Abnormal cortical brain integration of somatosensory afferents in ALS

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Abstract

Objectives. Infraclinical sensory alterations have been reported at early stages of amyotrophic lateral sclerosis (ALS). While previous studies mainly focused on early somatosensory evoked potentials (SEPs), late SEPs, which reflect on cortical pathways involved in cognitive-motor functions, are relatively underinvestigated. Early and late SEPs were compared to assess their alterations in ALS.

Methods. Median and ulnar nerves were electrically stimulated at the wrist, at 9 times the perceptual threshold, in 21 ALS patients without clinical evidence of sensory deficits, and 21 age- and gender-matched controls. SEPs were recorded at the Erb point using surface electrodes and using a needle inserted in the scalp, in front of the primary somatosensory area (with reference electrode on the ear lobe).

Results. Compared to controls, ALS patients showed comparable peripheral (N9) and early cortical component (N20, P25, N30) reductions, while the late cortical components (N60, P100) were more depressed than the early ones.

Conclusions. The peripheral sensory alteration likely contributed to late SEP depression to a lesser extent than that of early SEPs.

Significance. Late SEPs may provide new insights on abnormal cortical excitability affecting brain areas involved in cognitive-motor functions.

Highlights

- Subclinical peripheral sensory deficits may influence cortical excitability in ALS
- Sensory dysfunction may contribute to cortical excitability changes of motor and premotor areas
- Neural processing for cognitive-motor control may be intrinsically altered in ALS

Keywords

Somatosensory Evoked Potentials

Amyotrophic Lateral Sclerosis

Sensory impairment

Cortical excitability

Human

Abbreviations

ALS: amyotrophic lateral sclerosis

MT: motor threshold

MRI: magnetic resonance imaging

PT: perceptual threshold

S1: primary somatosensory area

S2: secondary somatosensory area

SD: standard deviation

SEP: somatosensory evoked potential

1. Introduction

Amyotrophic Lateral Sclerosis (ALS) is an adult-onset neurodegenerative condition characterized by the loss of motor neurons at cortical, brainstem and spinal levels (Kiernan *et al.*, 2011). ALS is widely considered as a pure motor degeneration; sensory impairment is not a recognised feature of ALS or regarded as secondary to motor impairment (Fincham and Van Allen, 1964; Feller *et al.*, 1966; Schulte-Mattler *et al.*, 1999). However, in addition to the 10 % patients describing frank paraesthesia and neuropathic pain, sensory impairments have been reported in up to 60 % patients, including abnormal vibration, cutaneous and heat thresholds. (Feller *et al.*, 1966; Dyck *et al.*, 1975; Kawamura *et al.*, 1981; Mulder *et al.*, 1983; Bosch *et al.*, 1985; Gregory *et al.*, 1993; Theys *et al.*, 1999; Hammad *et al.*, 2007; Isak *et al.*, 2016). Furthermore, several studies demonstrated a loss of large-caliber cutaneous afferents and dorsal column demyelination at lumbar level (Feller *et al.*, 1966; Dyck *et al.*, 1975; Tohgi *et al.*, 1977; Kawamura *et al.*, 1981; Bradley *et al.*, 1983; di Trapani *et al.*, 1986; Heads *et al.*, 1991; Shefner *et al.*, 1991; Hammad *et al.*, 2007; Vaughan *et al.*, 2015). Furthermore, post-mortem studies have also confirmed the degeneration of spinal ascending tracts (Swash *et al.*, 1988; Williams *et al.*, 1990; Takahashi *et al.*, 1992). In line with these data, multi-parametric spinal cord magnetic resonance imaging (MRI) has shown dorsal column alterations at the cervical level in ALS patients with limited motor deficits and no clinical signs of sensory impairment (Cohen-Adad *et al.*, 2013; Iglesias *et al.*, 2015), confirming that sensory alterations can manifest in parallel to motor impairment but to a lesser degree and with slower progression (Kawamura *et al.*, 1981; Mulder *et al.*, 1983; Cosi *et al.*, 1984; Zanette *et al.*, 1990; Heads *et al.*, 1991; Shefner *et al.*, 1991; Gregory *et al.*, 1993; Mondelli *et al.*, 1993; Theys *et al.*, 1999; Hammad *et al.*, 2007). Finally, recent studies using the superoxide dismutase 1 (SOD1) mouse model have reported disorganization of muscle spindles, primary and secondary sensory endings (group Ia and group II afferents) at early presymptomatic stages, and 50 % axonal loss in the dorsal roots at the late presymptomatic stage (Fischer *et al.*, 2005; Sábado *et al.*, 2014). These results further suggest that sensory impairment in ALS is unlikely to be merely a consequence of motor impairment.

Somatosensory evoked potentials (SEPs) by peripheral nerve stimulation are captured in electroencephalogram (EEG) and allow the quantitative assessment of the integrity of the somatosensory pathway. They are divided into three components, including i) the peripheral potential (N9) reflecting the afferent volley at the level of the plexus brachialis, ii) the early cortical SEPs (N20, P25, N30 and P35) whose activity sources are located in the primary somatosensory area (S1) and the motor cortex, and iii) the late cortical SEPs (P45, N60, P100

