



HAL
open science

Transient increase in recurrent inhibition in amyotrophic lateral sclerosis as a putative protection from neurodegeneration

Sina Sangari, Iseline Peyre, Alexandra Lackmy-Vallee, Eléonore Bayen,
Pierre-François Pradat, Veronique Marchand-Pauvert

► To cite this version:

Sina Sangari, Iseline Peyre, Alexandra Lackmy-Vallee, Eléonore Bayen, Pierre-François Pradat, et al..
Transient increase in recurrent inhibition in amyotrophic lateral sclerosis as a putative protection from
neurodegeneration. *Acta Physiologica*, 2022, pp.e13758. 10.1111/apha.13758 . hal-03534758

HAL Id: hal-03534758

<https://hal.sorbonne-universite.fr/hal-03534758>

Submitted on 19 Jan 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **TRANSIENT INCREASE IN RECURRENT INHIBITION IN AMYOTROPHIC**
2 **LATERAL SCLEROSIS AS A PUTATIVE PROTECTION FROM**
3 **NEURODEGENERATION**

4 Sina SANGARI^{1,2,3}, Iseline PEYRE¹, Alexandra LACKMY-VALLEE¹, Eléonore BAYEN^{1,4}, Pierre-François
5 PRADAT^{1,4}, Véronique MARCHAND-PAUVERT¹

6 ¹Sorbonne Université, INSERM, CNRS, Laboratoire d'Imagerie Biomédicale, LIB, F-75006, Paris,
7 France

8 ²Shirley Ryan AbilityLab, Chicago, Illinois, 60611

9 ³Department of Physical Medicine and Rehabilitation, Northwestern University, Chicago, Illinois,
10 60611

11 ⁴AP-HP, Hôpital Pitié-Salpêtrière, Maladies du Système Nerveux, F-75013, Paris, France

12 **Short title:** Renshaw cell adaptation in ALS

13 **Corresponding author:** Pr. Véronique MARCHAND-PAUVERT, PhD

14 Campus Cordeliers, LIB, Bât. A, 3^e étage

15 15 rue de l'Ecole de Médecine

16 75006 Paris

17 France

18 Email: veronique.marchand-pauvert@inserm.fr

19 Tel.: +33 1 42 16 11 20

20

21 **ABSTRACT**

22 **Aim:** Adaptive mechanisms in spinal circuits are likely involved in homeostatic responses to maintain
23 motor output in amyotrophic lateral sclerosis. Given the role of Renshaw cells in regulating the
24 motoneuron input/output gain, we investigated the modulation of heteronymous recurrent
25 inhibition.

26 **Methods:** Electrical stimulations were used to activate recurrent collaterals resulting in the
27 Hoffmann reflex depression. Inhibitions from soleus motor axons to quadriceps motoneurons, and
28 *vice versa*, were tested in 38 patients and matched group of 42 controls.

29 **Results:** Compared to controls, the mean depression of quadriceps reflex was larger in patients
30 while that of soleus was smaller suggesting that heteronymous recurrent inhibition was enhanced
31 in quadriceps but reduced in soleus. The modulation of recurrent inhibition was linked to the size
32 of maximal direct motor response and lower limb dysfunctions suggesting a significant relationship
33 with the integrity of the target motoneuron pool and functional abilities. No significant link was
34 found between the integrity of motor axons activating Renshaw cells and the level of inhibition.
35 Enhanced inhibition was particularly observed in patients within the first year after symptom onset
36 and with slow progression of lower limb dysfunctions. Normal or reduced inhibitions were mainly
37 observed in patients with motor weakness first in lower limbs and greater dysfunctions in lower
38 limbs.

39 **Conclusion:** We provide the first evidence for enhanced recurrent inhibition and speculate that
40 Renshaw cells might have transient protective role on motoneuron by counteracting
41 hyperexcitability at early stages. Several mechanisms likely participate including cortical influence
42 on Renshaw cell and reinnervation by slow motoneurons.

43 **Key words:** H-reflex, Interneurons, Motoneurons, Renshaw cells, Spinal cord, Spinal excitability

44

45 **1 | INTRODUCTION**

46 Amyotrophic lateral sclerosis (ALS) is the third most frequent neurodegenerative disorder,
47 characterized by a focal motor onset, which most often manifests in distal part of upper or lower
48 limbs¹ and rapidly spreads to other body regions. Cognitive dysfunctions are also reported in ~50 %
49 of cases². ALS has a relative low prevalence (~6/100 000)³ partly explained by its rapid progression
50 (respiratory failure leading to death within the 2-5 years after the disease onset) and the limited
51 effects of current therapy (riluzole prolonging the lifespan to only 3-6 months). About 90 % of cases
52 are sporadic, with heterogenous phenotype, but ALS hallmark is the progressive motor neuron loss,
53 including bulbar and spinal motoneurons and pyramidal cells in the primary motor cortex,
54 commonly termed as lower (LMNs) and upper motor neurons (UMNs), respectively⁴. Research has
55 mostly dealt with motor neuron dysfunctions which have been reported in presymptomatic ALS
56 mouse models and human patients at both levels⁵⁻¹¹, but non-cell autonomous pathogenic
57 mechanisms have also been reported, involving glial cells and interneurons¹²⁻¹⁴. To date, most of
58 the studies focused on glial cells and their interaction with motor neurons¹² and much less is known
59 on interneurons. However, it is commonly admitted that excitation/inhibition balance shifts
60 towards excitation, leading to hyperexcitability involved in neurodegeneration¹⁵, but studies
61 primarily focused on the motor cortex¹³ and much less is known on the spinal cord and its
62 pathophysiological plasticity. To better understand the mechanisms underlying ALS progression and
63 open new avenues for therapies, it is now crucial to consider the complex organization of the spinal
64 circuitry and its interaction with supraspinal structures^{14,16}.

65 Post mortem analyses in humans have evidenced that degeneration in the spinal grey matter
66 likely occurs first in the ventral horn (LMNs and interneurons) and, subsequently, in the
67 intermediate zone (interneurons); an hypothesis further supported by white matter analysis and
68 the progressive degeneration of propriospinal fibers^{17,18}. However, it has not been possible to
69 dissociate degeneration of LMNs and interneurons in the ventral horn¹⁹. In presymptomatic ALS

70 mouse models, specific subsets of glycinergic inhibitory interneurons in the intermediate zone and
71 ventral horn degenerate before LMNs^{20–22}, and it has been evidenced that alteration of glycinergic
72 interneurons in the ventral horn is not consecutive to LMN degeneration²³. More recently, it has
73 been shown that LMNs innervating fast-type motor units, which are among the first LMNs to
74 degenerate in ALS^{24–28}, receive more glycinergic inhibitory synaptic inputs from V1 interneurons
75 compared to more resistant LMNs innervating slow motor units²⁹. Furthermore, loss of inhibitory
76 synapses from V1 interneurons on fast LMNs precedes LMN degeneration and causes locomotor
77 dysfunctions²⁹. V1 interneurons include group Ia interneurons mediating reciprocal inhibition
78 between antagonists (glycinergic, located in intermediate zone and ventral horn), non-reciprocal
79 group Ib interneurons mediating autogenic inhibition (also termed as Ib interneurons; mostly
80 glycinergic but with some GABAergic synapses, same location as Ia interneurons), and Renshaw cells
81 mediating recurrent inhibition (glycinergic and GABAergic, located in the ventral horn)^{16,30–33}.

82 Renshaw cells have been particularly explored in ALS, liable to their activation by recurrent
83 collaterals from LMNs. Studies in both mouse models^{34,35} and humans^{36,37} revealed that recurrent
84 inhibition is particularly depressed in ALS, likely due to cell loss or reduced inhibitory action of
85 Renshaw cells secondary to a possible decreased excitation from cholinergic interneurons^{35,38}. The
86 depression of recurrent inhibition reported in patients has raised questions³⁹ *i)* for methodological
87 and physiological reasons, given that the reduced silent period after mixed nerve stimulation
88 reported in ALS⁴⁰, is not specific to Renshaw cell activity and the targeted LMNs (innervating intrinsic
89 hand muscles) have no recurrent collaterals^{41,42}, and *ii)* for pathophysiological reasons, since initial
90 wasting mostly occur in muscles innervated by LMNs without recurrent collaterals^{39,41,42}. These led
91 Mazzocchio and Rossi³⁹ to suggest that Renshaw cell impairment is not a general feature of ALS.
92 Altered connectivity between Renshaw cells and LMNs is indeed unlikely involved in the *primum*
93 *movens* of the human form of ALS, but maladaptive mechanisms at this level might contribute to
94 disease spread and progression¹⁶. More convincing results in patients were obtained by testing

95 Hoffmann reflex (H-reflex)^{36,43}, using the particular technique of H', enabling to evaluate the level
96 of homonymous recurrent inhibition (inhibition produced by recurrent collaterals from the target
97 LMNs)⁴⁴. However, the technique has not been properly implemented in ALS patients³⁹ and the
98 results have to be interpreted with caution when the LMN pool is already affected⁴⁴. Moreover, the
99 H' technique is not sufficient to test the hypothesis that altered recurrent inhibition might
100 contribute to disease progression. Testing heteronymous recurrent inhibition between different
101 LMN pools^{42,45-47}, with or without clinical signs of degeneration, has additional value to further
102 investigate the modulation of recurrent inhibition in humans and its putative role along the disease
103 course.

104 The present study was thus designed to further investigate the modulation of recurrent
105 inhibition in patients with ALS by testing heteronymous recurrent inhibition between soleus and
106 quadriceps LMNs^{46,47}. For this, we have examined the H-reflex evoked in the electromyogram (EMG)
107 of vastus lateralis (VL) head of quadriceps by femoral nerve (FN) stimulation, and its modulation
108 after stimulation of the posterior tibial nerve (PTN, activating soleus motor axons) at the optimal
109 interstimulus intervals (ISIs) for producing VL H-reflex depression due to recurrent inhibition in
110 quadriceps LMNs⁴⁴. Experiments were performed in patients with ALS without clinical signs of motor
111 degeneration in proximal muscles (quadriceps) but with or without distal muscle weakness, and in
112 age and gender-matched group of healthy controls. In a subgroup of participants, we also tested
113 the inhibition in soleus LMNs produced by activating quadriceps motor axons, by testing the
114 modulation of H-reflex in soleus EMG after FN stimulation, at the optimal ISIs for eliciting recurrent
115 inhibition in soleus LMNs⁴⁴. Lastly, we studied the link between the modifications of recurrent
116 inhibition and the patient clinical and electrophysiological profiles.

117 **2 | RESULTS**

118 **2.1 | Recurrent inhibition from soleus to quadriceps LMNs**

119 The first experiment consisted in testing the recurrent inhibition from soleus motor axons to

120 quadriceps LMNs (Fig. 1A). Figures 1B and C illustrate the mean VL EMG recordings without (FN
 121 stimuli delivered alone; test H-reflex) and with conditioning PTN stimuli (combined PTN and FN
 122 stimuli delivered at 15, 20 and 25-ms ISIs^{44,45}; conditioned H-reflexes) in 1 control (Fig. 1B) and 1
 123 patient (Fig. 1C). In both participants, the amplitude of H-reflex was smaller on combined stimuli.
 124 Figures 1D and E show the resulting mean level of inhibition in quadriceps LMNs plotted against the
 125 ISI between conditioning PTN and test FN stimuli. In both participants, PTN stimuli reduced
 126 significantly VL H-reflex between 30 and 60 % of its mean test size at the 3 ISIs investigated. Paired
 127 *t* test was performed at each ISI, in each participant: i) control: 15-ms ISI, $p = 0.02$; 20-ms ISI, $p =$
 128 0.007 ; 25-ms ISI, $p = 0.005$, and ii) ALS: 15-ms ISI, $p = 0.0003$; 20- and 25-ms ISIs, $p < 0.0001$. Figures
 129 1 F and G show the mean amplitude of the maximal direct motor response (Mmax) in VL and soleus
 130 EMG in the same control (Fig. 1F) and the same patient (Fig. 1G). Mmax in quadriceps was ~1 mV in
 131 both participants (0.85 ± 0.08 vs. 1.22 ± 0.01 mV in the control and the patient, respectively) while
 132 that in soleus was smaller in the patient (1.20 ± 0.01 mV) compared to the control (3.58 ± 0.04 mV).
 133 Lastly, the test H-reflex in VL EMG was of similar size in both participants, reaching on average 9.7
 134 ± 7.7 % of Mmax (in VL EMG) in the control and 10.3 ± 4.8 % in the patient (patient #25 in Table 1).

135 **Figure 1 near here**

136 For a reliable comparison of conditioned H-reflexes, the most important is to ensure that the
 137 test size of H-reflex (normalized to Mmax) and the peripheral volley in motor axons were
 138 comparable between groups^{44,46,48}. In the full group of participants (42 controls vs. 38 patients),
 139 Mmax in quadriceps was similar in controls and patients (Kolmogorov-Smirnov test, $p = 0.94$;
 140 Cohen's $d^{49,50} = 0.1$) while Mmax in soleus was significantly smaller in patients than in controls
 141 (Kolmogorov-Smirnov test, $p = 0.0246$; $d = 0.6$; Fig. 2A); none of the outliers in Figure 2A were
 142 detected as significant using the interquartile range (IQR) method. Despite this difference in soleus
 143 Mmax, the intensity of conditioning PTN stimuli, which was adjusted at the threshold intensity for
 144 Mmax, was not statistically different between groups: 60.4 ± 21.6 vs. 65.8 ± 23.9 mA in controls and

145 patients, respectively (Student *t* test, $p = 0.29$; $d = 0.2$). A particular care was taken to adjust the
 146 intensity of the test FN stimuli so as to produce a stable H-reflex in VL EMG and within a range that
 147 its amplitude has limited effect on the level of inhibition (*i.e.*, $\sim 25\%$ of Mmax as revealed for soleus
 148 H-reflex⁴⁸). However, the mean size of VL H-reflex in the control group was $12.9 \pm 11.9\%$ of Mmax.
 149 This result is consistent with previous studies^{51–53}: H-reflex in VL EMG is indeed generally much
 150 smaller than in soleus, hardly reaching 10–20% of Mmax. In the patient group, the test FN stimuli
 151 were adjusted the same way as in controls but the resulting mean H-reflex in VL EMG was
 152 significantly larger than in controls, reaching $22.3 \pm 15.3\%$ of Mmax (no significant outlier;
 153 Kolmogorov-Smirnov test, $p = 0.0038$; $d = 0.7$; Fig. 2B).

154

Figure 2 near here

155 The level of recurrent inhibition was first compared between groups at similar ISIs and Figure
 156 2C shows the data distribution in both groups (no significant outlier). The levels of recurrent
 157 inhibition were compared at the 3 ISIs (repeated measures) between groups (controls vs. ALS) by
 158 building a linear mixed model including the subjects as random effect and fixed effects were group
 159 (controls vs. ALS), ISI (15 vs. 20 vs. 25 ms), amplitude of conditioning Mmax in soleus and of test H-
 160 reflex in VL. The adjusted R^2 was 0.84 and variance analysis revealed a significant influence of ISI (p
 161 < 0.0001 ; f^2 ⁵⁴ = 0.49). The other regressors in the model had no significant influence: group ($p =$
 162 0.1176 ; $f^2 = 0.04$), conditioning Mmax ($p = 0.2335$; $f^2 = 0$) and test H-reflex ($p = 0.593$; $f^2 = 0$).
 163 Interestingly, the inhibition least mean square (*i.e.*, the best unbiased estimates of the marginal
 164 means of the model) was stronger in ALS than in controls (Fig. 2D), and a significant interaction was
 165 found between ISI and group ($p = 0.0394$; $f^2 = 0.04$). *Post-hoc* multiple comparisons revealed a
 166 significant difference between groups at ISI 25 ms (Student test, $p = 0.0269$; $d = 0.4$); at shorter
 167 intervals, the difference did not reach the statistically significant level (15-ms ISI: $p = 0.5441$, $d = 0$;
 168 20-ms ISI: $p = 0.1225$, $d = 0.3$). These results suggest that the inhibition from soleus motor axons to
 169 quadriceps LMNs was larger in patients with ALS than in controls and this was particularly true at ISI

170 25 ms.

171 According to previous studies^{44,45}, the 3 ISIs are within the range for optimal ISIs for recurrent
172 inhibition from soleus motor axons to quadriceps LMNs. They have been tested systematically in
173 each participant to ensure inhibition would occur at least once among the 3 ISIs. Interindividual
174 variability regarding participants' height and conduction velocity indeed influence the ISI (*e.g.*,
175 inhibition can manifest at shorter interval in small participants compared to tall ones). Indeed, the
176 inhibition has not manifested at all the 3 ISIs in all participants: i) 15-ms ISI: 27 (significant in 19)/42
177 controls vs. 23 (significant in 16)/38 patients, ii) 20-ms ISI: 35 (significant in 24)/42 controls vs. 33
178 (significant in 26)/38 patients, and iii) 25-ms ISI: 35 (significant in 24)/42 controls vs. 33 (significant
179 in 27)/38 patients. As we could expect, the optimal ISI for inhibition was not the same in each
180 participant. Therefore, we identified the ISI at which the level of recurrent inhibition was the
181 strongest in each participant. Maximal recurrent inhibition was indeed observed at ISI 15 ms in 6
182 controls and 3 patients, at ISI 20 ms in 19 controls and 9 patients, and at ISI 25 ms in 15 controls and
183 24 patients. Consistently with Figure 2C, maximal inhibition was thus mainly observed at the 20-
184 and 25-ms ISIs in both groups. Student *t* test was performed to compare maximal inhibition between
185 the 2 groups and it was found significantly greater in patients with ALS than in controls ($p = 0.0282$;
186 $d = 0.4$; Fig. 2E). This result further confirms that recurrent inhibition in quadriceps LMNs was
187 significantly increased in ALS group.

188 **2.2 | Recurrent inhibition from quadriceps to soleus LMNs, and reciprocally**

189 In the second half of the participants included in the study, we performed a second
190 experiment which consisted in testing recurrent inhibition from quadriceps motor axons to soleus
191 LMNs (Fig. 3A)^{44,45}. Accordingly, experiment 1 (inhibition from soleus to quadriceps; see 2.1) and
192 experiment 2 (inhibition from quadriceps to soleus) were successively performed during the same
193 experimental session in a subgroup of participants (17 controls vs. 17 patients).

194 **Figure 3 near here**

195 Figure 3B shows the distribution of Mmax amplitude in VL and soleus EMG in the subgroup of
 196 participants performing the 2 experiments (no significant outliers). The difference between controls
 197 and patients was not statistically significant (Kolmogorov-Smirnov test, $p = 0.7344$ [$d = 0.5$] and
 198 0.9539 [$d = 0.3$] for quadriceps and soleus, respectively). As observed in the full group of
 199 participants, H-reflex in VL EMG was significantly larger in patients than in controls (Welch ANOVA,
 200 $p = 0.0034$; $d = 1.1$; Fig. 3C). Similarly, H-reflex in soleus EMG was significantly larger in patients
 201 (Student t test, $p = 0.0042$; $d = 1.1$; no significant outliers; Fig. 3C). Lastly, the intensities of
 202 conditioning stimuli, adjusted at the threshold intensity for Mmax, were not statistically different
 203 between groups: *i*) in experiment 1 (PTN-induced depression of VL H-reflex), the mean intensity of
 204 conditioning PTN stimuli was 57.6 ± 31.4 vs. 65.3 ± 29.6 mA in controls and patients, respectively
 205 (Kolmogorov-Smirnov test, $p = 0.45$; $d = 0.2$) and *ii*) in experiment 2 (FN-induced depression of soleus
 206 H-reflex), the mean intensity of conditioning FN stimuli was 69.1 ± 29.8 vs. 78.8 ± 17.09 mA in
 207 controls and patients, respectively (Student t test, $p = 0.17$; $d = 0.5$).

