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

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

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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 limbs<sup>1</sup> and rapidly spreads to other body regions. Cognitive dysfunctions are also reported in ~50 %  
49 of cases<sup>2</sup>. ALS has a relative low prevalence (~6/100 000)<sup>3</sup> 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), respectively<sup>4</sup>. Research has  
55 mostly dealt with motor neuron dysfunctions which have been reported in presymptomatic ALS  
56 mouse models and human patients at both levels<sup>5-11</sup>, but non-cell autonomous pathogenic  
57 mechanisms have also been reported, involving glial cells and interneurons<sup>12-14</sup>. To date, most of  
58 the studies focused on glial cells and their interaction with motor neurons<sup>12</sup> 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 neurodegeneration<sup>15</sup>, but studies  
61 primarily focused on the motor cortex<sup>13</sup> 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<sup>14,16</sup>.

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<sup>17,18</sup>. However, it has not been possible to  
69 dissociate degeneration of LMNs and interneurons in the ventral horn<sup>19</sup>. In presymptomatic ALS

70 mouse models, specific subsets of glycinergic inhibitory interneurons in the intermediate zone and  
71 ventral horn degenerate before LMNs<sup>20–22</sup>, and it has been evidenced that alteration of glycinergic  
72 interneurons in the ventral horn is not consecutive to LMN degeneration<sup>23</sup>. 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<sup>24–28</sup>, receive more glycinergic inhibitory synaptic inputs from V1 interneurons  
75 compared to more resistant LMNs innervating slow motor units<sup>29</sup>. Furthermore, loss of inhibitory  
76 synapses from V1 interneurons on fast LMNs precedes LMN degeneration and causes locomotor  
77 dysfunctions<sup>29</sup>. 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)<sup>16,30–33</sup>.

82 Renshaw cells have been particularly explored in ALS, liable to their activation by recurrent  
83 collaterals from LMNs. Studies in both mouse models<sup>34,35</sup> and humans<sup>36,37</sup> 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<sup>35,38</sup>. The  
86 depression of recurrent inhibition reported in patients has raised questions<sup>39</sup> *i)* for methodological  
87 and physiological reasons, given that the reduced silent period after mixed nerve stimulation  
88 reported in ALS<sup>40</sup>, is not specific to Renshaw cell activity and the targeted LMNs (innervating intrinsic  
89 hand muscles) have no recurrent collaterals<sup>41,42</sup>, and *ii)* for pathophysiological reasons, since initial  
90 wasting mostly occur in muscles innervated by LMNs without recurrent collaterals<sup>39,41,42</sup>. These led  
91 Mazzocchio and Rossi<sup>39</sup> 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 progression<sup>16</sup>. More convincing results in patients were obtained by testing

95 Hoffmann reflex (H-reflex)<sup>36,43</sup>, 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)<sup>44</sup>. However, the technique has not been properly implemented in ALS patients<sup>39</sup> and the  
98 results have to be interpreted with caution when the LMN pool is already affected<sup>44</sup>. 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<sup>42,45-47</sup>, 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<sup>46,47</sup>. 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 LMNs<sup>44</sup>. 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 LMNs<sup>44</sup>. 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<sup>44,45</sup>; 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 ( $0.85 \pm 0.08$  vs.  $1.22 \pm 0.01$  mV in the control and the patient, respectively) while  
 132 that in soleus was smaller in the patient ( $1.20 \pm 0.01$  mV) compared to the control ( $3.58 \pm 0.04$  mV).  
 133 Lastly, the test H-reflex in VL EMG was of similar size in both participants, reaching on average  $9.7$   
 134  $\pm 7.7$  % of Mmax (in VL EMG) in the control and  $10.3 \pm 4.8$  % 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<sup>44,46,48</sup>. 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:  $60.4 \pm 21.6$  vs.  $65.8 \pm 23.9$  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-reflex<sup>48</sup>). 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<sup>51–53</sup>: 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  $< 0.0001$ ;  $f^2$  <sup>54</sup> = 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<sup>44,45</sup>, 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)<sup>44,45</sup>. 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  $57.6 \pm 31.4$  vs.  $65.3 \pm 29.6$  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  $69.1 \pm 29.8$  vs.  $78.8 \pm 17.09$  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*<sup>44</sup>, 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. Chi<sup>2</sup> 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 (Chi<sup>2</sup>,  $p = 0.0012$ ).

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 ALS<sup>1</sup>. The mean disease duration (time from symptom onset) was  $21.7 \pm 16.4$  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 living<sup>55</sup>, 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  $5.4 \pm 2.0$  (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  $0.5 \pm 0.5$  points/month (between 0.1 and 2.4, median at 0.4). Based on UMN and  
275 LMN scores<sup>56,57</sup>, 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. Chi<sup>2</sup> 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), Chi<sup>2</sup> 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)<sup>58,59</sup>. 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 Chi<sup>2</sup> 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 (Chi<sup>2</sup>,  $p = 0.0365$ ).

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:  $\leq 1$  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:  $\geq 40$  or  $< 40$  for total score and  $\geq 6$  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 (Chi<sup>2</sup> 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 (ALSF<sub>RS-r</sub> 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;  $\text{Chi}^2$ ,  $p < 0.01$ ) 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  $\text{Chi}^2$ ,  $p > 0.99$ ) 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 (1<sup>st</sup> 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 %;  $\text{Chi}^2$ ,  $p < 0.001$ ), and *ii*) dimension 2  
373 representing only 21.7 % ( $\text{Chi}^2$ ,  $p > 0.99$ ). 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 1<sup>st</sup> 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<sup>42,46</sup>. 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<sup>41,44,45</sup>.

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<sup>60,61</sup>. 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)<sup>44,62</sup>. 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 ones<sup>44</sup>, and it is interesting to note that this might also be the  
433 case when comparing homonymous recurrent inhibition using the H' technique<sup>41</sup>.