and N120) involving the secondary somatosensory area (S2) and associative areas (Giblin, 1964; Woolsey *et al.*, 1979; Drechsler, 1980; Shahani *et al.*, 1980, Small, 1980; Anziska and Cracco, 1983; Allison *et al.*, 1992; Mauguiere *et al.*, 1997, 1999; Urbano *et al.*, 1997; Torquati *et al.*, 2002; Mauguiere, 2005; Papadelis *et al.*, 2011; Aspell *et al.*, 2012; Saradjian *et al.*, 2013). Early SEPs mainly depend on peripheral inputs and are primarily involved in stimulus perception (intensity, location) (Halgren *et al.*, 1998; Torquati *et al.*, 2002; Lim *et al.*, 2012). Late SEPs have been less studied than early SEPs and consequently, much less characterized. However, it has been shown that late SEPs depend on the excitability of more complex cortico-subcortical networks, involved in higher-order functions (texture, shape recognition) and in cognitive and limbic projections through the insula, amygdala and hippocampus (Mauguiere *et al.*, 1997; Halgren *et al.*, 1998; Hari and Forss, 1999; Torquati *et al.*, 2002; Eickhoff *et al.*, 2006*a*, *b*; Papadelis *et al.*, 2011; Lim *et al.*, 2012). The evaluation of SEP in ALS gave rise to strikingly inconsistent results, due to differences in sample sizes, disease stages, the presence or absence of sensory impairment, and reference electrode placement (cephalic *vs*. extra-cephalic) (Bosch *et al.*, 1985; Facco *et al.*, 1989; Gregory *et al.*, 1993; Mondelli *et al.*, 1993; Theys *et al.*, 1999; de Carvalho and Swash, 2000). Abnormal early SEPs produced by upper limb nerve stimulation have been reported in 35 % patients (Matheson *et al.*, 1986; Radtke *et al.*, 1986; Constantinovici, 1993), and have been found delayed and/or reduced in amplitude (Pugdahl *et al.*, 2006; Hammad *et al.*, 2007). Moreover, altered amplitude has been reported at the latency of N60 without any associated modification of N9 or early cortical SEPs (Cosi *et al.*, 1984; Bosch *et al.*, 1985; Dasheiff *et al.*, 1985). This suggests that altered SEPs in ALS may result from sensory impairment and/or altered cortico-subcortical excitability, probably mediated by thalamus pathology, S1 or S2 atrophy at later stages of the disease (Smith, 1960; Kew *et al.*, 1994; Zanette *et al.*, 1996; Filippini *et al.*, 2010; Canu *et al.*, 2011; Li *et al.*, 2012; Bede *et al.*, 2013; Chapman *et al.*, 2014; Verstraete *et al.*, 2014; Devine *et al.*, 2015). Lastly, it is conceivable that the integrative properties of cortico-subcortical networks are altered in ALS, which leads to cortical excitability alterations, as reported during disease progression with incremental pyramidal cells and inhibitory interneurons pathology (Mills and Nithi, 1997; Eisen and Swash, 2001; Zanette *et al.*, 2002*a*, *b*; de Carvalho *et al.*, 2003; Mills, 2003; Vucic *et al.*, 2008; Floyd *et al.*, 2009; Menon *et al.*, 2015*a*, *b*).

The possible involvement of somatosensory alterations (deafferentation and altered integrative neural properties) is not commonly considered in the pathogenesis of ALS. We have recently reported a correlation between structural changes in ascending sensory

pathways at cervical level (using diffusion spinal imaging) and altered transmission of peripheral afferent inputs (depression of N9 and N20) in patients exhibiting weak distal motor dysfunctions without overt clinical signs of sensory impairment (Iglesias *et al.*, 2015). Furthermore, we identified altered transmission of muscle spindle sensory inputs to spinal motoneurons supplying clinically unaffected muscles, and we proposed that this could contribute to motoneuron hyperexcitability (Sangari *et al.*, 2016). In this present study, we have compared early and late SEPs induced by median and ulnar nerve stimulations in the same group of ALS patients in order to determine whether the changes observed at the cortical level are directly linked to the peripheral subclinical sensory defect and/or associated with perturbed cortico-subcortical integrative functions. Furthermore, given that early and late SEPs likely involve motor and non-motor areas, we wondered whether signals generated at these levels were altered to the same extent by comparing early and late SEPs. Late SEPs are considerably less investigated in ALS than early SEPs.

2. Materials & Methods

2.1. Ethics and participants

The study was performed in accordance with the Declaration of Helsinki, and has been approved by the ethics committee: Ile-de-France VI (Pitié-salpêtrière). All participants gave written consent. The data were collected from the same groups of subjects as described by Iglesias *et al*., 2015 and Sangari *et al*., 2016: 21 patients (1 familial case; 16 males; age 56.3 ± 2.1, 37-76 y.o.; see Table 1) and 21 controls (16 males; age 56.6 ± 2.1, 33-73 y.o.). The mean duration of clinical motor signs was 26.6 ± 3.6 months and 20/21 patients took riluzole (100 mg/day) and α-tocopherol (1000 mg/ day). The inclusion criteria were: 1) 'probable' or 'definite' ALS according to the El Escorial criteria, 2) weakness in hand muscles at least on one side, 3) absence of peripheral neuropathy and 4) no clinical signs of sensory deficits. Patients were investigated on the more affected side or on the dominant side when both sides were equally affected. Controls were stimulated on their dominant side (Oldfield, 1971). Table 1 shows ALSFRS-r scores reflecting the global functional disability, the ALSFRS-r sub-scores for hand functions (writing and feeding), and force measured using the manual muscle testing (MMT) for thumb abduction and distal phalanx flexion of finger III).

Insert Table 1

2.2. SEPs recordings

Percutaneous electrical stimulations were applied to median and ulnar nerves at the wrist level (bipolar electrodes, 0.5 cm^2, 1-cm apart; 1-ms rectangular electrical stimulation; DS7A, Digitimer Ltd, Hertfordshire, UK). The resulting afferent volley was recorded using surface electrodes placed in the supraclavicular fosses (Erb point): one ipsilateral to the stimulations and the other one, on the other side (reference). The brain signal was recorded using a needle electrode implanted in the skin, face to S1 area contralateral to peripheral nerve stimulations (4-cm lateral and 2-cm posterior to vertex point), and two reference electrodes were placed on each ear lobe. Signal capture was limited to 30-3000 Hz-bandwidths, and amplification was 10 000 (D360 8-Channel Patient Amplifier, Digitimer Ltd, Hertfordshire, UK). Peripheral and brain signals were digitalized (2-kHz sampling rate) for subsequent analysis (Power 1401 and Signal Software, CED, Cambridge, UK). The experiment started by evaluating the perceptual thresholds (PT) for both peripheral nerve stimulations, and conditioning stimulus intensity was fixed at 9 x PT for recordings. A total number of 600 stimuli were delivered to both nerves, distributed over 3 recording sequences; one recording sequence included 200 stimuli applied to median nerve and 200 to ulnar nerve, delivered randomly at 2 Hz.

2.3. Data analysis

Mean EEG signal from each participant was normalized to twice the standard deviation (2 x SD) calculated over a 100-ms period of the prestimulus activity, excluding stimulus artifacts (see Iglesias *et al.*, 2015). Peaks and onsets latencies of each potential were evaluated for each subject. Then, EEG signal was rectified and SEP area was measured to better appreciate their modulation. Area of each potential was estimated using a fixed analysis window determined according to the mean potential latency and duration estimated in each subject; the analysis window was similar for ALS and controls (see results). In order to further compare the changes in SEP area in ALS, for the different component (ALS patient area reduction), we calculated the mean area of each SEP component (N9, N20, P25, N30, N60 and P100) in controls ($\overline{controls}$) and the difference from this 'reference value' was calculated in each patient, and for each component. Then, the difference was expressed as a percentage of mean N9 ($\overline{N9}$) and of mean N20 ($\overline{N20}$) area calculated in controls, for peripheral (N9) and cortical SEPs, respectively. The level of SEP depression in ALS was thus calculated according to this equation:

$$\text{ALS patient area reduction} = \frac{\text{Mean } (\overline{\text{controls}} - \text{patient})}{\overline{\text{N9}} \text{ or } \overline{\text{N20}} \text{ controls}} \times 100$$

Analysis focused on N9, N20, P25, N30, N60 and P100 components, excluding P35 and N120 because their origin has not been characterized (Small, 1980; Anziska and Cracco, 1983; Bosch *et al.*, 1985; Urbano *et al.*, 1997; Hari and Forss, 1999; Mauguiere *et al.*, 1999; Hoshiyama and Kakigi, 2001; Mauguiere, 2005; Papadelis *et al.*, 2011).