208 Results of experiments 1 (inhibition from soleus to quadriceps) are illustrated in Figure 3D (no
 209 significant outlier) and the levels of inhibition at the 3 ISIs were compared as in 2.1. The adjusted R^2
 210 of the linear mixed model was 0.88 and only ISI had a significant influence on the level of inhibition
 211 ($p < 0.0001$; $f^2 = 0.23$). The other regressors had no significant influence: group ($p = 0.9783$, $f^2 = 0$),
 212 conditioning Mmax in soleus ($p = 0.091$, $f^2 = 0$) and test H reflex in VL ($p = 0.8021$, $f^2 = 0$). Contrariwise
 213 to the full group, the recurrent inhibition in quadriceps did not increase in the subgroup of patients:
 214 Figure 3E shows that the inhibition least mean square was comparable between groups and the
 215 interaction between ISI and group was not significant ($p = 0.1735$; $f^2 = 0$). Multiple *post hoc*
 216 comparisons were thus limited to ISI, showing a significantly greater inhibition at ISIs 20 and 25 ms,
 217 compared to ISI 15 ms (whatever the group): *i*) 15 vs. 20 ms: $p < 0.0001$, $d = 0.5$, *ii*) 15 vs. 25 ms: $p <$
 218 0.0001 , $d = 0.7$ and *iii*) 20 vs. 25 ms: $p = 0.2036$, $d = 0.1$.

219 Results of experiment 2 (inhibition from quadriceps to soleus) are illustrated in Figure 3G and,

220 as for experiment 1, a linear mixed model was built to compare the level of inhibition at the 3 ISIs
221 tested. The adjusted R^2 was 0.97 and ISI had a significant influence on the level of inhibition ($p =$
222 0.0102 ; $f^2 = 0$). The other regressors had no significant influence: group ($p = 0.0999$, $f^2 = 0$),
223 conditioning Mmax in VL ($p = 0.5758$, $f^2 = 0$) and test H reflex in soleus ($p = 0.2529$, $f^2 = 0$). Figure 3H
224 shows that the inhibition least mean square was lower in patients compared to controls, and the
225 interaction between ISI and group was significant ($p = 0.0275$; $f^2 = 0$). However, multiple *post hoc*
226 comparisons did not reveal any significant difference between groups tested at the same ISIs: *i*) ISI
227 5 ms: $p = 0.1367$, $d = 0.4$, *ii*) ISI 10 ms: $p = 0.1438$, $d = 0.4$ and *iii*) ISI 15 ms: $p = 0.0515$, $d = 0.7$. The
228 only significant difference between groups was found at ISI 5 ms in controls vs. ISI 15 ms in ALS
229 (inhibition being less in the latter; $p = 0.0475$, $d = 0.7$).

230 In each participant and each experimental paradigm, we retained for further analysis the
231 maximal amount of inhibition observed at the 3 ISIs tested. In experiment 1 (inhibition in quadriceps
232 LMNs), the inhibition was maximal at ISI 15 ms in 3 controls and 2 patients, at ISI 20 ms in 7 controls
233 and 4 patients and at ISI 25 ms in 7 controls and 11 patients. In experiment 2 (inhibition in soleus
234 LMNs), the maximal inhibition was observed at ISI 5 ms in 8 controls and 9 patients, at ISI 10 ms in
235 5 controls and 6 patients and at ISI 15 ms in 4 controls and 2 patients. Then, we compared the
236 maximal amount of inhibition in both LMN pools. We did not find any significant difference between
237 controls and patients when comparing the inhibition produced in quadriceps LMNs (experiment 1;
238 Student *t* test, $p = 0.8923$, $d = 0$; Fig. 3F) but we confirmed that the inhibition in soleus LMNs was
239 significantly depressed in patients (experiment 2; Kolmogorov-Smirnov test, $p = 0.0463$, $d = 0.5$; Fig.
240 3I).

241 **Figure 4 near here**

242 To determine whether the modulation of recurrent inhibition in patients was different
243 between LMN pools (quadriceps vs. soleus), independent from the fact that heteronymous
244 recurrent inhibition between both motor nuclei is stronger in soleus than in quadriceps LMNs *per*

245 *se*⁴⁴, we calculated the 95 % of confidence interval (CI95) of the mean level of maximal recurrent
 246 inhibition in the control group of which the lower and upper cut-offs were respectively 17.5 and
 247 31.6 % of the mean test H-reflex for quadriceps (n = 42 controls) and 52.8 and 79.5 % of the mean
 248 test H-reflex for soleus (n= 17 controls). Figure 4A shows the proportion of patients with reduced
 249 (below CI95), normal (within CI95) or increased (above CI95) maximal recurrent inhibition as a % of
 250 the total number of patients (n = 38 patients for quadriceps and 17 for soleus). In quadriceps LMNs,
 251 a much greater proportion of patients exhibited an increase in the level of recurrent inhibition
 252 compared to controls: in 50 % of the patients the inhibition was above the CI95 upper limit, 21.1 %
 253 had inhibition within CI95 and 28.9 %, below the CI95 lower limit. In soleus LMNs, the recurrent
 254 inhibition was almost equally distributed between values within CI95 (52.9 % of the patients) or
 255 below its lower limit (47.1 %); we did not find any patient with recurrent inhibition above the CI95
 256 upper limit. Chi² test revealed that the modulation of recurrent inhibition in the patient group was
 257 significantly different between quadriceps and soleus ($p = 0.0022$). It is important to notice that for
 258 quadriceps, we found the same repartition in the subgroup of 17 patients in whom recurrent
 259 inhibition was tested in both LMN pools: 53 % of the patients had inhibition in quadriceps above the
 260 CI95 upper limit, 23.5 % within the CI95 and 23.5 % below the lower limit (Chi², $p = 0.0012$).

261 **Table 1 near here**

262 **2.3 | Relationship between the modulation of recurrent inhibition, electrophysiological and** 263 **clinical features**

264 The clinical features of the patient group are detailed in Table 1. In most patients, the first
 265 clinical symptoms have manifested in upper (UL, 44.7 %) or lower limbs (LL, 36.8 %) and only 7/38
 266 patients have had bulbar signs at first (18.4 %), which corresponds to the classical repartition
 267 observed in ALS¹. The mean disease duration (time from symptom onset) was 21.7 ± 16.4 months,
 268 ranging between 5 and 72 months, median being 15.5 months. The mean score to revised ALS
 269 functional rating scale (ALSFRS-r), which measures disability in activities of daily living⁵⁵, was $40.0 \pm$

270 4.5 (ranging between 24 and 47, median at 40). We also calculated a sub-score for lower limb
271 functions, including walking and climbing stairs (maximal score being 8 indicated in bold caps in
272 Table 1), which was on average 5.4 ± 2.0 (ranging between 2 and 8, median at 6). No patients had
273 non-invasive ventilation nor gastrostomy. The mean progression rate, indicating ALSFRS-r decline
274 per month, was 0.5 ± 0.5 points/month (between 0.1 and 2.4, median at 0.4). Based on UMN and
275 LMN scores^{56,57}, we identified a greater proportion of patients with predominant UMN signs (60.5
276 %) than with predominant LMN signs (13.1 %) or mixed form (equal score for UMN and LMN signs,
277 26.4 %). The muscle strength on the investigated limb, evaluated by manual muscle testing and
278 rated using the cumulative Medical Research Council (MRC) scale, was normal (scored 5) in
279 quadriceps in all patients according to inclusion criteria, and was depressed in only 2 patients in
280 soleus (scored 3 in patient #2 and 2 in patient #35, Table 1); 13/38 patients had lower strength in
281 tibialis anterior (TA) and/or in extensor hallucis longus (EHL; in bold caps in Table 1). Lastly, almost
282 all patients were under riluzole therapy except 6 of them.

283

Table 2 near here

284 Table 2 summarises the electrophysiological profile of the patient group regarding quadriceps
285 and soleus. To smooth the intrinsic differences between quadriceps and soleus (electrophysiological
286 measures being systematically smaller in quadriceps than in soleus), we calculated the CI95s for
287 each measure in the control group, to evaluate their modulation in patients. We thus estimated that
288 the test H-reflex in the patient groups was particularly enhanced in both LMN pools: 63.2 and 70.6
289 % of the patients had larger H-reflexes in VL and soleus EMG, respectively, compared to controls.
290 While quadriceps H-reflex could be smaller in 21.0 % of the patients, we did not find any patient
291 with soleus H-reflex below the lower CI95 limit. Chi² test revealed a significantly different
292 distribution of the results between LMN pools ($p = 0.0297$). Then, we ran the same test in the
293 subgroup of patients performing the 2 experiments ($n = 17$ for quadriceps instead of 38), and while
294 the repartition was quite similar as in the full group (17.6 % below the lower limit, 17.7 % within the

295 CI95 and 64.7 % above the upper limits; to be compared with results for quadriceps H-reflex in Table
296 2), Chi² test was not anymore significant ($p = 0.095$). This result suggests that test H-reflex was larger
297 in both LMN pools in patients, compared to controls, with a tendency to be more enhanced in soleus
298 than in quadriceps.

299 The evaluation of Mmax, also termed as maximal compound muscle action potential (CMAP),
300 is included in routine electrodiagnosis for ALS and is used as a biomarker of muscle denervation
301 (LMN loss)^{58,59}. Moreover, its size is used to normalize the test H-reflex and to monitor the
302 consistency of conditioning stimuli. Therefore, we also calculated the proportion of patients
303 exhibiting significant depression of Mmax in VL and soleus EMG, according to CI95 in the control
304 group. Table 2 indicates that a greater proportion of patients had reduced Mmax in soleus (73.7 %)
305 than in quadriceps (39.5 %) and Chi² test indicated that the repartition was significantly different
306 between LMN pools ($p = 0.0026$) suggesting that Mmax in soleus was more depressed than in
307 quadriceps. We also ran the test in the subgroup of 17 patients performing the 2 experiments and
308 we found the same results: 41.2 % of the patients with depressed Mmax in quadriceps vs. 76.5 % of
309 for soleus (Chi², $p = 0.0365$).

310 The last part of the statistical analysis consisted in determining the possible link between the
311 patient profile and their modulation of recurrent inhibition (according to CI95 in controls) in the two
312 distinct target LMN pools (quadriceps vs. soleus). The parameters included in their clinical and
313 electrophysiological profiles were: the site of onset (lower limbs vs. other), the disease duration (2
314 classes: ≤ 1 year vs. > 1 year), the total and sub-score (lower limb functions) to ALSFRS-r (2 classes
315 according to the median scores in the group: ≥ 40 or < 40 for total score and ≥ 6 or < 6 for sub-score),
316 the progression (based on the total ALSFRS-r score; 2 classes according to the median score in the
317 group: slow vs. fast), the progression of lower limb dysfunctions (LL-progression; based on the
318 ALSFRS-r sub-score for lower limb functions; 2 classes according to the median score in the group:
319 slow vs. fast), the predominant form (UMN vs. LMN vs. mixed form), riluzole intake (yes vs. no), the

320 size of conditioning and test Mmax (according to CI95 in controls; 2 classes: not depressed vs.
321 depressed), and of H-reflex in the test and conditioning muscle (according to CI95 in controls; 3
322 classes: > upper limit vs. within CI95 vs. < lower limit).

323 The first step consisted in analysing the relationship between the modulation of recurrent
324 inhibition in patients (according to CI95 in controls) and each parameter, individually (analyse of
325 contingency tables and of the correspondences between the modulations of recurrent inhibition
326 and the modalities of each parameter). The results are summarized in Table 3 in which the clinical
327 and electrophysiological features are listed according to their statistical significance. We only found
328 3 significant parameters (Chi² tests; column 3, Table 3): *i*) the target LMN pool (LMNs inhibited by
329 Renshaw cells): as in 2.3, we found again a significant difference between quadriceps and soleus,
330 with more patients exhibiting increased recurrent inhibition in quadriceps and no modulation or
331 depressed inhibition in soleus (results of correspondence analysis in column 4, Table 3), *ii*) the test
332 Mmax: when Mmax in the test muscle (*e.g.*, Mmax in VL EMG when testing soleus-induced inhibition
333 in quadriceps LMNs) was not depressed compared to controls, recurrent inhibition was most often
334 increased, but when Mmax was depressed, the inhibition was more within the same range as
335 controls or depressed, and *iii*) the progression: recurrent inhibition was more within the normal
336 range in fast progressors. The influence of the other factors did not reach the statistically significant
337 level. However, the correspondence analysis revealed interesting associations between measures
338 for further analysis and Discussion (column 4, Table 3): *i*) inhibition was most often increased or not
339 modified in patients with predominant UMN or mixed form, *ii*) the size of H-reflex in the test and
340 conditioning muscles and that of Mmax in the conditioning muscle (*i.e.*, the motor axons we
341 stimulated to activate Renshaw cells; Figs. 1A and 3A) were not at all associated with the modulation
342 of recurrent inhibition, *iii*) inhibition was mostly increased in patients with mild or no motor
343 dysfunction in lower limbs, and not modified or decreased in those with greater lower limb
344 disabilities (ALSFERS-r sub-score), *iv*) inhibition was most often increased in patients with slow LL-

345 progression but normal or decreased in those with faster LL-progression, v) the patients with first
346 symptoms in lower limbs exhibited more often depressed inhibition, and at last vi) enhanced
347 recurrent inhibition was particularly observed in patients within the first year after the first
348 symptoms.

349 In the second step, we thus performed a multiple correspondence analysis (MCA) to evaluate
350 the relative links between clinical and electrophysiological features and the modulation of recurrent
351 inhibition. First, we performed MCA using the significant parameters in Table 3 (target LMN pool,
352 test Mmax and progression). The projection of each modality in a 2-dimension (2-D) plot is
353 illustrated in Figure 4B, and their partial contribution to inertia (*e.g.*, strength of the links between
354 variables) in each dimension, in Figure 4C. Dimension 1 (X-axis; Chi^2 , $p < 0.01$) represents 71.1 % of
355 the deviation from independence between variables (inertia). The target LMN pool and the
356 modification of test Mmax size particularly contribute to dimension 1 (Fig. 4C). Dimension 2 (Y-axis;
357 Chi^2 , $p > 0.99$) represents 28.9 % of the total inertia, and the progression (according to the total
358 ALSFRS-r score) particularly contributes to it (Fig. 4C). These results indicate that the modulation of
359 recurrent inhibition was significantly influenced by the origin of the target LMN pool (test muscle)
360 and the size of the test Mmax, and the 2-D plot indicates a strong link between an increase in
361 recurrent inhibition and the absence of depression of test Mmax, in quadriceps in particular.
362 Concerning the progression, the plot distinguishes slow from fast progressors but there is no
363 significant link with the other modalities and the modulation of recurrent inhibition; the
364 corresponding symbol (open triangles) are close to 0 in dimension 1 and dimension 2 did not reach
365 the statistically significant level ($p > 0.99$).

366 Lastly, we performed another MCA taking into account the results of the correspondence
367 analysis as well. We thus tested whether the modulation of recurrent inhibition was influenced by
368 the target LMN pool, the test Mmax size, the predominance of UMN signs or at least the equal
369 involvement of both UMNs and LMNs (yes [UMN + mixed forms] vs. no [LMN form]), the LL-

370 progression (slow vs. fast), the site of onset (lower limbs vs. others), the duration (1st year vs. > 1
371 year) and their respective links (Figs. 4DE). Figure 4D shows the 2D-plot with *i*) significant dimension
372 1 representing the most part of the total inertia (78.3 %; Chi^2 , $p < 0.001$), and *ii*) dimension 2
373 representing only 21.7 % (Chi^2 , $p > 0.99$). Here again, we found that the target LMN pool and the
374 test Mmax particularly contribute to dimension 1 together with LL-progression but to a much lesser
375 extent; onset site and duration also contribute to dimension 1 but to an even smaller extent than
376 LL-progression (Fig. 4E). The predominance of LMN signs (N in Fig. 4E) particularly contributes to
377 dimension 2; the corresponding symbol is outside the plot in Figure 4D given the scale used for
378 illustration (coordinates = 0.004 in dimension 1 and 0.66 in dimension 2). This result indicates the
379 predominance of LMN form has no significant link with the modulation of recurrent inhibition ($p >$
380 0.99), nor the fact that patients exhibit predominant UMN or mixed form. Even if the corresponding
381 symbol indeed appears in Fig. 4D (filled, right-orientated triangle ‘yes’), it is positioned in the middle
382 of dimension 1, almost at equal distant from the 3 modalities of modulation of recurrent inhibition.
383 The repartition of the other modalities on either side of dimension 1 indicates more links on the left
384 part of Figure 4D, between the enhanced recurrent inhibition and, from the strongest to the
385 weakest link, normal test Mmax, quadriceps, slow LL-progressors within the 1st year after the first
386 symptoms which did not manifest in lower limbs (filled circle ‘Other’ in Fig. 4D). The items on the
387 right part of Figure 4D are more spaced, indicating less links between unchanged or depressed
388 recurrent inhibition and the patient features, except the close link between the depression of
389 recurrent inhibition and the fact that the first symptoms have manifested in lower limbs (filled circle
390 ‘LL’ in Fig. 4D), suggesting that the inhibition was more depressed in patients with first symptoms in
391 lower limbs.

392 **3 | DISCUSSION**

393 This study has thus shown that heteronymous recurrent inhibition between lumbar LMNs is
394 modified in ALS, being particularly increased in quadriceps LMNs in the present cohort but not

395 reciprocally in soleus ones where inhibition was unchanged or decreased (compared to matched
396 control group). The modulations of recurrent inhibition were particularly linked to the size of the
397 test Mmax: the inhibition was particularly enhanced when the test Mmax was within the control
398 range, or it decreased to similar or lower levels than controls when the test Mmax was depressed
399 (see Supplemental Material 1AB: the inhibition increased with the size of test Mmax in both LMN
400 pools). On the contrary, the conditioning Mmax had no influence. These results suggest that the
401 level of recurrent inhibition likely depends on the integrity of the target LMN pool (test Mmax) but
402 not on the integrity of the motor axons activating Renshaw cells (conditioning Mmax). Lastly, the
403 modulation of recurrent inhibition was linked to the level of lower limb dysfunctions and their
404 progression: the inhibition was enhanced in patients within the first year after the first symptoms,
405 when onset site was not in lower limbs, and when the progression of lower limb disabilities was
406 slow (see Supplemental Material 1CD: the inhibition decreased with LL-progression in both LMN
407 pools but this was more pronounced in quadriceps LMNs). On the contrary, the inhibition was
408 particularly decreased in patients with first symptoms in lower limbs. These results suggest that the
409 level of recurrent inhibition likely depend on the integrity of the target LMNs and is associated to
410 the peripheral denervation of the corresponding muscles and the resulting functional disabilities.

411 **3.1 | Methodological considerations**

412 Heteronymous recurrent inhibition at both cervical and lumbar spinal levels has first been
413 described in humans by investigating the modulation of single motor unit discharge after peripheral
414 nerve stimulation. It has been shown that the depression observed in the resulting post-stimulus
415 time histograms always appeared and increased with the conditioning motor discharge (*i.e.*, the size
416 of the H-reflex and/or of the M response elicited in the conditioning EMG), was independent of the
417 conditioning stimulus intensity *per se* (a characteristic further supported by the present study since
418 the intensity of conditioning was similar in both groups and we found different modulations), and
419 had short latency and long duration^{42,46}. Then, heteronymous recurrent inhibition has been

420 assessed by testing the modulation of H-reflex, of on-going EMG activity and of motor evoked
421 potential, and its origin was confirmed using L-acetyl carnitine known to potentiate recurrent
422 inhibition^{41,44,45}.