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)<sup>41,63,64</sup>, which is shortened in  
436 patients with mild motor dysfunction and increased again with motor deficit progression<sup>65</sup>. There is  
437 thus a possibility that the increase of H' reflex in ALS<sup>36,43</sup>, 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)<sup>44,47</sup>. 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<sup>44,60</sup>) *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<sup>66,67</sup>. These ISIs are shorter  
452 than those for D1 method we used in Howells et al. 2020 (10 to 30-ms ISIs)<sup>68</sup> *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<sup>44–47,60</sup>. Lastly, it has been shown that presynaptic inhibition is depressed in ALS<sup>68–</sup>  
462 <sup>71</sup>. 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<sup>42,44,46,47,72</sup>. 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<sup>16,44,45,73,74</sup>. Beside their excitation by LMN discharge, Renshaw cells also  
477 receive polysynaptic excitation and inhibition from flexor reflex afferents (FRA)<sup>73</sup>. 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<sup>75,76</sup>. 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 movement<sup>77</sup>. However, during a  
483 weak tonic contraction, recurrent inhibition has been found increased<sup>77</sup>, 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 movement<sup>44</sup> and likely mostly serves as a  
487 variable gain regulator of the spinal motor output<sup>78</sup>. 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 progression<sup>16</sup>.

### 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<sup>29,35</sup>. However, none of these studies focused  
497 on Renshaw cells in particular, which mediate both glycinergic and GABAergic recurrent inhibitions  
498 to LMNs<sup>31,32</sup>. More recently, specific alteration of V1 interneurons has been reported<sup>29</sup> 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 interneurons<sup>79</sup>. 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 death<sup>34</sup>.

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 collaterals<sup>40</sup> and H' technique contaminated by AHP<sup>36,43,44</sup>. Accordingly, the possible alteration of  
513 recurrent inhibition and the implication of Renshaw cells in ALS has been quite rightly questioned<sup>34</sup>.  
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 ones<sup>37</sup>. 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<sup>80,81</sup>. 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.<sup>37</sup> 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<sup>8,82</sup>, 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 ALS<sup>83</sup> 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 syndrome<sup>44</sup>: *i)* it has been found  
539 increased in stroke and spinal cord injured patients<sup>84,85</sup>; *ii)* in patients with cerebral palsy, the  
540 inhibition was found unchanged<sup>86</sup> 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<sup>87,88</sup>, and *iii)*  
542 interestingly, it has been found in patients with hyperekplexia that recurrent inhibition is preserved,  
543 likely due to its GABAergic components<sup>89</sup>. 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 FRA<sup>73</sup> and it has been shown that  
556 recurrent inhibition is particularly reduced by group II afferents<sup>90</sup>. 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 deficits<sup>91</sup>. 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 rate<sup>92</sup>. 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 approaches<sup>91</sup>. 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 LMNs<sup>34</sup>. 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*)<sup>45,73</sup>. 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 excitations<sup>45</sup>. 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*)<sup>45</sup>. 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<sup>69–71,93</sup>. 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)<sup>78</sup>. 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)<sup>24–28,94,95</sup>, which generates large motor unit potentials in EMG<sup>96</sup>. 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.<sup>34</sup> 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<sup>5,6,97</sup> and we have shown that LMNs in symptomatic patients with sporadic ALS  
631 are normo-to-hypoexcitable (participants are common to the present study)<sup>57</sup>. It has been clearly  
632 stated in ALS mice that the equilibrium between opposite effects (excessive activity of the voltage-  
633 gated Na<sup>+</sup> and Ca<sup>2+</sup> 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 death<sup>98</sup>. 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<sup>14,16,98</sup>.

### 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.<sup>37</sup> 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.<sup>34</sup> 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 quadriceps<sup>44</sup>. 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 LMNs<sup>44</sup>, and manual muscle testing is not specific for EHL, but also includes intrinsic foot  
679 muscles without LMN recurrent collaterals<sup>41</sup>. 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 cells<sup>78</sup>, and it has been evidenced  
690 that PICs are enhanced in ALS<sup>98</sup>. 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 spasms<sup>99</sup>. 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<sup>100,101</sup> but its interest in ALS therapy  
700 was discarded by the disappointing results of clinical trials<sup>102</sup>. 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 ALS<sup>1</sup>. LMNs innervating these muscles have no recurrent collaterals<sup>41,42</sup> 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 pool<sup>103</sup>. 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<sup>14,16</sup> 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 muscles<sup>73</sup>. 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 lasted 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 inhibition<sup>104</sup>, 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  $61.5 \pm 9.7$  years old (mean  $\pm$  1 SD; ranging from 39 to 78), and  
749  $61.8 \pm 9.0$  (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 criteria<sup>105</sup>, 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 side<sup>106</sup>. 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-cm<sup>2</sup>  
782 brass plaque placed on the posterior aspect of the thigh (below the buttock) and anode, a 7-cm<sup>2</sup>  
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<sup>44,61</sup> (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 EMG<sup>48</sup>, 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<sup>44,60</sup>. 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<sup>56,57</sup> = reflex score (0, 1 or 2) + Babinski or Hoffmann sign (0 or 1) + Ashworth  $\geq 3$  (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  $\geq 3$  (*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  $\pm 1$  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  $d$ <sup>49,50</sup>  
861 when we compared 2 means, and using  $f^2$ <sup>54</sup>, 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<sup>45,46,51-53</sup>, 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, Chi<sup>2</sup>  
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. Chi<sup>2</sup> 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 Chi<sup>2</sup> 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.

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### 888 **CONFLICT OF INTEREST**

