and a slow conducting pathway which may be mono- or polysynaptic (Mills, 1995; Kohara et al., 1996; Weber et al., 2000). The occurrence of double peaks may be relatively specific to ALS; they are rarely seen in other UMN diseases such as multiple sclerosis or cerebrovascular disease (Boniface et al., 1991; Kohara et al., 1996, 1999). With progression of ALS the earlier peak may be lost, resulting in profoundly delayed onset to the primary peak (>31 ms) (Weber et al., 2000).

None of the abnormalities described in the PSTH for ALS patients are seen in Kennedy’s disease or spinal muscular atrophy, which do not have a UMN component (Kohara et al., 1996, 1999; Weber and Eisen, 1999). It is concluded that the abnormalities of the primary peak seen for ALS patients are due to supraspinal mechanisms. Further support for this comes from the observation that in the same spinal motor neuron that produces an abnormal response to corticospinal stimulation in ALS patients, the response to Ia afferent input is normal (Awiszus and Feistner, 1995; Nakajima et al., 1996).

**Slow conduction in D90A patients**

In our D90A patients, the PSTHs were characterized by profoundly delayed, desynchronized primary peaks, with the additional finding of a marked suppression phase which preceded the primary peak (see Fig. 1B). A period of inhibition preceding the primary peak has not been recorded in our non-hereditary ALS patients. Even though the onset latency of the primary peak in D90A patients was slightly longer compared with the delayed primary peaks in non-hereditary ALS patients, the threshold required to activate the motor neurons was the same in both groups. This suggests that the same subpopulation of slow conducting corticomotorneuronal or corticospinal connections was being stimulated in D90A patients and non-hereditary ALS cases with delayed primary peaks. The slightly but significantly longer onset of delayed primary peaks in the D90A patients can be explained on the basis of the marked inhibitory phase preceding the primary peak in these patients.

It is also possible that the delayed primary peaks seen in non-hereditary ALS and D90A patients could result from the very late arrival of D and I waves caused by faulty initiation of descending volleys (Mills, 1995). However, such long intervals between subsequent descending volleys have not been described and normally even late I waves occur within 6 ms after the D wave (Day et al., 1989; Boniface et al., 1991; Nakamura et al., 1997).

The most plausible explanation for delayed primary peaks in ALS, including D90A patients, is activation of a slow conducting corticospinal pathway. These have been demonstrated in the cat (Takakashi et al., 1965) and monkey (Lemon et al., 1993). Recently, a slow conducting pathway could be stimulated in patients with idiopathic paraparesis using high intensity electrical motor cortex and brainstem stimulation (Ugawa and Kanazawa, 1999).

At low stimulus intensity, transcortical magnetic stimulation preferentially activates large, fast conducting pyramidal cells (Rossini, 1988). At higher stimulus intensities, as was required in the D90A patients and in the cases of non-hereditary ALS patients who had delayed primary peaks, the smaller, slow
Role of cortical inhibition

In sporadic ALS there is evidence to indicate that there is dysfunction or demise of modulating inhibitory interneurons resulting in diminished inhibition (Caramia et al., 1991; Eisen et al., 1993; Prout and Eisen, 1994; Disiato et al., 1997; Mills and Nithi, 1997). Protection of the central motor pathways and their connections to the anterior horn cells, as clinically evident in the D90A patients, might be secondary to sparing, or relative sparing, of the inhibitory interneurons in the D90A mutation. Various cortical and spinal inhibitory phenomena responsible for the suppression phase that normally follows the primary peak have been revealed through cortical magnetic stimulation (Di Lazzaro et al., 1998; Gerloff et al., 1998; Pauvert et al., 1998). The inhibitory activity preceding the delayed primary peak in the D90A mutation was not seen in non-hereditary ALS patients. The onset of this suppression phase seems to occur at the expected onset latency of a normal primary peak. This may suggest that corticospinal activity in D90A patients is normal but exerts an abnormal inhibitory effect on spinal motor neurons. However, the estimated onset of the suppression phase (sum of mean onset latency plus mean duration of the primary peak) was 22.8 ms in controls, which almost equals the onset of the prominent suppression phase (22.1 ms) preceding the delayed primary peaks in the D90A patients. This favours the concept that cortical and possibly spinal inhibitory mechanisms are relatively intact in this mutation and that the excitation mediated through the fast monosynaptic corticomotoneuronal pathway is lost.

We and others have proposed that ALS results from selective vulnerability of the most recently expressed genes to environmental factors (Kimura and Kaji, 1997). The cell bodies of the slower conducting axons are considerably smaller than the large cell bodies of the fast corticomotoneuronal fibres. The metabolic demands of the smaller neurons are less, making them more resistant to the complex biochemical series of events that result in the demise of the corticomotoneurons and anterior horn cells. It is reasonable to speculate that in cases with the D90A mutation, additional resistance has been conferred on the small diameter corticomotoneurons through the interaction of a protective modifier acting on the inhibitory interneurons.

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