423 To limit the duration of the experiments for the comfort of the participants (we also tested
424 other spinal pathways in the same participants), we only tested 3 ISIs at which recurrent inhibition
425 was found maximal in previous studies^{60,61}. Indeed, the range of ISIs tested for each motor nucleus
426 corresponds to optimal ISIs for investigating Renshaw cell activity: i) it excludes the first 10 ms of
427 central delay during which disynaptic non-reciprocal group I inhibition (also termed as group Ib
428 inhibition) manifests and ii) it was limited to inhibition purely spinal in origin (< 12 ms duration;
429 excluding longer ISIs likely contaminated by transcortical effects)^{44,62}. Moreover, we found the
430 conditioned H-reflexes in soleus EMG more depressed than those in VL EMG. Similarly, it has been
431 previously reported that heteronymous recurrent inhibition between both nuclei is stronger in
432 soleus LMNs than that in quadriceps ones⁴⁴, and it is interesting to note that this might also be the
433 case when comparing homonymous recurrent inhibition using the H' technique⁴¹.

434 The depression of conditioned H-reflex assessed using the H' technique (homonymous
435 paradigm) is partly due to the post-spike after hyperpolarization (AHP)^{41,63,64}, which is shortened in
436 patients with mild motor dysfunction and increased again with motor deficit progression⁶⁵. There is
437 thus a possibility that the increase of H' reflex in ALS^{36,43}, interpreted as a result of depressed
438 recurrent inhibition, might be partly due to reduced AHP. The great advantage of the heteronymous
439 paradigms we used in the present study is that the depression of conditioned H-reflex is not
440 contaminated by AHP (conditioned and test stimuli not applied to the same nerve; inhibition
441 produced in post-stimulus time histograms, even without preceding monosynaptic group Ia
442 excitation; inhibition independent of the strength of preceding monosynaptic group Ia
443 excitation)^{44,47}. Additionally, modification in AHP cannot explain the enhanced inhibition in
444 quadriceps LMNs nor its depression with progressive lower limb dysfunctions.

445 Other spinal inhibitory mechanisms could have contributed to the H-reflex depression: i)
446 group Ib inhibition but this is unlikely at the ISIs we tested (see above), and ii) presynaptic inhibition
447 of group Ia terminals. In the experimental paradigms we used, presynaptic inhibition is assessed at
448 shorter ISIs than those we tested. Indeed, presynaptic inhibition between soleus and quadriceps is
449 estimated based on the modulation of heteronymous group Ia facilitation of H-reflex (which
450 precedes the long lasting inhibition^{44,60}) *i.e.*, about 5 ms ISI when testing conditioning PTN on VL H-
451 reflex and about -5 ms ISI when testing conditioning FN on soleus H-reflex^{66,67}. These ISIs are shorter
452 than those for D1 method we used in Howells et al. 2020 (10 to 30-ms ISIs)⁶⁸ *i)* due to the difference
453 in the peripheral afferent conduction time between PTN and FN, while this time is similar between
454 PTN and fibular nerve in the D1 method, and *ii)* because presynaptic inhibition between soleus and
455 quadriceps is estimated based on the modulation of heteronymous facilitation of H-reflex (adding
456 central processing) while the direct modification of H-reflex size by the conditioning is investigated
457 in the D1 method. Moreover, it has been previously shown that the inhibition reported in the
458 present study is evoked only when a motor volley is produced in the conditioning nerve,
459 independently of the intensity of the conditioning stimuli, which does not match the characteristics
460 of presynaptic inhibition for which the threshold intensity of the conditioning volley is below the
461 motor threshold^{44–47,60}. Lastly, it has been shown that presynaptic inhibition is depressed in ALS^{68–}
462 ⁷¹. Similarly, in our cohort of patients, we found presynaptic inhibition depressed, and we also found
463 the group Ib inhibition unchanged compared to controls (unpublished data). Accordingly, the
464 depression of conditioned H-reflexes in the present study was mostly due to Renshaw cell activity
465 and its modification in ALS, to modulation of recurrent inhibition.

466 **3.2 | Basics on Renshaw cells and recurrent inhibition**

467 The distribution of recurrent inhibition has been particularly well described in humans and
468 has been found closely linked to that of monosynaptic group Ia excitation of LMNs, as reported in
469 animal models (cats, baboons). However, the projections are less diffuse in human upper limbs

470 compared to cat forelimbs and, on the contrary, more diffuse in lower limbs compared to hindlimbs,
471 likely due to the development of the bipedal stance and gait, and the parallel release of the upper
472 limbs from their locomotor functions in humans^{42,44,46,47,72}. Renshaw cells are indeed activated by
473 axon collaterals from LMNs of different motor pools and they project onto homonymous and
474 synergistic (heteronymous) LMNs in the same or adjacent spinal segments; they also project onto
475 gamma LMNs (different Renshaw cells from those projecting onto alpha LMNs) and group Ia
476 reciprocal interneurons^{16,44,45,73,74}. Beside their excitation by LMN discharge, Renshaw cells also
477 receive polysynaptic excitation and inhibition from flexor reflex afferents (FRA)⁷³. Moreover,
478 transcranial magnetic stimulation over the primary motor cortex has been shown to reduce the level
479 of recurrent inhibition likely due to cortical suppression of a tonic excitatory drive from the reticular
480 formation rather than a direct corticospinal inhibition of Renshaw cells^{75,76}. The same way, recurrent
481 inhibition is primarily depressed during voluntary contractions, likely to reinforce reciprocal
482 inhibition between antagonists and assist muscle synergies during movement⁷⁷. However, during a
483 weak tonic contraction, recurrent inhibition has been found increased⁷⁷, suggesting a more complex
484 organization and control of Renshaw cell activity. The functional role of recurrent inhibition has
485 been debated for a long time and is still discussed. However, it is commonly accepted that it
486 participates in the control of muscle synergies during movement⁴⁴ and likely mostly serves as a
487 variable gain regulator of the spinal motor output⁷⁸. Accordingly, it has recently been proposed that
488 adaptative mechanisms at the level of Renshaw cells would take part in the homeostatic response
489 to maintain force output as long as possible during the course of ALS. Then, a gradual unbalanced
490 activity in local micro-circuitries linking different LMN pools would overwhelm the initial
491 homeostatic response and contribute to excitotoxicity participating in LMN degeneration and
492 disease progression¹⁶.

493 **3.3 | Modulations in ALS and adaptive mechanisms**

494 Glycinergic inhibition mediated by ventral horn interneurons has been found particularly

495 altered in mouse models of ALS and interneurons start to degenerate before LMNs; the authors
496 have speculated on the involvement of Renshaw cells^{29,35}. However, none of these studies focused
497 on Renshaw cells in particular, which mediate both glycinergic and GABAergic recurrent inhibitions
498 to LMNs^{31,32}. More recently, specific alteration of V1 interneurons has been reported²⁹ but Renshaw
499 cells constitute only a small part of V1 interneuron pool (9 %); the rest being associated to
500 proprioceptive interneurons, including group Ia interneurons⁷⁹. To our knowledge, only one study
501 has assessed specifically Renshaw cells and modulation of recurrent inhibition. This study has
502 evidenced an early (presymptomatic) downregulation of vesicular acetylcholine transporters in
503 recurrent collaterals and of cholinergic receptors, associated to major structural abnormalities of
504 motor axon synapses. However, the authors also reported a transient sprouting of Renshaw cells to
505 remaining LMNs. The synaptic disorganization between LMNs and Renshaw cells was followed by
506 retraction of motor collaterals but it was not clearly accompanied by any degeneration of Renshaw
507 cells which, for the most part, survived until the late stages. They concluded that the alteration of
508 LMN recurrent inhibition in ALS is likely due to synaptic pathology and not to interneuron cell
509 death³⁴.

510 In ALS patients, it has been proposed that recurrent inhibition is reduced but this assumption
511 relies on misinterpreted results based on mixed nerve silent period in LMNs without recurrent
512 collaterals⁴⁰ and H' technique contaminated by AHP^{36,43,44}. Accordingly, the possible alteration of
513 recurrent inhibition and the implication of Renshaw cells in ALS has been quite rightly questioned³⁴.
514 However, a recent study has shown that the inhibitory period in peristimulus frequencygram (PSF)
515 of single motor units is shortened in lumbar-affected ALS patients but unchanged in nonlumbar-
516 affected ones³⁷. The authors, who developed the method, have argued that this inhibition is mostly
517 due to recurrent inhibition of soleus LMNs produced by stimulation of PTN in the lateral part of the
518 popliteal fossa, which primarily produces M response in soleus EMG^{80,81}. They discussed the possible
519 involvement of other inhibitory mechanisms involving group I afferents from proprioceptors, and

520 argued on their minimal involvement. Their results in ALS patients are consistent with our
521 observations that the inhibition can be within the normal range or decreased according to the level
522 of lower limb disabilities. Accordingly, Ozyurt et al.³⁷ and the present study allow to reconsider the
523 possible role of Renshaw cells in ALS.

524 Several mechanisms well described in ALS pathophysiology can interfere with Renshaw cell
525 activity and can modulate the level of recurrent inhibition according to the neural network
526 connectivity within the spinal cord and its interaction with supraspinal structures and peripheral
527 afferents. Indeed, indirect electrophysiological techniques in humans do not allow to evaluate the
528 inhibitory post synaptic potentials (IPSPs) produced in LMNs; they only give an estimate of the net
529 motor output resulting from the conditioning-induced activity in the target spinal pathway (*e.g.*,
530 recurrent inhibition in the present study) and the tonic synaptic activity in surrounding neural
531 network.

532 **3.3.1 | Cortico-reticulo-spinal influence**

533 UMN degeneration in ALS manifests in the presymptomatic phase^{8,82}, which likely reduces the
534 inhibitory cortical influence on Renshaw cell activity and, thus, would likely contribute to enhance
535 recurrent inhibition. Furthermore, degeneration in reticular formation, and alteration of
536 serotonergic neurons in particular, likely contribute to pyramidal signs in ALS⁸³ and would thus
537 lead to depressed inhibitory descending influence on Renshaw cells. Recurrent inhibition has
538 previously been tested in other pathologies with pyramidal syndrome⁴⁴: *i)* it has been found
539 increased in stroke and spinal cord injured patients^{84,85}; *ii)* in patients with cerebral palsy, the
540 inhibition was found unchanged⁸⁶ as in some patients with hereditary spastic paraplegia but, in the
541 latter, inhibition could also be depressed but this was attributed to modification in AHP^{87,88}, and *iii)*
542 interestingly, it has been found in patients with hyperekplexia that recurrent inhibition is preserved,
543 likely due to its GABAergic components⁸⁹. In the present study, we did not find any significant link
544 between the modulations of recurrent inhibition and UMN sign manifestation likely because most

545 of the patients have predominant UMN or mixed form of ALS, and different modulations were
546 observed according to the disease duration and the progression of lower limb dysfunctions.
547 Enhanced inhibition was indeed particularly observed at early disease stage *i.e.*, within the first year
548 after the first symptoms, but the inhibition was within the normal range or depressed when the
549 disease was more advanced (depressed test Mmax, link with onset site in lower limbs, functional
550 disability and its progression in lower limbs). Therefore, UMN degeneration and the consecutive
551 changes in the cortico-reticulo-spinal influence on Renshaw cell activity can likely account for the
552 enhanced recurrent inhibition but other mechanisms likely interfere with it, leading to progressive
553 depression of recurrent inhibition.

554 **3.3.2 | Modulation by peripheral afferents**

555 Renshaw cells can receive excitation and inhibition from FRA⁷³ and it has been shown that
556 recurrent inhibition is particularly reduced by group II afferents⁹⁰. It is commonly admitted that
557 sensory deficits in ALS are secondary mechanisms, occurring at late stages of the disease, and early
558 clinical evidences for sensory defects exclude ALS from diagnosis. However, using spinal diffusion
559 MRI, we evidenced early microstructural alteration in the dorsal columns, correlated with depressed
560 peripheral afferent volleys, in patients without any clinical signs of sensory deficits⁹¹. These results
561 are in accordance with those in ALS mice exhibiting presymptomatic disorganization of muscles
562 spindles and specific alteration of group Ia and group II muscle afferents, concomitantly with LMN
563 degeneration but developing at a slower rate⁹². The clinical evaluation of sensory deficits is not
564 specific enough to discriminate a specific alteration of muscle spindles and of their resulting
565 afferents inputs, including group II afferents, which likely explains why early sensory deficits in ALS
566 can only be detected using experimental approaches⁹¹. Depression of muscle spindle group II inputs
567 releases Renshaw cells from peripheral inhibition but this might have little impact at early stages of
568 the disease, when there is no clinical evidence for muscle weakness and LMN alteration, and thus
569 little contribution to enhanced recurrent inhibition. Additionally, this cannot account for the

570 depression of recurrent inhibition at later stages. Without fully discarding the group II hypothesis,
571 we assume that this might have little role in the modulation of Renshaw cell activity in ALS.

572 **3.3.3 | Influence of LMNs**

573 For obvious reasons, we can expect that LMN dysfunctions and degeneration have strong
574 impact on Renshaw cells. Indeed, a specific loss of their collaterals has been found from ventral
575 horns with extensive loss of LMNs³⁴. However, this does not fully match the present results in
576 patients because: *i*) while we found a significant reduction of soleus Mmax (conditioning Mmax) in
577 the full group, the inhibition in quadriceps LMNs was significantly increased, and *ii*) we did not find
578 any significant link with the size of H-reflex and Mmax in the conditioning EMG *i.e.*, with the motor
579 inputs to Renshaw cells, whatever the target LMN pools. On the contrary, we found the modulation
580 of recurrent inhibition closely linked to the test Mmax *i.e.*, with the integrity of the target LMN pool.
581 These results raise questions on the origin of the Renshaw cells mediating heteronymous recurrent
582 inhibition, on the source of their motor axon inputs, on their intrinsic excitability and the
583 repercussion of early LMN dysfunctions.

584 The *proximity hypothesis* (short projections of Renshaw collaterals), for the diffuse pattern of
585 recurrent inhibition, has been discarded by showing that recurrent inhibition occurs between
586 synergistic LMNs (but not between pure antagonists) whatever their location in the spinal cord
587 (*functional hypothesis*)^{45,73}. However, it is not known whether homonymous and heteronymous
588 recurrent inhibitions are mediated by the same Renshaw cells or by different subsets. In the sub-
589 group of participants in whom inhibition was tested reciprocally in the 2 LMN pools, we only found
590 6/17 patients in whom the recurrent inhibition was modulated the same way in quadriceps and
591 soleus LMNs (decreased in 3 patients and within the control range in the 3 remaining ones). This
592 observation does not help to distinguish between the 2 hypotheses (same interneurons vs. different
593 subsets) but if the modulation of recurrent inhibition were due to intrinsic changes of Renshaw cell
594 activity, and if the inhibitions were mediated by the same subsets of interneurons, one would have

595 expected systematic parallel changes in soleus and quadriceps LMNs.

596 The pattern of recurrent inhibition strongly correlates with the distribution of group Ia
597 monosynaptic excitations⁴⁵. If homonymous and heteronymous collaterals converge onto the same
598 Renshaw cells, one would thus expect that the resulting recurrent inhibition would be greater from
599 homonymous motor axons than heteronymous ones. This possibility would explain the link between
600 the modulation of recurrent inhibition and the test Mmax: the global inhibition (from homonymous
601 and heteronymous motor axons) would be particularly depressed when the target (homonymous)
602 LMNs and their motor outputs are particularly altered. However, the rule Ia connections-recurrent
603 inhibition is not exclusive since recurrent inhibition without preceding Ia excitation has also been
604 reported (*extended recurrent inhibition*)⁴⁵. Additionally, it is not possible to argue on the size of H-
605 reflexes and the strength of monosynaptic Ia excitations since the larger H-reflex amplitude in
606 patients, compared to controls, are likely due to a change in presynaptic inhibition of group Ia
607 terminals^{69–71,93}. Furthermore, the change in H-reflex size in both muscles does not match the
608 change in recurrent inhibition in their respective LMN pool. However, it is important to keep in mind
609 that while we tested heteronymous recurrent inhibition, its level likely depends on the tonic level
610 of homonymous inhibition, and if the latter was depressed due to degeneration of target LMNs, we
611 could expect less recurrent inhibition.

612 Several alternative mechanisms would also explain the link between the modulation of
613 recurrent inhibition and the test Mmax, and the enhanced inhibition at early stages. I) On one hand,
614 Renshaw cells receive stronger inputs from fast LMNs than from slow ones but, on the other hand,
615 the level of recurrent inhibition (in resting condition) is greater in slow LMNs compared to fast ones
616 (due to intrinsic properties of LMNs)⁷⁸. Since fast LMNs are among the first to degenerate in ALS<sup>24–
617 28</sup>, their loss would have a strong impact on Renshaw cell activity and would thus greatly depress
618 the level of recurrent inhibition at early disease stages, which does not correspond to our
619 observations. In addition, we should have observed a link with the conditioning Mmax. Accordingly,

620 we assume this possibility has little role in the modulation of recurrent inhibition. Moreover, the
621 loss of fast LMNs is accompanied by peripheral reinnervation by resistant slow LMNs (peripheral
622 nerve sprouting)^{24–28,94,95}, which generates large motor unit potentials in EMG⁹⁶. Therefore,
623 inhibition of slow LMNs would lead to greater depression of H-reflex amplitude (due to suppression
624 of large motor unit potentials) in patients compared to controls, which might account for the
625 enhanced recurrent inhibition in quadriceps. However, we did not find any increase of recurrent
626 inhibition in soleus LMNs. II) Wootz et al.³⁴ have revealed transient axon sprouting at the level of
627 Renshaw collaterals at early disease stages, projecting onto surviving LMNs. If this result can be
628 transposed to humans, this would contribute to reinforce recurrent inhibition of resilient (slow)
629 LMNs. III) Excitability of LMNs has been found to progress from hyper to hypo-excitability along the
630 course of the disease^{5,6,97} and we have shown that LMNs in symptomatic patients with sporadic ALS
631 are normo-to-hypoexcitable (participants are common to the present study)⁵⁷. It has been clearly
632 stated in ALS mice that the equilibrium between opposite effects (excessive activity of the voltage-
633 gated Na⁺ and Ca²⁺ channels mediating persistent inward currents [PICs] vs. increase in cell size and
634 membrane conductance) is disrupted at the time of peripheral denervation leading to LMN hypo-
635 excitability and death⁹⁸. It would be particularly interesting to investigate the IPSPs from Renshaw
636 cells under these conditions, in different LMN pools (to assess homonymous and heteronymous
637 inhibitions) and at different stages of the disease. Indeed, both intrinsic and extrinsic LMN
638 mechanisms, including Renshaw cells, might contribute to the excessive homeostatic response of
639 LMNs but this has to be further investigated in animal models to open new avenues for
640 therapy^{14,16,98}.

641 **3.4 | Pathophysiological role in ALS**

642 The present results suggest that recurrent inhibition is enhanced in LMNs without evidence
643 for peripheral denervation (link with test Mmax), when there was no or only weak muscle weakness,
644 within the first year after symptom onset in particular. This increase was particularly observed in

645 patients with slow functional progression in the target limbs. On the contrary, the inhibition was
646 within the control range or even decreased when there were electrophysiological and clinical
647 evidences for LMN degeneration in the target motor pool, and particularly in patients with first
648 symptoms in lower limbs. These results are in accordance with Ozyurt et al.³⁷ and suggest that
649 recurrent inhibition likely transiently increases at early stages of ALS, before decreasing when the
650 target LMNs degenerate. This hypothesis is supported by Wootz et al.³⁴ who showed a transient
651 sprouting of Renshaw collaterals on resilient (slow) LMNs (with strong recurrent inhibition), which
652 would reinforce recurrent inhibition. Changes along the disease course should be confirmed by
653 longitudinal study. In the present cohort, we had the opportunity to test recurrent inhibition in
654 quadriceps twice in one patient: patient #13 (Table 1) was evaluated 6 and 30 months after the first
655 symptoms, and recurrent inhibition was within the control range during the first evaluation (data
656 retained for the group analysis) but strongly reduced during the second visit (Supplemental material
657 2A). Between both visits, the patient conditions have worsened (Supplemental material 2B) with
658 depression of Mmax in both VL and soleus EMG, losing 7 points to total ALSFRS-r and 4, to lower
659 limb ALSFRS-r sub-score, MRC score in quadriceps and soleus was still 5 but respectively 1 and 3 in
660 TA and EHL, and patient #13 exhibited a predominant UMN form during the first evaluation but LMN
661 predominant form the next time.