2.4. Statistical analysis

Data distribution and variances were checked for parametric testing; alternatively, non-parametric tests were used. First, the level of EEG pre-stimulus activity was compared between controls and ALS patients with unpaired *t* test. Then, two-way ANOVA was performed to compare the onset and peak latencies of early and late SEPs between patients and controls (SEPs x groups). Subsequently, the SEP size between groups (controls *vs*. ALS) and the level of SEP depression in patients were compared using Kruskall-Wallis *H* test, and *post-hoc* Tukey test was used for pairwise comparisons. Pearson product moment correlation was performed to determine whether changes in SEP size were correlated. Finally, multivariate analyses were performed to investigate the change in SEPs according to motor dysfunctions. A probability (*P*) value of < 0.05 was considered statistically significant. Results were expressed as mean ± standard error of the mean (SEM).

3. Results

Figure 1 illustrates the averaged waveform recorded at the Erb point and over S1 area (normalized to 2 x SD prestimulus activity) in 2 individuals. The mean waveforms (N = 600 stimuli), conditioned by median nerve stimulation, in one control (dark line) and in one ALS patient (grey line; Fig. 1A) show that the size of N9 and of cortical SEPs were reduced in the patient, compared to the control. The grand average pictured in Figure 1B shows similar results in the group of ALS patients, compared to the group of controls. All potentials were reduced in the patient group compared to the control group, regardless of the conditioning stimuli (median or ulnar nerve stimulation).

Insert Figure 1

Table 2 shows the level of prestimulus activity and the latency of each potential observed in controls (dark) and in ALS patients (grey) for the median and ulnar nerves. No difference in the level of prestimulus activity (in μV ± SEM) was observed between groups,

in either nerve (Table 2; unpaired *t* test, Erb point: $0.07 < P < 0.08$; S1 area: $0.09 < P < 0.12$). The onset and peak latencies (in ms ± SEM) for each component were not significantly different between groups, in either nerve (Table 2; two-way ANOVA, group (ALS vs. controls): $0.09 < P < 0.98$; SEPs (N9 vs. N20 vs. P25 vs. N30 vs. N60 vs. P100: $P < 0.001$; interaction (group x SEP): $0.52 < P < 0.96$). The SEP modulation was not associated with changes in background EEG activity and component latency. Moreover, conduction time through the somatosensory pathway was found to be unaltered (see Iglesias *et al.*, 2015).

Insert Table 2

Since we did not observe any difference in peak onset and duration for each SEP component between the study groups, the area of each potential was estimated using a fixed analysis window. Box plot charts in Figure 2 represent the data distribution for potential area (normalized to 2 x SD prestimulus activity) in controls (dark) and in ALS patients (grey) for median and ulnar nerves. N9 and cortical SEP mean areas were found significantly reduced in the patient group compared to controls (Kruskal-Wallis, $P < 0.001$ for median and ulnar nerves). In controls, we observed that SEP area increased gradually from N9 to early cortical potentials (N20, P25, N30) while late cortical potentials (N60, P100) exhibited a significant increment. Moreover, we noted that in ALS patients late cortical potentials (N60, P100) exhibited a more significant reduction compared to N9 and early cortical potentials. These findings suggest that all SEP components were altered in ALS patients, and it seems that late SEPs (N60 and P100) were particularly reduced compared to N9, N20, P25 and N30.

Insert Figure 2

To investigate this notion in more detail, the level of SEP change observed in each patient has been estimated for each component and each nerve, by using the mean results in control group as reference values (see equation in Methods; Fig. 3). The difference from control data, for N9 and cortical SEPs in patient, was then expressed as a percentage of mean N9 (dark) or mean N20 (grey) in controls, respectively. Figure 3 shows that depression in patients was significantly different between components, for each nerve (Kruskal-Wallis, $P < 0.001$ and < 0.01 for median and ulnar nerves, respectively). *Post-hoc* comparisons revealed that, in each nerve, N9 and early cortical SEPs (N25, P30) were similarly reduced as N20 (Tukey, $P > 0.05$), while late SEPs (N60, P100) were significantly more reduced than N20 (Tukey, $P < 0.05$). Thus, the depression of early cortical SEPs was likely linked to that of peripheral afferent volley (N9). Conversely, the depression of late cortical SEPs seemed less dependent of peripheral afferent volley reductions.

Insert Figure 3

In addition, we tested whether the level of depression was correlated between each component. The results were similar for both nerves. The correlation coefficient was not significant when testing the possible correlation between the reduction size of N9 and that of cortical SEPs (Pearson product moment correlation; -0.05 < correlation coefficient < 0.28, 0.06 < P < 0.9). On the contrary, the depression of N20 area was correlated to that of P25 and N30 (0.4 < correlation coefficient < 0.6, 0.001< P < 0.01) but not to that of N60 and P100 (0.3 < correlation coefficient < 0.4, 0.08 < P < 0.1). Finally, the depression of P25, N30, N60 and P100 also were correlated: 0.5 < correlation coefficient < 0.8 (P < 0.01). We have observed smaller correlation coefficients between P25, N60 and P100, than between P25 and N30. Overall, we did not observe any correlation between the changes in peripheral and cortical SEPs, but we found the depression of cortical SEPs with a latency > 25 ms correlated.