889 The authors declare no conflict of interest.

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## 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	<b>3</b>	<b>4</b>	<b>3</b>	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	<b>2</b>	<b>3</b>	5	x
5	LL	15	36	3	0,80	0,33	0	2	UMN	5	5	5	5	-
6	LL	16	44	<b>8</b>	0,25	0,00	0	0	Mixed	5	5	5	5	x
7	UL	17	45	<b>8</b>	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	<b>3</b>	5	5	x
10	LL	26	38	3	0,38	0,19	3	1	UMN	5	5	<b>4</b>	5	x
11	LL	14	43	4	0,36	0,29	3	2	UMN	5	5	5	5	x
12	UL	9	39	<b>8</b>	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	<b>8</b>	0,36	0,00	0	0	Mixed	5	5	5	5	x
16	UL	16	45	<b>8</b>	0,19	0,00	0	0	Mixed	5	5	5	5	-
17	LL	24	41	3	0,29	0,21	0	2	LMN	5	<b>1</b>	<b>0</b>	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	<b>4</b>	5	5	-
21	UL	13	47	<b>8</b>	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	<b>3</b>	<b>3</b>	5	x
24	UL	59	34	5	0,24	0,05	3	1	UMN	5	5	<b>4</b>	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	<b>3</b>	<b>4</b>	5	x
30	Bulbar	48	43	<b>8</b>	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	<b>4</b>	<b>3</b>	5	x
32*	LL	12	42	3	0,50	0,42	2	2	Mixed	5	5	5	5	x
33	UL	7	42	<b>8</b>	0,86	0,00	3	0	UMN	5	5	5	5	x
34	UL	7	44	<b>8</b>	0,57	0,00	3	1	UMN	5	5	5	5	x
35	LL	41	39	2	0,22	0,15	0	4	LMN	<b>2</b>	<b>0</b>	<b>0</b>	5	x
36	Bulbar	63	36	4	0,19	0,06	4	0	UMN	5	5	<b>3</b>	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	<b>3</b>	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**

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

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 (Chi<sup>2</sup> 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. <sup>†</sup>  $p < 0.01$  and <sup>†</sup>  
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  $\pm 1$  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 (1<sup>st</sup> quartile, Q1), the upper limit, the 75th percentile (3<sup>rd</sup> 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



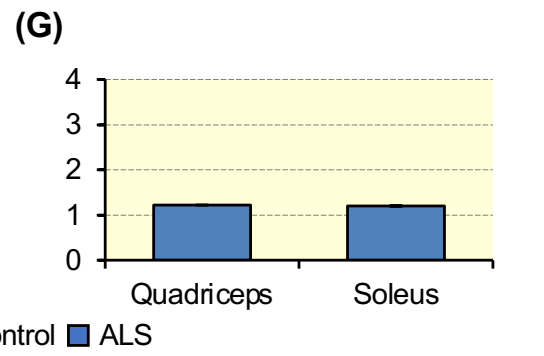
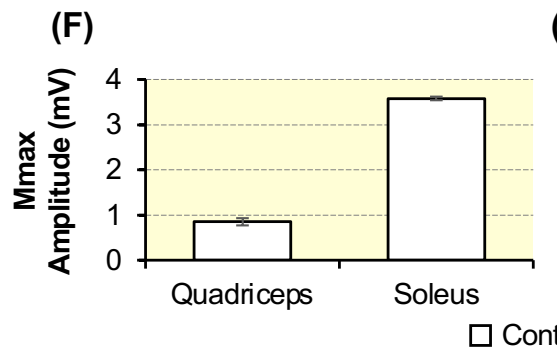
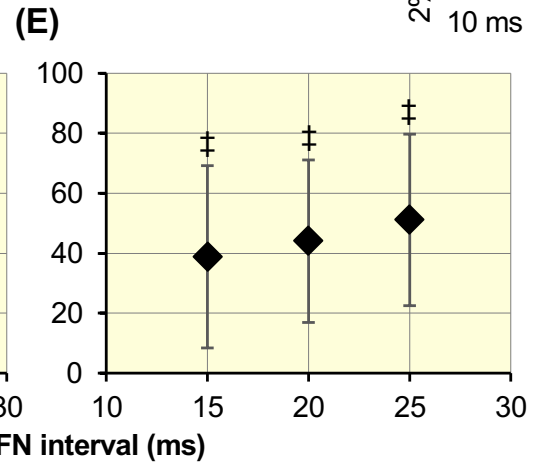
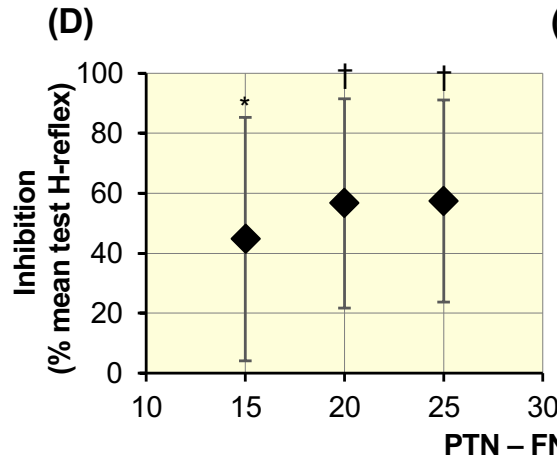
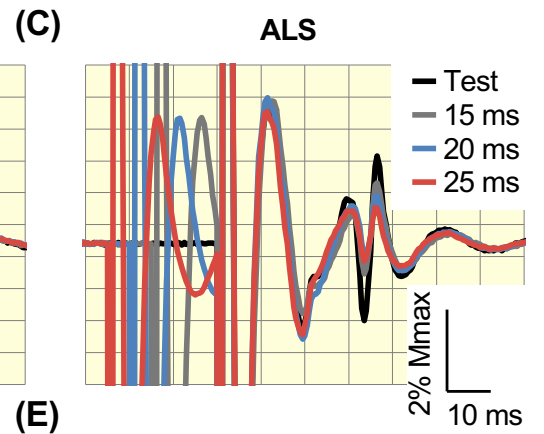
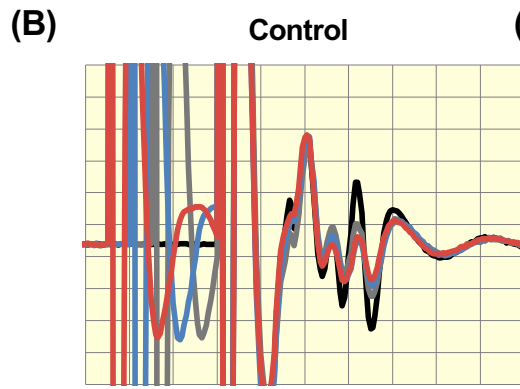
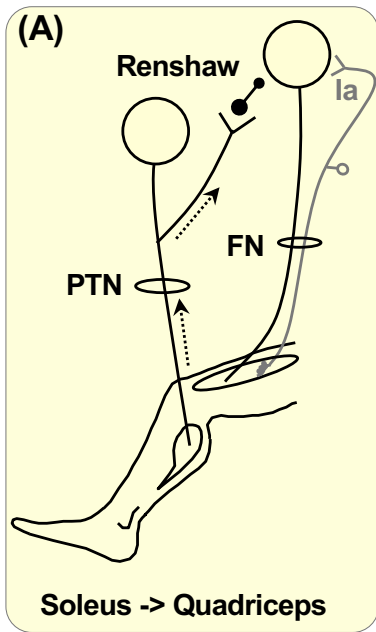
1238 columns represent the repartition of the patients (% of full group; n = 38 participants for quadriceps  
 1239 and 17 for soleus) according to their modulation of maximal recurrent inhibition (RI) in quadriceps  
 1240 (left column) and soleus (right column) LMN pools, compared to the 95 % of confidence 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 ( $\lambda$ ; 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