662 The results in quadriceps clearly indicate that recurrent inhibition can increase at early stage
663 of the disease but likely decreases progressively with time and degeneration of target LMNs.
664 However, similar increase was not revealed in soleus LMNs. Several mechanisms can explain the
665 difference between both motor pools: I) We found again in both controls and patients that recurrent
666 inhibition was greater in soleus than in quadriceps⁴⁴. Since the conditioning stimuli were adjusted
667 so as to produce Mmax in the corresponding EMG, there is a possibility that inhibitions were
668 saturated, and possibly more in soleus LMNs than in quadriceps ones, which makes it difficult the
669 detection of small variations (increase) of recurrent inhibition. However, inhibitions in both LMN

670 pools were evaluated the same way, and inhibition in quadriceps was likely at saturation too,
671 making this hypothesis less plausible. II) According to MRC scores, the patients exhibited distal
672 muscle weakness, affecting soleus in only 2/38 patients, but TA and/or EHL in 13/38 (Table 1), and
673 in patient #13 we found that reduced recurrent inhibition in quadriceps was accompanied by the
674 development of muscle weakness in TA and EHL (Supplemental material 2B). There is thus a
675 possibility that reduced inhibition from distal LMNs, including TA and EHL, might affect the level of
676 recurrent inhibition but this is unlikely since no recurrent inhibition from pretibial muscles (including
677 TA and EHL; by stimulating the deep peroneal nerve) have been observed in both quadriceps and
678 soleus LMNs⁴⁴, and manual muscle testing is not specific for EHL, but also includes intrinsic foot
679 muscles without LMN recurrent collaterals⁴¹. III) Quadriceps LMN pool is more heterogeneous than
680 soleus, including both fast and slow LMNs while soleus mainly includes slow LMNs. There is thus a
681 possibility that structural reorganisation at both spinal and peripheral levels, and the global
682 homeostatic response, affect more the level of inhibition in quadriceps than in soleus and/or
683 enhanced inhibition in soleus manifests at even earlier (presymptomatic) stages of the disease. Here
684 again, investigating recurrent inhibition in different LMN pools, with different proportions of slow
685 and fast LMNs, and the characteristics of their homeostatic response would be particularly
686 interesting in ALS mice, to determine the possible mechanism at pre- and post-synaptic levels
687 underlying the modulations reported here in patients.

688 Several mechanisms have been identified in the regulation of the input/output gain across
689 LMN pools, including PICs and recurrent inhibition from Renshaw cells⁷⁸, and it has been evidenced
690 that PICs are enhanced in ALS⁹⁸. A theoretical model has allowed to show that recurrent inhibition,
691 and its GABAergic component in particular, is particularly efficient to control PICs and regulate LMN
692 recruitment. It has thus been suggested that modulation of the strength and kinetics of GABAergic
693 currents could provide treatment strategies for uncontrollable spasms⁹⁹. Therefore, we assume that
694 adaptive mechanisms in spinal circuitry involving Renshaw cells and enhanced recurrent inhibition

695 might be particularly efficient to limit PICs amplification and maintain LMN homeostasis in ALS. Such
696 mechanisms, as long as they are effective, would be protective from neurodegeneration. In line with
697 this, we particularly observed enhanced recurrent inhibition in patients with slow worsening of
698 lower limb functions. Moreover, lithium, which among other effects increases the number of
699 Renshaw cells, had a neuroprotective effect in ALS mouse model^{100,101} but its interest in ALS therapy
700 was discarded by the disappointing results of clinical trials¹⁰². Last important point, distal hand and
701 foot muscles are among the first to manifest clinical symptoms of weakness and wasting in most
702 cases of ALS¹. LMNs innervating these muscles have no recurrent collaterals^{41,42} and, consequently,
703 cannot benefit from any protective role from Renshaw cells. This might participate in their greater
704 sensitivity to ALS. On the other hand, recurrent collaterals are only scarce in the most resistant
705 oculomotor LMN pool¹⁰³. However, this does not rule out the putative protective role of Renshaw
706 cells in ALS and further suggests that several other mechanisms likely participate in the homeostatic
707 response, making it difficult the pharmacological approach to slow down progression.

708 **3.5 | Conclusion and perspectives**

709 The present study provides the first experimental evidence for enhanced activity in spinal
710 circuitry involving Renshaw cells and further confirms that recurrent inhibition is modulated and
711 progressively depressed with LMN degeneration. Our results allow to reconsider the role of
712 recurrent inhibition in ALS and in the LMN homeostatic response, and suggest that Renshaw cells
713 likely have a transient putative protective role on LMNs from neurodegeneration. Several
714 mechanisms likely participate in the adaptive mechanisms, including cortical influence on Renshaw
715 cells and reinnervation by slow LMNs. Accordingly, our study gives strong support to hypotheses
716 recently raised on the role of spinal circuitry organization in the homeostatic response, with
717 enhanced inhibition counteracting PICs amplification, and in the disease progression^{14,16} given the
718 diffuse distribution of heteronymous projections supporting muscle synergies and likely
719 participating in the spread of local alterations to other regions, in particular between proximal

720 muscles⁷³. These results in humans cannot help to determine the exact mechanisms underlying
721 those changes at spinal level, due to limited methodological approaches, but encourage further
722 studies, in both animal models and patients in parallel, to deepen the knowledge on spinal network
723 plasticity in ALS, its functional role in homeostatic response to maintain LMN functions and in
724 disease progression.

725 **4 | MATERIALS AND METHODS**

726 **4.1 | Ethics**

727 The present study is part of a large study aiming at studying the spinal excitability in patients
728 with ALS (SpinalBioMark-SLA) during which we assessed different spinal circuitries using indirect
729 electrophysiological tools. The full study and the experimental procedures, including those in the
730 present paper, conform to the lastest revision of the Code of Ethics of the World Medical Association
731 (Declaration of Helsinki) and were approved by the ethic committee of INSERM (protocol n°C14-21)
732 and by the national ethical authorities (CPP Ile de France, Paris 6 - Pitié-Salpêtrière, CPP/16-15; RCB
733 2014-201-A01240-47). It has been registered in a public registry (<https://clinicaltrials.gov>,
734 NCT02429492). The experiments were performed with the written informed consent of each
735 participant. The data that support the findings of this study are available on request from the
736 corresponding author; they are not publicly available due to ethical restrictions.

737 **4.2 | Participants**

738 Based on dataset from previous studies in the laboratory (mean inhibition in quadriceps and
739 variance), and for minimum difference of 10 % between groups, we estimated that 40 controls and
740 40 ALS patients had to be included in the present study to obtain a statistical power with an alpha
741 risk (type I error) of 5% and beta risk (type II error) of 10%. Accordingly, 45 patients and 49 controls
742 were included but the experiments could be performed in 38 patients and 42 controls because i) H-
743 reflex could not be produced in VL EMG in 3 patients and 3 controls and hardly evoked making it
744 unusable for the experiments in 3 other patients and 3 other controls, ii) the conditioning stimuli

745 applied to PTN was not selective for soleus in 1 patient, activating pretibial flexors whose spindle
746 afferents produce spinal excitation in quadriceps LMNs and no recurrent inhibition¹⁰⁴, and iii) 1
747 control was too sensitive to electrical stimulation. In both groups, 7 women were tested and the
748 mean age in the patient group was 61.5 ± 9.7 years old (mean \pm 1 SD; ranging from 39 to 78), and
749 61.8 ± 9.0 (from 40 to 77) in the control group.

750 The inclusion criteria for controls included the absence of prior or current neurological illness.
751 Those for patients included 1) probable or definite ALS according to the El Escorial criteria¹⁰⁵, 2) no
752 clinical signs of motor deficits with normal clinical EMG examination in quadriceps, 3) absence of
753 peripheral neuropathy, and 4) no comorbid neurological conditions. Patients were screened and
754 tested for the 4 most common ALS-causing mutations (SOD1, FUS, C9orf72 and TDP43; DNA
755 extraction was performed by Genethon, Evry, France; DNA analysis was carried out at the University
756 of Tours, France), and all were negative except 2 (C9orf72 in patient #13 and SOD1 in patient #32;
757 Table 1). Table 1 resumes the main clinical features; MRC scores were those obtained on the
758 investigated side. Patients were explored on their less affected side which explains why some of
759 them had normal MRC score while the onset site was in lower limbs *i.e.*, on the non-investigated
760 side.

761 All participants were indeed tested on one side, preferably the dominant side¹⁰⁶. When
762 patients had motor deficits in quadriceps on the dominant side, we explored their non-dominant
763 side and, in both groups, the non-dominant side was tested in case of orthopaedic trauma on the
764 dominant side: *i*) right-handers tested on the right (dominant) side: 36 controls vs. 26 patients, *ii*)
765 left-handers tested on the left (dominant) side: 4 controls vs. 3 patients, *iii*) right-handers tested on
766 the left (non-dominant) side: 1 control vs. 8 patients, and *iv*) left-handers tested on the right (non-
767 dominant) side: 1 control vs. 1 patient.

768 **4.3 | Materials**

769 EMG activities were recorded using single-use bipolar surface electrodes (sticky foam

770 electrodes with solid gel; 2-cm apart; FIAB, Florence, Italy) that were secured on the skin, over i) the
771 vastus lateralis (VL) head of the quadriceps femoris, on the antero-lateral part of the thigh, ~15cm
772 above the patella and ii) the soleus, on the posterior part of the leg, ~5cm below the insertion of
773 gastrocnemius muscles. In our experience, H-reflex in quadriceps is larger when recording
774 electrodes are over VL head. However, in the participants in whom there was no H-reflex in VL EMG,
775 we tried unsuccessfully other electrode positions, on rectus femoris and vastus medialis. The signals
776 were amplified and filtered ($\times 1,000$ -5,000; 0.1-1kHz bandpass; D360 8-channel Patient Amplifier,
777 Digitimer Ltd, Hertfordshire, UK) before being digitally stored on a personal computer (2-kHz
778 sampling rate; Power 1401 controlled by Signal Software 6.05; CED, Cambridge, UK) for offline
779 analysis.

780 Percutaneous electrical stimulations (1-ms duration rectangular pulse; DS7A, Digitimer Ltd,
781 Hertfordshire, UK) were applied to the i) FN trough monopolar electrodes: cathode being a 21-cm²
782 brass plaque placed on the posterior aspect of the thigh (below the buttock) and anode, a 7-cm²
783 brass hemisphere placed in the femoral triangle, and ii) PTN with similar electrodes: the cathode
784 was placed above the patella and the anode, in the medial part of the popliteal fossa. Stimulating
785 electrodes were covered by wet sponge tissue and their positions were checked according to motor
786 response evoked in VL and soleus EMG, respectively, and clinically, by tendon palpation.

787 **4.4 | Experimental protocols**

788 The participants were comfortably seated in a reclining armchair, with head support, and the
789 tested leg was positioned in a device fixed to the chair and adaptable so that the hip was semi-flexed
790 (~80°), the knee semi-extended (~130°) and the ankle in semi-plantarflexion (~100°). The skin was
791 first cleaned using exfoliating cream before positioning recording electrodes. All during the
792 experimental protocol, the participants were asked to relax as much as possible and the recordings
793 were performed at rest. Experiment 1 was performed in all the participants and aimed at evaluating
794 the level of recurrent inhibition produced in quadriceps LMNs by activating recurrent collaterals of

795 soleus motor axons (Fig. 1A). Test stimuli were applied to FN to produce H-reflex in VL EMG and
796 conditioning stimuli, to PTN. First, the maximal amplitude of Mmax was evaluated in VL EMG by
797 testing FN stimuli at different intensities between H-reflex threshold and suprathreshold intensity
798 for Mmax (N = 5 stimuli/intensity; H/M recruitment curve). Then, the intensity of FN-test stimuli
799 was adjusted so as to produce a measurable and stable H-reflex in VL EMG. The intensity of PTN-
800 conditioning stimuli was adjusted at the threshold intensity for evoking Mmax in soleus EMG. The
801 effects of PTN-conditioning stimuli on quadriceps H-reflex were tested at 3 ISIs; the PTN-
802 conditioning stimuli being delivered 15, 20 and 25 ms before the FN-test stimuli *i.e.*, at the optimal
803 ISIs for producing recurrent inhibition in quadriceps LMNs^{44,61} (Figs. 1B-E; Fig. 5). Experiment 2 was
804 performed in the second half of each group: 17 ALS patients (2 women; mean age in the group: 62.7
805 \pm 9.5 years old) vs. 17 controls (3 women; 60.0 \pm 10.8 years old) during the same experimental
806 session as experiment 1. Basically, the experimental procedure followed the reverse design of
807 experiment 1: the test stimuli were applied to PTN and the conditioning to FN, to evaluate the level
808 of recurrent inhibition produced in soleus LMNs by activating quadriceps motor axon recurrent
809 collaterals (Fig. 3A). The intensity of PTN-test stimuli was adjusted to produce H-reflex of \sim 25% of
810 Mmax in soleus EMG⁴⁸, and that of FN-conditioning stimuli, at the threshold intensity for producing
811 Mmax in VL EMG. The ISIs between FN and PTN stimulations were set at 5, 10 and 15 ms *i.e.*, optimal
812 for recurrent inhibition in soleus LMNs^{44,60}. In both experiments, one run of acquisition consisted in
813 testing 1 ISI between conditioning and test stimuli, with 20 isolated test stimuli vs. 20 combined
814 (conditioning + test) stimuli randomly alternated (0.3-Hz stimulation frequency rate). The size of
815 conditioning Mmax was monitored throughout the experiment to ensure the stability of
816 conditioning stimuli (Fig. 5B).

817 **Figure 5 near here**

818 **4.5 | Analysis**

819 The peak-to-peak amplitude of H-reflexes (test EMG; Fig. 5A) and Mmax (conditioning EMG;

820 Fig. 5B) were evaluated; in Figure 5, VL EMG was the test EMG, and soleus EMG, the conditioning
 821 one. For each run of acquisition, we evaluated the mean amplitude of the test H-reflex, which was
 822 expressed as a % of the corresponding (test) Mmax for interindividual comparison. H-reflex in VL
 823 EMG can be hardly evoked without preceding M response (Figs. 1B, Fig. 5A), and both can overlap
 824 making it difficult their distinction especially at intensity producing $\sim H_{max}/2$ and above. A particular
 825 attention was thus taken to determine the amplitude of quadriceps H-reflex in each participant,
 826 according to their own H/M recruitment curve. The amplitude of each conditioned H-reflex was
 827 normalized to the mean amplitude of the test H-reflex. The mean difference between the mean test
 828 H-reflex and conditioned H-reflexes, expressed as a % of the mean test H-reflex, was calculated to
 829 evaluate the level of recurrent inhibition produced in the test LMN pool (Figs. 1DE).

830 UMN and LMN scores, and their difference (UMN – LMN scores), were calculated to determine
 831 whether the patients exhibited predominant UMN (difference > 0), or LMN (difference < 0) or a
 832 mixed form (difference = 0) at the time at inclusion:

833 - UMN score^{56,57} = reflex score (0, 1 or 2) + Babinski or Hoffmann sign (0 or 1) + Ashworth ≥ 3 (0 or
 834 1)

835 Here, the reflex score is based on tendon reflexes in soleus and quadriceps: score is 0 when reflexes
 836 were normal or absent, 1, when present in wasted muscle, and 2, when brisk. When Babinski reflex
 837 was absent, the score is 0 and 1 when present. If grade from the modified Ashworth scale was < 3,
 838 the score is 0, and if the grade was ≥ 3 (*i.e.*, with high possibility of muscle clonus), the score is 1.

839 - LMN score = atrophy (0 or 1) + fasciculation (0 or 1) + MRC (0, 1 or 2)

840 Here, when atrophy was absent, the score is 0, and 1 when present. When fasciculations were
 841 absent, the score is 0, and 1 when present. Lastly, when MRC grade was 5, the score is 0, when MRC
 842 was 4 or 3, the score is 1, lastly if MRC was between 2 and 0, the score is 2.

843 4.6 | Statistics

844 Statistical analyses were performed using JMP® Pro 16.0.0 (SAS Institute JMP, Brie Comte

845 Robert, France). The alpha significance level was fixed at 0.05 and the results were considered
846 statistically significant only if $p < 0.05$. Mean values are indicated ± 1 standard deviation (SD).

847 Descriptive data in groups of participants are illustrated using box plot charts (Figs. 2 and 3).
848 The lower limit of the box indicates the 25th percentile (1st quartile, Q1), the upper limit, the 75th
849 percentile (3rd quartile, Q3), the continuous line within the box, the median and the cross, the
850 mean. The lines that extend from the box (whiskers) are limited to minimum and maximum data
851 values; values above or below the end of the whiskers are outliers. Homoscedasticity (Levene
852 median test) and normality (Shapiro-Wilk test) were first verified to allow parametric analyses
853 (Student t test) to compare electrophysiological parameters and the level of maximal recurrent
854 inhibition between controls and ALS. Alternatively, Welch ANOVA (normal distribution with
855 heterogenous variances) or non-parametric methods were used (Kolmogorov-Smirnov test).
856 Outliers were detected using the inter-quantile range (IQR) method. Linear mixed models were built
857 and ANOVA were performed to test the difference between controls and ALS, taking into account
858 electrophysiological metrics significantly different between groups and that could have influenced
859 the level of recurrent inhibition (ISI, H-reflex and Mmax sizes in test and conditioning EMG). *Post*
860 *hoc* analyses were performed using Student tests. Effect size was measured using Cohen's d ^{49,50}
861 when we compared 2 means, and using f^2 ⁵⁴, when we performed multivariate analysis (linear mixed
862 model). Effect size is very small when d or $f^2 = 0$, small when $d = 0.2$ and $f^2 \geq 0.15$, medium when d
863 $= 0.5$ and $f^2 \geq 0.15$ and large when $d \geq 0.8$ and $f^2 \geq 0.35$.

864 Given the intrinsic differences in the size of electrophysiological metrics and the level of
865 recurrent inhibition between quadriceps and soleus^{45,46,51–53}, we calculated the CI95 in controls and
866 metrics in ALS were classified according to the lower and upper limits of CI95 in controls. Then, Chi²
867 tests were performed to compare the LMN pools in ALS. The resulting categorial data were also
868 used to evaluate the link between the modulation of recurrent inhibition in ALS and the patient
869 phenotype including their clinical and electrophysiological features. Chi² tests and correspondence

870 analyses were first performed to evaluate the influence of each parameter individually on the
871 modulation of recurrent inhibition. Then, multiple correspondence analysis (MCA) and Chi² tests
872 were undertaken to identify the associations between modalities of clinical and electrophysiological
873 parameters and the level of recurrent inhibition. Similar to other multivariate methods, MCA is a
874 dimension reducing method, representing the data as points in 2 or 3-D space (Figs. 4B and D). For
875 clarity, the statistical tests and the parameters included in each test are specifically indicated in
876 Results.

877 **ACKNOWLEDGEMENT**

878 This work was generously supported by grants from ARSLA (VMarchand/2013), AFM-Telethon
879 (DdT1 2015-2; CTL/SS/2016-0029/n°16597), and Fondation Thierry Latran (FTL AAP7/2015). During
880 his PhD supervised by V. Marchand-Pauvert, S. Sangari was supported by grants from The French
881 Ministry of Higher Education, Research and Innovation delivered by University Pierre et Marie Curie
882 / Paris 6 (now Sorbonne University; 2012-2015) and AFM-Téléthon (2015-2016). Similarly, the
883 authors wish to express their grateful to Dr. Patrick Vourc'h who performed the genetic analyses
884 (Dpt of Biochemistry and Molecular Biology, University of Tours, France). They also thank Pr. Rose
885 Katz for her valuable support and advices, and Drs. Alain Giron and Mélanie Péligrini-Issac for their
886 advices for statistical analysis. Finally, they also thank the Paris ALS referent centre and all the
887 participants.

