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1 **SYNAPTIC DYNAMICS LINKED TO WIDESPREAD ELEVATION OF H-REFLEX**
2 **BEFORE PERIPHERAL DENERVATION IN AMYOTROPHIC LATERAL SCLEROSIS**

3
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14 **Running head:**

15 ALS-related rise in H-reflex

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27 **ABSTRACT**

28 Changes in Hoffmann reflex (H-reflex) exhibit heterogeneity among patients with amyotrophic
29 lateral sclerosis (ALS), likely due to phenotype diversity. Current knowledge primarily focuses
30 on soleus H-reflex, which may demonstrate an initial increase before subsequent decline
31 throughout the disease course. The main objective was to investigate other muscles, to
32 determine whether H-reflex changes could be associated with patient phenotype (onset site,
33 functional disabilities). Additional experiments were performed to elucidate the
34 neurophysiological mechanisms underlying H-reflex modifications. In age- and sex-matched
35 groups of controls and patients, we compared H-reflex recruitment curves in soleus,
36 quadriceps, and forearm flexors. Additionally, we examined H-reflex and motor evoked
37 potential (MEP) recruitment curves in quadriceps. Last, to assess potential changes in
38 monosynaptic excitatory post-synaptic potentials (EPSPs) of both peripheral and cortical
39 origins, we analyzed peri-stimulus time histograms (PSTH) and peristimulus frequencygrams
40 (PSF) of single motor units, along with H-reflex occurrence after paired pulse stimuli. The ratio
41 between maximal amplitudes of H-reflex and direct motor response increased in all muscles,
42 irrespective of disease onset, and was found positively correlated with exaggerated
43 osteotendinous reflexes and spasticity, but depressed in patients on-riluzole. This finding was
44 accompanied by a reduction in MEP size and no changes in PSTH, PSF, and paired-pulse H-
45 reflex probability. It is speculated that spinal interneurons may compensate for potential
46 depression of monosynaptic EPSPs in ALS. From a clinical perspective, while the added value
47 of H-reflex to osteotendinous reflex evaluation may be limited, it can serve as a valuable
48 quantitative biomarker of pyramidal dysfunction in clinical trials.

49 **NEW & NOTEWORTHY**

50 Without significant evidence of peripheral denervation, H-reflex enhancement appears to be
51 a widespread phenomenon, regardless of disease onset site. This increase is likely associated
52 with a decrease in inhibitory control over presynaptic transmission of the synapse between
53 muscle group Ia afferents and motoneurons. Although the link to exaggerated osteotendinous
54 reflexes and spasticity implies a restricted role in identifying a pyramidal syndrome, its
55 quantitative aspect positions the H-reflex as a valuable biomarker in clinical trials.

56 **Key words:** H-reflex, MEP, monosynaptic EPSPs, Interneurons, ALS

57 INTRODUCTION

58 Amyotrophic lateral sclerosis (ALS) is a relentlessly progressive neurodegenerative
59 disease of the human motor system (1). The delay for definite diagnosis is about one year, due
60 to heterogenous clinical presentations contributing to misdiagnoses (2, 3). With the
61 emergence of novel treatment options (4), the importance of promptly identifying and
62 diagnosing ALS has become more critical than ever. Consequently, there has been a consistent
63 increased interest in biomarkers relevant to ALS over the past few decades (5, 6). Developing
64 biomarkers for ALS faces a substantial challenge due to the considerable clinical
65 heterogeneity, encompassing factors such as genetic origins, site of onset, rate of decline,
66 cognitive impairment, and the varying extent of brain cortex (upper motor neurons, UMN)
67 and bulbospinal motor neuron impairment (lower motor neurons, LMN; Turner et al. 2013).
68 Although the diverse underlying biochemical pathways may appear unrelated, they converge
69 to produce a shared clinical syndrome characterized by the progressive degeneration of both
70 UMN and LMN (1). This supports the emphasis on neurophysiological biomarkers and the
71 remaining recognition of electrodiagnosis as the gold standard in ALS. To broaden the scope
72 of electromyogram (EMG) examination, several studies have explored the features of the
73 Hoffman reflex (H-reflex) and motor evoked potential (MEP) in ALS (8, 9). However, the
74 predominant focus in the majority of these studies has been on specific muscle groups, driven
75 by methodological and clinical considerations.

76 H-reflexes can be elicited in nearly all muscles containing muscle spindles, yet the
77 majority of muscles may require reinforcement (such as voluntary contraction) to exhibit the
78 response in EMG. In resting condition, H-reflex is readily produced in soleus EMG of most
79 healthy individuals, whereas it is more challenging in the EMG of other muscles (*e.g.*,
80 quadriceps and forearm flexors; Burke 2016). Consequently, many studies examining H-
81 reflexes have predominantly concentrated on soleus (11), particularly in the context of ALS
82 (9). Findings consistently indicated that the H-reflex was more easily induced in patients with
83 ALS compared to healthy elderly subjects, considering the age-related decline in H-reflex (12).
84 However, the ratio between maximum amplitudes of H-reflex and the direct motor (M)
85 response (H_{max}/M_{max}) remained unchanged (13–17). This observation seems unforeseen
86 considering the early-stage hyperreflexia in ALS (10, 18). Moreover, a recent *in vivo*
87 electrophysiological investigation on isolated motoneurons in the SOD1-mutated mouse

88 model unveiled a reduction in monosynaptic excitatory post-synaptic potentials (EPSPs) of
89 both peripheral and descending origins, contrary to the anticipated hyperexcitability (19).
90 Therefore, we have undertaken additional evaluations of peripheral and corticospinal
91 monosynaptic excitations in various motor nuclei and motor units that innervate the upper
92 and lower limbs in ALS patients.

93 Some years ago, we undertaken an extensive study to assess spinal interneurons in ALS
94 by investigating conditioned H-reflexes and MEPs (<https://clinicaltrials.gov>, NCT02429492). In
95 the initial experiments focusing on quadriceps in patients exhibiting normal clinical
96 examination at this level, we quickly noticed that H-reflex was more easily elicited in patients
97 with ALS, and the H_{max}/M_{max} ratio was higher compared to controls (20). We hypothesized
98 that this outcome might be more dependable than findings reported in the soleus (9).
99 Leveraging this protocol, we systematically investigated the recruitment curve of H-reflex and
100 M response in soleus, quadriceps, and flexors in the forearm, to determine whether ALS-
101 related alterations were consistent across these muscle groups.

102 The increase in the H_{max}/M_{max} ratio can be attributed to various mechanisms, including
103 alterations in M_{max} (a decrease in M_{max} , for a similar H-reflex size, increases the ratio), and/or
104 re-innervation of denervated motor plates by resilient motoneurons resulting in larger motor
105 unit potentials (21–23). These slow type units are the first to be activated by the reflex
106 pathway (consistent with Henneman's principle; Henneman and Mendell 1981) and may
107 consequently cause an artificial increase in the reflex. Another potential factor is a reduction
108 in presynaptic inhibition of group Ia sensory afferents mediating H-reflex to motoneurons (13,
109 15, 25). To discern the contribution of these different phenomena to H-reflex size, a
110 comparative analysis with MEP can be insightful (26). The MEP size typically diminishes over
111 the course of the disease, particularly in the lower limbs (8). To the best of our knowledge,
112 changes in H-reflex and MEP have not been systematically examined in the same patients. The
113 NCT02429492 protocol provided an opportunity for such a comparison, and we evaluated
114 changes in both H-reflex and MEP in the quadriceps.

115 Finally, numerous studies have been conducted on ALS patients to evaluate excitations
116 produced in isolated motoneurons using the peri-stimulus time histogram (PSTH) technique
117 (8, 27). Only one study specifically examined peripheral excitation mediated by group Ia
118 afferents and indicated no alteration in the peaks produced in PSTHs (28). More emphasis has

119 been placed on transcranial magnetic stimulation (TMS)-induced excitation, particularly in
120 motor units isolated from the EMG of intrinsic hand muscles. Globally, corticospinal inputs led
121 to a smaller but more fragmented early peak in the PSTHs (longer duration, multiple sub-
122 peaks) compared to controls. This reflects a desynchronized discharge of motoneurons in
123 response to corticospinal inputs; a phenomenon that intensifies over the course of the disease
124 (29–33). These findings deviate from recent reports in mouse models where monosynaptic
125 EPSPs are diminished (19). However, PSTH studies in patients have not precisely examined the
126 monosynaptic part of the peaks in PSTH, limited to the first 0.6 ms (34, 35). Additionally, PSTH
127 well characterizes the rise time of EPSPs but is limited to distinguish multiple EPSPs and to
128 evaluate their duration, unlike the PSF technique (36, 37). Despite their usefulness and
129 contribution to the study of EPSPs, the PSF and paired-pulse H-reflex methods (38, 39), have
130 not been employed in ALS to date (except PSF to assess spinal recurrent inhibition in ALS;
131 Özyurt et al. 2020). Consequently, we used these two techniques to offer novel insights into
132 monosynaptic EPSPs of peripheral and descending origin in patients with ALS, focusing on
133 muscle groups that are minimally affected or even unaffected in the patients we have
134 investigated.

135 **MATERIALS AND METHODS**

136 **Ethics**

137 This study is part of a larger one on spinal excitability in patients with ALS
138 (SpinalBioMark-SLA) during which we have evaluated several spinal circuitries using indirect
139 electrophysiology (20, 41). The experimental procedures conform to the latest revision of the
140 Code of Ethics of the World Medical Association (Declaration of Helsinki) and were approved
141 by the ethic committee of INSERM (protocol n° C14-21) and by the national ethical authorities
142 (CPP Ile de France, Paris 6 - Pitié-Salpêtrière, CPP/16-15; RCB 2014-201-A01240-47). It has
143 been registered in a public registry (<https://clinicaltrials.gov>, NCT02429492). All participants
144 have given their written informed consent before the experiments. The data are available on
145 request from the corresponding author; they are not publicly available due to ethical
146 restrictions.

147 **Participants**

148 A total of 55 patients (11 women; mean age \pm 1 standard deviation [SD]: 62.5 \pm 9.5 years
149 old) and 56 controls (12 women; 62.8 \pm 8.9 years old) have participated in this study but not
150 all have performed all the experiments (see below, demographic details for each Experiment).
151 The main inclusion criterion for controls was the absence of prior or current neurological
152 illness. Those for patients included probable or definite ALS according to the El Escorial criteria
153 (42), no peripheral neuropathy and no comorbid neurological conditions. Patients were
154 screened and tested for the 4 most common ALS-causing mutations (SOD1, FUS, C9orf72 and
155 TDP43; DNA extraction was performed by Genethon, Evry, France; DNA analysis was carried
156 out at the University of Tours, France), and all were negative except 1 (C9orf72 in patient 37;
157 Table 1). Table 1 resumes the main clinical features. Patients were explored on their non or
158 less affected side, according to the muscle explored in each experiment. This explains why
159 some of them had normal MRC score in the muscle investigated while the onset site was in
160 the corresponding limbs (Table 1: for lower limbs [LL], patients 8, 22-24, 28, 31-33, 37, 45, 46,
161 49, 52 and 55, and for upper limbs [UL], patients 21, 25, 27, 29 34, 39, 44 and 53).

Table 1 near here

163 All participants were tested preferably on the dominant side (43). When patients had
164 motor deficits on this side, we explored their non-dominant side and, in both groups, the non-
165 dominant side was tested in case of orthopedic trauma on the dominant side: i) right-handers
166 tested on the right (dominant) side: 48 controls vs. 40 patients, ii) left-handers tested on the
167 left (dominant) side: 4 controls vs. 3 patients, iii) right-handers tested on the left (non-
168 dominant) side: 1 control vs. 10 patients, and iv) left-handers tested on the right (non-
169 dominant) side: 3 controls vs. 2 patients.

170 Recordings

171 EMGs were recorded using single-use bipolar surface electrodes (sticky foam electrodes
172 with solid gel; 2-cm apart; FIAB, Florence, Italy) secured on the skin cleaned beforehand
173 (exfoliating cream and alcohol) over i) the vastus lateralis (VL) head of the quadriceps femoris,
174 on the antero-lateral part of the thigh, \sim 15 cm above the patella, ii) the soleus, on the
175 posterior part of the leg, \sim 5 cm below the insertion of gastrocnemius muscles, iii) the flexor
176 carpi radialis (FCR), on the medial part of the palmar aspect of the forearm, \sim 8-10 cm distal
177 from the elbow, vi) the extensor carpi radialis (ECR), on the medial part of the dorsal aspect
178 of the forearm, \sim 4 cm distal from the elbow, and v) the tibialis anterior (TA), on the antero-

179 medial part of the leg, ~10-15 cm below the patella. For investigating the discharge of single
180 motor units in ECR and TA, intramuscular EMG recordings were also performed using paired
181 hook wire electrodes (40-cm polytetrafluoroethylene insulated stainless-steel wire, 0.08-mm
182 diameter, 40G) threaded through a hypodermic needle. In the needle, the tips of wires were
183 positioned so that 2 mm of one wire and 5 mm of the second wire protruded from the needle.
184 The first wire was stripped 2 mm, while the second wire was insulated 3 mm and stripped 2
185 mm. The protruding ends were bent at 180° (SGM d.o.o., Split, Croatia). The needle was
186 inserted in ECR and TA to implant the fine wires, and it was subsequently removed.

187 VL, soleus and FCR EMG were amplified and filtered (x 1,000-5,000; 0.1-1kHz bandpass;
188 D360 8-channel Patient Amplifier, Digitimer Ltd, Hertfordshire, UK). For ECR and TA EMG, both
189 surface electrodes and fine wires were plugged to wifi connectors that transmitted the signals
190 to a zero-wire system (Cometa Srl, Milan, Italy); EMG were amplified and filtered (x 5,000 and
191 10-500-Hz bandpass for surface EMG; x 1,000 and 10-1000-Hz bandpass for intramuscular
192 EMG). EMG activities were digitally stored on a personal computer (2-kHz sampling rate;
193 Power 1401 controlled by Signal 6.03 [Experiments 1 & 3] or Spike2 8.07 [Experiments 2 & 4],
194 CED, Cambridge, UK) for off-line analysis.

195 Percutaneous electrical stimulations (1-ms duration rectangular pulse; DS7A, Digitimer
196 Ltd, Hertfordshire, UK) were applied to i) the femoral nerve (FN) through monopolar electrodes,
197 the cathode being a 21-cm² brass plaque placed on the posterior aspect of the thigh (below
198 the buttock) and the anode, a 3-cm diameter brass hemisphere placed in the femoral triangle,
199 ii) the posterior tibial nerve (PTN) using similar electrodes as FN: the cathode was placed
200 above the patella and the anode, in the medial part of the popliteal fossa, iii) the median nerve
201 using bipolar electrodes (3-cm diameter brass hemispheres separated in their center by ~5
202 cm): the 2 electrodes were placed one above the other on the anterior aspect of the arm along
203 the median nerve trajectory, just above the elbow, with the anode at the most proximal site
204 from spinal cord, iv) the radial nerve using bipolar electrodes as median nerve: the cathode
205 was placed ~5 cm above the elbow, anterior to the humerus, and the anode, above the
206 cathode but posterior to the humerus, and v) the common peroneal nerve (CPN) using bipolar
207 electrodes placed on the either side of the neck of the fibula, with the cathode ~5 cm below
208 the patella and the cathode, near the outer edge of the popliteal fossa. Stimulating electrodes
209 were covered by wet sponge tissue and their positions were checked according to motor

210 response evoked in the corresponding EMG, respectively VL for FN, soleus for PTN, FCR for
211 median nerve, ECR for radial nerve and TA for CPN. Clinical responses were also checked by
212 tendon palpation. Sketches in Figure 1AB resume the experimental set ups.