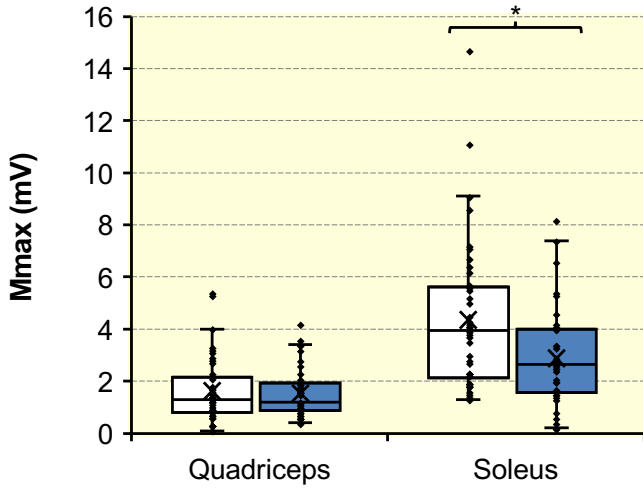
1262 ISIs for producing recurrent inhibition in quadriceps LMNs: 15 ms (upper trace), 20 ms (middle trace)  
1263 and 25 ms (lower trace).

1264 **PHYSIOLOGICAL RELEVANCE**

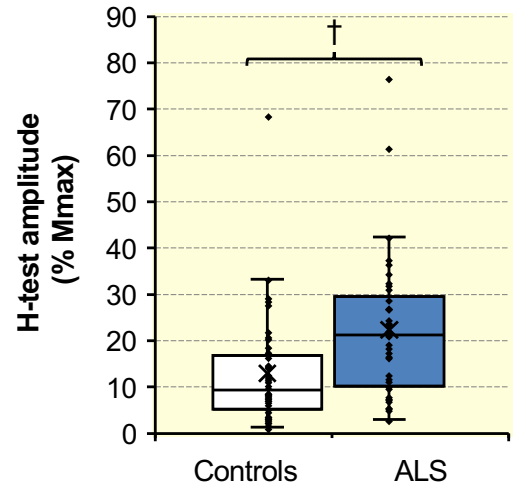
1265 The present study allows to reconsider the role of Renshaw cells in the pathophysiology of ALS and  
1266 shows that adaptative mechanisms transiently enhance recurrent inhibition of LMNs at early  
1267 disease stage, which can counteract PICs amplification, and likely contribute to maintain  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