888 **CONFLICT OF INTEREST**

889 The authors declare no conflict of interest.

890 **ORCID**

891 Eléonore Bayen: <https://orcid.org/0000-0002-0286-5521>

892 Véronique Marchand-Pauvert: <https://orcid.org/0000-0002-0226-7169>

893 Sangari Sina: <https://orcid.org/0000-0002-7800-9959>

894 **REFERENCES**

- 895 1 Gromicho M, Figueiral M, Uysal H et al. Spreading in ALS: The relative impact of upper and lower
896 motor neuron involvement. *Ann Clin Transl Neurol.* 2020;7(7):1181–1192.
- 897 2 Pender N, Pinto-Grau M, Hardiman O. Cognitive and behavioural impairment in amyotrophic
898 lateral sclerosis. *Curr Opin Neurol.* 2020;33(5):649–654.
- 899 3 Talbott EO, Malek AM, Lacomis D. The epidemiology of amyotrophic lateral sclerosis. *Handb Clin*
900 *Neurol.* 2016;138:225–238.
- 901 4 Masrori P, Van Damme P. Amyotrophic lateral sclerosis: a clinical review. *Eur J Neurol.*
902 2020;27(10):1918–1929.
- 903 5 Leroy F, Lamotte d’Incamps B, Imhoff-Manuel RD, Zytnicki D. Early intrinsic hyperexcitability does
904 not contribute to motoneuron degeneration in amyotrophic lateral sclerosis. *Elife.* 2014;3.
905 doi:10.7554/eLife.04046.
- 906 6 Delestrée N, Manuel M, Iglesias C, Elbasiouny SM, Heckman CJ, Zytnicki D. Adult spinal
907 motoneurons are not hyperexcitable in a mouse model of inherited amyotrophic lateral
908 sclerosis. *J Physiol (Lond).* 2014;592(7):1687–1703.
- 909 7 Fogarty MJ, Mu EWH, Noakes PG, Lavidis NA, Bellingham MC. Marked changes in dendritic
910 structure and spine density precede significant neuronal death in vulnerable cortical pyramidal
911 neuron populations in the SOD1(G93A) mouse model of amyotrophic lateral sclerosis. *Acta*
912 *Neuropathol Commun.* 2016;4(1):77.
- 913 8 Ozdinler PH, Benn S, Yamamoto TH, Güzel M, Brown RH, Macklis JD. Corticospinal motor neurons
914 and related subcerebral projection neurons undergo early and specific neurodegeneration in
915 hSOD1^{G93A} transgenic ALS mice. *J Neurosci.* 2011;31(11):4166–4177.
- 916 9 Neuwirth C, Barkhaus PE, Burkhardt C et al. Motor Unit Number Index (MUNIX) detects motor
917 neuron loss in pre-symptomatic muscles in Amyotrophic Lateral Sclerosis. *Clin Neurophysiol.*
918 2017;128(3):495–500.
- 919 10 De Vocht J, Blommaert J, Devrome M et al. Use of Multimodal Imaging and Clinical
920 Biomarkers in Presymptomatic Carriers of C9orf72 Repeat Expansion. *JAMA Neurol.*
921 2020;77(8):1008–1017.
- 922 11 Vucic S, Nicholson GA, Kiernan MC. Cortical hyperexcitability may precede the onset of
923 familial amyotrophic lateral sclerosis. *Brain.* 2008;131(Pt 6):1540–1550.
- 924 12 Harten ACMV, Phatnani H, Przedborski S. Non-cell-autonomous pathogenic mechanisms in
925 amyotrophic lateral sclerosis. *Trends in Neurosciences.* 2021;0(0).
926 doi:10.1016/j.tins.2021.04.008.
- 927 13 Kiernan MC, Ziemann U, Eisen A. Amyotrophic lateral sclerosis: Origins traced to impaired
928 balance between neural excitation and inhibition in the neonatal period. *Muscle Nerve.*
929 2019;60(3):232–235.
- 930 14 Falgairolle M, O’Donovan MJ. Motoneuronal Spinal Circuits in Degenerative Motoneuron
931 Disease. *Front Mol Neurosci.* 2020;13:74.
- 932 15 Fogarty MJ. Driven to decay: Excitability and synaptic abnormalities in amyotrophic lateral
933 sclerosis. *Brain Res Bull.* 2018;140:318–333.
- 934 16 Brownstone RM, Lancelin C. Escape from homeostasis: spinal microcircuits and progression
935 of amyotrophic lateral sclerosis. *J Neurophysiol.* 2018;119(5):1782–1794.
- 936 17 Oyanagi K, Ikuta F, Horikawa Y. Evidence for sequential degeneration of the neurons in the
937 intermediate zone of the spinal cord in amyotrophic lateral sclerosis: a topographic and
938 quantitative investigation. *Acta Neuropathol.* 1989;77(4):343–349.

- 939 18 Oyanagi K, Makifuchi T, Ikuta F. The anterolateral funiculus in the spinal cord in amyotrophic
940 lateral sclerosis. *Acta Neuropathol.* 1995;90(3):221–227.
- 941 19 Stephens B, Guiloff RJ, Navarrete R, Newman P, Nikhar N, Lewis P. Widespread loss of
942 neuronal populations in the spinal ventral horn in sporadic motor neuron disease. A
943 morphometric study. *J Neurol Sci.* 2006;244(1–2):41–58.
- 944 20 Martin LJ, Liu Z, Chen K et al. Motor neuron degeneration in amyotrophic lateral sclerosis
945 mutant superoxide dismutase-1 transgenic mice: mechanisms of mitochondriopathy and cell
946 death. *J Comp Neurol.* 2007;500(1):20–46.
- 947 21 Chang Q, Martin LJ. Glycinergic innervation of motoneurons is deficient in amyotrophic
948 lateral sclerosis mice: a quantitative confocal analysis. *Am J Pathol.* 2009;174(2):574–585.
- 949 22 Martin LJ, Chang Q. Inhibitory synaptic regulation of motoneurons: a new target of disease
950 mechanisms in amyotrophic lateral sclerosis. *Mol Neurobiol.* 2012;45(1):30–42.
- 951 23 Lim SM, Guiloff RJ, Navarrete R. Interneuronal survival and calbindin-D28k expression
952 following motoneuron degeneration. *J Neurol Sci.* 2000;180(1–2):46–51.
- 953 24 Frey D, Schneider C, Xu L, Borg J, Spooren W, Caroni P. Early and selective loss of
954 neuromuscular synapse subtypes with low sprouting competence in motoneuron diseases. *J*
955 *Neurosci.* 2000;20(7):2534–2542.
- 956 25 Schaefer AM, Sanes JR, Lichtman JW. A compensatory subpopulation of motor neurons in a
957 mouse model of amyotrophic lateral sclerosis. *J Comp Neurol.* 2005;490(3):209–219.
- 958 26 De Winter F, Vo T, Stam FJ et al. The expression of the chemorepellent Semaphorin 3A is
959 selectively induced in terminal Schwann cells of a subset of neuromuscular synapses that display
960 limited anatomical plasticity and enhanced vulnerability in motor neuron disease. *Mol Cell*
961 *Neurosci.* 2006;32(1–2):102–117.
- 962 27 Pun S, Santos AF, Saxena S, Xu L, Caroni P. Selective vulnerability and pruning of phasic
963 motoneuron axons in motoneuron disease alleviated by CNTF. *Nat Neurosci.* 2006;9(3):408–419.
- 964 28 Hegedus J, Putman CT, Gordon T. Time course of preferential motor unit loss in the SOD1
965 G93A mouse model of amyotrophic lateral sclerosis. *Neurobiol Dis.* 2007;28(2):154–164.
- 966 29 Allodi I, Montaña-Rosell R, Selvan R, Löw P, Kiehn O. Locomotor deficits in a mouse model
967 of ALS are paralleled by loss of V1-interneuron connections onto fast motor neurons. *Nat*
968 *Commun.* 2021;12(1):3251.
- 969 30 Jankowska E. Interneuronal relay in spinal pathways from proprioceptors. *Prog Neurobiol.*
970 1992;38(4):335–378.
- 971 31 Schneider SP, Fyffe RE. Involvement of GABA and glycine in recurrent inhibition of spinal
972 motoneurons. *J Neurophysiol.* 1992;68(2):397–406.
- 973 32 González-Forero D, Alvarez FJ. Differential postnatal maturation of GABAA, glycine receptor,
974 and mixed synaptic currents in Renshaw cells and ventral spinal interneurons. *J Neurosci.*
975 2005;25(8):2010–2023.
- 976 33 Alaynick WA, Jessell TM, Pfaff SL. SnapShot: spinal cord development. *Cell.* 2011;146(1):178-
977 178.e1.
- 978 34 Wootz H, Fitzsimons-Kantamneni E, Larhammar M et al. Alterations in the motor neuron-
979 renschow cell circuit in the Sod1(G93A) mouse model. *J Comp Neurol.* 2013;521(7):1449–1469.
- 980 35 Ramírez-Jarquín UN, Lazo-Gómez R, Tovar-Y-Romo LB, Tapia R. Spinal inhibitory circuits and
981 their role in motor neuron degeneration. *Neuropharmacology.* 2014;82:101–107.
- 982 36 Raynor EM, Shefner JM. Recurrent inhibition is decreased in patients with amyotrophic
983 lateral sclerosis. *Neurology.* 1994;44(11):2148–2153.
- 984 37 Özyurt MG, Topkara B, İşak B, Türker KS. Amyotrophic lateral sclerosis weakens spinal
985 recurrent inhibition and post-activation depression. *Clin Neurophysiol.* 2020;131(12):2875–2886.
- 986 38 Casas C, Herrando-Grabulosa M, Manzano R, Mancuso R, Osta R, Navarro X. Early
987 presymptomatic cholinergic dysfunction in a murine model of amyotrophic lateral sclerosis. *Brain*

- 988 *Behav.* 2013;3(2):145–158.
- 989 39 Mazzocchio R, Rossi A. Role of Renshaw cells in amyotrophic lateral sclerosis. *Muscle Nerve.*
- 990 2010;41(4):441–443.
- 991 40 Shefner JM, Logigian EL. The mixed nerve silent period in normal subjects and patients with
- 992 amyotrophic lateral sclerosis. *Electromyogr Clin Neurophysiol.* 1998;38(8):505–510.
- 993 41 Rossi A, Mazzocchio R. Presence of homonymous recurrent inhibition in motoneurons
- 994 supplying different lower limb muscles in humans. *Exp Brain Res.* 1991;84(2):367–373.
- 995 42 Katz R, Mazzocchio R, Pénicaud A, Rossi A. Distribution of recurrent inhibition in the human
- 996 upper limb. *Acta Physiol Scand.* 1993;149(2):183–198.
- 997 43 Drory VE, Kovach I, Groozman GB. Electrophysiologic evaluation of upper motor neuron
- 998 involvement in amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Other Motor Neuron*
- 999 *Disord.* 2001;2(3):147–152.
- 1000 44 Pierrot-Deseilligny E, Burke D. Recurrent Inhibition (Chapter 4). In: *The circuitry of the human*
- 1001 *spinal cord.* Cambridge University Press: New York, USA, 2005, pp 151–196.
- 1002 45 Katz R, Pierrot-Deseilligny E. Recurrent inhibition in humans. *Prog Neurobiol.*
- 1003 1999;57(3):325–355.
- 1004 46 Meunier S, Pierrot-Deseilligny E, Simonetta-Moreau M. Pattern of heteronymous recurrent
- 1005 inhibition in the human lower limb. *Exp Brain Res.* 1994;102(1):149–159.
- 1006 47 Meunier S, Penicaud A, Pierrot-Deseilligny E, Rossi A. Monosynaptic Ia excitation and
- 1007 recurrent inhibition from quadriceps to ankle flexors and extensors in man. *J Physiol.*
- 1008 1990;423:661–675.
- 1009 48 Crone C, Hultborn H, Mazières L, Morin C, Nielsen J, Pierrot-Deseilligny E. Sensitivity of
- 1010 monosynaptic test reflexes to facilitation and inhibition as a function of the test reflex size: a
- 1011 study in man and the cat. *Exp Brain Res.* 1990;81(1):35–45.
- 1012 49 Cohen J. *Statistical power analysis for the behavioral sciences.* 2nd ed. Lawrence Erlbaum
- 1013 Associates: Hillsdale, NJ, 1988.
- 1014 50 Rosnow R, Rosenthal R. Computing contrasts, effect sizes, and counternulls on other
- 1015 people's published data: General procedures for research consumers. *Psychological methods.*
- 1016 1996;1(4):331.
- 1017 51 Marchand-Pauvert V. Suppression of the H reflex in humans by disynaptic autogenetic
- 1018 inhibitory pathways activated by the test volley. *The Journal of physiology.* 2002.
- 1019 doi:10.1113/jphysiol.2002.021683.
- 1020 52 Iglesias C, Nielsen JB, Marchand-Pauvert V. Corticospinal inhibition of transmission in
- 1021 propriospinal-like neurones during human walking. *Eur J Neurosci.* 2008;28(7):1351–1361.
- 1022 53 Marchand-Pauvert V. Beyond muscular effects: depression of spinal recurrent inhibition
- 1023 after botulinum neurotoxin A. *The Journal of physiology.* 2013.
- 1024 doi:10.1113/jphysiol.2012.239178.
- 1025 54 Selya AS, Rose JS, Dierker LC, Hedeker D, Mermelstein RJ. A Practical Guide to Calculating
- 1026 Cohen's $f(2)$, a Measure of Local Effect Size, from PROC MIXED. *Front Psychol.* 2012;3:111.
- 1027 55 Cedarbaum JM, Stambler N, Malta E et al. The ALSFRS-R: a revised ALS functional rating scale
- 1028 that incorporates assessments of respiratory function. BDNF ALS Study Group (Phase III). *J Neurol*
- 1029 *Sci.* 1999;169(1–2):13–21.
- 1030 56 Simon NG, Lin CS-Y, Lee M et al. Segmental motoneuronal dysfunction is a feature of
- 1031 amyotrophic lateral sclerosis. *Clin Neurophysiol.* 2015;126(4):828–836.
- 1032 57 Marchand-Pauvert V, Peyre I, Lackmy-Vallee A et al. Absence of hyperexcitability of spinal
- 1033 motoneurons in patients with amyotrophic lateral sclerosis. *J Physiol.* 2019;597(22):5445–5467.
- 1034 58 de Carvalho M. Electrodiagnosis of Amyotrophic Lateral Sclerosis: A Review of Existing
- 1035 Guidelines. *J Clin Neurophysiol.* 2020;37(4):294–298.
- 1036 59 Gunes T, Sirin NG, Sahin S, Kose E, Isak B. Use of CMAP, MScan fit-MUNE, and MUNIX in

- 1037 understanding neurodegeneration pattern of ALS and detection of early motor neuron loss in
 1038 daily practice. *Neurosci Lett*. 2021;741:135488.
- 1039 60 Meunier S, Mogyoros I, Kiernan MC, Burke D. Effects of femoral nerve stimulation on the
 1040 electromyogram and reflex excitability of tibialis anterior and soleus. *Muscle Nerve*.
 1041 1996;19(9):1110–1115.
- 1042 61 Iles JF, Pardoe J. Changes in transmission in the pathway of heteronymous spinal recurrent
 1043 inhibition from soleus to quadriceps motor neurons during movement in man. *Brain*. 1999;122 (Pt 9):1757–1764.
- 1044 62 Barbeau H, Marchand-Pauvert V, Meunier S, Nicolas G, Pierrot-Deseilligny E. Posture-related
 1045 changes in heteronymous recurrent inhibition from quadriceps to ankle muscles in humans. *Exp Brain Res*. 2000;130(3):345–361.
- 1046 63 Bussel B, Pierrot-Deseilligny E. Inhibition of human motoneurons, probably of Renshaw
 1047 origin, elicited by an orthodromic motor discharge. *J Physiol*. 1977;269(2):319–339.
- 1048 64 Hultborn H, Pierrot-Deseilligny E, Wigström H. Recurrent inhibition and
 1049 afterhyperpolarization following motoneuronal discharge in the cat. *J Physiol*. 1979;297(0):253–
 1050 266.
- 1051 65 Piotrkiewicz M, Hausmanowa-Petrusewicz I. Motoneuron afterhyperpolarisation duration in
 1052 amyotrophic lateral sclerosis. *J Physiol (Lond)*. 2011;589(Pt 11):2745–2754.
- 1053 66 Hultborn H, Meunier S, Morin C, Pierrot-Deseilligny E. Assessing changes in presynaptic
 1054 inhibition of Ia fibres: a study in man and the cat. *J Physiol*. 1987;389:729–756.
- 1055 67 Meunier S, Pierrot-Deseilligny E. Cortical control of presynaptic inhibition of Ia afferents in
 1056 humans. *Exp Brain Res*. 1998;119(4):415–426.
- 1057 68 Howells J, Sangari S, Matamala JM, Kiernan MC, Marchand-Pauvert V, Burke D. Interrogating
 1058 interneurone function using threshold tracking of the H reflex in healthy subjects and patients
 1059 with motor neurone disease. *Clin Neurophysiol*. 2020;131(8):1986–1996.
- 1060 69 Schieppati M, Poloni M, Nardone A. Voluntary muscle release is not accompanied by H-reflex
 1061 inhibition in patients with upper moto neuron lesions. *Neurosci Lett*. 1985;61(1–2):177–181.
- 1062 70 Morin C, Pierrot-Deseilligny E. [Spinal mechanism of the antispastic action of TRH in patients
 1063 with amyotrophic lateral sclerosis]. *Rev Neurol (Paris)*. 1988;144(11):701–703.
- 1064 71 Sangari S, Iglesias C, El Mendili M-M, Benali H, Pradat P-F, Marchand-Pauvert V. Impairment
 1065 of sensory-motor integration at spinal level in amyotrophic lateral sclerosis. *Clin Neurophysiol*.
 1066 2016;127(4):1968–1977.
- 1067 72 Hongo T, Lundberg A, Phillips CG, Thompson RF. The pattern of monosynaptic Ia-connections
 1068 to hindlimb motor nuclei in the baboon: a comparison with the cat. *Proc R Soc Lond B Biol Sci*.
 1069 1984;221(1224):261–289.
- 1070 73 Baldissera F, Hultborn H, Illert M. Integration in spinal neuronal systems. In: *Handbook of*
 1071 *Physiology*. American Physiological Society: Bethesda, MA, US, 1981, pp 508–595.
- 1072 74 Baret M, Katz R, Lamy JC, Pénicaud A, Wargon I. Evidence for recurrent inhibition of
 1073 reciprocal inhibition from soleus to tibialis anterior in man. *Exp Brain Res*. 2003;152(1):133–136.
- 1074 75 Mazzocchio R, Rossi A, Rothwell JC. Depression of Renshaw recurrent inhibition by activation
 1075 of corticospinal fibres in human upper and lower limb. *J Physiol*. 1994;481 (Pt 2):487–498.
- 1076 76 Iles JF. Recruitment of the vastus lateralis motor pool by corticospinal volleys in man during
 1077 the activation of recurrent inhibition. *J Physiol*. 1996;494:P65.
- 1078 77 Hultborn H, Pierrot-Deseilligny E. Changes in recurrent inhibition during voluntary soleus
 1079 contractions in man studied by an H-reflex technique. *J Physiol*. 1979;297(0):229–251.
- 1080 78 Hultborn H, Brownstone RB, Toth TI, Gossard J-P. Key mechanisms for setting the input-
 1081 output gain across the motoneuron pool. *Prog Brain Res*. 2004;143:77–95.
- 1082 79 Alvarez FJ, Jonas PC, Sapir T et al. Postnatal phenotype and localization of spinal cord V1
 1083 derived interneurons. *J Comp Neurol*. 2005;493(2):177–192.
- 1084
 1085