213 **Experimental protocols**

214 The participants were comfortably seated in a reclining armchair, with head support.
215 For upper limb experiments (FCR and ECR EMG), the palmar side of the tested forearm rested
216 on the arm of the chair, with the hand in pronation, shoulder in $\sim 20^\circ$ abduction, elbow in
217 $\sim 100^\circ$ extension and the wrist in neutral position. For lower limb experiments, the tested leg
218 was positioned in a device fixed to the chair and adaptable so that the hip was semi-flexed
219 ($\sim 80^\circ$), the knee semi-extended ($\sim 130^\circ$) and the ankle in semi-plantarflexion ($\sim 100^\circ$). For H-
220 reflex investigations (quadriceps, soleus and FCR), the participants were asked to relax as
221 much as possible and the recordings were performed at rest. MEPs and the discharge of
222 isolated motor units were studied during tonic contraction of the target muscles (quadriceps
223 for MEP, ECR and TA for motor units) which force was estimated according to the mean EMG
224 level during the maximal voluntary contraction.

225

Figure 1 near here

226 ***Experiment 1: H-reflexes in upper and lower limbs***

227 Twenty controls and 19 patients were enrolled in Experiment 1 but we only retained for
228 analysis those in whom we have been able to plot the full recruitment curves of the direct
229 motor M response and of the H-reflex in quadriceps, soleus and FCR. Accordingly, we have
230 excluded: i) 4 controls: no data in FCR in 2 of them (no H-reflex in one and consent withdrawal
231 in the other one), no H-reflex in VL in the third one, and the last one was too sensitive to the
232 stimulations to perform the experiments, and ii) 4 patients: no FCR H-reflex in 2 of them, no
233 soleus H-reflex in the third one and the recruitment curve of soleus was incomplete in the last
234 one. The comparison was thus possible in 16 controls (controls #1-16; 3 women; 60.2 ± 10.6
235 years old) and 15 patients (patients #1-15; 2 women; 62.7 ± 10.2 years old). The 3 muscles
236 were tested during the same experimental session. The intensity of the peripheral nerve
237 stimulations was varied between the threshold for H-reflex and M response and the
238 supramaximal intensity for M response in each individual (Fig. 1AF); 5 trials were performed
239 at each intensity. The stimulation rate was fixed at 0.33 Hz. The intensity was normalized to

240 the threshold intensity of the direct M response in the corresponding EMG (motor threshold,
241 MT) to plot the M response and H-reflex recruitment curves (Figs. 2A-F).

242 ***Experiment 2: Paired-pulse H-reflex in soleus***

243 Twelve controls and 9 patients were enrolled in Experiment 2 but we only retained for
244 further analysis those in whom less than 13 % of the conditioning pulse produced an H-reflex
245 in soleus EMG when given alone (38, 39), which occurred in 2 controls. The group analysis was
246 thus performed in 10 controls (control #4, 15, 17-24; 5 women; 66.0 ± 7.8 years old) and 9
247 patients (patients #16-24; 2 women; 65.4 ± 8.7 years old). Two distinct stimulators were
248 plugged to the same stimulating electrodes thanks to a home-made electronic device we
249 developed according to the recommendations of Digitimer Ltd. This device, linked to 2
250 stimulators, has enabled us to deliver either single or paired pulses through the same pair of
251 electrodes placed over the PTN. First of all, both maximal amplitude of H-reflex (H_{max}) and M
252 response (M_{max}) were evaluated at 0.33-Hz stimulation rate (single pulse). Then, the threshold
253 intensity for H-reflex (1/10 stimuli) was estimated at 1-Hz stimulation rate (single pulse). For
254 the paired pulse paradigm, the intensity of the (first) conditioning pulse was adjusted at the
255 threshold intensity for H-reflex (1-Hz rate) and the intensity of the (second) test pulse was set
256 at 90, 95, 100, 105 and 110 % of the conditioning stimulus intensity. The interstimulus interval
257 (ISI) between paired pulses was 5, 10, 15, 20, 25, 30, 40 and 50 ms. One session of acquisition
258 consisted in alternating series of 10 isolated test and conditioning pulses (5 of each; 1-Hz
259 stimulation rate; white boxes in Fig. 1/), intercalated with series of 20 paired pulse delivered
260 at 1 Hz (blue boxes in Fig. 1/). So, one acquisition included 40 isolated conditioning stimuli (8
261 series x 5 stimuli), 40 isolated test stimuli (8 series x 5 stimuli) and 160 paired pulses (20 stimuli
262 x 8 ISIs). During one session, the intensity of the test stimuli was fixed but it was changed
263 randomly from one session to another (between 90 and 110 % of the single-pulse H-reflex
264 threshold intensity). Five sessions of acquisition were thus performed in each individual and
265 the order of the investigated test intensity was randomized. The ISI between paired pulse was
266 randomized from one series of paired pulses to another. The resulting sequence of tested ISIs
267 during one session of acquisition was determined at the beginning of the experiment and kept
268 constant for the 5 sessions in each participant but the randomization was done for each
269 individual.

270 ***Experiment 3: H-reflex and MEP in VL***

271 Twenty-nine controls and 27 patients were enrolled in Experiment 3 (none in common
272 with other experiments) but i) TMS experiment could not be done in one control because the
273 MEP threshold was too high to study the MEP recruitment curve and ii) one patient was unable
274 to support FN stimulations. Experiment 3 was thus fully undertaken in 28 controls (controls
275 #25-51; 3 women; 62.3 ± 8.9 years old) and 26 patients (patients #25-50; 4 women; 60.3 ± 9.7
276 years old). MEP, M response and H-reflex were tested during the same experimental session.
277 The procedure for M response and H-reflex was similar as in Experiment 1. For the MEP
278 recruitment curve, we first evaluated the mean EMG activity in VL during maximal voluntary
279 contraction. Then, the participants were asked to perform tonic contraction of quadriceps
280 during TMS so as to produce 10 % of the mean EMG level during maximal voluntary
281 contraction (visual feedback). TMS (Rapid Stim, Magstim Company Ltd, Whitland, UK) was
282 delivered through double cone coil placed at the hot spot for MEP in VL EMG; the voluntary
283 tonic contraction helped to focus TMS on quadriceps cortical motor area and to get reliable
284 MEPs in quadriceps EMG. Stimulator output was changed from MEP threshold intensity and
285 the supramaximal intensity for maximal MEP (MEP_{max}), using steps of 2 % of maximal
286 stimulator output (MSO); 10 trials were performed at each intensity. The intensity of FN
287 stimulation was normalized to the motor threshold (MT, see Experiment 1) and that of TMS
288 was normalized to the threshold for MEP (active motor threshold, AMT) to plot the H-reflex
289 and MEP recruitment curves (Fig. 5AB).

290 ***Experiment 4: Monosynaptic excitation in upper and lower limb motoneurons.***

291 Twenty-three controls (control #1-3, 5-14, 16-18, 52-56; 4 women; 61.6 ± 9.6 years old)
292 and 12 patients (patient #11-15, 17, 24, 51-55; 4 women; 65.8 ± 8.2 years old) were enrolled
293 in Experiment 4 (some of which have participated in the previous Experiments). Participants
294 were asked to perform tonic contraction of ECR or TA (2-5 % of maximal voluntary contraction)
295 so as to extract one single motor unit potential off-line and investigate its probability of
296 occurrence in EMG and frequency rate around stimulation applied to i) the corresponding
297 peripheral nerve (radial nerve for ECR, and CPN for TA) or to ii) the primary motor cortex using
298 TMS, with a 9-cm round coil for ECR or cone-coil for TA, both localized at the hot spot for MEP
299 in the corresponding EMG. Intensity of peripheral nerve stimulations was adjusted at 1 x the
300 motor threshold (MT) and 100 stimuli were delivered à 2-Hz frequency rate during 1 session
301 of recording. TMS intensity was set at 0.9 the active motor threshold (AMT; same level of

302 contraction as during motor unit recordings) and 50 stimuli were delivered at 0.8-Hz frequency
303 rate during 1 session of recording. In both cases, peripheral nerve stimulations or TMS, 4
304 sessions of recordings were repeated in each participant.

305 **Analysis**

306 For Experiment 1 and 3, peak-to-peak amplitude of H-reflexes and M responses (Fig. 1C-
307 E) and size of rectified MEPs (Fig. 1JL) were measured and their mean were plotted against
308 stimulus intensity in each individual (Figs. 1F-H,L). For MEP analysis, we have estimated the
309 extra-activity due to TMS, in addition to voluntary tonic contraction (10 % of maximal
310 voluntary contraction). For this, we have calculated i) the area of rectified MEP limited to its
311 duration (Fig. 1K), ii) the area of pre-stimulus EMG activity during 80 ms, and iii) the
312 background activity was first estimated from the 80-ms area of pre-stimulus activity and then
313 related to the same duration as MEP (related background activity). MEP size was then
314 estimated as followed:

$$315 \quad MEP = \frac{\text{MEP area} - \text{Related background activity}}{M_{max} \text{ area}}$$

316 Then, data for each individual were fitted with the following 3-parameter sigmoid
317 function (Fig. 1 F-H,L; Carroll et al. 2001; Devanne et al. 1997; Klimstra and Zehr 2008):

$$318 \quad \text{Amplitude} = \frac{\text{Asymptote}}{\left(1 + e^{\frac{I_{50}-I}{k}}\right)}$$

319 where amplitude of H-reflex (within the ascending phase of the H-reflex recruitment curve;
320 Fig. 1C-E), M response and MEP is related to its maximal size (upper asymptote of the
321 recruitment curve), I_{50} , *i.e.* the intensity producing half the asymptote (crossover point of the
322 sigmoid) and k , the growth rate. The peak slope of the linear part of the sigmoid was calculated
323 to provide an indication of the maximal rate of increase in the magnitude of H-reflex, M
324 response or MEP with stimulus intensity (44):

$$325 \quad \text{Peak slope} = \frac{\left(\frac{1}{k}\right) \text{Asymptote}}{4}$$

326 For group analysis, M response, H-reflex and MEP were normalized to M_{max} (peak-to-peak
327 amplitude for M response and H-reflex, and its area for MEP). The intensity of peripheral nerve

328 stimulations and TMS was normalized to motor threshold (\times MT for peripheral nerve, and \times
329 AMT for TMS; Figs. 2A-F, Fig. 5AB).

330 For Experiment 2, H-reflex was detected if its amplitude exceeded the $\pm 5 \times$ SD limits of
331 the level of the 400-ms pre-stimulus activity. In paired-pulse series, we paid attention that the
332 conditioning stimulus did not produce H-reflex. The probability of paired pulse H-reflex
333 corresponds to its occurrence on the total number of paired-pulse stimulations; it was
334 calculated for each ISI (5, 10, 15, 20, 25, 30, 40 and 50 ms) and each test intensity (90, 95, 100,
335 105 and 110 % of the single-pulse H-reflex threshold intensity). Paired pulse H-reflex
336 probability was plotted against ISI and test intensity (3D-distribution; Fig. 4AB) and iso-
337 response curves as a function of ISI and test intensity were calculated using locally weighted
338 scatterplot smoothing (LOWESS, MATLAB R2022, The MathWorks, Natick, MA; (47); 2D-
339 distribution, Fig. 4CD). Then, to quantify the rate of decay of population excitatory
340 postsynaptic potentials (EPSPs), an exponential function was fit to the data of the normalized
341 stimulus current amplitude as a function of the ISI (time constant = x-intercept of the tangent
342 at 0 of the 1st derivative). The average of time constants calculated from the 7 iso-probability
343 contours from 0.4 to 0.7 (with a 0.05 interval) was then calculated to quantify the time course
344 of the population EPSPs (38, 39).

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

$$348 \quad \text{UMN score} = \textit{reflex score} + \textit{Babinski or Hoffmann sign score} + \textit{MAS score}$$

349 where the *reflex score* reflecting tendon reflexes indicates 0 when normal or absent, 1 when
350 present in wasted muscle, and 2 when brisk; the *Babinski or Hoffmann sign score* indicates 0
351 when absent or 1 when present; and the *MAS score* indicates 0 if the Modified Ashworth Scale
352 (MAS) < 3 , and 1 of ≥ 3 (i.e., with high possibility of muscle clonus).

$$353 \quad \text{LMN score} = \textit{atrophy score} + \textit{fasciculation score} + \textit{MRC score}$$

354 where the *atrophy score* indicates 0 when absent or 1 when present; the *fasciculation score*
355 indicates 0 when absent or 1 when present; and the *MRC score* indicates 0 when the Medical
356 Research Council (MRC) grade was 5, 1 if the grade was 4 or 3, and 2 if the grade was between
357 2 and 0.

358 Spike sorting was performed using Spike2 8.07, to extract single motor unit potentials
359 from EMG. Then, peristimulus time histograms (PSTH, 0.2-ms bins; Awiszus 1997) and
360 frequencygrams (PSF; Norton et al. 2008; Türker and Powers 2005) were constructed still
361 using Spike2 8.07 (script PSF 03e.s2s) to investigate motor unit discharge around stimulation
362 (Fig. 6AD). We have evaluated the latency and duration of the peak in PSTH, the total number
363 of bins (% of number of stimulations) within the peak and during its 0.6 first ms (corresponding
364 to purely monosynaptic EPSP; Pauvert et al. 1998; Pierrot-Deseilligny et al. 1981), the latency
365 and duration of PSTH cumulative sum (CUSUM), and the latency and duration of PSF CUSUM
366 corresponding to the first peak in PSTH (both CUSUM calculated using Excel 16.78.3).

367 **Statistics**

368 Statistical analyses were performed using JMP® Pro 16.0.0 (SAS Institute JMP, Brie
369 Comte Robert, France). The alpha significance level was fixed at 0.05 and the results were
370 considered statistically significant only if $p < 0.05$. Mean values are indicated ± 1 SD.
371 Parametric or non-parametric tests were undertaken according to homoscedasticity (assessed
372 using the Levene median test) and normality (assessed using the Shapiro-Wilk test). Significant
373 outliers were detected using the inter-quantile range (IQR) method. Effect size was measured
374 using i) Cohen's d (50, 51) for 2-mean comparisons, ii) Cohen's f^2 (52) for mixed models and
375 iii) Cohen's w (Cohen 1988) for χ^2 . Alternatively, mean and 95 % confidence interval (CI_{95})
376 are indicated. Linear mixed model analyses on repeated measures were used to perform
377 multivariate analysis and *post hoc* analyses were performed using Student t tests on least-
378 squares means (marginal means). Given the differences in electrophysiological metrics, we
379 calculated the CI_{95} in controls and metrics in ALS were classified according to the lower and
380 upper limits of CI_{95} in controls. Then, χ^2 tests, multiple correspondence analysis and analysis
381 of variance (ANOVA) were undertaken to evaluate the link between electrophysiological
382 results and clinical features; χ^2 was also use to compare the proportion of motor units
383 retained for analysis in controls and patients (Experiment 4). For clarity, the statistical tests
384 and the parameters included in each test are specifically indicated in Results.