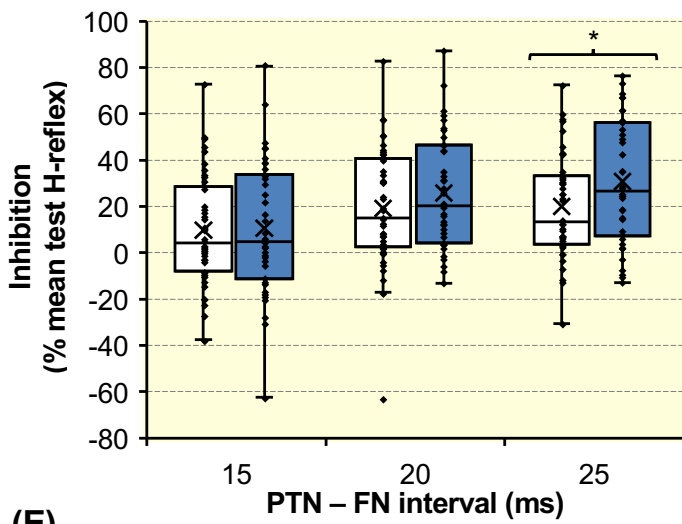
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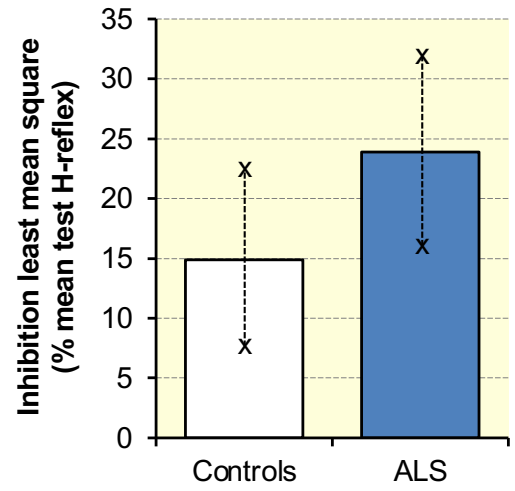
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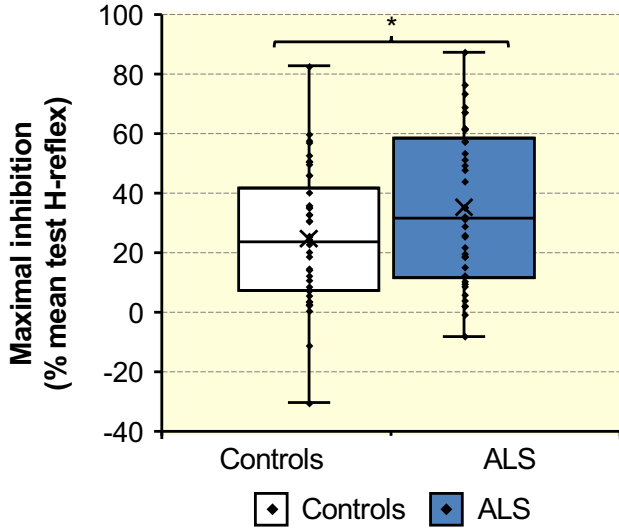
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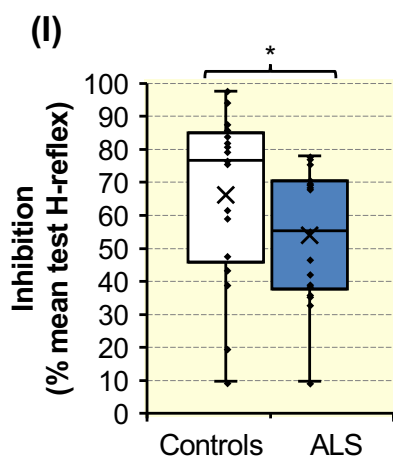