- 1086 80 Özyurt MG, Shabsog M, Dursun M, Türker KS. Optimal location for eliciting the tibial H-reflex
1087 and motor response. *Muscle Nerve*. 2018;58(6):828–833.
- 1088 81 Özyurt MG, Piotrkiewicz M, Topkara B, Weisskircher H-W, Türker KS. Motor units as tools to
1089 evaluate profile of human Renshaw inhibition. *J Physiol*. 2019;597(8):2185–2199.
- 1090 82 Querin G, Bede P, El Mendili MM et al. Presymptomatic spinal cord pathology in c9orf72
1091 mutation carriers: A longitudinal neuroimaging study. *Ann Neurol*. 2019;86(2):158–167.
- 1092 83 Dentel C, Palamiuc L, Henriques A et al. Degeneration of serotonergic neurons in
1093 amyotrophic lateral sclerosis: a link to spasticity. *Brain*. 2013;136(Pt 2):483–493.
- 1094 84 Katz R, Pierrot-Deseilligny E. Recurrent inhibition of alpha-motoneurons in patients with
1095 upper motor neuron lesions. *Brain*. 1982;105(Pt 1):103–124.
- 1096 85 Shefner JM, Berman SA, Sarkarati M, Young RR. Recurrent inhibition is increased in patients
1097 with spinal cord injury. *Neurology*. 1992;42(11):2162–2168.
- 1098 86 Rossi A, Decchi B, Vecchione V. Supraspinal influences on recurrent inhibition in humans.
1099 Paralysis of descending control of Renshaw cells in patients with mental retardation.
1100 *Electroencephalogr Clin Neurophysiol*. 1992;85(6):419–424.
- 1101 87 Mazzocchio R, Schieppati M, Scarpini C, Rossi A. Enhancement of recurrent inhibition by
1102 intravenous administration of L-acetylcarnitine in spastic patients. *J Neurol Neurosurg Psychiatry*.
1103 1990;53(4):321–326.
- 1104 88 Mazzocchio R, Rossi A. Involvement of spinal recurrent inhibition in spasticity. Further insight
1105 into the regulation of Renshaw cell activity. *Brain*. 1997;120 (Pt 6):991–1003.
- 1106 89 Floeter MK, Andermann F, Andermann E, Nigro M, Hallett M. Physiological studies of spinal
1107 inhibitory pathways in patients with hereditary hyperekplexia. *Neurology*. 1996;46(3):766–772.
- 1108 90 Windhorst U. On the role of recurrent inhibitory feedback in motor control. *Prog Neurobiol*.
1109 1996;49(6):517–587.
- 1110 91 Iglesias C, Sangari S, El Mendili M-M, Benali H, Marchand-Pauvert V, Pradat P-F.
1111 Electrophysiological and spinal imaging evidences for sensory dysfunction in amyotrophic lateral
1112 sclerosis. *BMJ Open*. 2015;5(2):e007659.
- 1113 92 Vaughan SK, Kemp Z, Hatzipetros T, Vieira F, Valdez G. Degeneration of proprioceptive
1114 sensory nerve endings in mice harboring amyotrophic lateral sclerosis-causing mutations. *J Comp
1115 Neurol*. 2015;523(17):2477–2494.
- 1116 93 Marchand-Pauvert V. Interrogating interneurone function using threshold tracking of the H
1117 reflex in healthy subjects and patients with motor neurone disease. *Clinical neurophysiology :
1118 official journal of the International Federation of Clinical Neurophysiology*. 2020.
1119 doi:10.1016/j.clinph.2020.03.028.
- 1120 94 Bruneteau G, Simonet T, Bauché S et al. Muscle histone deacetylase 4 upregulation in
1121 amyotrophic lateral sclerosis: potential role in reinnervation ability and disease progression.
1122 *Brain*. 2013;136(Pt 8):2359–2368.
- 1123 95 Bruneteau G, Bauché S, Gonzalez de Aguilar JL et al. Endplate denervation correlates with
1124 Nogo-A muscle expression in amyotrophic lateral sclerosis patients. *Ann Clin Transl Neurol*.
1125 2015;2(4):362–372.
- 1126 96 de Carvalho M, Turkman A, Swash M. Sensitivity of MUP parameters in detecting change in
1127 early ALS. *Clin Neurophysiol*. 2014;125(1):166–169.
- 1128 97 Devlin A-C, Burr K, Borooah S et al. Human iPSC-derived motoneurons harbouring TARDBP
1129 or C9ORF72 ALS mutations are dysfunctional despite maintaining viability. *Nat Commun*.
1130 2015;6:5999.
- 1131 98 Kuo S-W, Binder MD, Heckman CJ. Excessive Homeostatic Gain in Spinal Motoneurons in a
1132 Mouse Model of Amyotrophic Lateral Sclerosis. *Sci Rep*. 2020;10(1):9049.
- 1133 99 Venugopal S, Hamm TM, Crook SM, Jung R. Modulation of inhibitory strength and kinetics
1134 facilitates regulation of persistent inward currents and motoneuron excitability following spinal

- 1135 cord injury. *J Neurophysiol.* 2011;106(5):2167–2179.
- 1136 100 Fornai F, Longone P, Cafaro L et al. Lithium delays progression of amyotrophic lateral
1137 sclerosis. *Proc Natl Acad Sci U S A.* 2008;105(6):2052–2057.
- 1138 101 Pasquali L, Longone P, Isidoro C, Ruggieri S, Paparelli A, Fornai F. Autophagy, lithium, and
1139 amyotrophic lateral sclerosis. *Muscle Nerve.* 2009;40(2):173–194.
- 1140 102 Gamez J, Salvado M, Martínez de la Ossa A, Badia M. Lithium for treatment of amyotrophic
1141 lateral sclerosis: much ado about nothing. *Neurologia.* 2016;31(8):550–561.
- 1142 103 Sasaki K. Electrophysiological studies on oculomotor neurons of the cat. *Jpn J Physiol.*
1143 1963;13:287–302.
- 1144 104 Simonetta-Moreau M, Marque P, Marchand-Pauvert V, Pierrot-Deseilligny E. The pattern of
1145 excitation of human lower limb motoneurons by probable group II muscle afferents. *J Physiol*
1146 *(Lond).* 1999;517(1):287–300.
- 1147 105 Brooks BR, Miller RG, Swash M, Munsat TL, World Federation of Neurology Research Group
1148 on Motor Neuron Diseases. El Escorial revisited: revised criteria for the diagnosis of amyotrophic
1149 lateral sclerosis. *Amyotroph Lateral Scler Other Motor Neuron Disord.* 2000;1(5):293–299.
- 1150 106 Oldfield RC. The assessment and analysis of handedness: the Edinburgh inventory.
1151 *Neuropsychologia.* 1971;9(1):97–113.

1152

1153

1154 TABLES

1155 Table 1: Clinical features

	Site of Onset	Duration	ALSFRS-r				UMN score	LMN score	Predominant form	MRC				
			total	sub-score	Progression	LL-progression				Soleus	TA	EHL	Quadriceps	Riluzole
1	UL	11	45	7	0,27	0,09	2	0	UMN	5	5	5	5	x
2	UL	72	40	4	0,11	0,06	1	2	LMN	3	4	3	5	x
3	UL	15	40	6	0,53	0,13	2	1	UMN	5	5	5	5	x
4	UL	28	40	3	0,29	0,18	4	2	UMN	5	2	3	5	x
5	LL	15	36	3	0,80	0,33	0	2	UMN	5	5	5	5	-
6	LL	16	44	8	0,25	0,00	0	0	Mixed	5	5	5	5	x
7	UL	17	45	8	0,18	0,00	0	0	Mixed	5	5	5	5	x
8	LL	5	36	6	2,40	0,40	0	0	Mixed	5	5	5	5	x
9	LL	21	39	4	0,43	0,19	2	1	UMN	5	3	5	5	x
10	LL	26	38	3	0,38	0,19	3	1	UMN	5	5	4	5	x
11	LL	14	43	4	0,36	0,29	3	2	UMN	5	5	5	5	x
12	UL	9	39	8	1,00	0,00	0	0	Mixed	5	5	5	5	-
13*	LL	6	47	7	0,17	0,17	2	2	Mixed	5	5	5	5	-
14	Bulbar	7	44	6	0,57	0,29	3	0	UMN	5	5	5	5	-
15	UL	25	39	8	0,36	0,00	0	0	Mixed	5	5	5	5	x
16	UL	16	45	8	0,19	0,00	0	0	Mixed	5	5	5	5	-
17	LL	24	41	3	0,29	0,21	0	2	LMN	5	1	0	5	x
18	Bulbar	23	36	5	0,52	0,13	0	1	LMN	5	5	5	5	x
19	LL	20	42	4	0,30	0,20	4	0	UMN	5	5	5	5	x
20	UL	14	40	6	0,57	0,14	3	1	UMN	5	4	5	5	-
21	UL	13	47	8	0,08	0,00	2	0	UMN	5	5	5	5	-
22	UL	18	40	7	0,44	0,06	2	0	UMN	5	5	5	5	x
23	LL	7	47	7	0,14	0,14	0	1	LMN	5	3	3	5	x
24	UL	59	34	5	0,24	0,05	3	1	UMN	5	5	4	5	x
25	UL	14	33	7	1,07	0,07	2	0	UMN	5	5	5	5	x
26	UL	22	38	7	0,45	0,05	2	1	UMN	5	5	5	5	x
27	Bulbar	33	39	4	0,27	0,12	0	0	Mixed	5	5	5	5	x
28	Bulbar	14	24	3	1,71	0,36	3	1	UMN	5	5	5	5	x
29	LL	11	34	3	1,27	0,45	3	1	UMN	5	3	4	5	x
30	Bulbar	48	43	8	0,10	0,00	3	0	UMN	5	5	5	5	x
31	LL	14	38	3	0,71	0,36	3	0	UMN	5	4	3	5	x
32*	LL	12	42	3	0,50	0,42	2	2	Mixed	5	5	5	5	x
33	UL	7	42	8	0,86	0,00	3	0	UMN	5	5	5	5	x
34	UL	7	44	8	0,57	0,00	3	1	UMN	5	5	5	5	x
35	LL	41	39	2	0,22	0,15	0	4	LMN	2	0	0	5	x
36	Bulbar	63	36	4	0,19	0,06	4	0	UMN	5	5	3	5	x
37	UL	46	41	6	0,15	0,04	0	0	Mixed	5	5	5	5	x
38	Bulbar	13	41	3	0,54	0,38	2	1	UMN	5	5	3	5	x

1156

1157 Site of onset: location of first symptoms in upper limb (UL), lower limb (LL) or bulbar LMNs (Bulbar);

1158 Duration: time since first symptoms (months); ALSFRS-r: total score (maximal score = 48) and sub-

1159 score for lower limb functions (walking and climbing stairs; maximal score = 8 indicated in bold);

1160 Progression: lost points to ALSFRS-r/month; LL-progression: lost points to ALSFRS-r sub-score for

1161 lower limbs (LL)/months; UMN: upper motor neuron score (see Methods); LMN: lower motor

1162 neuron score (see Methods); Predominant form in UMN, or LMN, or mixed form, according to the

1163 difference between UMN and LMN scores. MRC: muscle strength evaluated on the investigated

1164 limb, in soleus, tibialis anterior (TA), extensor hallucis longus (EHL) and quadriceps; Taking riluzole

1165 (x) or not (-). * Patients with genetic mutation: C9orf72 in patient #13 and SOD1 in patient #32.

1166

1167 **Table 2: Electrophysiological profile of the patient group**

		Quadriceps	Soleus
H-reflex	< lower limit	8/38 21.0 % 6.2 ± 1.0 %	0/17 0 % -
	Within CI95	6/38 15.8 % 12.1 ± 2.4 %	5/17 29.4 % 20.3 ± 2.6 %
	> upper limit	24/38 63.2 % 30.2 ± 13.8 %	12/17 70.6 % 53.8 ± 18.3 %
Mmax	Not depressed	23/38 60.5 % 2.3 ± 1.3 mV	10/38 26.3 % 5.4 ± 1.5 mV
	Depressed	15/38 39.5 % 0.8 ± 0.2 mV	28/38 73.7 % 2.0 ± 0.9 mV

1168 CI95s of the mean H-reflex and Mmax in quadriceps and soleus were calculated in the group of
1169 controls. Row 1 indicates the title of the column corresponding to the results for quadriceps and for
1170 soleus. Row 2 indicates when H-reflex was below the CI95 lower limit, row 3, when it was within the
1171 CI95 and row 4, when it was above the CI95 upper limit. Row 5 indicates when Mmax was not
1172 depressed (within or above the CI95 upper limit) and row 6, when it was depressed (below the CI95
1173 lower limit). In each cell, rows 1 and 2 indicate the corresponding proportion of patients (ratio
1174 between the number of patients with results corresponding to the cell and the total number of
1175 patients and below, the corresponding %), and row 3 indicates the mean size of the response ± 1 SD
1176 (in % of Mmax for H-reflex and in mV for Mmax).

1177

1178 **Table 3: Link between modulation of recurrent inhibition and patients features**

	r^2	p value	Correspondence
Motoneuron pool[†]	0.15	0.0012	<ul style="list-style-type: none"> • ↑ RI in quadriceps • ↔/↓ RI in soleus
Test Mmax[†]	0.09	0.0055	<ul style="list-style-type: none"> • ↔ Mmax: ↑ RI • ↓ Mmax: ↔/↓ RI
Progression[†]	0.08	0.0093	<ul style="list-style-type: none"> • Fast progressors: ↔ RI
Predominant form	0.07	0.0929	<ul style="list-style-type: none"> • UMN/mixed: ↑/↔ RI
H-reflex in conditioning muscle	0.04	0.5019	<ul style="list-style-type: none"> • ↑ H-reflex: ↔ RI
ALSFRS-r sub-score (lower limb functions [LL])	0.03	0.1397	<ul style="list-style-type: none"> • Score ≥ 6: ↑ RI • Score < 6: ↓ RI
LL-Progression	0.02	0.3239	<ul style="list-style-type: none"> • ↑ RI in slow progressors • ↔/↓ RI in fast progressors
Site of onset	0.02	0.3415	<ul style="list-style-type: none"> • LL: ↓ RI • Other: ↔/↑ RI
H-reflex in test muscle	0.02	0.7303	None
Conditioning Mmax	0.01	0.4304	<ul style="list-style-type: none"> • ↔ Mmax: ↔ RI
Total ALSFRS-r	0.005	0.7572	<ul style="list-style-type: none"> • Score ≥ 40: ↑/↓ RI • Score < 40: ↔ RI
Duration	0.002	0.8579	<ul style="list-style-type: none"> • 1st year: ↑ RI • > 1 year: ↔/↓ RI
Riluzole	0.002	0.8792	<ul style="list-style-type: none"> • On riluzole: ↔/↓ RI

1179 Column 1: patient parameters tested to evaluate their relationship with the modulation of recurrent
1180 inhibition (RI) expressed according to CI95 in controls. Column 2: r^2 indicating the strength of the
1181 regression. Column 3: p value (Chi² test). Column 4: result of the correspondence analysis: ↑ for
1182 increase (value superior to the upper limit of the CI95 in controls), ↓ for decrease (value inferior to
1183 the lower limit of the CI95 in controls) and ↔ for value within the CI95 in controls. [†] $p < 0.01$ and [†]
1184 $p < 0.001$.

1185 **LEGENDS TO FIGURES**

1186 **Figure 1: Recurrent inhibition from soleus to quadriceps. A**, Schematic representation of recurrent
1187 collaterals of soleus motor axons projecting onto Renshaw cells mediating recurrent inhibition to
1188 quadriceps LMNs. Dashed arrows indicate the trajectory of antidromic volley in soleus motor axons
1189 after PTN stimulation. Grey line represents group Ia afferent inputs after FN stimulation, mediating

1190 monosynaptic excitation to quadriceps LMNs producing H-reflex in VL EMG. **BC**, Superimposition of
 1191 mean VL EMG after isolated test FN stimulation (Test, black line) and after combined stimuli
 1192 (conditioned PTN + test FN stimuli) delivered at 15-ms ISI (grey line), 20-ms ISI (blue line) and 25-ms
 1193 ISI (red line) in one control (**B**) and one patient (**C**; N = 20 stimuli in each condition). **DE**, The mean
 1194 inhibition (= the mean difference between the mean test H-reflex and the conditioned H-reflexes in
 1195 VL EMG, expressed as % of the mean test H-reflex) produced in quadriceps LMNs in the same control
 1196 (**D**) and the same patient (**E**) as in **BC**, is plotted against the ISI (ms) between PTN (conditioning) and
 1197 FN (test) stimuli. **FG**, Mean amplitude of Mmax (mV) produced in quadriceps (VL head; left column)
 1198 and soleus EMG (right column) by FN (test) and PTN (conditioning), respectively, in the same control
 1199 (white columns; **F**) and the same patient (blue columns; **G**) as illustrated in (**BD**) and (**CE**),
 1200 respectively. Vertical bars are ± 1 SD. * $p < 0.05$, † $p < 0.01$ and ‡ $p < 0.001$.

1201 **Figure 2: Modulation of recurrent inhibition in quadriceps. A**, Box plots illustrating the distribution
 1202 of Mmax amplitude produced in VL (quadriceps, left part) and soleus EMG (right part; mV) in the
 1203 group of controls (white box and black diamonds; N = 42 participants) and patients with ALS (blue
 1204 box and black diamonds; N = 38 participants). The lower limit of the box indicates the 25th percentile
 1205 (1st quartile, Q1), the upper limit, the 75th percentile (3rd quartile, Q3), the continuous line within
 1206 the box, the median and the cross, the mean. The lines that extend from the box (whiskers) are
 1207 limited to minimum and maximum data values; values above or below the end of the whiskers are
 1208 outliers. **B**, Box plots representing the mean amplitude of test H-reflex in VL EMG (% Mmax in VL
 1209 EMG; same legend as in **A**) in controls (white box on the left) and ALS (blue box on the right). **C**, Box
 1210 plots (as in **ABC**) illustrating the distribution of recurrent inhibition (% of mean test H-reflex) in both
 1211 groups at the ISIs 15, 20 and 25 ms between conditioning PTN and test FN stimuli. **D**, Recurrent
 1212 inhibition least mean square calculated using the mixed linear model (marginal inhibition
 1213 conditioned by group, ISI, Mmax in soleus and H test in VL used as fixed effects in the model, and

1214 subject as random effect) in controls (white bar) and patients with ALS (blue bar). Upper and lower
 1215 crosses indicate respectively the upper and lower limits of the 95 % of confidence interval (CI95),
 1216 and the interrupted line the CI95. **E**, Distribution of the mean maximal amount of recurrent
 1217 inhibition (% of mean test H-reflex; box plots as in ABC) in each group. * $p < 0.05$, † $p < 0.01$.