385 **RESULTS**

386

<i>Table 2 near here</i>

387 **H-reflex and M response recruitment curves in upper and lower limbs**

388 First, we have compared the motor threshold between groups when stimulating PTN
389 (soleus), FN (quadriceps) and median nerves (FCR; Experiment 1). Mixed model for repeated
390 measures was built, including the group (controls vs. ALS) as between-subject factor, and the
391 peripheral nerve (FN vs. PTN vs. median nerve) as within-subject factors. The model (adjusted
392 $r^2 = 0.61$) did not reveal any significant influence of the group (ALS vs. controls; $p = 0.44$, $f^2 =$
393 0.10) and the interaction between factors (group x peripheral nerve) was not significant either
394 ($p = 0.09$, $f^2 = 0.10$; no significant outliers, IQR method; Table 2). We then compared the
395 maximal amplitude of M response and H-reflex still using mixed model analysis and we did
396 not find any difference between groups ($p = 0.44$, $f^2 = 0.02$) and no significant interaction
397 between factors (group x response type [M response vs. H-reflex] x muscles [FCR vs. soleus vs.
398 quadriceps]; $p = 0.83$, $f^2 = 0.19$; no significant outliers, IQR method; Table 2). The absence of
399 significant differences validates normalization of stimulus intensity to the motor threshold (x
400 MT) and of both responses to the maximal amplitude of M response (M_{max}), to plot the
401 recruitment curves (17, 54–56).

402 **Figure 2 near here**

403 Figure 2 illustrates the mean amplitude of H-reflex and M response (normalized to M_{max})
404 plotted against the stimulation intensity (normalized to the motor threshold; x MT), in FCR
405 (Fig. 2AB), soleus (Fig. 2CD) and quadriceps (Fig. 2EF), in the group of controls (Fig 2A,C,E) and
406 of patients with ALS (Fig 2B,D,F). The most striking differences between the groups were the
407 leftward shift and higher asymptote of the H-reflex recruitment curves in ALS. The asymptotes
408 of H-reflex recruitment curves were compared using mixed model for repeated measures
409 (adjusted $r^2 = 0.53$), including group (controls vs. ALS) as between-subject factor, and muscle
410 (FCR vs. soleus vs. quadriceps) as within-subject factors. The results confirmed a significant
411 difference between groups ($p < 0.01$, $f^2 = 0.01$; no significant outliers, IQR method) and
412 between muscles ($p < 0.01$, $f^2 = 0.01$) but no significant interaction between factors (group x
413 muscle; $p = 0.94$, $f^2 = 0.01$) indicating that the asymptote of the normalized H-reflex
414 recruitment curves was greater in ALS whatever the muscle tested (Fig. 2G; *post hoc* analyses
415 to compare the marginal means between controls and ALS using Student t tests: $p < 0.01$ for
416 FCR, $p < 0.05$ for soleus and quadriceps). Similar analyses were performed to compare I_{50} and
417 peak slopes of both H-reflex and M response recruitment curves (no significant outliers, IQR
418 method; Table 2). The model for comparing I_{50} (adjusted $r^2 = 0.94$) has revealed significant

419 influence of muscle (FCR vs. soleus vs. quadriceps; $p < 0.01$, $f^2 = 1.44$), response type (H-reflex
420 vs. M response, $p < 0.001$, $f^2 = 1.48$) and significant interactions between muscle x response
421 ($p < 0.001$, $f^2 = 0.19$) and between group x muscle x response type ($p < 0.05$, $f^2 = 1.49$). Figure
422 2H shows that I_{50} for H-reflex was indeed systematically lower in ALS than in controls but the
423 *post hoc* pairwise comparisons of marginal means failed to reveal any significant differences
424 between groups for each muscle (Student t test with $0.18 < p < 0.31$), and I_{50} for M response
425 was only significantly lower in FCR of ALS as compared to controls ($p < 0.05$). Regarding the
426 peak slope, the model (adjusted $r^2 = 0.23$) revealed a significant difference between groups (p
427 < 0.01 , $f^2 < 0.02$) and a significant interaction between group x response type ($p < 0.05$, $f^2 <$
428 0.02). Figure 2J shows that the peak slope for H-reflex recruitment curve was steeper in ALS
429 than in controls (*post hoc* Student t test, $p < 0.001$; Table 2), whatever the muscle tested
430 (interaction between group x response x muscle, $p = 0.82$, $f^2 < 0.02$). Accordingly, we found a
431 significant increase of the ratio between the peak slopes of H-reflex and M response
432 recruitment curves whatever the muscle tested (adjusted $r^2 = 0.07$, p value for group < 0.05
433 [$f^2 = 0.01$], p value for muscle = 0.51 [$f^2 = 0.01$], p value for interaction between group x muscle
434 = 0.53 [$f^2 = 0.01$]; marginal means 1.04 ± 0.57 vs. 2.87 ± 0.59 for controls and ALS, respectively).
435 All these results indicate that normalized H-reflex was enhanced and more excitable in ALS
436 than in controls (left shift, steeper linear increase, higher maximal amplitude either estimated
437 using recruitment curve asymptote or empirical H_{\max}/M_{\max} ratio: Student t test to compare H-
438 reflex asymptote and H_{\max}/M_{\max} ratio, $p = 0.94$, $d = 0.001$; Table 2).

439 **Figure 3 near here**

440 We calculated the number of patients with increased, similar or decreased H-reflex
441 asymptote, according to CI_{95} in the control group, for each muscle (Fig. 3A). χ^2 analysis
442 revealed no significant difference between muscles ($p = 0.41$, $w = 0.3$), further confirming that
443 the normalized H-reflex was significantly increased in ALS, whatever the muscle tested.
444 Possible link between normalized H-reflex enhancement in ALS (asymptote, peak slope ratio)
445 and clinical features (onset site, ALSFRS-r, progression, score to MRC scale, UMN and LMN
446 scores, spasticity [yes or no]), hyperreflexia [yes or no], and riluzole [on or off]) was assessed
447 using ANOVA taking the muscle origin into account. We only found significant links between
448 asymptote and (p -values adjusted for muscle factor) i) spasticity ($p < 0.001$, $d = 1.6$): the mean
449 asymptote was 41.7 ± 25.1 % of M_{\max} in non-spastic patients vs. 72.6 ± 11.8 % in spastic

450 patients (Fig. 3C), ii) hyperreflexia ($p < 0.05$, $d = 0.7$): the mean asymptote was 38.2 ± 13.9 %
451 of M_{\max} in patients with either normal or abolished osteotendinous reflex vs. 54.8 ± 25.8 % in
452 patients with exaggerated osteotendinous reflex (Fig. 3D), and iv) with riluzole intake ($p < 0.05$,
453 $d = 0.6$): the mean asymptote was 53.8 ± 28.6 % of M_{\max} in patients off-riluzole vs. 38.8 ± 19.3
454 % in patients on-riluzole (Fig. 3E). Lastly, we found significantly higher asymptote in patients
455 exhibiting reduced M_{\max} in comparison to those with M_{\max} size falling within the range
456 observed in the control group (CI_{95} limits in the control group; $p < 0.001$ adjusted for the
457 muscle factor, $d = 1.1$): 38.8 ± 26.1 % of M_{\max} in patients with M_{\max} unchanged vs. 62.8 ± 18.5
458 % M_{\max} in patients with reduced M_{\max} . In each muscle, when M_{\max} was unchanged in patients,
459 the mean asymptote was above that of controls (continuous lines in Fig. 3B). When M_{\max} was
460 reduced in patients, the difference between groups was even greater and the mean ratio in
461 patients (and its SD) above the range of controls (Fig. 3B). It is interesting to note that in
462 controls, no significant correlation was observed between the asymptote of normalized H-
463 reflex and M_{\max} ($p = 0.19$, adjusted for the muscle factor) while a significant negative
464 correlation was identified in individuals with ALS ($p < 0.02$). These results indicate that the H-
465 reflex maximal amplitude in ALS was particularly enhanced in spastic muscles, in presence of
466 hyperreflexia, and in patients off-riluzole. Moreover, maximal H-reflex was related to M_{\max}
467 changes, being even more increased when M_{\max} was reduced.

468 **Figure 4 near here**

469 **Paired-pulse H-reflex in soleus**

470 The objective of Experiment 2 was to investigate the probability of occurrence of H-
471 reflex in soleus EMG after paired PTN stimulation, to estimate the duration of population
472 EPSPs. First of all, we compared the amplitude of M_{\max} and H_{\max} in soleus EMG (as in
473 Experiment 1) and we found again that: i) M_{\max} did not differ between groups (3.0 ± 1.7 vs.
474 3.5 ± 2.3 mV in ALS and controls, respectively; Student t test, $p = 0.67$, $d = 0.2$) and the
475 H_{\max}/M_{\max} ratio was significantly greater in ALS (50.9 ± 6.2 vs. 26.2 ± 15.5 % of M_{\max} , in ALS
476 and controls, respectively; Kolmogorov-Smirnov test, $p < 0.01$, $d = 2.1$). Then, H-reflex
477 probability after paired PTN stimulation was plotted against the ISI between pulses and the
478 test stimulus intensity. Figure 4AB shows the resulting 3D plot in one control (A) and one
479 patient with ALS (B). The 2D plots in Figure 4CD shows the corresponding iso-probability
480 curves in the same participants and the exponential decreasing between the test intensity and

481 the ISI, especially when the H-reflex probability was between 0.4 and 0.7 (yellow to cyan
482 curves).

483 At the group level, we first compared the proportion of stimulating conditions (among
484 the 40 tested) which led to produce an H-reflex with $p \geq 0.5$. Figure 4E illustrates the result in
485 each individual and Kolmogorov-Smirnov test indicates that the proportion of patients with p
486 ≥ 0.5 was significantly greater compared to controls ($p < 0.05$, Cohen $d = 1.5$; no significant
487 link with clinical features). This result was not accompanied by a significant difference in the
488 time constant of the population EPSPs between groups (Kolmogorov-Smirnov test, $p = 0.47$, d
489 $= 0.9$; Fig. 4E). We did not find any significant outliers (IQR method) and the 2 patients (#17
490 and #21) with the largest time constant were not different from the rest of the group.

491 These results suggest that while the probability to produce H-reflex after paired
492 stimulations was greater in this group of ALS patients, the time course of population EPSPs
493 was unchanged compared to controls.

494 **H-reflex and MEP recruitment curves in quadriceps**

495 In Experiment 3, we did not find any significant differences between controls and ALS
496 when comparing i) M_{max} in quadriceps (1.42 ± 1.29 mV in controls vs. 1.62 ± 1.09 mV in ALS;
497 Kolmogorov-Smirnov, $p = 0.13$, $d = 0.2$), ii) the background quadriceps EMG activity during
498 TMS (1.03 ± 0.55 $\mu\text{V}/\text{ms}$ in controls vs. 1.17 ± 0.64 $\mu\text{V}/\text{ms}$ in ALS; Kolmogorov-Smirnov, $p =$
499 0.51 , $d = 0.2$), and iii) the motor threshold: threshold for M response after FN stimulation (36.3
500 ± 11.8 mA in controls vs. 32.3 ± 12.4 mA in ALS; Student t test, $p = 0.24$, $d = 0.3$), and active
501 motor threshold (AMT) to TMS (44.8 ± 10.7 % of maximal stimulator output in controls vs.
502 48.7 ± 8.1 % in ALS; Student t test, $p = 0.15$, $d = 0.4$).

503 **Figure 5 near here**

504 Figure 5AB illustrates the mean normalized H-reflex (A) and MEP (B) recruitment curves
505 in both groups. The adjusted r^2 of the 3-parameter sigmoid fitting ranged between: i) 0.51 and
506 0.99 (0.82 ± 0.15) for the H-reflex in controls, ii) 0.43 and 1.0 (0.87 ± 0.13) for the H-reflex in
507 ALS, iii) 0.87 and 1.0 (0.96 ± 0.04) for the MEP in controls, and iv) 0.63 and 0.99 (0.89 ± 0.10)
508 for the MEP in ALS. Mixed models for repeated measures were built to compare the
509 asymptote, peak slope and I_{50} taking into account the group (controls vs. ALS) as between-
510 subject factor and the response type (H-reflex vs. MEP) as within-subject factors. The models

511 revealed a significant interaction between group and the asymptote (adjusted $r^2 = 0.08$, $p <$
512 0.0001 , $f^2 = 0.20$), I_{50} (adjusted $r^2 = 0.13$, $p < 0.05$, $f^2 = 0.18$), and peak slope (adjusted $r^2 = 0.09$,
513 $p < 0.01$, $f^2 = 0.20$). *Post hoc* analysis on least-squares means confirmed that i) H-reflex
514 asymptote was significantly larger in ALS than in controls (Student t test, $p < 0.05$) while that
515 for MEP_{max} was significantly decreased in ALS ($p < 0.001$; Fig. 5C). ii) The I_{50} for MEP was
516 decreased in ALS ($p < 0.01$) while that for H-reflex was not modified ($p = 0.52$; Fig. 5D). And iii)
517 the peak slope of H-reflex recruitment curve was significantly steeper in ALS ($p < 0.01$) but
518 there was no difference between groups for MEP ($p = 0.37$; Fig. 5E). These results highlight
519 the different modulations of H-reflex and MEP in ALS, the former being enhanced (asymptote,
520 peak slope; similar results as Experiment 1) while the latter is depressed. However, it is
521 interesting to note that half size of maximal MEP is produced at lower intensity in ALS (I_{50}).

522 According to CI_{95} in the control group, we counted the number of patients with larger,
523 similar or smaller H-reflex and MEP asymptotes. Figure 5F illustrates the contingency table
524 and we found a significant difference in the proportion of patients according to H-reflex and
525 MEP modifications (χ^2 , $p < 0.001$, $w = 0.7$). In almost 2/3 of the patients, H-reflex asymptote
526 was increased (65.4 %) while almost all patients had smaller MEP asymptote (92.3 %)
527 compared to control group. This outcome, increased H-reflex and decreased MEP, aligned
528 with the findings observed in 13 out of 26 patients; in all patients with enhanced H-reflex rise,
529 the MEP was depressed except one. Only 8/26 patients had a smaller M_{max} (according to CI_{95}
530 in control group): 6/8 exhibited a larger H-reflex and only one a larger MEP as well, the second
531 patient with a larger MEP, had an M_{max} within the control range and a depressed H-reflex.

532 We then performed a multiple correspondence analysis to evaluate the relative links
533 between neurophysiological changes and clinical features. Response (H-reflex vs. MEP), onset
534 site (upper vs. lower limbs vs. bulbar vs. axial), total score to ALSFRS-r (more or less affected,
535 according to the median score), progression rate (slow vs. faster progressors), ALS form
536 (predominant UMN, LMN or mixed), riluzole (on vs. off) and spasticity (yes vs. no) were
537 included in the MCA. The projection of each modality in a 2-dimension plot is illustrated in
538 Figure 5G. The type of response (H-reflex vs. MEP) particularly contributes to dimension 1
539 which represents 64.3 % of the deviation from the independence between variables (inertia)
540 further confirming the difference between both responses: according to the distance between
541 response type and the modulation of their amplitude, the MEP was decreased and the H-

542 reflex more increased in this group of patients (Chi^2 , $p < 0.01$). The LMN form of ALS and the
543 unchanged amplitude of neurophysiological metrics particularly contributed to the dimension
544 2 which was not significant (35.7 % of the inertia; Chi^2 , $p > 0.05$). This analysis primarily
545 confirms the different modulation of H-reflex (particularly enhanced) and MEP (depressed) in
546 this group of ALS.