Additionally, we have also explored the relationship between the level of SEP change observed in patients and their hand-related motor disability. For this, we used the ALSFRS-r sub-score for hand functions, including writing and feeding (functional score). Moreover, we have added the MMT scores for thumb abduction and distal phalanx flexion of finger III (Table 1). We performed multivariate analyses taking into account the following factors: nerve, SEP latency, hand functional (ALSFR-S sub-score) and MMT scores. We did not find any significant difference between nerves, whose results were thus mixed in the following analysis. Furthermore, the force level and the functional score were moderately correlated (correlation coefficient = 0.5, P < 0.05). The factor 'latency' had a significant influence (P < 0.01) and the link with motor deficits was quite different according to the clinical scale used. Indeed, the functional score for hand movements (ALSFRS-r sub-score) did not have significant influence and the interaction with the factor 'latency' was not significant either. On the contrary, the muscle strength had no significant influence but the interaction with the factor 'latency' was significant (0.01 < P < 0.05). These results further confirm that the change in SEP area in patients was different according to the SEP latency, and this was particularly related to the depression of hand force but not to the alteration of hand functions. Therefore, to further investigate the relationships with alteration of functional tasks, we performed the same multivariate analyses taking the full score to ALSFRS-r, and we found a significant influence of this score (P < 0.05) and a significant interaction with SEP latencies (ALSFR-S x latency, P < 0.001). Figure 4 illustrates the change in SEP area according to the force level (A), the functional clinical score for hand movements (B) or the total ALSFRS-r

score (C). The modification of the different SEP components (early SEPs represented by the grey symbols, and late SEP, by the black ones) overlapped whatever the hand functional score (Fig. 4B), while late SEPs were more depressed than early ones when the force level was depressed (compare score 5-6 to 8-9; Fig. 4A) or with alteration of day life functions (ALSFR-S < 38-40; Fig. 4C).

Insert Figure 4

4. Discussion

The present study brings new evidence for late cortical SEP alterations induced by median and ulnar nerves stimulation in ALS. Indeed, N60 and P100 were more depressed than the peripheral component N9 and early cortical responses such as N20, P25 and N30. Moreover, the degree of depression correlated only for SEPs with latencies above 25 ms. Finally, the SEP alteration was linked to the depression of hand muscle force and global alteration of day life functions, especially for late SEPs, but not to specific alteration of hand functions.

4.1. SEP alteration in ALS

SEP investigation performed in ALS to date mainly focused on early components such as N9, N20 and P25 and by performing median nerve stimulation only. These studies have revealed abnormalities in 35 % patients (Matheson *et al.*, 1986; Radtke *et al.*, 1986; Constantinovici, 1993). The reported alterations included a delayed peak latency with reduction of central and peripheral conduction velocity for 40 % and 20 % patients, respectively, and reduced amplitude for 20 % patients (Anziska and Cracco, 1983; Cosi *et al.*, 1984; Dasheiff *et al.*, 1985; Matheson *et al.*, 1986; Subramaniam and Yiannikas, 1990; Zanette *et al.*, 1990; Shefner *et al.*, 1991; Gregory *et al.*, 1993; Georgesco *et al.*, 1994; Pugdahl *et al.*, 2006; Hammad *et al.*, 2007). In this present study, we did not find any difference in peak onset and latency between groups, reflecting a normal conduction velocity through large-diameter fibres and normal mean conduction velocity, respectively (Parain and Delapierre, 1991; Tanosaki *et al.*, 1999; Shiga *et al.*, 2001). However, we confirm evidence of consistently reduced SEPs, especially the late ones that have been understudied in ALS to date. Moreover, we showed that SEPs were altered for both median and ulnar nerves, the later seldom tested when studying SEPs. The differences compared to previous studies, in terms of proportion of patients with altered SEPs (60 % in the present group, reaching 80 % when

coupling with spinal diffusion MRI; Iglesias *et al*., 2015), could be explained by i) the extra-cephalic reference (ear lobe) we used, which is free from EEG distortions (Stephenson and Gibbs, 1951; Facco *et al.*, 1989; Essl and Rappelsberger, 1998; Hu *et al.*, 2012), and ii), EEG normalization (to 2 x SD pre-stimulus activity), which avoids any bias due to recording conditions that is known to influence the inter- and intra-individual comparisons of electrophysiological recordings (Iglesias *et al.*, 2015). Moreover, our recordings indicate that the cortical background activity was similar in both groups, which may have also influenced the results of previous studies (Arieli *et al.*, 1996; Klistorner and Graham, 2001; You *et al.*, 2012). As stated in Iglesias *et al.*, 2015, it appears that SEP alteration in ALS has been underestimated.

4.2. Neural origins of early and late SEPs

N9 reflects the stimulation-induced afferent volley captured at the level of the plexus brachialis (Small, 1980; Anziska and Cracco, 1983; Mauguiere *et al.*, 1999; Mauguiere, 2005). As shown previously in the same group of patients (Iglesias *et al.*, 2015), N9 reductions were associated with sensory fibre alterations, likely proprioceptive in origin. Indeed, the depression of N9 was correlated to MRI diffusion changes in the dorsal columns at the cervical level.

N20 results from the activation of thalamo-cortical projections reaching S1 area. Then, S1 neural networks project through thalamus and parietal posterior area (associative area) to motor and premotor areas to generate P25, and onto the primary and supplementary motor areas to produce N30 (Small, 1980; Anziska and Cracco, 1983; Bosch *et al.*, 1985; Urbano *et al.*, 1997; Mauguiere *et al.*, 1999; Hoshiyama and Kakigi, 2001; Mauguiere, 2005; Papadelis *et al.*, 2011). Given that the depression of N9 and early cortical SEPs (N20, P25 and N30) were similar, we assumed that alterations of early cortical components were primarily driven by impaired peripheral sensory afferents. However, given that inputs/outputs relationships reach a plateau at 2 x MT (Lesser *et al.*, 1979; Huttunen, 1995; Torquati *et al.*, 2002), N20 was likely less sensitive than N9 to the reduction of afferent volley produced at 9 x PT (corresponding to an intensity a bit higher than 2 x MT, approximately). This suggests that the reduction of early cortical SEPs may involve supplementary alteration, other than the impairment of sensory inputs only. According to the correlation we found between SEP alterations, this might be particularly true for SEPs with latency above 25 ms, which makes sense given the alteration of motor excitability in ALS (see below). Moreover, atrophy of thalamus, S1 area, as well as motor cortex have been consistently reported in ALS (Smith,

1960; Nihei *et al.*, 1993; Kew *et al.*, 1994; Zanette *et al.*, 1996; Graham *et al.*, 2004; Thivard *et al.*, 2007; Filippini *et al.*, 2010; Verstraete *et al.*, 2010, 2014; Canu *et al.*, 2011; Mochizuki *et al.*, 2011; Chapman *et al.*, 2012; Li *et al.*, 2012; Bede *et al.*, 2013; Chiò *et al.*, 2014; Devine *et al.*, 2015). Furthermore, P25 alteration observed in our study could be associated with motor cortex pathology, which is supported in this patient group by their higher threshold to transcranial magnetic stimulation compared to controls (Sangari *et al.*, 2016). All of these observations suggest that the reduction of cortical SEPs, likely those with latency above 25 ms, may not only result from the loss of peripheral sensory afferents. Indeed, part of the depression was likely due to impaired neural processing of sensory inputs at the cortical and subcortical levels.