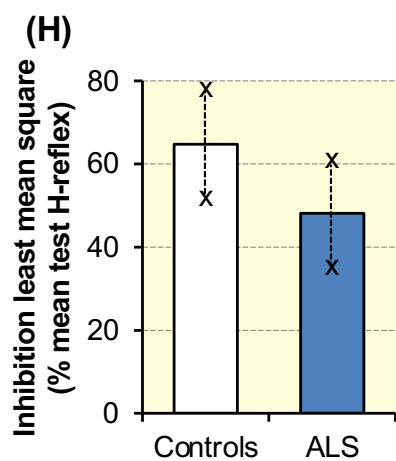
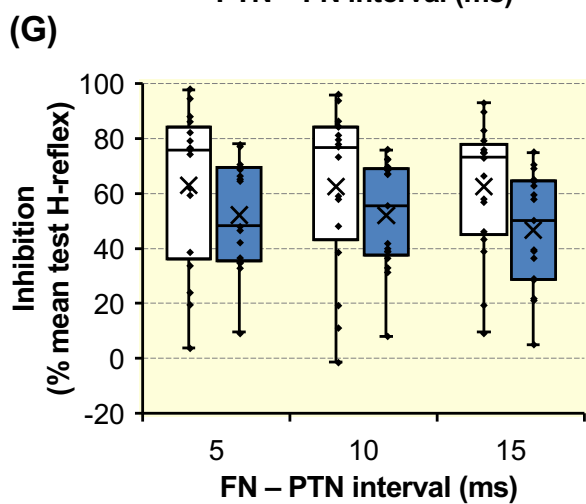
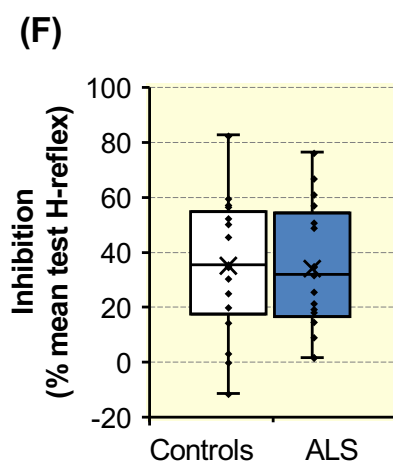
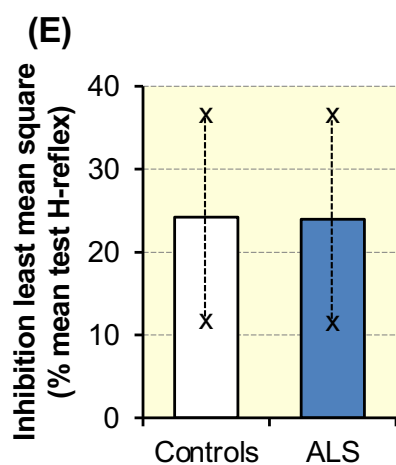
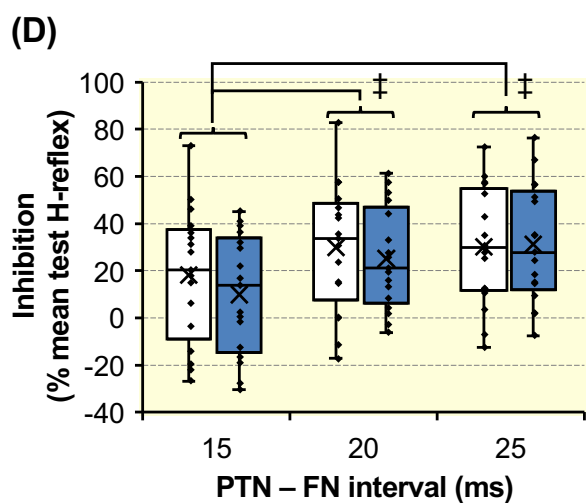
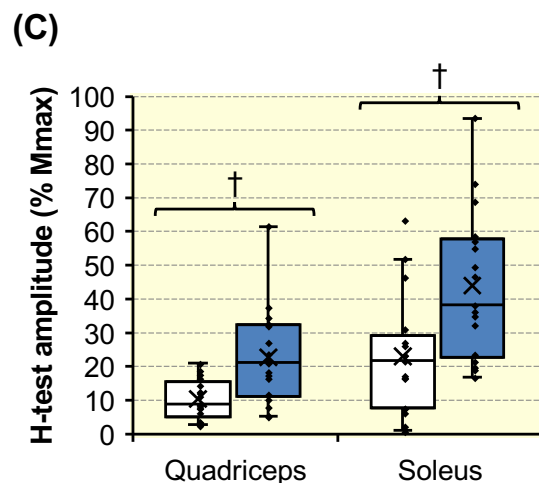
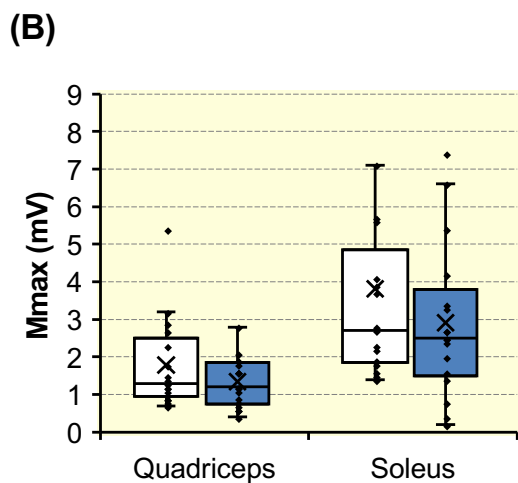
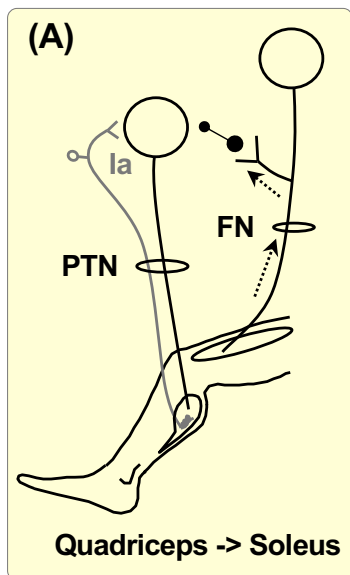
(D)