547 Interestingly, the 2D-plot shows that spastic patients and those off-riluzole are close to
548 increased amplitude of H-reflex (Fig. 5G). Accordingly, and based on the results from
549 Experiment 1, we explored potential associations between asymptote and spasticity,
550 hyperreflexia, and riluzole intake using the Wilcoxon sign rank test. Regarding the H-reflex, we
551 only observed a significant association with hyperreflexia ($p < 0.01$, $d = 1.5$), but not with
552 spasticity ($p = 0.08$, $d = 0.8$) nor with riluzole intake ($p = 0.80$, $d = 0.2$). Last, we found the MEP
553 asymptote significantly associated with spasticity ($p < 0.05$, $d = 0.2$), while no significant
554 associations were found with hyperreflexia ($p = 0.45$, $d = 0.2$) or riluzole intake ($p = 0.49$, $d =$
555 0.6).

556 All these findings suggest that within this patient group, the H-reflex in quadriceps was
557 found again mostly enhanced while the MEP was particularly depressed. Statistically,
558 alterations in maximal response size were associated with spasticity (compared to Experiment
559 1, we only found a tendency for H-reflex being linked to spasticity in this group of patients),
560 and the H-reflex rise was linked to the manifestation of hyperreflexia.

561 **Corticospinal and peripheral excitations in isolated motoneurons**

562 A total of 190 motor units could be extracted from EMGs in Experiment 4: i) ECR/radial
563 nerve stimulation: 30 units in 22 controls with significant PSTH peak in 13 units (43.3 %; 11
564 participants) vs. 20 units in 12 ALS with peak in 7 units (35.0 %; 6 participants; Chi^2 to compare
565 controls and patients, $p = 0.55$, $w = 0.1$), ii) ECR/TMS: 26 units in 19 controls with significant
566 PSTH peak in 17 units (65.4 %; 15 participants) vs. 21 units in 12 ALS with peak in 8 units (38.1
567 %; 6 participants; $p = 0.06$, $w < 0.1$), iii) TA/CPN stimulation: 31 units in 23 controls with
568 significant PSTH peak in 10 units (32.3 %; 10 participants) vs. 16 units in 11 ALS with peak in
569 13 units (81.3 %; 8 participants; $p < 0.01$, $w < 0.1$), and iv) TA/TMS: 30 units in 23 controls with
570 significant PSTH peak in 13 units (43.3 %; 13 participants) vs. 16 units in 11 ALS with peak in 3
571 units (18.6 %; 3 participants; $p = 0.09$, $w < 0.1$). We thus observed a greater proportion of
572 significant peaks in TA PSTHs after peripheral stimulation but there were no clinical features

573 that could explain this result (e.g., more altered TA compared to ECR but this was not the case;
574 Table 1).

575 *Figure 6 near here*

576 *Table 3 near here*

577 Figure 6*AB* illustrates PSTHs and their CUSUM from one single ECR motor unit around
578 stimulation (radial nerve) in one control and one ALS patient, and their corresponding PSFs
579 and CUSUM are shown in Figure 6*CD*. In both participants, we observed an increased in motor
580 unit discharge at about 35 ms after stimulation (the peak in PSTH and PSF has longer latency
581 as compared to H-reflex in ECR due to motor unit trigger delay). Table 3 resumes all the data
582 on motor units and their peaks in PSTHs and PSFs that were analyzed in controls and patients
583 with ALS.

584 At the group level, we first compared the threshold intensity for stimulation-induced
585 motor response in the target muscle between controls and ALS: i) radial nerve stimulation:
586 $MT = 12.2 \pm 5.9$ mA in controls vs. 12.2 ± 2.5 mA in ALS (Kolmogorov-Smirnov test, $p = 0.78$, d
587 $= 0$), ii) CPN stimulation: $MT = 7.4 \pm 2.6$ mA in controls vs. 6.2 ± 1.9 mA in ALS (Student t test,
588 $p = 0.23$, $d = 0.5$), iii) TMS over ECR motor cortex: $AMT = 45.1 \pm 8.4$ % of MSO in controls vs.
589 48.7 ± 10.9 % of MSO in ALS (Student t test, $p = 0.42$, $d = 0.4$), and iv) TMS over TA motor
590 cortex: $AMT = 46.5 \pm 9.0$ % of MSO in controls vs. 44.7 ± 9.6 % of MSO in ALS (Student t test,
591 $p = 0.79$, $d = 0.2$). Then, we performed multivariate analyses using linear mixed models on
592 repeated measures to determine whether the characteristics of motor unit responses in PSTHs
593 and PSFs were significantly different between groups. For all parameters in Table 3, we tested
594 the group (controls vs. ALS) as between-subject factor and, as within-subject factors, the
595 muscle (ECR vs. TA) and the stimulation type (peripheral nerve stimulation vs. TMS). We did
596 not find any significant results (Table 3). Figure 6*EF* show the grand average of the initial 0.6-
597 ms of the peaks in PSTH and that of PSF duration, revealing no difference between groups.

598 All these results suggest that single motor unit response to peripheral nerve stimulation
599 and TMS was unchanged in ALS, compared to controls.

600 **DISCUSSION**

601 In this study, we explored EMG in muscle groups where the majority of patients
602 displayed no clinical dysfunction as evidenced by the MRC scores. This was further supported

603 by similar mean M_{max} amplitudes in controls and patients. The recruitment curves of H-reflex
604 and M response were systematically investigated in FCR, soleus, and quadriceps, and they
605 revealed specific enhancement of H-reflex in all the 3 muscles (increased asymptote and peak
606 slope, decreased I_{50}). In quadriceps, this result was accompanied with a reduction in MEP, and
607 these changes were associated with spasticity. The H-reflex rise was also linked to the
608 presence of hyperreflexia, and particularly observed in patients off-riluzole. Lastly, this study
609 revealed that both unitary and population EPSPs, respectively assessed through single motor
610 unit discharge and paired-pulse H-reflex, were not modified in ALS.

611 **Methodological and physiological considerations**

612 The first studies on ALS-related H-reflex changes date back only to the 1990s, revealing
613 an initial increase in the H_{max}/M_{max} ratio, followed by a decline as the disease progressed.
614 Despite the absence of a clear link with the clinical phenotype, patients with a higher ratio
615 exhibited a better prognosis (16, 57, 58). However, subsequent studies have not confirmed
616 the rise phase, and have mainly reported that the ratio was decreased or unchanged (13, 14,
617 17). In many of these studies, M_{max} amplitude was either not reported or was decreased in
618 patients, making it difficult to interpret ratio changes. Clinically, the patient groups were
619 relatively comparable across studies in terms of disease duration, functional status (ALSFRS-
620 r), and UMN involvement, resembling the present group of patients. However, none of
621 previous studies documented the clinical assessment of the muscle involved (soleus). Yet,
622 considering the decline in M_{max} , it can be inferred that soleus was impaired.

623 Seemingly straightforward, the H-reflex technique conceals nuances that demand
624 stringent experimental protocols and meticulous data interpretation. Important
625 methodological principles include the study of the recruitment curve and the normalization
626 of the H-reflex to M_{max} , and of the intensity to the motor threshold (x MT), all estimated under
627 the same experimental conditions to prevent any distortion of the ratios and potential
628 misinterpretation (56). M_{max} is indeed considered as a physiological constant, *i.e.* the maximal
629 compound muscle action potential (CMAP) evoked in EMG when all LMNs are activated. It is
630 commonly used to normalize other CMAPs like H-reflex or MEP, for a more reliable intra- and
631 inter-individual comparisons (59, 60). However, a small M_{max} value results in a larger ratio,
632 irrespective of any alteration in the potential of interest (H-reflex or MEP). This arithmetical
633 bias emphasizes the importance of comparing M_{max} values between groups, and the validity

634 of such group comparisons hinges on the comparability of M_{max} . In the control group of the
635 present study, there was no correlation between the M_{max} size and the H_{max}/M_{max} ratio
636 (estimated using the asymptote of the normalized H-reflex recruitment curve), suggesting that
637 the reflex volley has activated the same proportion of LMNs in all healthy participants. In
638 contrast, in the patient group, we found a negative correlation between M_{max} and the ratio.
639 However, the ratio was increased even in patients whose M_{max} was within the same range of
640 the control group. This result confirms that irrespective of M_{max} size, the H-reflex was
641 enhanced in patients with no functional impairment of the target muscle, either clinically
642 (MRC score) or electrophysiologically (M_{max} amplitude). In patients with reduced M_{max} , the
643 ratio was even more increased, which raises questions. Indeed, the peripheral denervation
644 increases the probability of collision between the orthodromically-conducted H-reflex
645 discharge and the antidromically-conducted impulses in motor axons (17), which likely
646 underlies the reduction of the mean H-reflex amplitude with disease progression (16). In the
647 aforementioned study, the authors did not standardize the H-reflex in relation to M_{max} .
648 Instead, they examined variations in its amplitude based on the M response amplitude. They
649 found that, for a comparable M response amplitude, the reflex diminished with disease
650 progression (16). M_{max} normalization is a valuable method for comparing responses across
651 groups, whether H-reflexes or MEPs. Yet, the interpretability depends on the comparability of
652 M_{max} size among the groups and the level of peripheral denervation. Accordingly, we also
653 investigated the recruitment curves of non-normalized (raw) H-reflex, M response and MEP,
654 and we found similar results as normalized data: i) M_{max} tend to decrease in ALS (something
655 that one could expect), and the H_{max} , to increase, but the difference between groups was not
656 significant, ii) their ratio was decreased in ALS, specifically in spastic and hyperreflexic
657 patients, and those off-riluzole and iii) the MEP_{max} was significantly depressed in ALS groups
658 (Supplemental data 1).

659 Examining other parameters of the H-reflex recruitment curve, such as I_{50} and peak
660 slope, offers insights into the excitability of the reflex pathway, regardless of the amplitude of
661 EMG responses. However, normalizing the stimulation intensity can also distort the results.
662 The peripheral nerve stimulation is usually normalized to the motor threshold ($\times MT$). With
663 the peripheral denervation affecting primarily large diameter motor axons in ALS (22, 61), the
664 motor threshold increases with ALS progression (Table 3). Since more resilient (slow) LMNs

665 are first activated by the reflex pathway (Henneman size principle; Henneman and Mendell
666 1981), the threshold for H-reflex might be unchanged, and this interferes with the I_{50} and peak
667 slope estimation. Analyzing recruitment curves using raw non-normalized intensity did not
668 reveal any change in I_{50} and peak slope (Table 1 in Supplemental data 1). However, we found
669 again a steeper increase of H-reflex size when plotting the normalized H-reflex against the
670 intensity in mA (Table 2 in Supplemental data 1).

671 Whatever the method used to analyze the results, we consistently found the H-reflex
672 enhanced in the patient groups we studied.-This finding aligns with our experience that the H-
673 reflex was more easily evoked in patients compared to controls, especially in the elderly (>
674 70), and results of Experiment 3 (Fig. 4E).

675 **Corticospinal excitability and degeneration**

676 While previous researches primarily concentrated on the soleus H-reflex, reporting
677 heterogeneous results likely associated with LMN involvement, our study reveals that among
678 muscles in which H-reflex is easily and reliably elicited (soleus, FCR, quadriceps; Burke 2016),
679 patients exhibit an increased H_{max}/M_{max} ratio, irrespective of the site of the first clinical
680 symptoms. In the early stages of the disease, LMN loss is offset by collateral reinnervation,
681 preserving muscle strength (62, 63), and motor unit potentials are enlarged, disrupting the
682 relationship between EMG power and force (41, 64, 65). Such neuromuscular plasticity thus
683 allows the preservation of M_{max} amplitude for a while, as the enlargement of motor unit
684 potentials conceal the loss of LMNs. Considering that resilient LMNs are among the first ones
685 activated by the reflex afferent volley (22, 24, 61), the increased in H_{max}/M_{max} ratio in ALS may
686 stem from the activation of motor units with larger amplitude compared to those activated in
687 controls. This results in a larger H-reflex, even though the proportion of motor units could be
688 equal or potentially lower. Nevertheless, in a groundbreaking approach, our study
689 systematically examined the H-reflex and MEP in the same participants, during the same
690 experimental session. Contrary to H-reflex, the MEP size was found reduced. Even if it has
691 been established that for a valid comparison, H-reflex and MEP should be assessed under
692 consistent conditions (either at rest or during contractions; Morita et al. 2000), we assume
693 that both the peripheral afferent volley and the TMS-induced corticospinal outputs have
694 engaged LMNs within the same pool (low-threshold, resilient LMNs; Henneman's size
695 principle). Furthermore, the findings on individual motor units provide additional backing for

696 the notion that both the peripheral and corticospinal volleys have activated resilient LMNs in
697 a similar manner in both groups. Consequently, there is a minimal likelihood that the
698 intensified H-reflex in ALS can solely be attributed to the activation of large motor unit
699 potentials.

700 Various neurophysiological mechanisms may account for the rise in the H-reflex, among
701 which LMN hyperexcitability; a hypothesis that has been historically prominent in ALS.
702 However, this idea has been challenged by recent findings in both mouse models and humans,
703 demonstrating that resilient LMNs or those about to die are normo-to-hypoexcitable (41, 67–
704 69). Another possibility is an imbalance in the spinal excitation/inhibition ratio controlling
705 LMN excitability, consequently influencing the H-reflex. Despite its monosynaptic origin, the
706 H-reflex is indeed modulated by several inhibitory mechanisms that limit its magnitude (26,
707 70). Previous studies have reported impairment of these mechanisms in ALS (13, 20, 25, 40,
708 71–73). Nonetheless, the inconsistency in findings between H-reflex and MEP results cannot
709 be clarified by reduced post-synaptic inhibitory mechanisms such as reciprocal and recurrent
710 inhibitions, or after-hyperpolarization. Instead, a decline in presynaptic mechanisms, including
711 inhibition of group Ia terminals and post-activation depression, would affect H-reflex
712 amplitude but not the MEP size (74, 75). Presynaptic inhibition is indeed particularly
713 depressed in ALS (13, 25, 76, 77), and can affect both homonymous and heteronymous group
714 Ia monosynaptic excitation to LMNs (78, 79). The alteration of post-activation depression has
715 been studied to a much lesser extent in ALS but it has also been found depressed in both
716 mouse models and patients (40, 71, 73).

717 To further assess the transmission of peripheral and descending inputs to LMNs, we
718 examined peaks in PSTHs, and their initial 0.6 ms to narrow the investigation to the purely
719 monosynaptic portion of the EPSPs (34, 35). We did not find any difference between controls
720 and patients with ALS (for all parameters tested). We also investigated the PSFs, which give a
721 more reliable estimation of unitary EPSPs (36, 37). Here again, there were no distinctions
722 between groups following both peripheral nerve stimulation and TMS. Several mechanisms
723 may explain why the results of CMAPs (H-reflex and MEP) and isolated motor units do not
724 align. Firstly, CMAPs result from the activation of a number of LMNs and are therefore more
725 sensitive to the repercussions of LMN loss and changes in the properties of suffering LMNs
726 (presumably hypo-excitable; Marchand-Pauvert et al. 2019; Martínez-Silva et al. 2018).