Late SEPs include N60 generated in S2 area and P100 produced in central and temporal cortex (Bosch *et al.*, 1985; Mauguiere, 2005). The depression of late SEPs in ALS was stronger compared to that of N9 and early cortical SEPs. The stimulus intensity we used for conditioning was 1.5-2 x above the one producing the maximal size of late SEPs (plateau level), according to the inputs/outputs relationships (Lesser *et al.*, 1979; Huttunen, 1995; Torquati *et al.*, 2002). Therefore, similarly to early cortical components, the greater depression of late SEPs was likely not only related to the impaired sensory afferent inputs and S2 area atrophy (Smith, 1960; Filippini *et al.*, 2010; Verstraete *et al.*, 2014). Late SEPs are involved in higher-order sensorimotor and cognitive functions through insula, and in limbic function through amygdala and hippocampus (Mauguiere *et al.*, 1997; Halgren *et al.*, 1998; Hari and Forss, 1999; Torquati *et al.*, 2002; Eickhoff *et al.*, 2006*a*, *b*; Papadelis *et al.*, 2011; Lim *et al.*, 2012). The limbic system is known to influence autonomy and sensorimotor functions (Gloor, 1975; Baltadzhieva *et al.*, 2005). Besides, hippocampus atrophy has been reported in ALS (Takeda *et al.*, 2009; Bede *et al.*, 2013; Westeneng *et al.*, 2015). Moreover, results of neuropsychological tests revealed cognitive impairment in more than 50 % patients (Charles and Swash, 2001; Kiernan *et al.*, 2011; Bede *et al.*, 2013; Ravits, 2014; Vucic *et al.*, 2014; Murphy *et al.*, 2016). Thus, S2 area impairment could manifest in higher-order sensorimotor and cognitive functions alteration. While acknowledging that altered peripheral inputs may impact on the size of late SEPs, we propose that late SEP reductions primarily derive from extra-motor and cortico-subcortical circuitry dysfunction in ALS.

4.3. Cortico-subcortical alterations

We found that the depression of SEP area in ALS was similar for N9, reflecting the stimulation-induced peripheral afferent volley, and for the early components of cortical SEPs

(N20, P25, N30) but we found it stronger for the late components (N60, P100). These results suggest an abnormal relationship between peripheral inputs and cortical outputs. The association between stimulus intensities, *i.e*. the level of sensory inputs, and the size of SEPs have been previously studied in healthy subjects for SEPs using median nerve stimulation. These studies focused on N9 and N20 only or on both early and late SEPs but without discriminating these components (Giblin, 1964; Lesser *et al.*, 1979; Gandevia and Burke, 1984; Huttunen, 1995; Torquati *et al.*, 2002). Moreover, a variety of stimulus intensities were utilised, expressed in volts, amps or other composite measures of perceptual and motor thresholds (MT)**,** making it difficult to compare modulation of SEPs according to the stimulus intensity. However, the results can be synthesised as follows: i) N9 increases linearly with the stimulus intensity due to the progressive intensity-related increased of peripheral afferent volley; and ii) the inputs/outputs relationships for N20 and late SEPs reach a plateau level at about 2 and 1.5 x MT, respectively, *i.e*. at lower stimulus intensities compared to N9. It has been proposed that this result was likely due to a differential recruitment of peripheral fibres and/or an occlusive phenomenon occurring at the central level, in the sub-cortical nuclei relaying the sensory inputs to the cerebral cortex (Lesser *et al.*, 1979; Gandevia and Burke, 1984; Parain and Delapierre, 1991; Huttunen, 1995; Torquati *et al.*, 2002; Lim *et al.*, 2012). Moreover, a loss of N60 has been reported with a relative conservation of N9 potential and early cortical potentials in ALS (Cosi et al., 1984; Bosch et al., 1985; Dasheiff et al., 1985). In this present study, we have identified a correlation between the levels of depression only for SEPs with latency above 25 ms. All these results suggest that the sensory inputs likely have a smaller impact on late cortical components, whose size may thus mainly depend on the activity of cortico-subcortical networks underlying the late cortical responses to sensory inputs. The exact relationship between late SEPs and peripheral inputs remains to be established. Further studies are required using combined electrophysiology and neuroimaging to fully characterise the specific contribution of sensory inputs and cortico-subcortical networks to late cortical responses.

4.4. Cortical excitability in ALS and link with motor dysfunctions

Alteration of cortical excitability in ALS has been mainly studied in motor cortex using transcranial magnetic stimulation. It is now well established that cortical excitability impacts on motor thresholds and intra-cortical inhibition (Mills and Nithi, 1997; Eisen and Swash, 2001; Zanette *et al.*, 2002*a*, *b*; de Carvalho *et al.*, 2003; Mills, 2003; Vucic *et al.*, 2008; Floyd *et al.*, 2009; Menon *et al.*, 2015*a*, *b*). It has been reported that resting motor

threshold was unaltered, increased or decreased, associated with an unchanged, reduced or reinforced intra-cortical inhibition, respectively. These results led to the notion that cortical excitability evolves from hyper- to hypoexcitability during the progression from the presymptomatic to the symptomatic stage (Eisen *et al.*, 1992, 1996, 1998; Yokota *et al.*, 1996; Ziemann *et al.*, 1997; Weber and Eisen, 2000; Weber *et al.*, 2000; Stewart *et al.*, 2006; Wittstock *et al.*, 2007; Schmied and Attarian, 2008; Attarian *et al.*, 2009; Vucic *et al.*, 2009, 2013; Bae *et al.*, 2014). Moreover, in addition to structural abnormalities affecting the motor cortex, fMRI investigations in ALS patients have revealed functional connectivity impairments through areas of enhanced activation and enhanced connectivity (Douaud *et al.*, 2011; Prell and Grosskreutz, 2013; Chiò *et al.*, 2014; Schmidt *et al.*, 2014; Shen *et al.*, 2015). In particular, both cerebral activity and connectivity have been found to increase in motor and premotor areas, likely due to depressed inhibitory cortical influences (Lloyd *et al.*, 2000; Maekawa, 2004; Turner, 2005; Watanabe *et al.*, 2009; Verstraete *et al.*, 2010; Douaud *et al.*, 2011; Luo *et al.*, 2012; Prell and Grosskreutz, 2013; Shen *et al.*, 2015). Taken together, all of these studies strongly support the concept of depressed synaptic inhibition and excitability changes at the brain level, and motor cortex in particular. The present study brings new evidence for specific alteration of late SEPs, mediated by S2 and associative areas (cf. supra), which suggests that alterations of connectivity, excitability and integrative properties extend to non-motor areas in ALS, as suggested by Mochizuki *et al.*, 2011. The alterations of extra-motor areas responsible for higher-order sensori-motor control are likely to be closely linked to motor dysfunction. Indeed, the patients we investigated exhibited mild motor defects that were particularly associated with the alteration of late SEPs: the depression of late SEPs was found to be more constant (and stronger) than early ones, in patients with a more severe hand weakness and/or the lowest global scores to ALSFRS-r, while the change in early SEPs was more uniform whatever the clinical evaluation. Interestingly, the link disappeared when focusing the analysis on specific hand tasks (writing and feeding). These results suggest that the alteration of higher-order cortical pathways involved in cognitive-motor functions (motor programing including prediction of peripheral biofeedback) are not specific to hand muscles, and affect all motor regions implicated in ALS.