1218 **Figure 3: Modulation of recurrent inhibition in quadriceps and soleus. A**, Schematic representation
 1219 of recurrent collaterals of quadriceps motor axons projecting onto Renshaw cells mediating
 1220 recurrent inhibition in soleus LMNs. Dashed arrows indicate the trajectory of antidromic volley in
 1221 quadriceps motor axons after FN stimulation. Grey line represents group Ia afferent inputs after
 1222 PTN stimulation, mediating monosynaptic excitation to soleus LMNs producing H-reflex in soleus
 1223 EMG. **B**, Box plots (as in Fig. 2) showing the distribution of amplitude of Mmax (mV) produced in
 1224 quadriceps (VL head; left side) and soleus EMG (right side) by FN and PTN respectively, in the
 1225 subgroup of controls (n = 17; white boxes and black diamonds) and of patients (n = 17; blue boxes
 1226 and black diamonds). **C**, Box plots showing the distribution of amplitude of test H-reflex produced
 1227 by FN stimuli in quadriceps (VL head) and by PTN stimuli in soleus EMG in the control and patient
 1228 groups (same groups and same legend as in **B**). **D,G**, Box plots (same legend as in **BC**) illustrating the
 1229 distribution of the level of recurrent inhibition (% of mean test H-reflex) in the control and patient
 1230 groups in quadriceps LMNs at the ISIs 15, 20 and 25 ms between conditioning PTN and test FN
 1231 stimuli (**D**) and in soleus LMNs at the ISIs 5, 10 and 15 ms between conditioning FN and test PTN
 1232 stimuli (**G**). **E,H**, Recurrent inhibition least mean square (as in Fig. 2D) in controls (white bar) and
 1233 patients with ALS (blue bar). Upper and lower crosses, and the interrupted line delimit the 95 % of
 1234 confidence interval (CI95; as in Fig. 2D). **F,I**, Box plots (as in **C,D,G**) showing the distribution of
 1235 maximal amount of recurrent inhibition (% of mean test H-reflex) in each group, in quadriceps and
 1236 soleus LMNs. * $p < 0.05$, † $p < 0.01$ and ‡ $p < 0.001$.

1237 **Figure 4: Link between the modulation of recurrent inhibition and patient features. A**, The

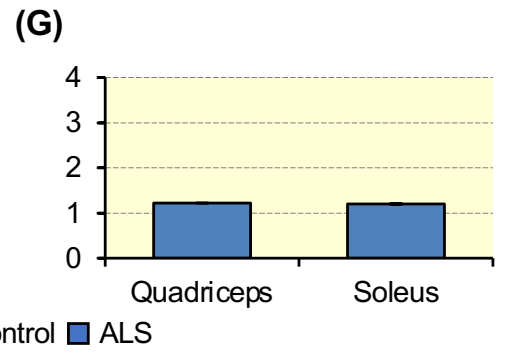
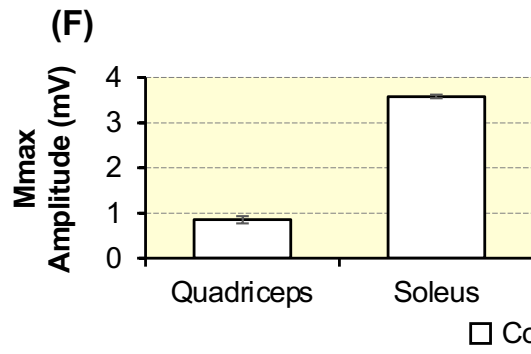
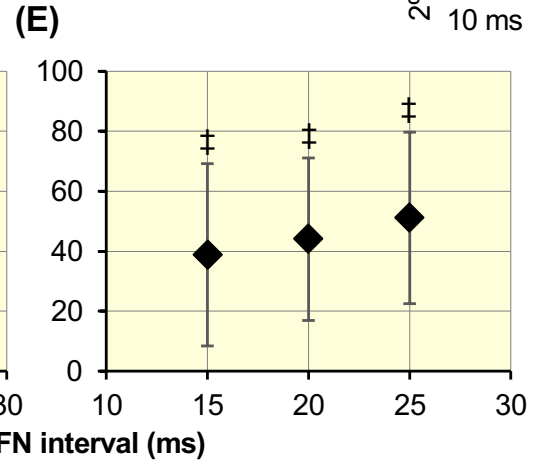
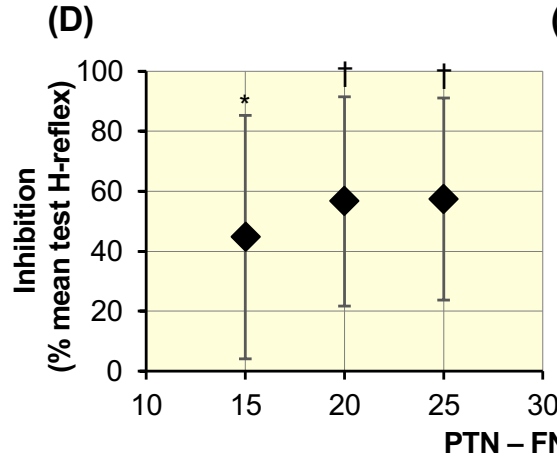
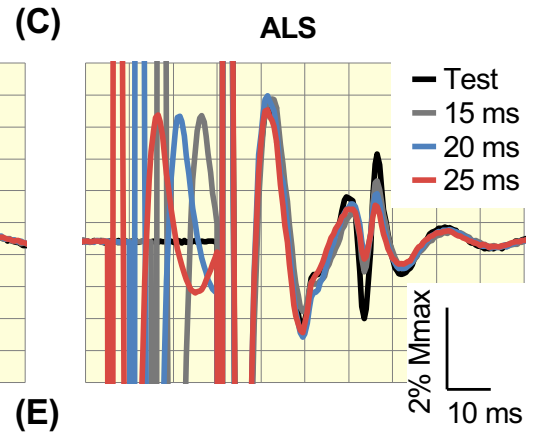
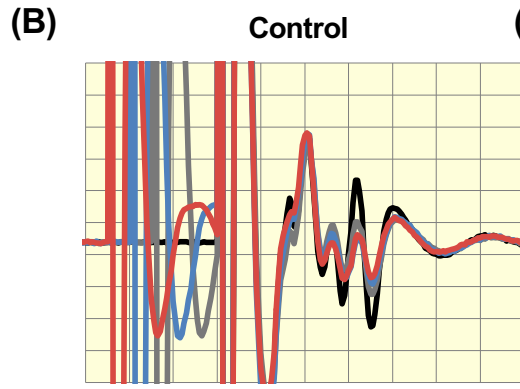
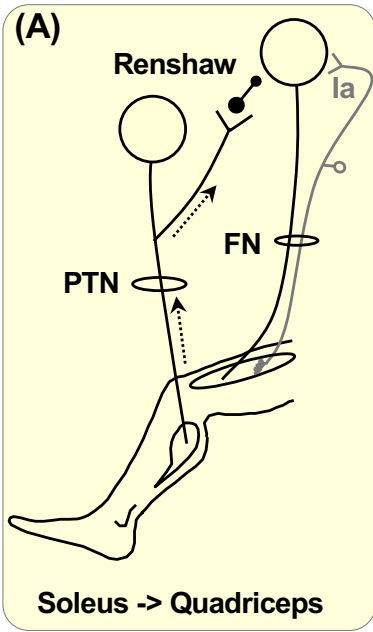
1238 columns represent the repartition of the patients (% of full group; n = 38 participants for quadriceps
 1239 and 17 for soleus) according to their modulation of maximal recurrent inhibition (RI) in quadriceps
 1240 (left column) and soleus (right column) LMN pools, compared to the 95 % of confidence interval
 1241 (CI95) of the mean inhibition in the control group. The number of patients (% of the full group of
 1242 participants in the corresponding muscle) exhibiting a mean maximal recurrent inhibition below the
 1243 CI95 in controls are in grey, those within the CI95, in blue, and those above the CI95, in white. **B,D**,
 1244 Plots illustrating the projection of variable modalities in 2 dimensions corresponding to X and Y axis,
 1245 according to their inertia (λ ; deviation from independence: the greater the value the larger the
 1246 dependency). Modalities include the modulation of recurrent inhibition (RI, black diamonds; \leftrightarrow for
 1247 inhibition within the CI95 in controls, \uparrow for inhibition above the upper limits of CI95 in controls, and
 1248 \downarrow for inhibition below the lower limits of CI95 in controls; **BD**), the target LMN pool (grey squares;
 1249 soleus and quadriceps; **BD**), the size of test Mmax (blue triangles; \leftrightarrow and \downarrow as for inhibition; **BD**),
 1250 progression type according to total ALSFRS-r score (red X; slow and fast progressors; **B**), onset site
 1251 in lower limbs (LL) or in other regions (Other = upper limbs and bulbar regions; blue circles; **D**),
 1252 duration (blue squares; \leq 1 year [y.] or $<$ 1 year; **D**), progression in lower limbs (LL-progression; slow
 1253 vs. fast; red diamonds; **D**) and clinical manifestation of UMN signs (Yes; right-orientated red cross;
 1254 **D**). **C,E**, Bars illustrate the partial contribution of each modality of each parameter in dimension 1
 1255 (white bars) and in dimension 2 (blue bars). Bars illustrate the lines of the contingency table *i.e.*,
 1256 target LMN pool (So. for soleus and Quad. or Q for quadriceps; **CE**), progression (**C**), and test Mmax
 1257 (\leftrightarrow for not depressed and \downarrow for depressed; **CE**), duration ($>$ 1 year and \leq 1 year; **E**), LL-progression
 1258 (Sl. for slow and F for fast; **E**), and UMN signs (N for no and Y for yes; **E**).

1259 **Figure 5: EMG recordings in one control.** Mean EMG activities in vastus lateralis (VL) head of
 1260 quadriceps (N = 20 stimuli; left panels) and in soleus (right panel) in one participant (control), when
 1261 FN-test stimuli were delivered alone (top trace), and on combined stimuli (PTN + FN) at the 3 optimal

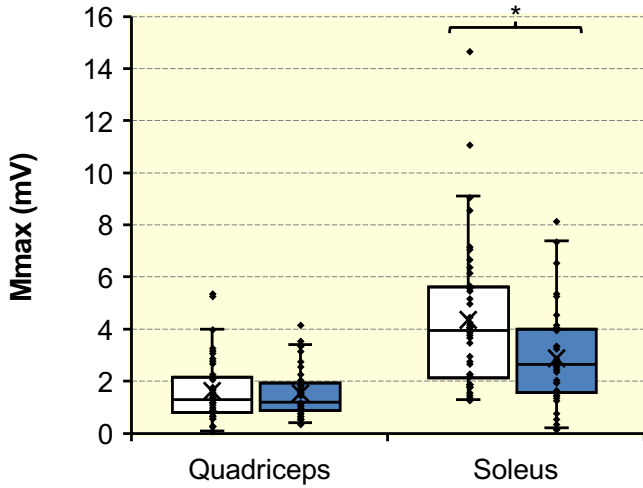
1262 ISIs for producing recurrent inhibition in quadriceps LMNs: 15 ms (upper trace), 20 ms (middle trace)
1263 and 25 ms (lower trace).

1264 **PHYSIOLOGICAL RELEVANCE**

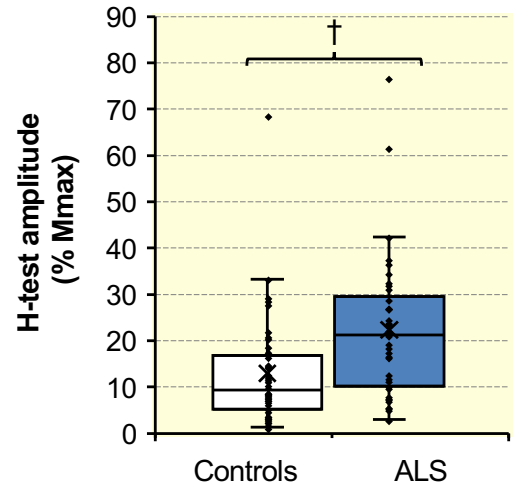
1265 The present study allows to reconsider the role of Renshaw cells in the pathophysiology of ALS and
1266 shows that adaptative mechanisms transiently enhance recurrent inhibition of LMNs at early
1267 disease stage, which can counteract PICs amplification, and likely contribute to maintain
1268 homeostasis and motor output before degeneration.



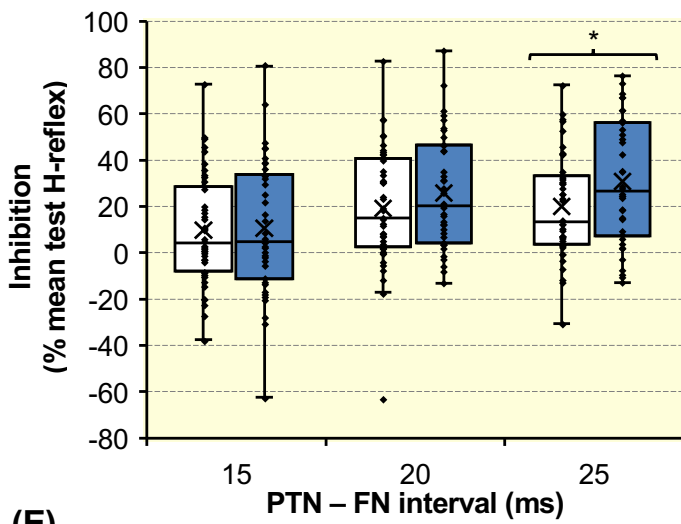
(A)



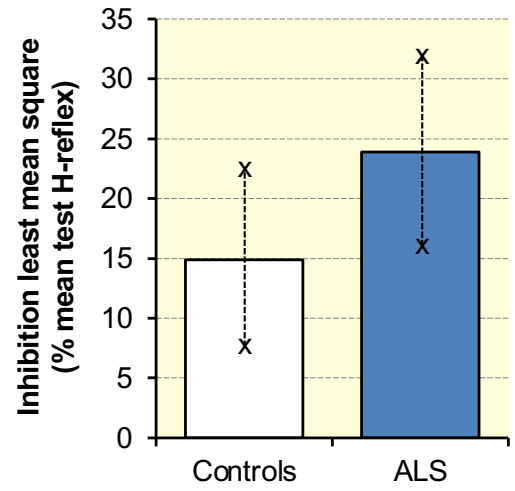
(B)



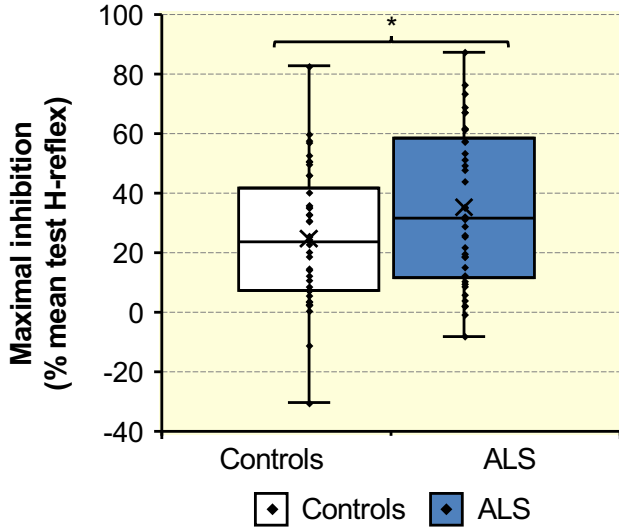
(C)



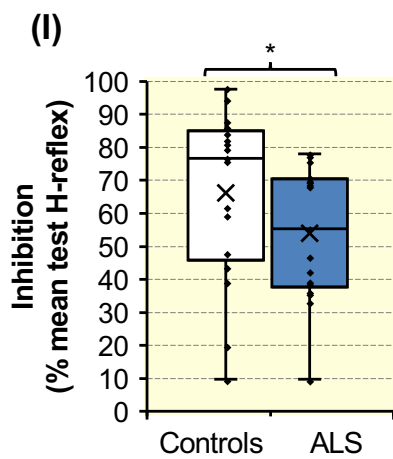
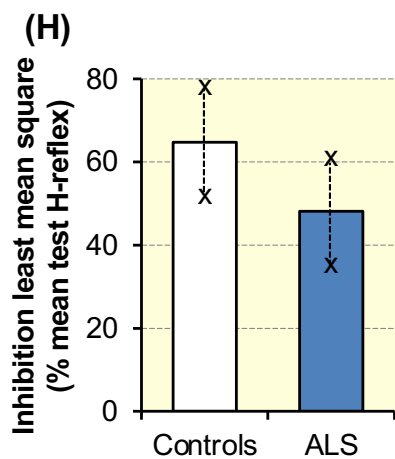
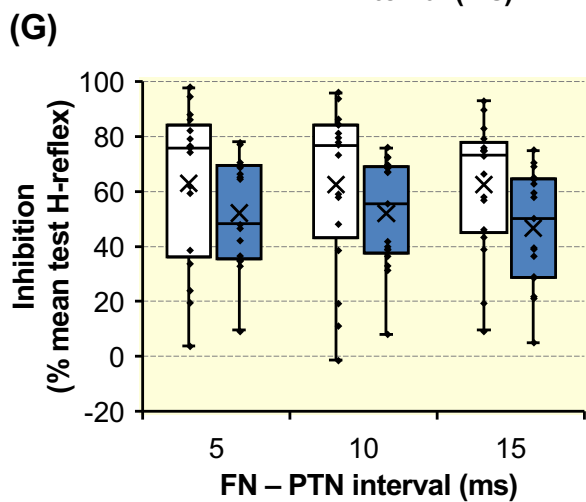
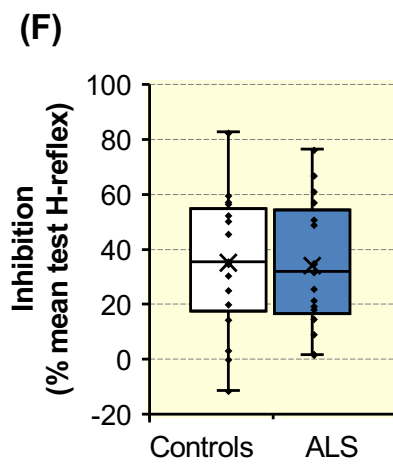
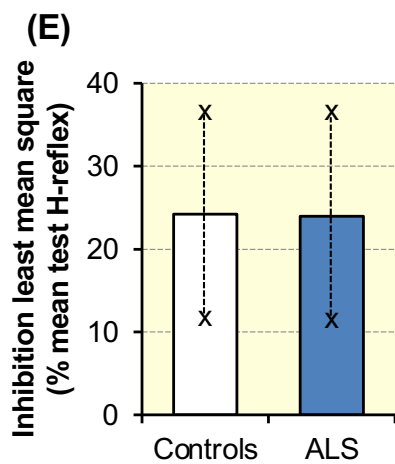
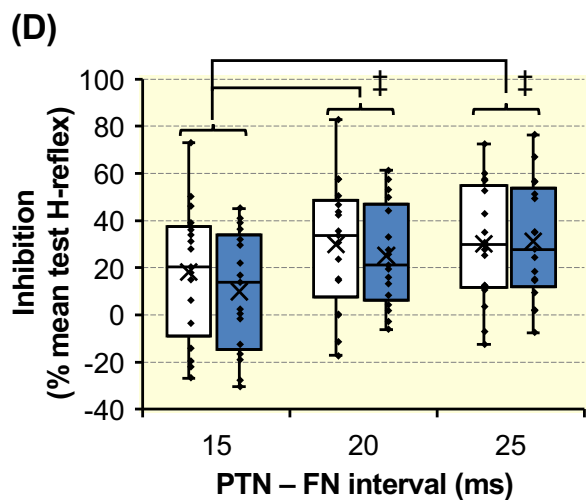
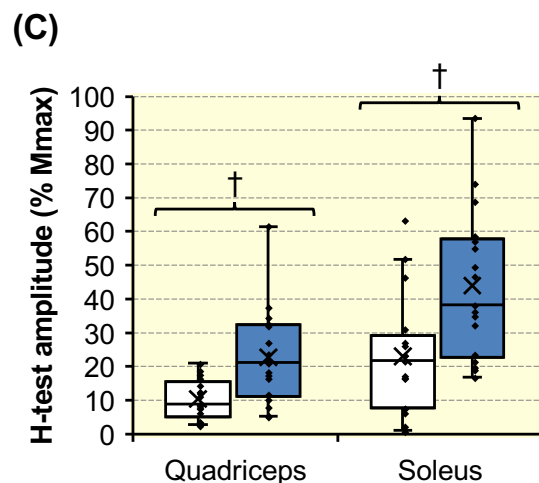
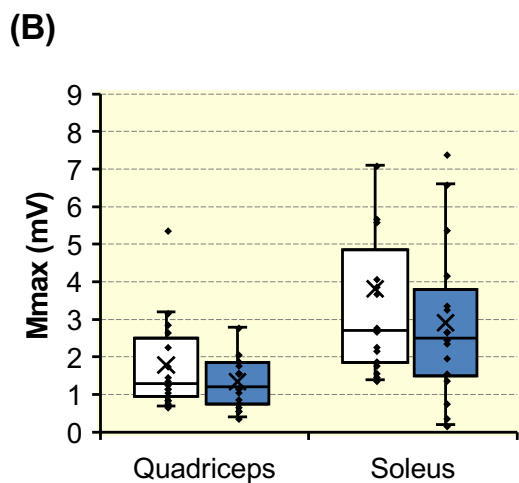
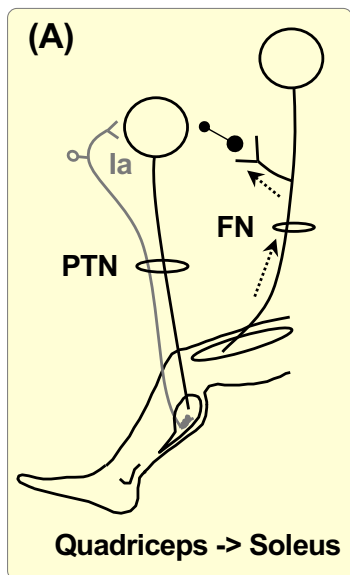
(D)