727 However, the paired-pulse H-reflex technique, used to assess population EPSPs (38, 39), failed
728 to reveal any difference between the groups either. These results suggest that the amplitude
729 of the rising phase of unitary EPSPs and the time course of population EPSPs were not
730 modified in patients. Given that presynaptic inhibition and post-activation depression are
731 diminished in ALS patients, we would have expected to observe results supporting enhanced
732 group Ia-induced EPSPs. However, this was not the case as reported previously in PSTHs from
733 patients with ALS (28, 80). Given that presynaptic inhibition and post-activation depression
734 can individually govern the magnitude of the H-reflex, it is conceivable that these spinal
735 mechanisms might similarly impact the size of EPSPs, rather than solely affecting the time
736 course of the EPSPs (39). Translating recent findings in mice showing a decrease in
737 monosynaptic EPSPs (19), a reduction in presynaptic inhibition and post-activation depression
738 might have compensated for a possible EPSP decrease in patients, making the net output of
739 LMNs comparable to controls.

740 Regarding corticospinal EPSPs, a decrease in monosynaptic EPSPs and descending drive
741 due to ALS-related pyramidal dysfunction support the decrease in MEPs but not the absence
742 of peak changes in the PSTH. One possible hypothesis is the involvement of interneurons in
743 the transmission of the descending command *en route* to LMNs, particularly propriospinal
744 interneurons (34, 81–84). Non-monosynaptic corticospinal transmission must indeed be
745 considered, depending on the muscle groups under study. This is particularly relevant to the
746 targeted muscle groups in the present study, in contrast to the intrinsic muscles of the hand,
747 which are predominantly, if not exclusively, controlled by the direct cortico-motoneuron
748 pathway (85–87). While the propriospinal relay has indeed a greater impact on the
749 transmission of the descending command compared to sensory feedback (MEP amplitude is
750 modified to a greater extent than that of H-reflex; Nicolas et al. 2001), the involvement of
751 these interneurons may compensate for the hypo-activation of LMNs, stemming from altered
752 corticospinal inputs and likely reduced EPSPs (19). However, this compensation does not
753 elucidate why MEPs experienced a significant reduction. It has been demonstrated that the
754 distribution of propriospinal inputs is heterogeneous within the LMN pool, countering
755 recruitment based on the Henneman's principle (89, 90). This heterogeneity allows for a fast
756 activation of high-threshold LMNs crucial for ballistic movements. These LMNs are particularly
757 vulnerable in ALS and are among the first to degenerate (22, 61). This ALS-related type-specific

758 LMN degeneration may clarify our observation of a distinct decrease in MEPs with no
759 concurrent change in unitary EPSPs since only resistant low-threshold LMNs were investigated
760 using PSTH/PSF.

761 Several studies have been conducted to monitor ALS-related changes in TMS responses.
762 As the disease progresses, the motor threshold increases, the MEP size decreases, especially
763 in lower limbs, and the primary peak in PSTH is reduced but exhibits a longer duration along
764 with the emergence of multiple sub-peaks (desynchronization; De Carvalho and Swash 2023;
765 Grapperon et al. 2021; Weber et al. 2000; Weber and Eisen 2000). Our PSTH results differ from
766 those reported previously since the latency, duration and size of the primary peak were
767 comparable between groups. In particular, we did not observe multiple peaks, a reliable
768 feature of TMS PSTH primary peak in ALS, which intensified as the disease progresses (33).
769 This might be explained by the fact that most studies focused on hand muscles, which were
770 likely more altered than the muscles investigated here. In addition, the corticospinal inputs to
771 hand muscle LMNs are transmitted through the direct cortico-motoneuron pathway, not
772 allowing compensation by interneurons. Moreover, it has been demonstrated that PSTH is an
773 unreliable method for assessing multiple EPSPs in single LMNs, whereas PSF has proven to be
774 more reliable for evaluating consecutive EPSPs (36, 37). Therefore, the current PSF data do
775 not support a change in corticospinal EPSPs in ALS patients, at least in the muscle groups
776 investigated which were not clinically affected. Otherwise, we would have anticipated a
777 decrease in PSF CUSUM duration in patients, attributable to the truncation of the first EPSP
778 by subsequent ones.

779 Interestingly, we found that MEP I_{50} was increased in the patient group which might be
780 related to the specific influence of cortical excitability in MEP threshold (92, 93), and cortical
781 hyperexcitability in ALS (94).

782 **Clinical impact**

783 The present study has shown that the H_{max}/M_{max} ratio was increased in patients, in all
784 the 3 muscles tested. To our knowledge, it is the first study in ALS in which H-reflex was tested
785 systematically in different muscles taking into account the state of the targeted muscle, from
786 clinical and electrophysiological viewpoints. We found a recent abstract reporting increased
787 H_{max}/M_{max} ratio in soleus (with reduced M_{max}) together with presence of H-reflex in abductor
788 pollicis brevis (APB), abductor digiti minimi (ADM), abductor hallucis (AH), FCR and extensor

789 digitorum (ED); H-reflexes in hand and forearm muscles were only observed in patients with
790 ALS (95). All these results suggest that the rise in H-reflex in ALS is likely to be a general
791 phenomenon, not just limited to certain muscle groups. Furthermore, our study revealed that
792 the increased H-reflex correlates with exaggerated osteotendinous reflexes and spasticity, but
793 may be reduced when patients are on riluzole (an effect not consistently found in the
794 subgroups tested). These findings are entirely consistent with what might be expected in case
795 of pyramidal syndrome (Álvarez et al. 2018; Delwaide 1989).

796 Can the H-reflex be used as a marker? From a practical standpoint, its evaluation in a
797 clinical setting appears no more informative than the conventional motor neurological
798 examination. However, the H_{max}/M_{max} ratio is a reliable quantitative measure that proves
799 useful in clinical trials, provided that normalization by M_{max} allows valid interpretation
800 according to patient status; the reported variability in results across previous studies likely
801 arises from the clinical heterogeneity. Despite its seemingly straightforward nature, the
802 method harbors several pitfalls, especially when applied to muscles other than the soleus, for
803 which the technique is relatively easy. In muscles like FCR and quadriceps, the delay between
804 the M response and the H-reflex may not always be sufficient to adequately distinguish
805 between the two responses. If the M response has a lower threshold than the reflex response,
806 which is often the case in FCR and quadriceps, the overlap between the two responses can
807 lead to misinterpretation of the observed EMG responses (*e.g.*, mistaking the tail of the M
808 response for the H-reflex). Moreover, caution is warranted when dealing with H-reflex in hand
809 muscles. In a distinct clinical context, our team identified, at the threshold of the M response,
810 a reflex-like response in ADM in a completely deafferented patient (Supplemental data 2),
811 likely resulting from F-wave activation. Considering the increased occurrence frequency and
812 enhanced amplitude of F waves in ALS (Fang et al. 2015), it is prudent to restrict the study of
813 reflexes in hand muscles to intensities below the M response threshold, using a double-pulse
814 protocol with an ISI of 5 ms to facilitate the production of H-reflex response in the EMG.

815 Regarding TMS, similar to the H-reflex, caution is warranted when interpreting
816 normalization by M_{max} ; an observed increase in MEP_{max}/M_{max} ratio may be solely attributable
817 to a decrease in M_{max} . Extensive literature exists on the role of TMS in assessing corticospinal
818 transmission and changes in cortical excitability (8, 91, 96). However, the progression of the
819 disease is marked by a gradual rise in motor threshold and the disappearance of MEPs linked

820 to both UMN and LMN degeneration. This limits the applicability of TMS to more advanced
821 disease stages and its relevance for longitudinal follow-up studies. Hence, it becomes crucial
822 to explore alternatives to TMS that are less reliant on patients' responsiveness to stimuli and
823 LMN involvement. Modalities such as EEG or MEG present promising avenues for identifying
824 new biomarkers that could offer more robust insights into disease progression (5, 97).

825 **CONCLUSION**

826 CMAPs including M_{max} , H-reflex and MEPs change over the disease course and the
827 normalization to M_{max} currently used in neurophysiological studies may lead to
828 misinterpretation due to LMN degeneration. In muscles where there is no clinical nor
829 electrophysiological evidence indicating significant peripheral involvement, H-reflex is
830 enhanced and the MEP is decreased. These changes were linked to exaggerated
831 osteotendinous reflexes and spasticity; two clinical manifestations associated with pyramidal
832 syndrome. However, changes in the H reflex and MEP are not paralleled with changes in
833 monosynaptic PPSEs transmitted by group Ia proprioceptive afferents and corticospinal
834 inputs. Compensatory mechanisms likely involving interneurons maintain the homeostasis of
835 resilient motoneurons (98), masking a possible depression of purely monosynaptic EPSPs as
836 reported in the mouse model, but this hypothesis needs to be confirmed with longitudinal
837 studies. H-reflex does not add value to the classic neurological motor assessment. However,
838 its normalized amplitude, if not misinterpreted, provides a reliable quantitative marker for
839 clinical trials for which quantitative assessment of hyperreflexia or spinal hyperexcitability is
840 necessary. The lack of association with disease onset makes it sufficient to limit the
841 assessment to soleus, given its ease of use and the fact that it is not among the first muscles
842 typically affected.

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856 **DISCLOSURE**

857 None

858 **AUTHOR CONTRIBUTIONS**

859 VMP conceptualized the research. PFP selected the patients and performed the clinical
860 evaluation. SS, ALV, IP and VMP performed the electrophysiological recordings. AP developed
861 the script in Matlab to analyze the paired pulse H-reflexes and performed the analysis. VMP
862 performed the statistical analysis and drafted the manuscript. All the authors have revised
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1168 TABLES

1169 Table 1: ALS group

	Site	Duration	ALSFRS-r	Progression	MRC					UMN	LMN
					Sol	Q	TA	FCR	ECR		
1	UL	18	40	0,4	5	5	5	4	5	2	0
2	LL	7	47	0,1	5	5	3	5	5	0	1
3	UL	59	34	0,2	5	5	5	2	3	3	1
4	UL	14	33	1,1	5	5	5	3	0	2	0
5 [∇]	UL	22	38	0,5	5	5	5	3	3	2	1
6	Bulb.	33	39	0,3	5	5	5	5	5	0	0
7 [∇]	Bulb.	14	24	1,7	5	5	5	5	4	3	1
8 [∇]	LL	11	34	1,3	5	5	3	4	4	3	1
9	Bulb.	48	43	0,1	5	5	5	5	5	3	0
10	LL	14	38	0,7	5	5	4	5	5	3	0
11*	UL	7	42	0,9	5	5	5	3	3	3	0
12*	UL	7	44	0,6	5	5	5	4	4	3	1
13*	LL	41	39	0,2	2	1	0	5	5	0	4
14* [∇]	Bulb.	63	36	0,2	5	5	5	4	5	4	0
15*	UL	46	41	0,2	5	5	5	4	3	0	0
16	LL	10	40	0,8	2	3	1	5	5	0	4
17	LL	9	43	0,6	5	5	4	5	4	0	0
18	UL	25	44	0,2	5	5	5	3	2	1	1
19*	Bulb.	10	44	0,4	5	5	5	5	5	3	0
20	Bulb.	6	47	0,2	5	5	5	5	5	3	5
21*	UL	27	44	0,1	5	5	5	5	5	0	0
22* [∇]	LL	9	35	1,4	5	5	5	5	5	4	2
23	LL	2	40	4,0	5	5	5	5	5	0	0
24*	LL	46	36	0,3	5	5	5	4	4	3	0
25*	UL	11	45	0,3	5	5	5	5	5	2	0
26*	UL	72	40	0,1	3	5	4	4	3	1	1
27*	UL	15	40	0,5	5	5	5	5	5	2	1
28*	LL	16	44	0,3	5	5	5	5	2	2	0
29* [∇]	UL	17	45	0,2	5	5	5	5	5	2	0
30*	LL	23	42	0,3	4	5	0	5	5	2	0
31*	LL	5	36	2,4	5	5	5	3	3	0	1
32*	LL	21	39	0,4	5	5	5	4	5	2	1
33*	LL	14	43	0,4	5	5	5	5	5	2	2
34*	UL	9	45	0,3	5	5	5	5	5	0	0
35	UL	9	39	1,0	5	5	5	3	3	0	0
36	UL	3	33	5,0	5	5	5	4	5	2	2
37 [∅]	LL	6	47	0,2	5	5	5	5	5	2	0
38*	UL	25	39	0,4	5	5	5	3	2	0	0
39	UL	16	45	0,2	5	5	5	5	5	0	0
40*	LL	24	41	0,3	5	1	5	5	5	0	2
41*	Bulb.	23	36	0,5	5	5	5	5	5	2	1
42	UL	13	47	0,1	5	5	5	4	5	2	0
43* [∇]	LL	21	38	0,5	3	5	1	5	5	2	0
44* [∇]	UL	28	40	0,3	5	5	2	5	5	4	1
45	LL	27	39	0,3	5	5	5	4	5	0	1
46* [∇]	LL	14	38	0,7	5	5	5	5	5	4	1
47	Axial	14	43	0,4	5	5	5	5	5	0	1
48 [∇]	Bulb.	7	44	0,6	5	5	5	5	5	3	0
49* [∇]	LL	20	42	0,3	5	5	5	5	5	3	1
50 [∇]	UL	14	40	0,6	5	5	4	5	3	3	1

51*	LL	70	40	0,1	5	5	3	5	5	0	3
52* [∇]	Bulb.	13	41	0,5	5	5	5	5	5	3	4
53*	UL	12	40	0,7	5	2	5	5	5	3	2
54	Bulb.	11	45	0,3	5	5	5	4	5	0	2
55*	LL	15	42	0,4	5	5	5	5	5	2	0

1170 Columns, from left to right, indicate the number of the patient with * when ON riluzole (50
1171 mg twice a day), [∇] when spastic and [∅] when genetic form (C9orf72), site of onset (upper limbs
1172 UL, lower limbs LL, bulbar Bulb. and Axial), time since first symptoms (Duration in months),
1173 score to ALSFRS-r (maximal score 48), mean progression rate since the first symptoms (=
1174 ALSFRS-r score – 48 / duration), score to muscle testing according to MRC scale (normal score
1175 5), upper motoneuron score (UMN) and lower motor neuron score (LMN).
1176

1177 **Table 2:** Recruitment curve parameters for H-reflex and M response in the 3 muscles tested

	FCR		Soleus		Quadriceps	
	Controls	ALS	Controls	ALS	Controls	ALS
Motor Threshold (mA)	17.3 ± 10.9	20.8 ± 11.3	17.3 ± 10.9	20.8 ± 11.3	29.8 ± 10.7	36.9 ± 14.5
M_{max} (mV)	2.5 ± 1.5	1.7 ± 1.0	3.8 ± 3.4	3.0 ± 2.1	1.9 ± 1.2	1.4 ± 0.6
H_{max} (mV)	0.3 ± 0.2	0.5 ± 0.3	1.3 ± 1.5	1.4 ± 1.2	0.5 ± 0.3	0.7 ± 0.4
H_{max}/M_{max} (%M _{max})	14.3 ± 18.8	37.2 ± 21.8	32.8 ± 22.8	54.3 ± 27.1	32.9 ± 20.4	53.1 ± 27.5
H-reflex asymptote (%M _{max})	14.9 ± 19.4	37.7 ± 22.5	32.4 ± 23.1	52.1 ± 26.4	33.7 ± 20.7	53.7 ± 27.9
M response I₅₀ (xMT)	2.0 ± 1.1	1.4 ± 0.5	2.5 ± 1.0	2.6 ± 1.0	2.1 ± 0.9	1.7 ± 0.6
M response peak slope	138.9	181.9	93.4	121.9	124.6	167.2
	± 55.4	± 169.8	± 52.4	± 109.8	± 79.8	± 103.0
M curve adjusted r²	0.97 ± 0.03	0.97 ± 0.02	0.97 ± 0.02	0.98 ± 0.01	0.98 ± 0.03	0.96 ± 0.03
H-reflex I₅₀ (xMT)	1.5 ± 0.7	1.1 ± 0.4	1.7 ± 0.9	1.4 ± 0.5	1.7 ± 0.6	1.4 ± 0.5
H-reflex peak slope	82.6	317.5	151.3	271.8	90.3	301.1
	± 106.9	± 495.3	± 229.9	± 299.1	± 125.2	± 516.0
H curve adjusted r²	0.92 ± 0.10	0.96 ± 0.03	0.92 ± 0.11	0.98 ± 0.01	0.95 ± 0.03	0.96 ± 0.05
Peak slope ratio	0.7 ± 0.9	1.9 ± 3.1	1.6 ± 2.0	2.9 ± 2.2	0.8 ± 0.9	3.9 ± 8.6

1178 Columns, from left to right, measurements (mean ± 1 SD) for each group (Controls, ALS) and
 1179 each muscle tested (FCR, soleus, quadriceps). Lines, indicate the measurements with from top
 1180 to bottom: the threshold intensity for M-response (Motor threshold in mA) in FCR, soleus and
 1181 quadriceps after median nerve, PTN and FN stimulations, respectively; Maximal amplitude of
 1182 M response produced in the corresponding EMG (M_{max} in mV); Maximal amplitude of H-reflex
 1183 produced in the corresponding EMG (H_{max} in mV); Ratio between maximal amplitudes
 1184 normalized to M_{max} (H_{max}/M_{max} as a % of M_{max}); Asymptote of the H-reflex recruitment curve
 1185 (% of M_{max}); I₅₀ of the M response recruitment curves (normalized to the motor threshold,
 1186 xMT); Peak slope of the M response recruitment curve and below its mean adjusted r²; I₅₀ of
 1187 the H-reflex recruitment curves (normalized to the motor threshold, xMT); Peak slope of the
 1188 H-reflex recruitment curve and below its mean adjusted r²; Last row, ratio of the peak slopes
 1189 of H-reflex and M response recruitment curves.