5. Conclusions

Sensory impairment and altered neural processing in motor and extra-motor areas are likely contributors to ALS pathogenesis. We have demonstrated that alteration of SEPs may also result from an impairment of cortico-subcortical network, involving extra-motor cortical

network. Moreover, sensory deficits could lead to cortical excitability changes through impaired cortico-subcortical integrative properties, similarly those reported at spinal level (van Zundert *et al.*, 2008; Jiang *et al.*, 2009; Martin and Chang, 2012; Sangari *et al.*, 2016). Thus, ALS should not be considered a pure motor disease, but a complex sensory-motor disease. However, future investigations including neuroimaging are required to further explore the relationship between sensory inputs and cortico-subcortical activities, taking into account the anatomical characteristics of brain structures (underlying the early and late SEPs) and the longitudinal evolution of motor dysfunction.

Figure legends

Figure 1: Somatosensory evoked potentials.

A) The mean signal (N = 600 frames) is expressed as a % 2 x SD the prestimulus activity, and plotted against the latency (ms) after the triggering of median nerve stimulation at wrist level (9 x PT). On the left side, the signal was collected at the Erb point and on the right side, over S1 area (EEG signal, contralateral to the stimulation site). The dark lines illustrate the signals from one control subject and the grey lines, the signals recorded in one ALS patient; the signals from the same control subject and the same patient are illustrated for the Erb point and S1 recordings. The SEPs are indicated on the traces: N9 is indicated on the signal from the Erb point (left side), and the cortical components N20, P25, N30, N60, and P100, are reported on the mean EEG signal (right side). B) Similar recordings as in A were performed in all the 21 patients and the 21 controls, for both median and ulnar nerves. The traces illustrated in B represent the grand average of signals collected in the group of ALS patients (grey line) and in controls (dark line), at the Erb point (left side) or face to S1 area (right side), after median nerve stimulation (upper figurines) and after ulnar nerve stimulation (lower figurines).

Figure 2: Somatosensory evoked potentials area.

Box plot charts showing the data distribution for SEPs area (% 2 x SD prestimulus) in controls (dark) and in ALS patients (grey): the top and bottom line of the box correspond to the 95 % confidence interval and the line in the box correspond to the median. The two bars extend from the maximum and minimum value. The cross within the box indicates the arithmetical mean which are significantly different between groups for each nerve ($p < 0.001$).

Figure 3: Somatosensory evoked potentials area reduction in ALS patients.

Changes in SEP area in ALS patients for the different component evoked from median and ulnar nerves stimulation are expressed in percentage of mean N9 (dark) or N20 (grey) area from controls group. * $p < 0.05$.

Figure 4: Relationships between SEP alteration and motor dysfunctions.
Changes in SEP area in ALS patients (as in Fig. 3) are plotted against the score to clinical evaluation of motor functions including the hand muscle force (muscular testing; A), the ALFR-S sub-score for hand tasks (B), and ALSFR-S total score (C); the lower the score, the stronger the motor dysfunctions.

Table 1: Clinical data
ALSFR-S sub-score for hand functions (/8): sub-score of the revised ALS functional resting scale for hand functions, including writing and feeding with maximal score 8 (4 for each item); ALSFR-S: total score of ALSFR-S (maximal score 48); Hand muscle force (/10): manual muscular testing (MMT) including thumb abduction and distal phalanx flexion of finger III with maximal score 10 (5 for each muscle).

Table 2: Somatosensory evoked potentials latencies and EEG prestimulus activity.
Mean onset and peak latencies (in ms ± SEM) of each SEPs and mean EEG prestimulus activity (in µV ± SEM) recorded at Erb point and S1 area are reported in controls (dark) and patients (grey) group for median and ulnar nerves.

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Table 1

Patient	ALSFR-S sub-score for hand functions (/8)	ALSFR-S	Hand muscle force (/10)
1	4	42	8
2	4	42	5
3	8	41	8
4	6	34	7
5	6	43	9
6	5	39	8
7	8	39	8
8	8	40	9
9	8	34	8
10	3	37	6
11	3	31	8
12	4	30	6
13	6	41	6
14	7	45	8
15	7	46	8
16	7	44	9
17	6	40	7
18	5	33	8
19	6	40	8
20	6	45	8
21	6	40	8