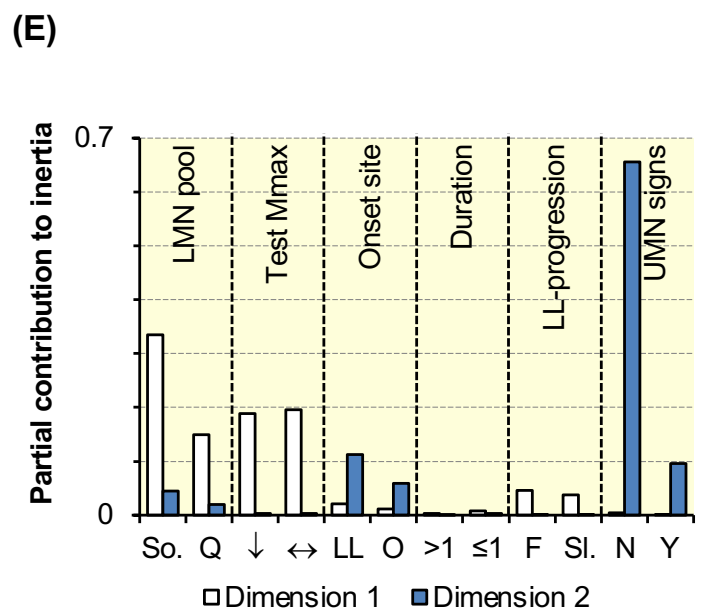
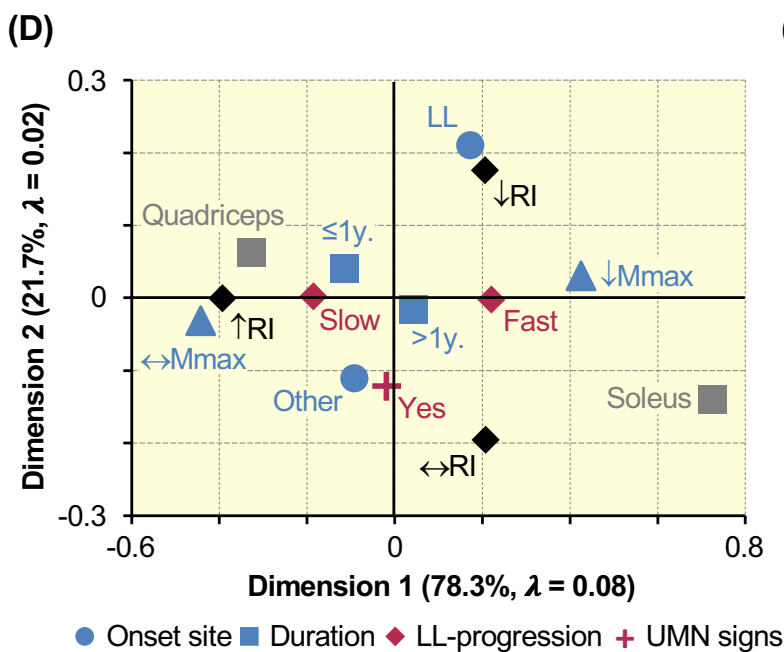
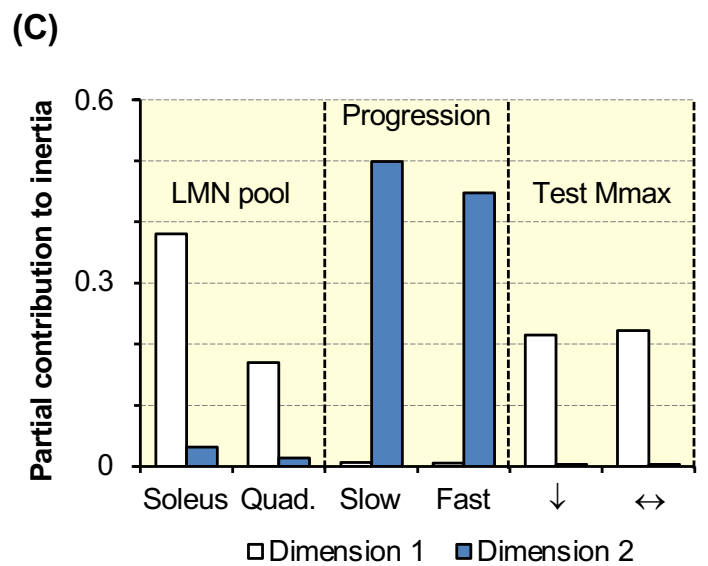
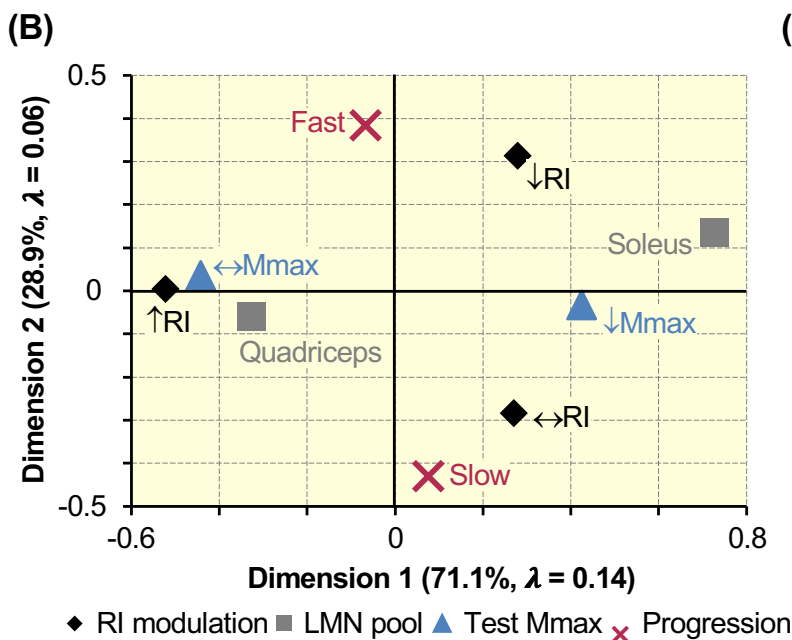
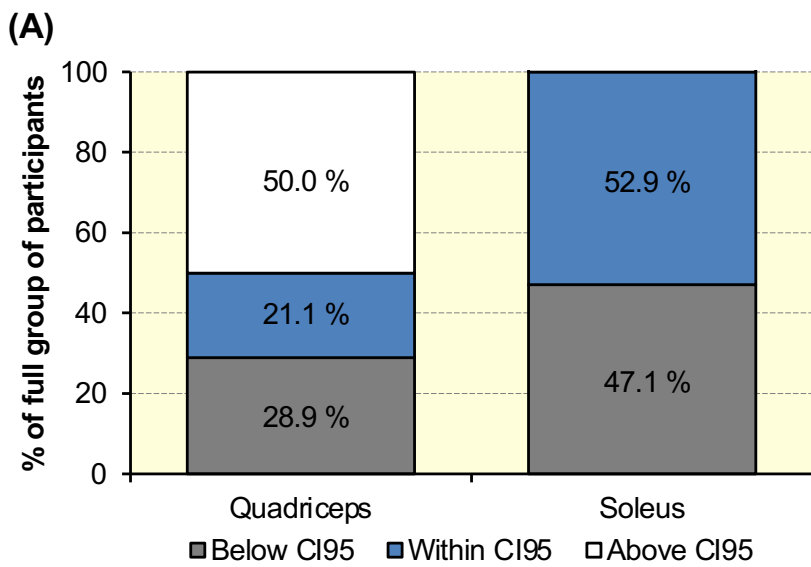
(E)