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1191

1192 **Table 3:** Characteristics of PSTH and PSF from isolated motor units

		ECR				TA			
		Peripheral		TMS		Peripheral		TMS	
		CTRL	ALS	CTRL	ALS	CTRL	ALS	CTRL	ALS
	Number of units/individuals	13/11	7/6	17/15	8/6	10/10	13/8	13/13	3/3
PSTH	Peak latency (ms)	19.2 ± 10.5	18.3 ± 6.1	17.6 ± 3.3	18.0 ± 5.6	36.8 ± 5.2	29.9 ± 4.9	34.8 ± 6.0	29.1 ± 6.1
		$r^2 = 0.97, p_{group} = 0.22 (f^2 = 0), p_{interaction} = 0.71 (f^2 = 0)$							
	Peak duration (ms)	2.5 ± 0.8	2.4 ± 1.1	2.7 ± 1.3	3.0 ± 1.7	3.4 ± 1.4	3.1 ± 1.2	3.1 ± 1.4	3.0 ± 0.9
		$r^2 = 0.14, p_{group} = 0.88 (f^2 = 0.31), p_{interaction} = 0.73 (f^2 = 0.56)$							
	Total counts (%)	17.0 ± 8.8	30.4 ± 25.8	19.5 ± 9.7	15.1 ± 8.8	24.8 ± 7.3	17.1 ± 9.2	14.9 ± 4.3	13.5 ± 5.8
		$r^2 = 0.90, p_{group} = 0.99 (f^2 = 0), p_{interaction} = 0.13 (f^2 = 0)$							
	0.6-ms counts (%)	4.9 ± 4.8	5.8 ± 4.2	4.9 ± 3.2	3.8 ± 2.3	4.4 ± 5.1	3.8 ± 2.9	3.3 ± 1.1	3.6 ± 2.1
		$r^2 = 0.56, p_{group} = 0.92 (f^2 = 0.01), p_{interaction} = 0.48 (f^2 = 0.04)$							
	CUSUM latency (ms)	18.9 ± 4.2	17.9 ± 6.5	17.2 ± 3.3	17.1 ± 5.1	36.7 ± 5.4	29.8 ± 5.0	33.0 ± 6.2	29.2 ± 6.1
		$r^2 = 0.98, p_{group} = 0.13 (f^2 = 0), p_{interaction} = 0.85 (f^2 = 0.01)$							
	CUSUM duration (ms)	3.0 ± 1.2	3.5 ± 1.4	3.2 ± 1.3	5.4 ± 2.9	4.9 ± 2.3	8.1 ± 15.3	5.3 ± 4.0	3.1 ± 1.4
		$r^2 = 0.94, p_{group} = 0.69 (f^2 = 0.02), p_{interaction} = 0.18 (f^2 = 0)$							
PSF	CUSUM latency (ms)	19.9 ± 5.5	18.2 ± 6.5	16.4 ± 3.0	17.9 ± 6.1	37.2 ± 5.1	30.1 ± 5.2	30.7 ± 14.0	30.2.3 ± 5.2
		$r^2 = 0.98, p_{group} = 0.91 (f^2 = 0.02), p_{interaction} = 0.99 (f^2 = 0.06)$							
	CUSUM duration (ms)	18.0 ± 10.8	49.8 ± 34.3	22.9 ± 14.1	27.3 ± 17.6	20.6 ± 21.0	32.3 ± 17.9	30.8 ± 20.6	25.5 ± 16.6
		$r^2 = 0.15, p_{group} = 0.07 (f^2 = 0.02), p_{interaction} = 0.77 (f^2 = 0)$							

1193 Columns, from left to right, indicate the method (PSTH and PSF), the mean results for each
 1194 group (CTRL = control vs. ALS) and muscle (ECR and TA) after peripheral nerve stimulation
 1195 (radial nerve for ECR and CPN for TA) and TMS over the primary motor cortex (at the hotspot
 1196 for the corresponding muscle). Below each condition, number of motor units
 1197 investigated/number of individuals. Lines indicates, from top to bottom, the PSTH peak

1198 latency, the PSTH peak duration, the total number of counts in PSTH peak (% of total number
1199 of stimulations), the number of counts in PSTH peak within its 0.6-ms first bins (% of total
1200 number of stimulations), PSTH CUSUM latency and duration, and the two last lines indicate
1201 the PSF CUSUM latency and duration. Mean value \pm 1 SD for each condition is indicated in
1202 each corresponding cell and the line below include the statistics with adjusted r^2 of the model,
1203 p value for group factor (p_{group}), for the interaction between factors group x muscle x
1204 stimulation type ($p_{interaction}$), and effect size (f^2).
1205

1206 **FIGURE LEGENDS**

1207 **Figure 1.** *AB*: sketches illustrating the experimental paradigms in upper (*A*) and lower limbs
1208 (*B*). *C-E*: mean H-reflex and M response ($n = 5$) in one control in FCR (*C*), soleus (*D*) and
1209 quadriceps (*E*) at 4 representative stimulus intensities between threshold intensity and
1210 intensity for H_{max} and M_{max} . Vertical dotted lines indicate the window for determining peak-
1211 to-peak amplitude of M response (red dots) and of H-reflex (blue dots). *F-H*: mean amplitudes
1212 (mV) plotted against the stimulus intensity (mA) in FCR (*F*), soleus (*G*) and quadriceps (*H*) in
1213 the same control, with blue and red dots representing the empirical measures of H-reflex and
1214 M response, respectively, and the line, the 3-parameter sigmoid fitting in blue for H-reflex
1215 (only the ascending phase of its recruitment curve) and red for M response. *I*: Design for
1216 paired-pulse H reflex (Experiment 2), starting with a series of 10 single pulses with sequential
1217 alternation of 5 test and 5 conditioning pulses (1-Hz stimulation rate) followed by the first
1218 series of 20 paired pulses at a given ISI (ISI_{1-8}) randomly determined. We repeated the same
1219 sequence 8 times to test the 8 ISIs between paired pulses. Accordingly, one acquisition session
1220 consisted of this sequence of 16 alternating blocks of single and paired pulses. *JK*: mean raw
1221 ($n = 10$; *J*) and corresponding rectified MEP (*K*) produced in VL EMG at 4 representative TMS
1222 intensities (% MSO) between threshold intensity and intensity for MEP_{max} , in one control;
1223 vertical dotted lines in *K* define the window of analysis. *L*: mean MEP size (normalized to M_{max}
1224 area) plotted against TMS intensity (% of MSO) in the same participant with the line
1225 representing the 3-parameter sigmoid fitting.

1226 **Figure 2.** *A-F*: mean H-reflex (blue lines) and M response (red lines) recruitment curves in the
1227 group of controls ($n = 16$; left part) and the group of ALS ($n = 15$; right part), in FCR (*AB*), soleus
1228 (*CD*) and quadriceps (*EF*), plotted using the mean of the 3 parameters of the sigmoid fitting in
1229 each group (k , I_{50} and asymptote) extracted from the recruitment curves in each participant
1230 (with H-reflex and M response amplitudes normalized to M_{max} and the intensity of the
1231 peripheral nerve stimulation, to the motor threshold, $\times MT$). The confidence bands have been
1232 calculated using the CI_{95} of each parameter, in each group: the darkest band was based on the
1233 CI_{95} of each parameter around the mean of the other parameters and the lightest band, by
1234 changing all the parameters in their CI_{95} simultaneously. The Y-axis indicates the amplitude of
1235 the responses (as % of M_{max}) and the X-axis, the stimulus intensity ($\times MT$). *G-J*: Marginal means
1236 estimated from the mixed models representing the prediction for H-reflex maximal amplitude

1237 (asymptote % M_{max} ; G), for the H-reflex I_{50} (H) and M response I_{50} (x MT; I), and the peak slope
1238 of the H-reflex and M response recruitment curves, whatever the muscle tested (J). * $p < 0.05$,
1239 ** $p < 0.001$, *** $p < 0.0001$.

1240 **Figure 3.** *A:* number of patients with normalized H-reflex asymptote (% of M_{max}) $>$ to the upper
1241 limit of CI_{95} in controls (\uparrow increased; orange), within the limits (\leftrightarrow unchanged; yellow) and $<$
1242 to the lower limit (\downarrow decreased; green). *B:* mean normalized H-reflex asymptote (% of $M_{max} \pm$
1243 SD) in FCR (blue), soleus (green), and quadriceps (Quad., red) in the subgroup of patients with
1244 unchanged (left part of the figurine) or reduced M_{max} (right part) according to the CI_{95} lower
1245 limit in controls. Mean normalized H-reflex asymptotes in controls are illustrated by
1246 continuous line in each muscle (same color legend) \pm SD illustrated by interrupted lines (same
1247 color legend). *C-D:* box plots illustrating the variability of normalized H-reflex asymptote (% of
1248 M_{max}) in spastic and non-spastic muscles (C), in muscles with normal/abolished or exaggerated
1249 osteotendinous reflexes (D) and in the subgroup of patients OFF or ON riluzole (E): the lower
1250 limit of the box indicates the 25th percentile (1st quartile, $Q1$), the upper limit, the 75th
1251 percentile (3rd quartile, $Q3$), the continuous line within the box, the mean and the cross, the
1252 median. The lines that extend from the box (whiskers) are limited to minimum and maximum
1253 values and those above or below the end of the whiskers are outliers. * $p < 0.05$, ** $p < 0.001$,
1254 *** $p < 0.0001$.

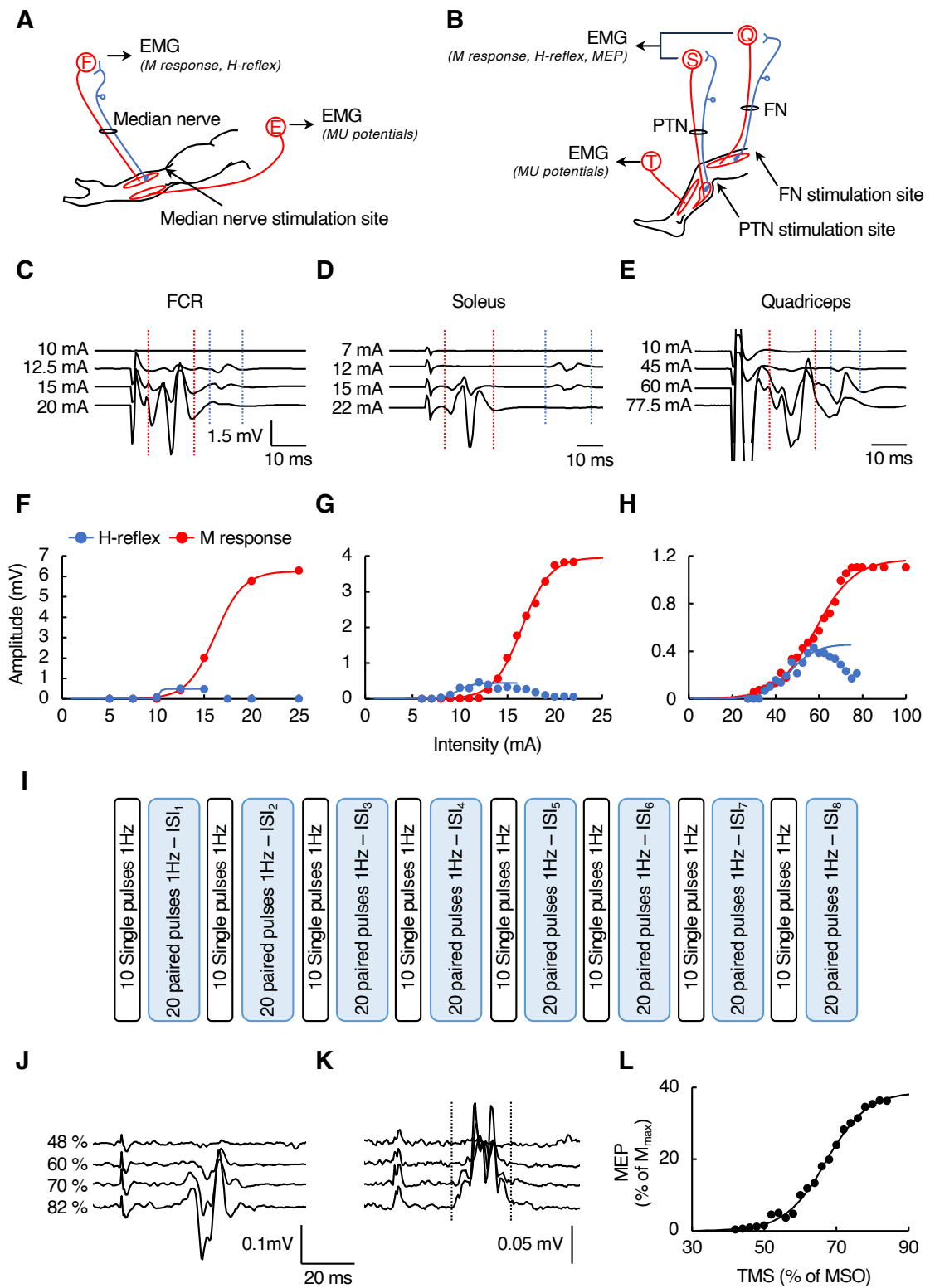
1255 **Figure 4.** *A-B:* probability of occurrence of H-reflex in soleus EMG after paired pulse PTN
1256 stimulation plotted against intensity of the second (test) pulse (x H-reflex threshold) and ISI
1257 between paired pulses (ms), in one control (A) and one ALS (B). The jet color map illustrates
1258 the probability from 0 in dark blue to 1 in dark red. *A-B:* iso-probability curves in the same
1259 participants, according to the jet color gradient as in AB , plotted against the decreasing
1260 intensity (x H-reflex threshold) and the ISI between paired pulses (ms). *E:* proportion of H-
1261 reflex occurring with $p \geq 0.5$ after paired pulse PTN stimulation; each black dot represents one
1262 control and each red dot, one patient with ALS. *F:* mean time constant (ms), in each control
1263 and each patient with ALS (black and red dots as in E). * $p < 0.05$.