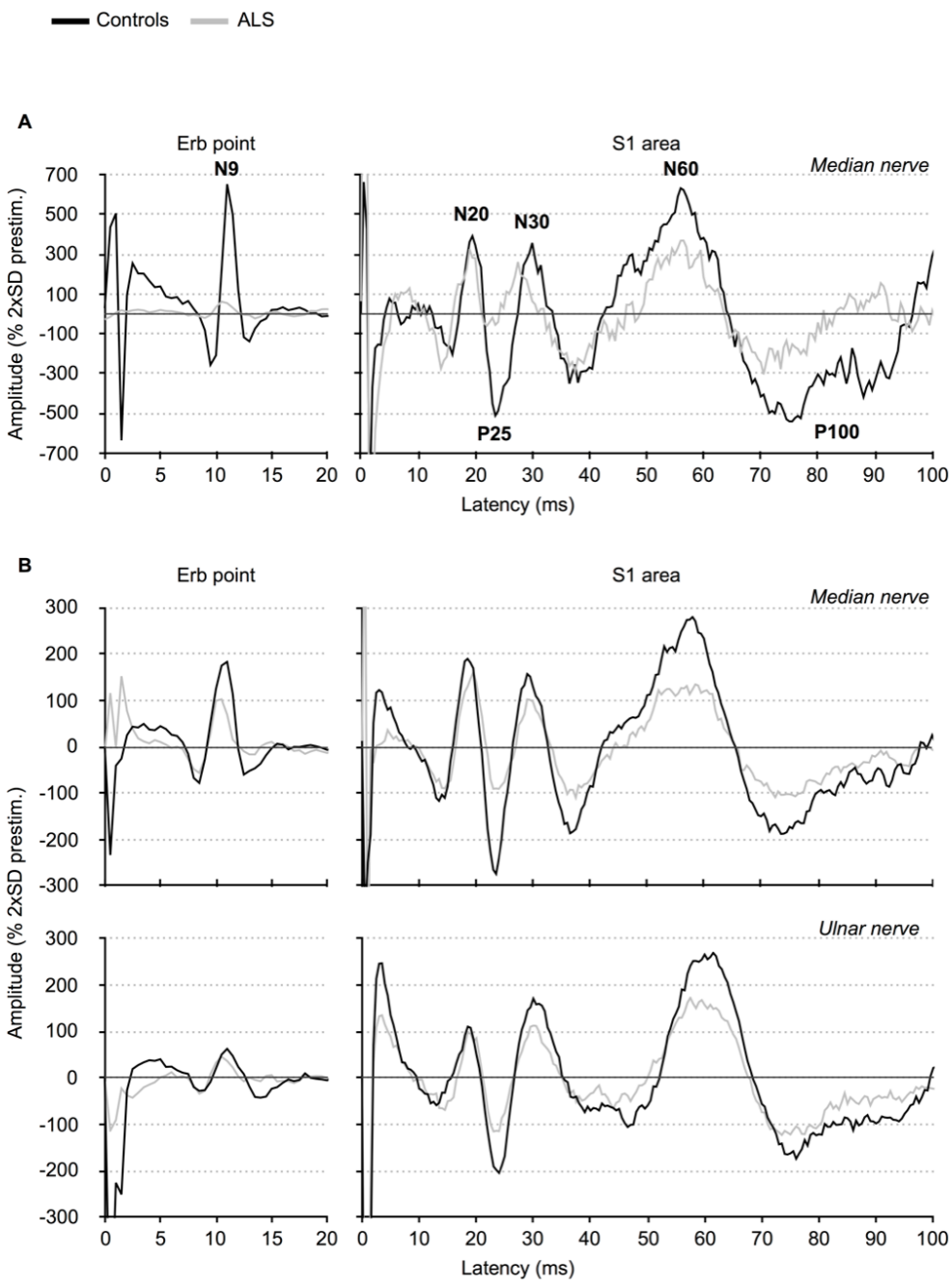
Table 2

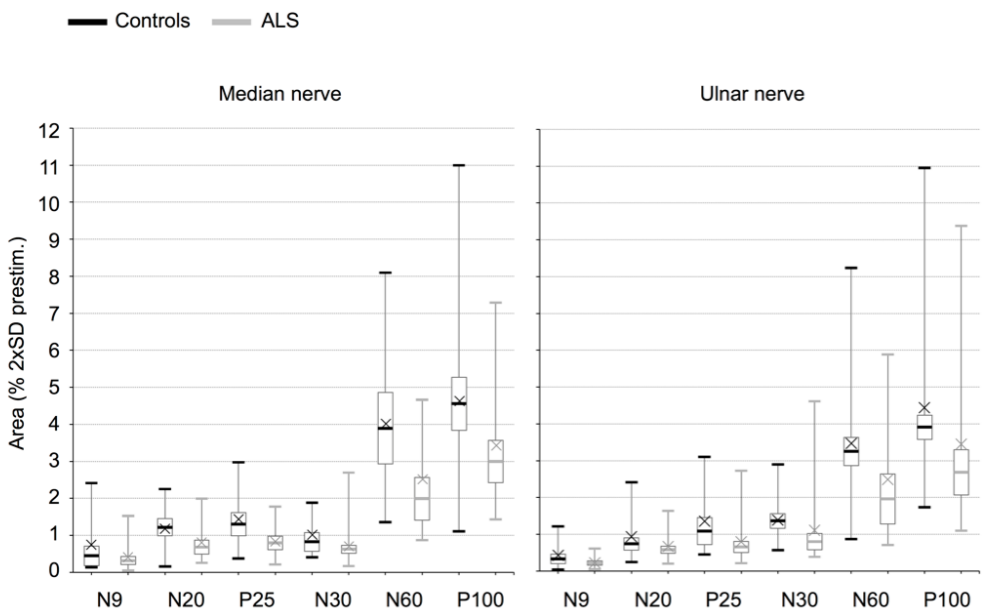
Latency (ms)

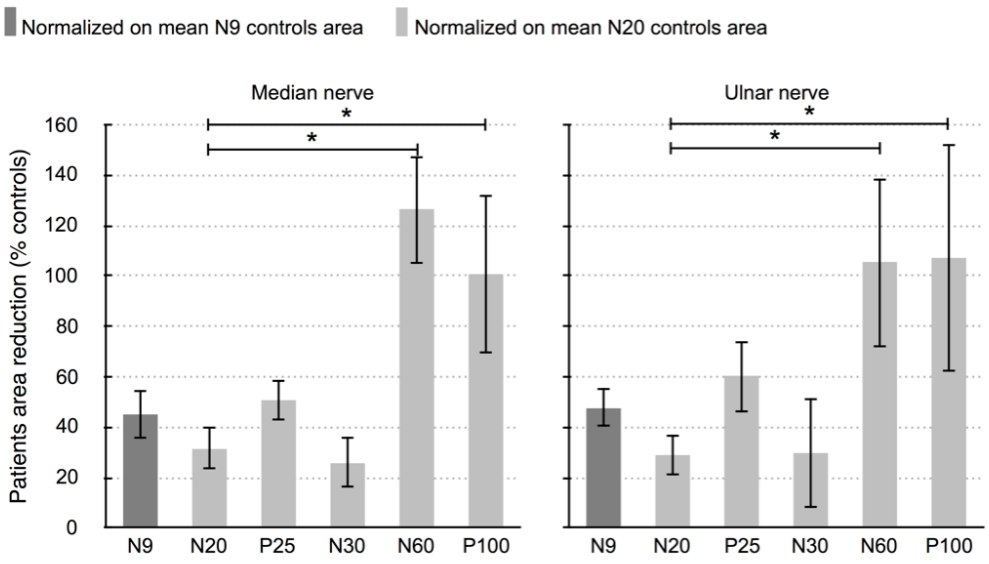
	Median nerve		Ulnar nerve	
	onset	peak	onset	peak
N9	9.1±0.2 9.2±0.2	10.7±0.2 10.7±0.2	9.6±0.2 9.7±0.2	11.2±0.2 11.0±0.2
N9 end	12.4±0.2 12.7±0.3		12.9±0.3 12.9±0.3	
N20	16.0±0.2 16.4±0.2	19.0±0.4 19.4±0.3	16.2±0.3 16.7±0.3	18.7±0.4 19.1±0.4
P25	21.2±0.3 21.9±0.3	23.5±0.4 24.0±0.4	21.2±0.4 22.2±0.4	23.5±0.4 23.9±0.5
N30	26.6±0.4 27.2±0.6	29.1±0.4 30.2±0.5	27.0±0.5 27.5±0.5	30.1±0.6 30.6±0.7
N30 end	33.0±0.5 33.3±0.4		35.7±0.7 35.1±0.7	
N60	43.8±0.9 46.3±1.2	55.0±1.2 55.7±1.1	51.8±07 50.0±1.2	60.8±0.9 60.0±1.0
P100	65.7±0.9 65.5±1.0	76.3±1.9 79.0±1.8	68.7±0.8 67.9±0.6	78.3±1.7 77.9±1.3
P100 end	100.7±1.2 99.8±1.5	113.5±2.8 111.4±1.9	102.3±1.4 103.3±1.5	113.1±1.8 117.7±2.0