◆ Controls ◆ ALS



◆ Controls ◆ ALS

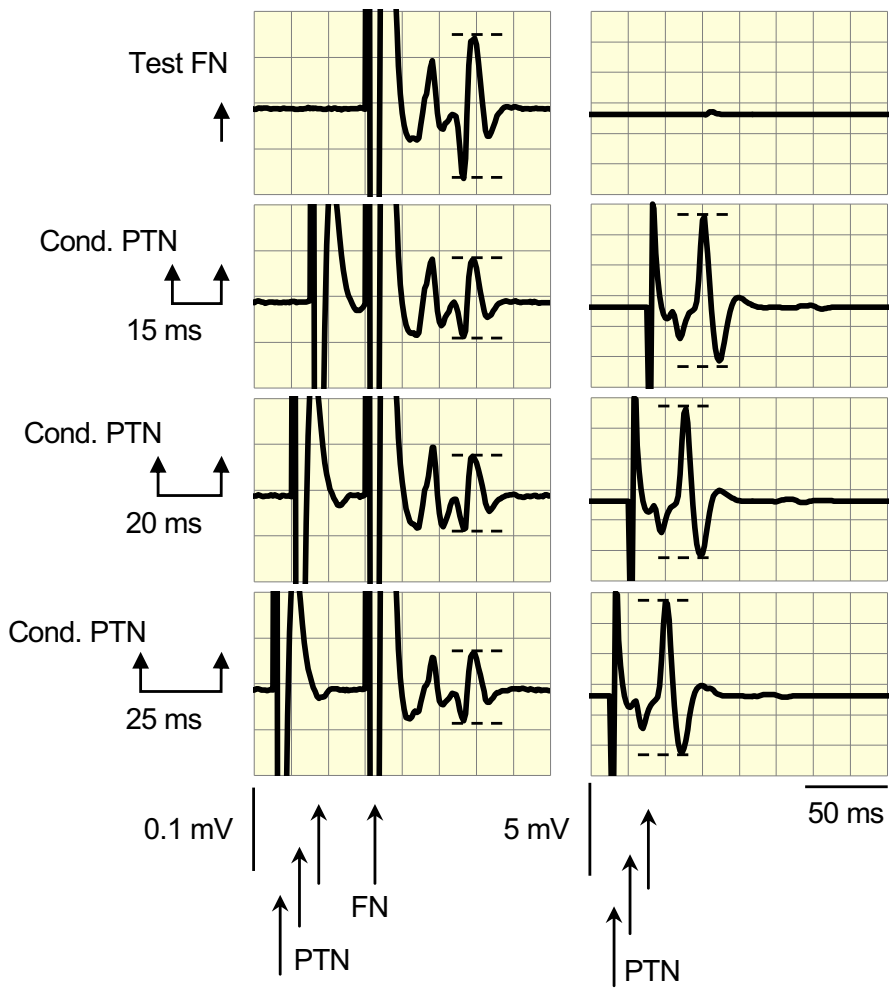


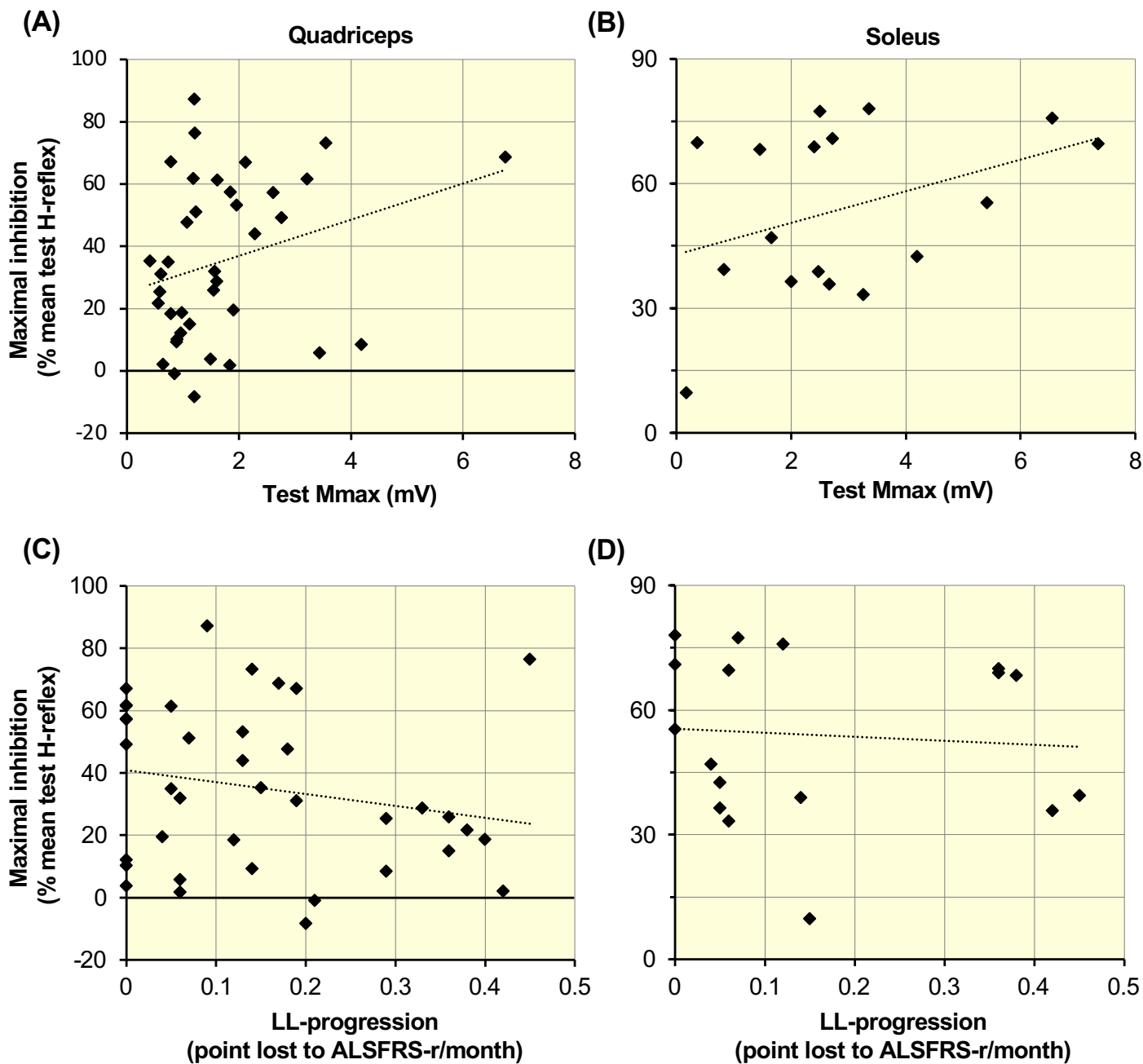
(A)

(B)

VL EMG

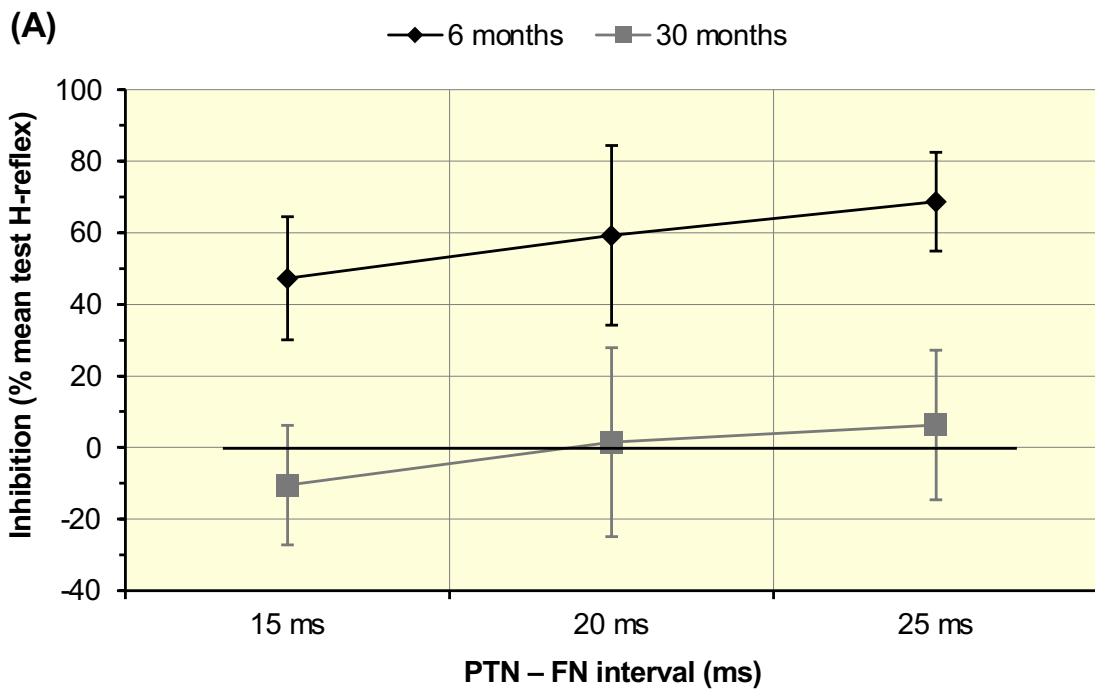
Soleus EMG





**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  $\pm 1$  SD. **B**, Table summarizing the following measures in patient #13, 6 (left column) and 30 (right column) months after the first symptoms (from 1<sup>st</sup> to 9<sup>th</sup> row): the mean amplitude ( $\pm 1$  SD) of test Mmax in VL EMG (mV), of test H-reflex in VL EMG (% Mmax;  $\pm 1$  SD), of conditioning Mmax in soleus EMG (mV;  $\pm 1$  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.