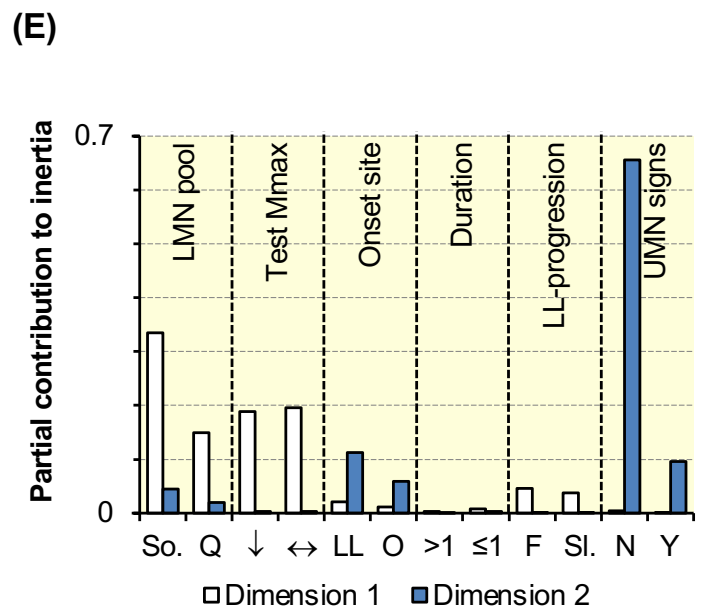
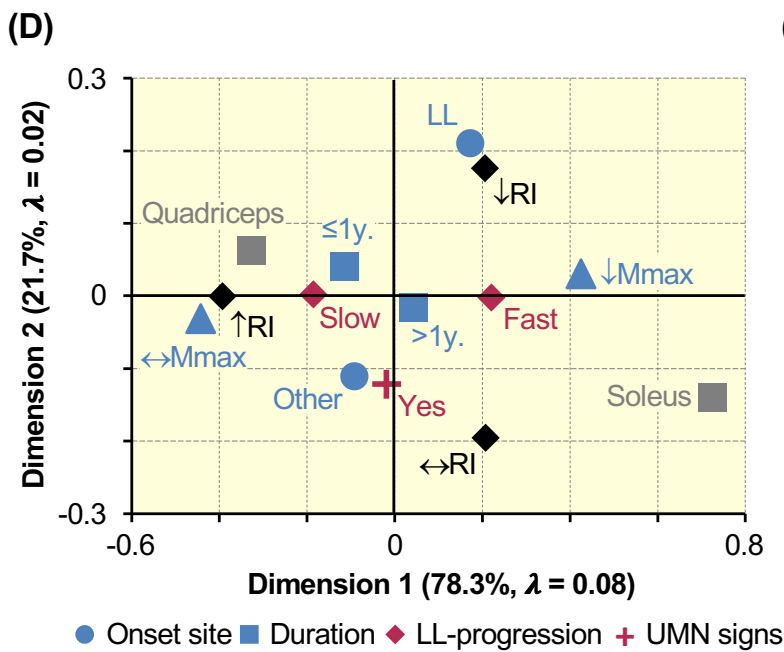
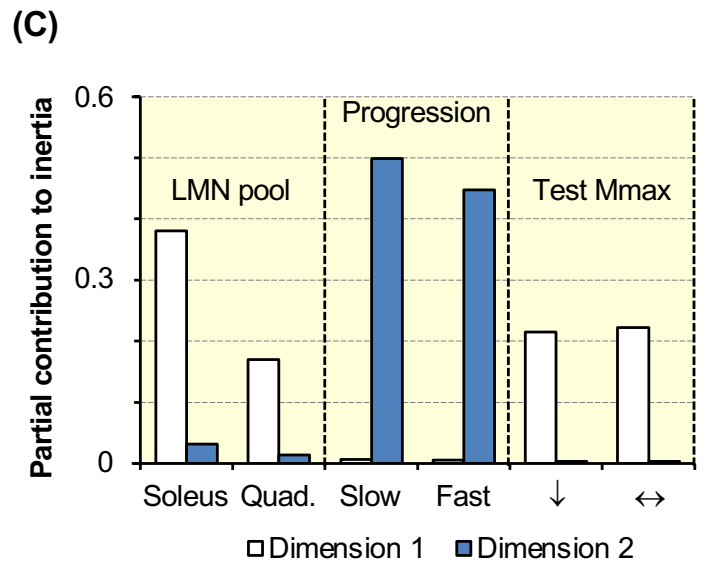
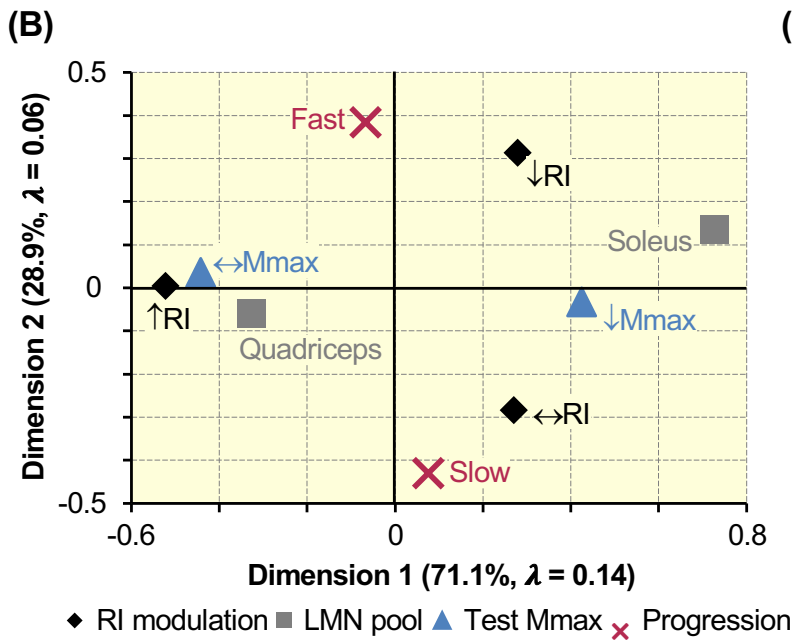
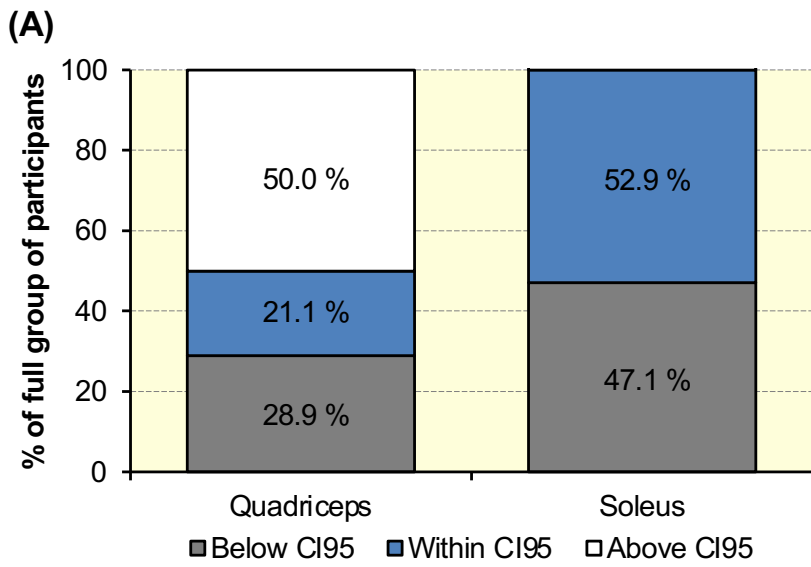
(E)

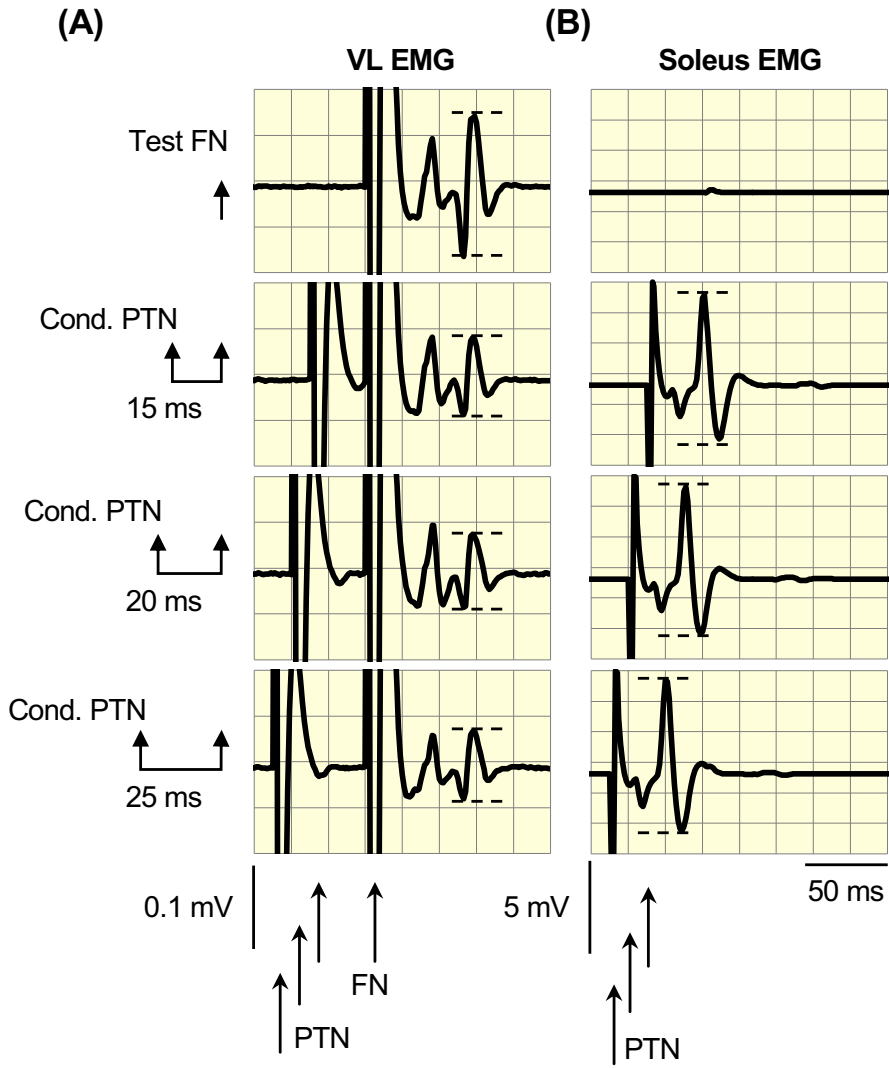


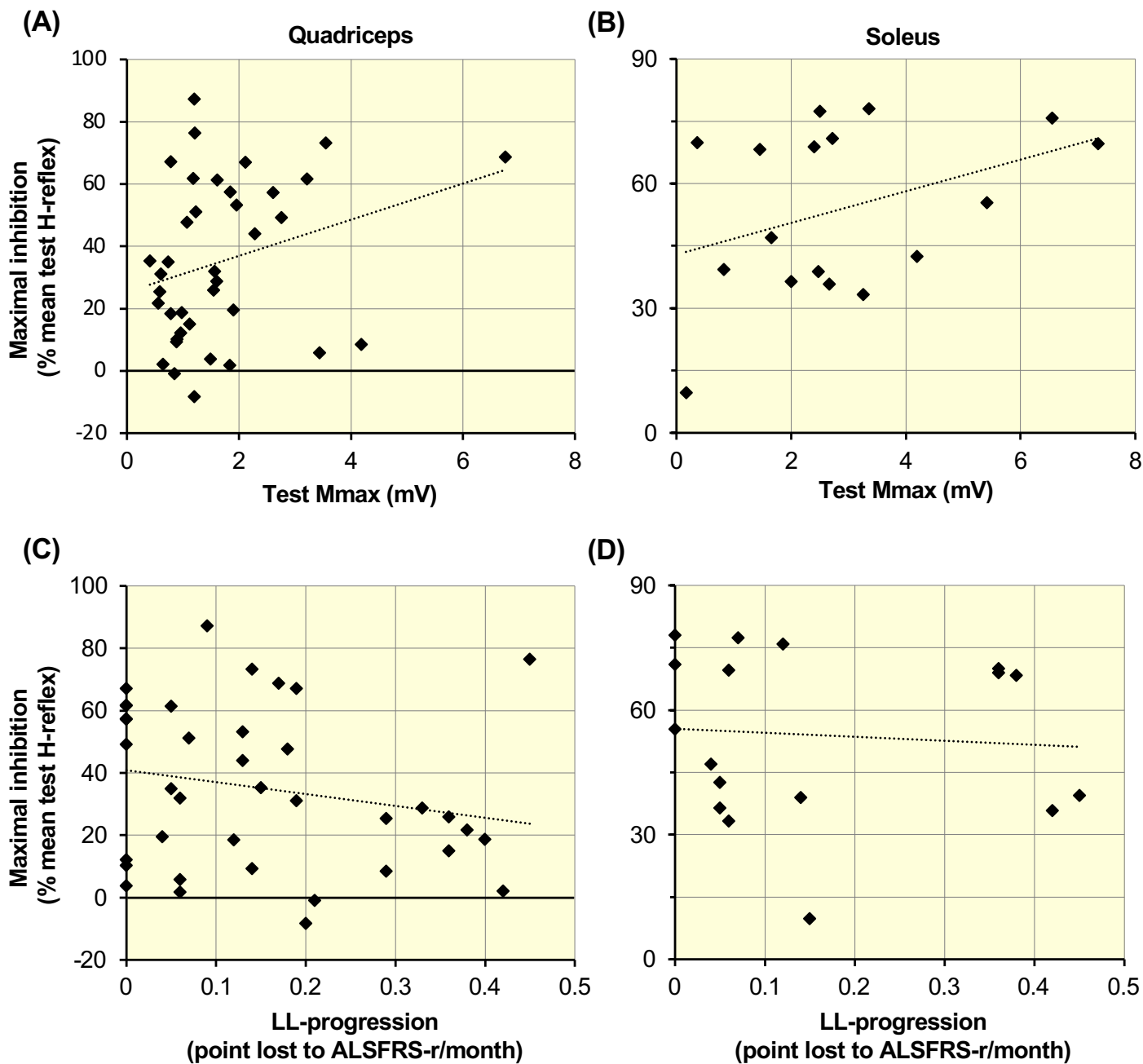
◆ Controls ◆ ALS



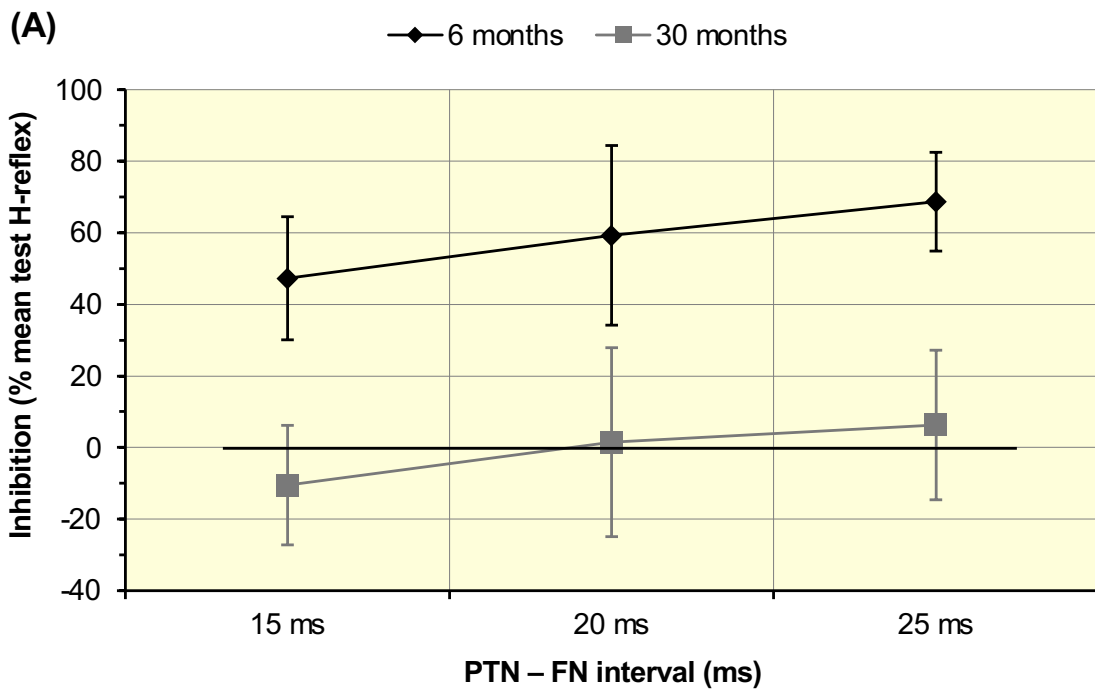
◆ Controls ◆ ALS







Supplemental material 1. Maximum inhibition (% mean test H-reflex) is plotted against the test Mmax (mV; AB) or the progression of motor dysfunctions in lower limbs (LL; mean loss of points to ALSFRS-r/month; CD). AC show the results obtained in quadriceps (38 patients, test Mmax in VL EMG). BD show the results obtained in soleus (17 patients; test Mmax in soleus EMG). Each dot represents one patient. Interrupted lines represent the linear regression curves.



(B)

	6 months	30 months
Mmax VL (mV)	0.68 ± 0.02	0.50 ± 0.03
VL H-reflex (% Mmax)	11.49 ± 5.72	22.43 ± 5.35
Mmax soleus (mV)	2.84 ± 0.14	0.08 ± 0.003
ALSFRS-r (total)	47	40
Sub-score ALSFRS-r	7	3
Quadriceps	5	5
Soleus	5	5
TA	5	1
EHL	5	3

Supplemental material 2. A, Mean inhibition (% mean test H-reflex) plotted against the ISI between conditioned PTN stimuli and test FN stimuli (ms) in patient #13 investigated at 24-month interval: 6 months (black diamonds and line) and 30 months after the first symptoms (grey squares and line). Vertical bars are ± 1 SD. **B**, Table summarizing the following measures in patient #13, 6 (left column) and 30 (right column) months after the first symptoms (from 1st to 9th row): the mean amplitude (± 1 SD) of test Mmax in VL EMG (mV), of test H-reflex in VL EMG (% Mmax; ± 1 SD), of conditioning Mmax in soleus EMG (mV; ± 1 SD), and scores to ALSFRS-r (total), to the items for lower limb functions in ALSFRS-r (sub-score for lower limbs), to muscle testing (according to MRC scale) in quadriceps, soleus, tibialis anterior (TA) and extensor hallucis longus (EHL) muscles.