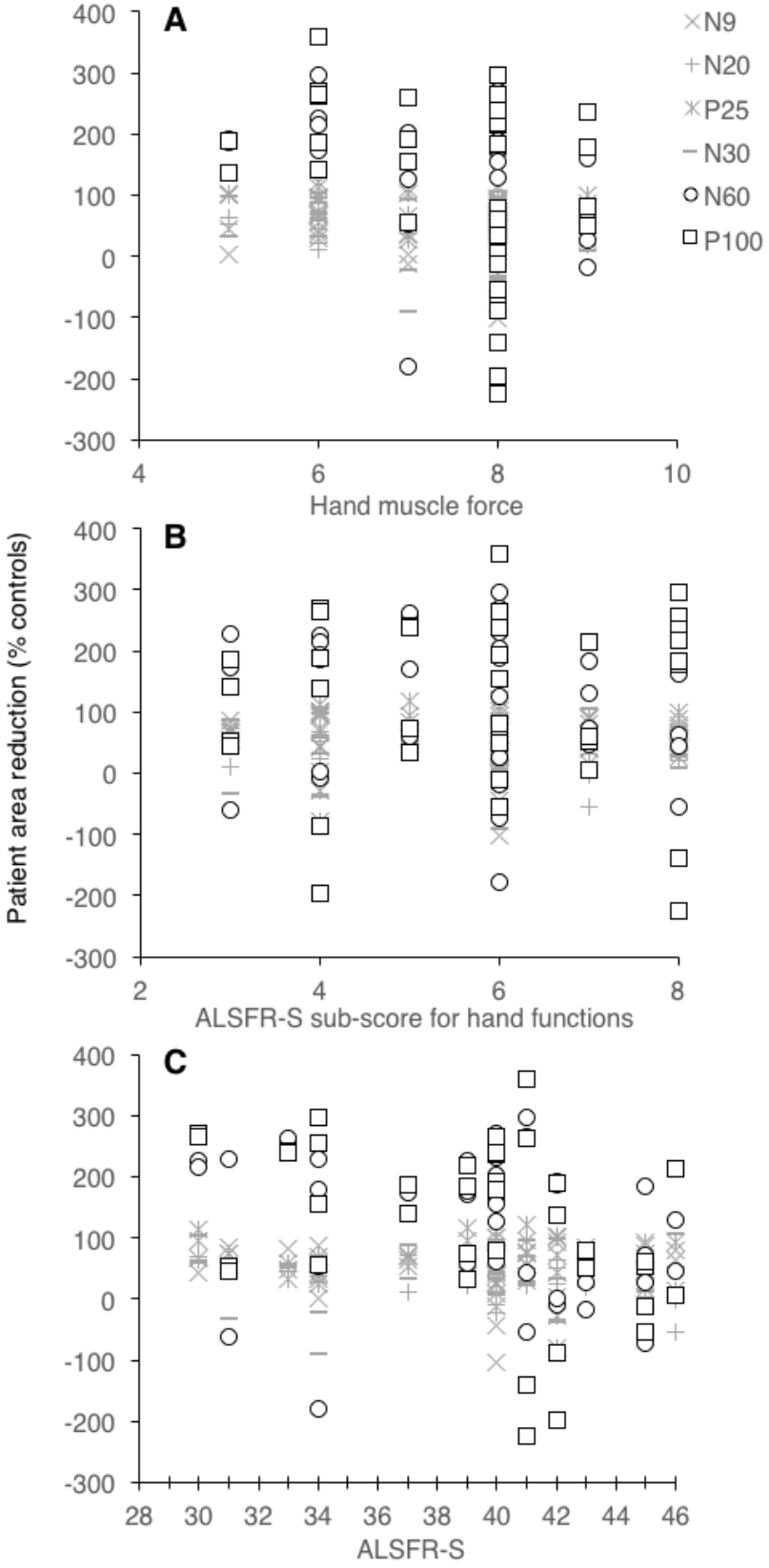
Prestimulus activity (μV)

Erb point	1.3±0.2 2.1±0.4	1.2±0.2 2.5±0.7
S1 area	0.4±0.03 0.5±0.05	0.4±0.03 0.5±0.04


Controls
ALS
A
Erb point
S1 area
Median nerve
N9
N20
N30
N60
P25
P100
Amplitude (% 2xSD prestim.)
Latency (ms)
B
Erb point
S1 area
Median nerve
Ulnar nerve
Amplitude (% 2xSD prestim.)
Latency (ms)


Controls
ALS
Median nerve
Ulnar nerve
Area (% 2xSD prestim.)
0
1
2
3
4
5
6
7
8
9
10
11
12
N9
N20
P25
N30
N60
P100


Normalized on mean N9 controls area
Normalized on mean N20 controls area
Median nerve
Ulnar nerve
Patients area reduction (% controls)
160
140
120
100
80
60
40
20
0
*
*
*
*
N9
N20
P25
N30
N60
P100
N9
N20
P25
N30
N60
P100


A
B
C
N9
N20
P25
N30
N60
P100
Patient area reduction (% controls)
Hand muscle force
ALSFR-S sub-score for hand functions
ALSFR-S
400
300
200
100
0
-100
-200
-300
4
6
8
10
2
28
30
32
34
36
38
40
42
44
46