1264 **Figure 5.** *A-B:* mean normalized (% of M_{max}) quadriceps H-reflex (A) and MEP (B) recruitment
1265 curves in the group of controls ($n = 28$; black) and the group of ALS ($n = 26$; red), plotted using
1266 the mean of the 3 parameters of the sigmoid fitting in each group (k , I_{50} and asymptote)
1267 extracted from the recruitment curves in each participant (with H-reflex and MEP amplitudes

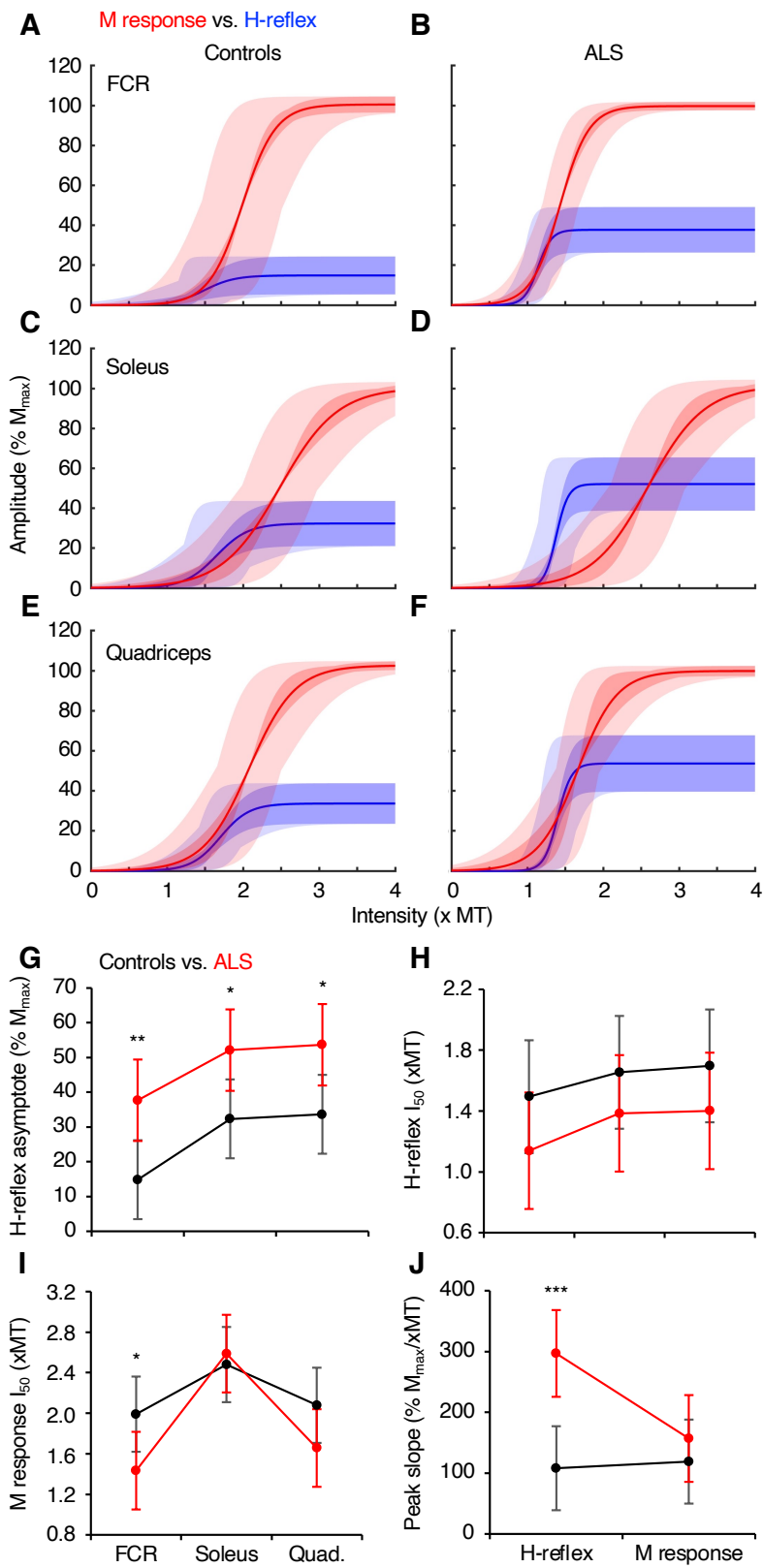
1268 normalized to M_{max}) and the stimulation intensities normalized to motor threshold (x MT for
1269 the FN stimulation, A ; x AMT for TMS, B). The confidence bands as in Fig. 2. C-E, marginal
1270 means (\pm CI₉₅) of asymptote (C), I_{50} (D) and peak slope (E) for H-reflex (left part of the figure)
1271 and MEP (right part of the figure). F : number of patients with increased (orange), similar
1272 (yellow) or decreased (green) H_{max}/M_{max} and MEP_{max}/M_{max} (% of M_{max}), according to the CI₉₅
1273 upper and lower limits in the control group. G : plots illustrating the projection of variable
1274 modalities in two dimensions corresponding to X and Y axes, according to their inertia (λ ;
1275 deviation from independence: the greater the value the larger the dependency). Modalities
1276 include the changes in normalized H_{max} and MEP_{max} (red diamonds; Unchanged, Increased,
1277 Decreased), response type (blue dots; H-reflex vs. MEP), site of onset (black dots; UL for upper
1278 limbs, LL for lower limbs, B for bulbar, axial), qualitative ALSFRS-r according to the median
1279 score in the group (pink dots; A+ for more affected = patients with score < 41 and A- for less
1280 affected = patients with score \geq 41), qualitative progression rate according to the median rate
1281 in the group (purple dots; slow progressors = patients with mean progression rate \leq 0.35 and
1282 fast progressors = patients with mean progression rate > 0.35), predominant form of ALS
1283 (UMN for upper motor neuron predominant form, LMN for lower motor neuron predominant
1284 form, Mixed for mixed form), riluzole (on vs. off), spasticity (yes if score to MAS \geq 1, no if score
1285 = 0). * $p < 0.05$, ** $p < 0.01$.

1286 **Figure 6.** A-B: PSTHs (0.2-ms bins) from one single unit extracted from ECR EMG in one control
1287 (A) and one patient (B) plotted around stimulation applied to radial nerve. The number of
1288 motor unit potentials (counts) in each bin are normalized to the total number of stimulations
1289 ($n = 150$). The upper trace illustrates the PSTH CUSUM. CD: PSFs of the corresponding units
1290 with their CUSUM (upper trace). EF: box plots (as in Fig. 2) illustrating the number of counts
1291 (% of number of stimulations) within the 3 first bins (0.6 ms) of the PSTH peak (E) and the
1292 duration of PSF CUSUM corresponding to the peak in PSTH (F), in the group of motor units
1293 from controls (in black) and from ALS (in red; see details in Table 4).

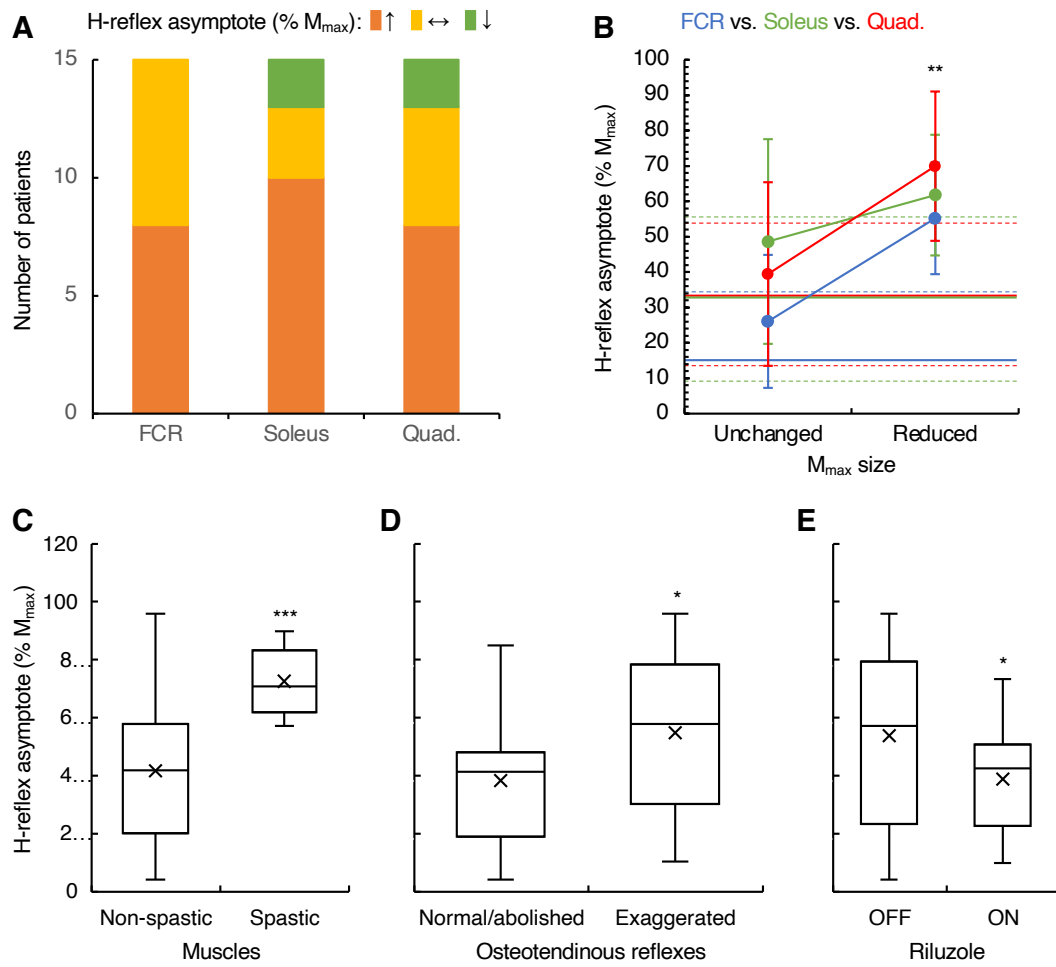
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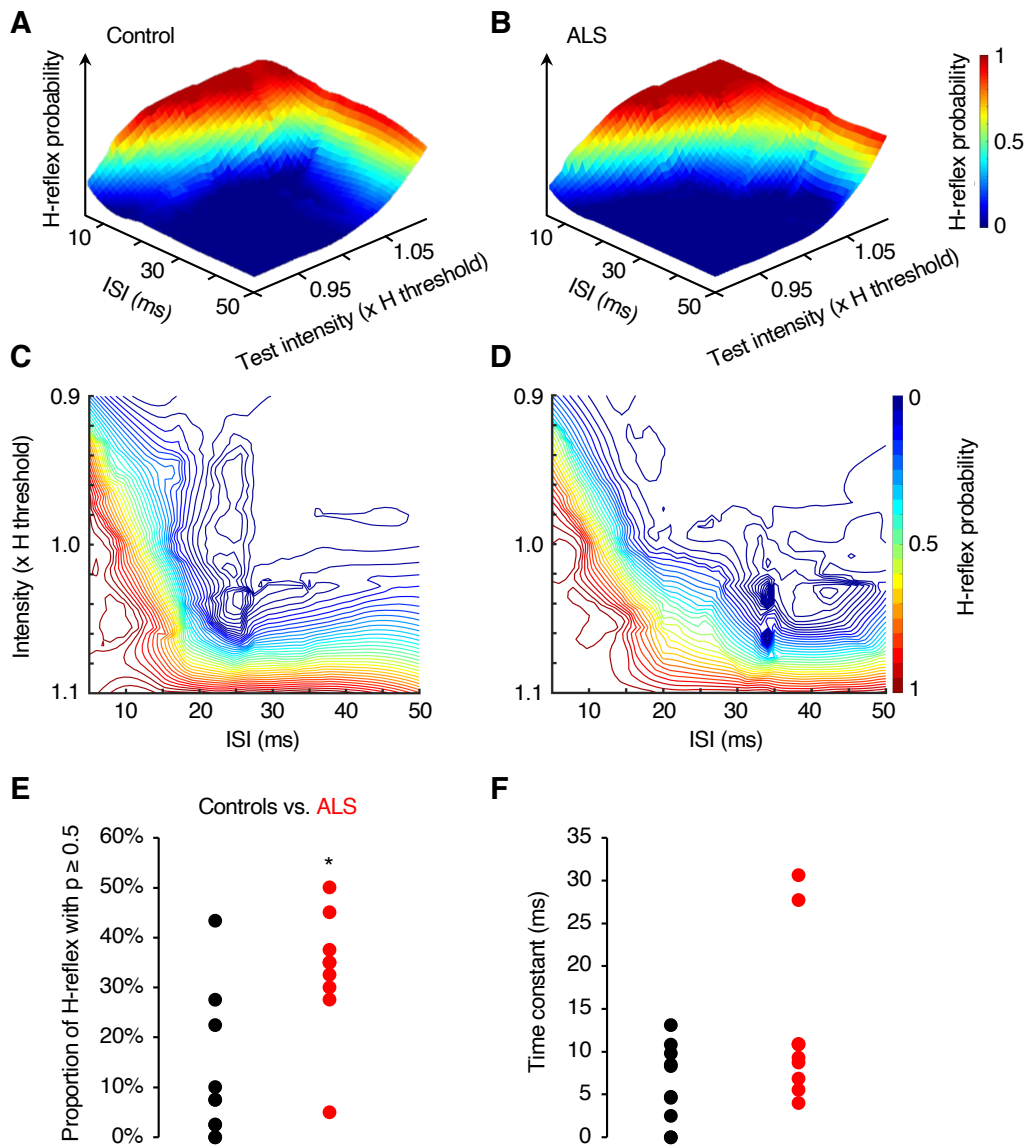
Sangari et al. _ Figure 1



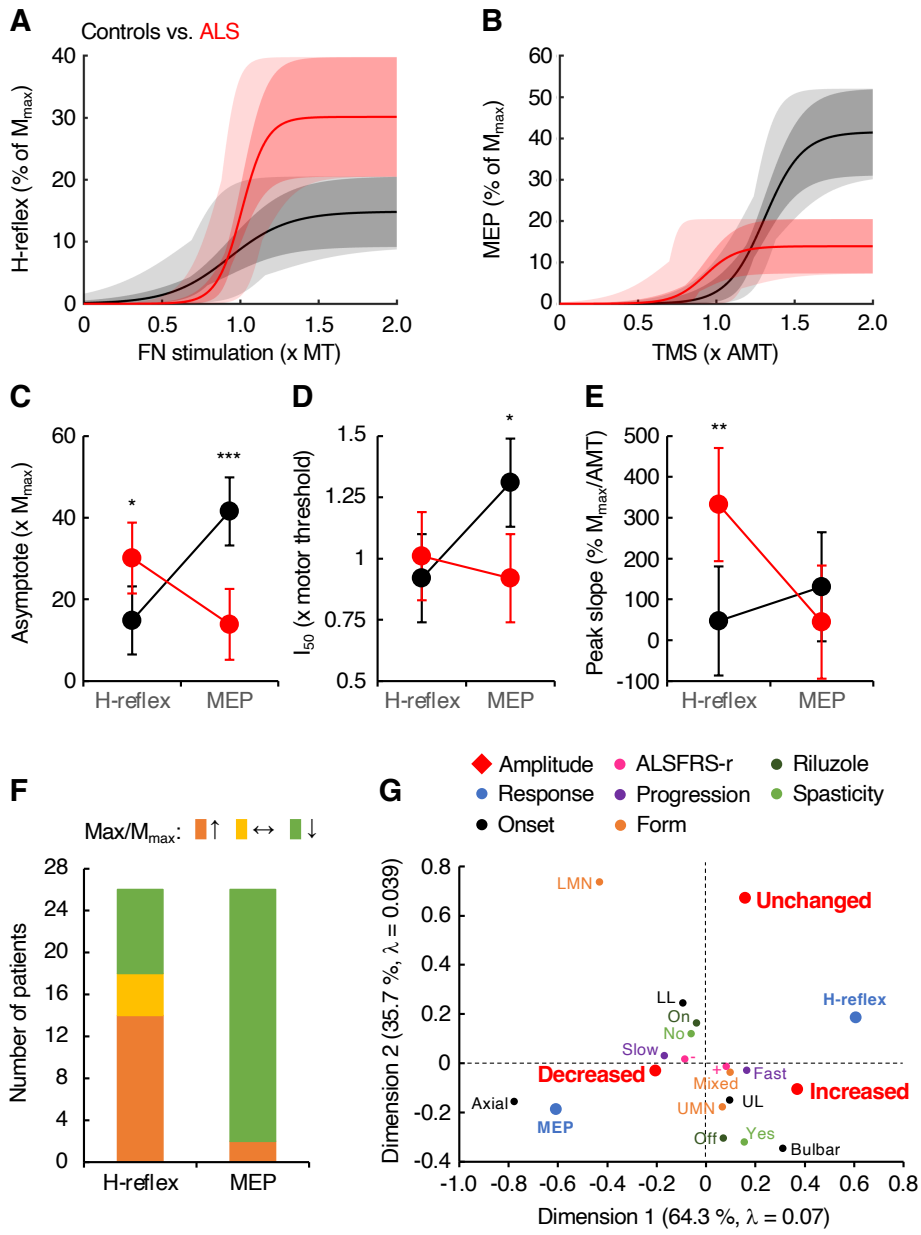
Sangari et al. _ Figure 2



Sangari et al. _ Figure 3



Sangari et al. _ Figure 4

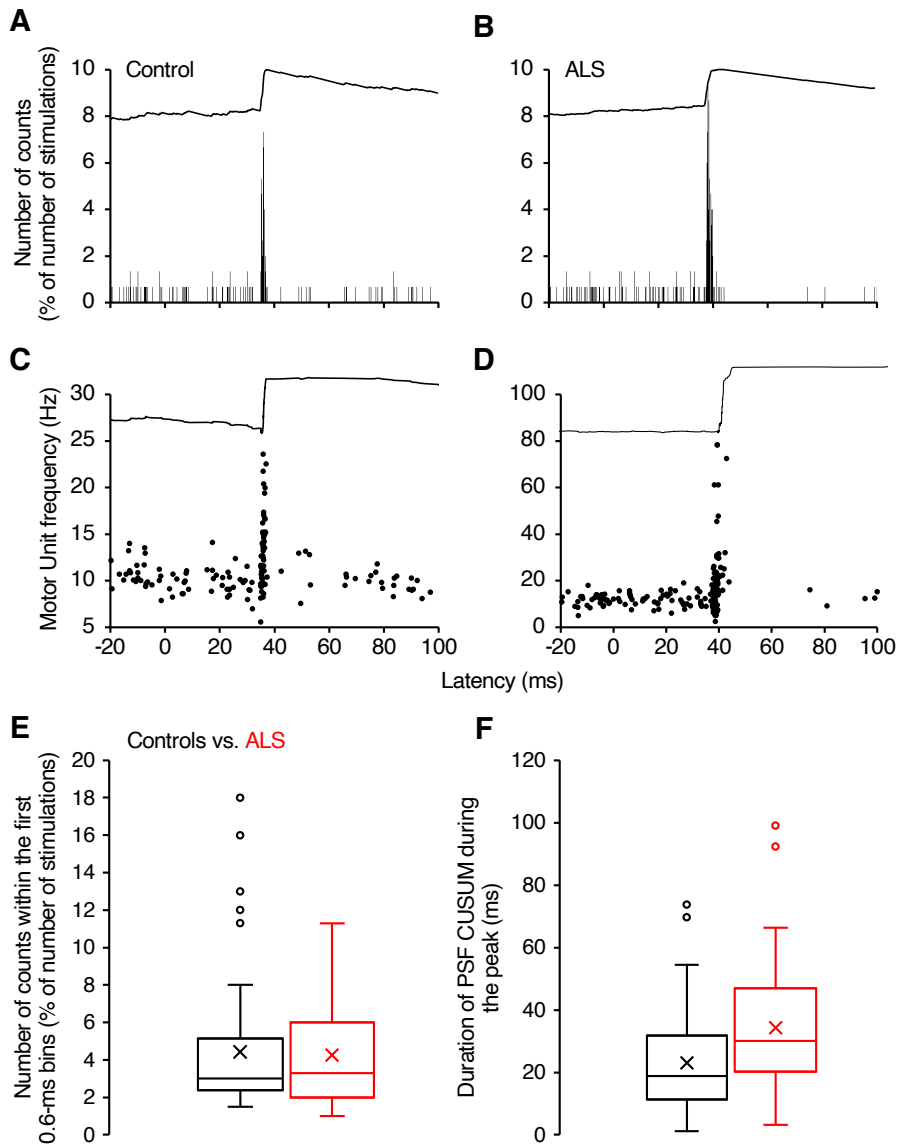


Sangari et al. _ Figure 5

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Sangari et al. _ Figure 6

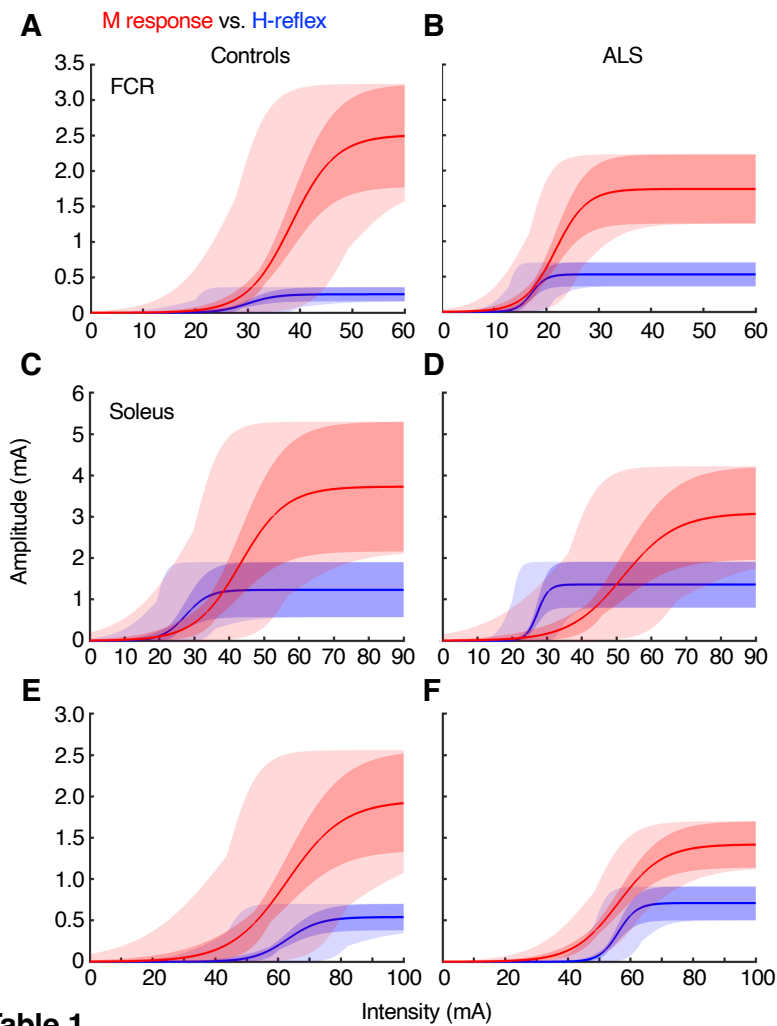


Table 1

P value (r^2)	Asymptote	I_{50}	Peak slope
group	0.42 (0.02)	0.32 (0.14)	0.59 (0.05)
muscle	< 0.0001 (0.21)	< 0.0001 (0.66)	< 0.05 (0.08)
response	< 0.001 (0.17)	< 0.0001 (0.66)	0.11 (0.09)
group x response	< 0.05 (0.19)*	= 0.81 (0.69)	= 0.96 (0.10)
group x muscle x response	0.86 (0.19)	0.06 (0.69)	0.31 (0.10)

**Post hoc* Student t test to compare marginal means Controls vs. ALS: H-reflex $p = 0.58$ and M response $p = 0.06$

Table 2

P value (r^2)	Ratio H/M	
	Asymptote	Peak slope
group	< 0.001 (0.01)	< 0.05 (0)
group x muscle	0.87 (0.01)	0.64 (0)

Link with clinical features:

Asymptote ratio increased in spastic patients ($p < 0.001$), in hyperreflexic ($p < 0.05$) and off-riluzole ($p < 0.05$)

Peak slope ratio increased off-riluzole ($p < 0.05$)

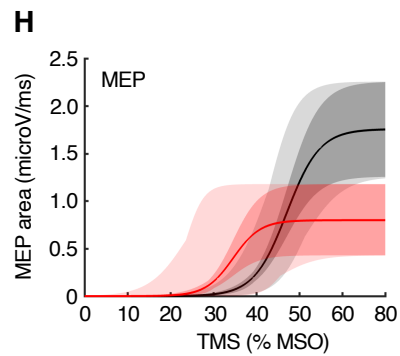
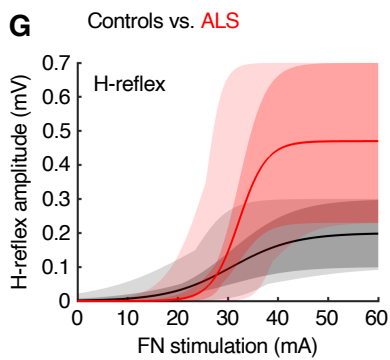


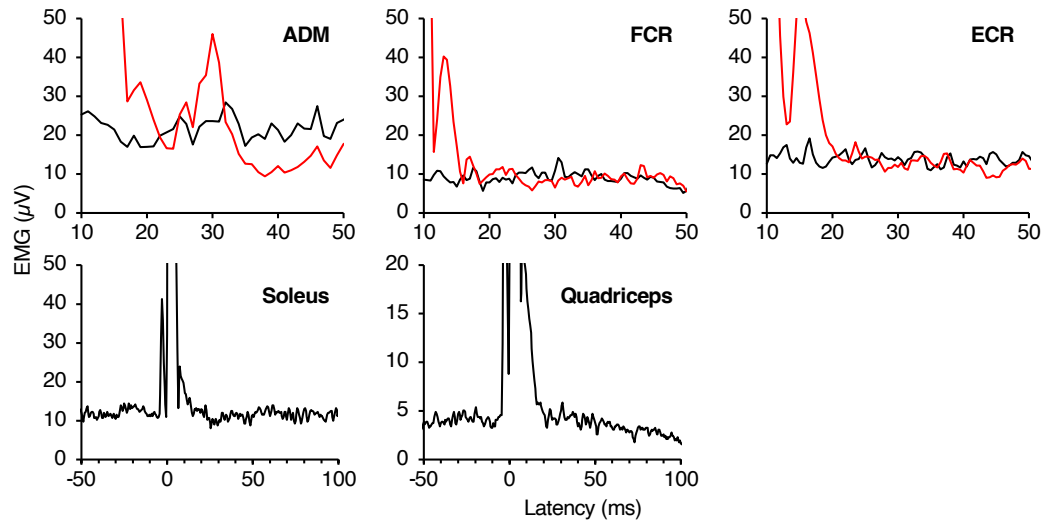
Table 3

P values (r^2)	Asymptotes	I_{50}	Peak slope
group	0.052 (0.36)	0.18 (0.09)	0.20 (0.09)
Response	< 0.001 (0.36)	< 0.001 (0.09)**	0.27 (0.09)
group x response	< 0.001 (0.36)*	0.058 (0.09)	0.06 (0.09)

**Post hoc* Student t test to compare marginal means Controls vs. ALS: H-reflex $p = 0.27$ and MEP $p < 0.001$

** *Post hoc* Student t test to compare marginal means Controls vs. ALS: H-reflex $p = 0.7865$ and MEP $p < 0.05$

Absence of monosynaptic excitation in a deafferented patient except in hand muscle



After two episodes of extensive sensory polyneuropathy, the patient exhibited a complete loss of touch, vibration, pressure, and kinaesthetic senses, along with the absence of tendon reflexes in all four limbs (deafferented patient GL). In collaboration with Prs. E. Pierrot-Deseilligny, H. Hultborn, and J.B. Nielsen, V. Marchand-Pauvert conducted a series of electrophysiological experiments in this patient 20 years ago (unpublished data). The present data originates from EMG in abductor digiti minimi (ADM), flexor carpi radialis (FCR), extensor carpi radialis (ECR), soleus and quadriceps recorded during isolated, tonic, voluntary contractions at 20% of the maximum force. Electrical stimuli (1-ms pulse duration, 1-Hz frequency, $1.5 \times MT$) were applied to the ulnar nerve at wrist level (ADM), the median nerve at elbow level (FCR), the radial nerve at arm level (ECR), and the tibial nerve for soleus and quadriceps (heteronymous monosynaptic excitation; *Meunier S. et al. Exp Brain Res. 1993;96(3):534-44*). In 2004 (upper limb), a Labview-NI program was used to record EMG over predefined windows, which randomly alternated recording with and without stimulation (control in black vs. conditioned EMG in red). In 2007 (lower limb), a Notocord-hem program was used to record EMG continuously (compare pre- vs. post-stimulus EMG). Any early facilitation corresponding to group Ia monosynaptic excitation was not observed in all muscles except ADM. Considering the afferent and efferent conduction times (estimated with the distance between the stimulation site and the C6 root, and the conduction velocity in group Ia fibers and motor axons), a monosynaptic excitation could be expected at around 20 ms in ADM. An early facilitation was consistently observed in ADM that aligned with the latency of a monosynaptic group Ia excitation. This facilitation occurred at the threshold intensity for M response and above (it was also observed at rest; data not recorded). Since this response couldn't be an H-reflex, it was likely the result of motor axon activation mediating F-wave.

Sangari et al. _ Supplemental data_2