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1 **Title: Regional T₁ mapping of the whole cervical spinal cord using an optimized**
2 **MP2RAGE sequence**

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59 **Abstract**

60 While T_1 measurements present multiple challenges (robustness, acquisition time), the
61 recently-proposed MP2RAGE sequence (Magnetization Prepared 2 Rapid Acquisition
62 Gradient Echoes) has opened new perspectives to characterize tissue microstructure changes
63 occurring in pathological or developmental context. Extensively used for brain studies, it was
64 herein adapted to investigate the cervical spinal cord (SC) at 3T.

65 By integrating Bloch equations, the MP2RAGE sequence parameters were chosen to
66 optimize SC gray matter / white matter (GM/WM) T_1 contrast with sub-millimetric resolution,
67 a scan time <10 min, a reliable T_1 determination with minimal B_1^+ variation effect, within a
68 range of values compatible with different pathologies and surrounding structures. The
69 residual B_1^+ -effect on T_1 values was corrected using a look-up-table approach and B_1^+
70 mapping. The accuracy of B_1^+ -corrected T_1 measurements was assessed on phantom with
71 respect to conventional inversion-recovery (IR). In vivo MP2RAGE acquisitions were
72 performed on five young (28.8 ± 4.3 yo) and five elderly (60.2 ± 2.9 yo) volunteers and analyzed
73 using a template-based approach.

74 Phantom experiments led to high agreements between IR-SE and MP2RAGE-based T_1
75 values ($R^2=0.997$). In vivo T_1 values for cervical WM, anterior GM, posterior sensory (PST)
76 and lateral motor tracts (LMT) were: 917 ± 29 ms, 934 ± 33 ms, 920 ± 37 ms and 877 ± 35 ms,
77 respectively, all subjects and cervical levels considered. Significant differences were observed
78 between anterior GM and LMT, and between LMT and PST, in agreement with the literature.
79 Repeated T_1 measurements demonstrated high reproducibility of the MP2RAGE in the SC
80 (variation coefficient < 5% in all regions of interest). Finally, preliminary assessment of age-
81 related SC tissue microstructure variation additionally showed evidences of SC atrophy and
82 slight trends of T_1 decrease with age in all regions.

83 Overall, this study shows that fast, robust and accurate sub-millimetric resolution T_1 -
84 mapping in the cervical SC using the MP2RAGE sequence is possible, paving the way for
85 future multi-centric and longitudinal clinical studies investigating the pathological cord.

86
87 **Keywords**

- | | | | |
|----|------------------|----|-------------------------|
| 88 | • Spinal cord; | 92 | • White matter; |
| 89 | • T_1 mapping; | 93 | • Gray matter; |
| 90 | • MP2RAGE; | 94 | • Spinal cord template. |
| 91 | • Relaxometry; | | |

96 **Abbreviations**

<i>(a)GM</i>	<i>(anterior) Gray matter</i>
<i>ANOVA</i>	<i>Analysis of variance</i>
<i>COV</i>	<i>Coefficient of variation</i>
<i>CSA</i>	<i>Cross-sectional area</i>
<i>CSF</i>	<i>Cerebrospinal Fluid</i>
<i>EPI</i>	<i>Echo Planar Imaging</i>
<i>FLASH</i>	<i>Fast low-angle shot</i>
<i>FOV</i>	<i>Field of view</i>
<i>GRE</i>	<i>Gradient Echo</i>
<i>IR-SE</i>	<i>Inversion-Recovery Spin-Echo</i>
<i>LUT</i>	<i>Look-up-table</i>
<i>MP2RAGE</i>	<i>Magnetization Prepared 2 Rapid Acquisition Gradient Echoes</i>
<i>MR(I)</i>	<i>Magnetic Resonance (Imaging)</i>
<i>PVE</i>	<i>Partial volume effect</i>
<i>RF</i>	<i>Radiofrequency</i>
<i>RMSE</i>	<i>Root Mean square error</i>
<i>ROI</i>	<i>Region of Interest</i>
<i>SC</i>	<i>Spinal Cord</i>
<i>STD</i>	<i>Standard deviation</i>
<i>VFA</i>	<i>Variable Flip Angle</i>
<i>WM</i>	<i>White Matter</i>

97

98

99 **Highlights**

- 100 • Fast and sub-millimetric T_1 mapping of the entire cervical spinal cord at 3T.
- 101 • Efficient and robust B_1^+ inhomogeneity correction using B_1^+ mapping and look-up-
- 102 table approach.
- 103 • Accurate and reproducible T_1 quantification of spinal cord GM and postero-lateral
- 104 WM sub-regions.
- 105 • Preliminary age- and tract-related T_1 variations in the cervical spinal cord.
- 106 • Feasibility of morphological measurements on MP2RAGE T_1 map.

107 **1. Introduction**

108 Quantitative mapping of Magnetic Resonance (MR) longitudinal relaxation time, referred
109 to as T_1 relaxometry, is not only important for the determination of adequate MR sequence
110 parameters, it also offers tremendous perspectives for structural and pathological tissue
111 characterization in different organs^{1,2}. The T_1 relaxation time, or spin-lattice relaxation time,
112 is indeed tissue- and field-specific, and a growing amount of work supports the use of T_1 -
113 mapping as an *in vivo* marker of tissue microstructure in clinical and neuroscientific research,
114 probing for instance disease-related tissue changes³⁻⁵ or developmental plasticity and ageing⁶⁻
115 ⁸.

116 Over the years, numerous MR techniques have been proposed and used to estimate T_1
117 values⁹. These include saturation-recovery¹⁰ and inversion-recovery (IR)¹¹ based
118 measurements, Look-Locker¹² techniques and faster methods based on variable flip angles
119 (VFA), such as the VFA ultrashort echo time¹³ and the Driven Equilibrium Single Pulse
120 Observation of T_1 (DESPOT1) method¹⁴. The gold standard method for T_1 -mapping remains
121 the inversion-recovery spin-echo (IR-SE) technique using multiple inversion times (TIs).
122 However, IR-SE acquisition times are prohibiting and hence not suitable for clinical routine.
123 Consequently, many efforts have been made to shorten the T_1 mapping acquisition times. In
124 this context, the modified Look-Locker (MOLLI) methods¹⁵, a common variation of the IR
125 technique with multiple RF pulses applied during the longitudinal recovery to allow for curve-
126 fitting T_1 estimation in a relatively short scan time, are well-established T_1 -mapping
127 techniques, particularly suited for cardiac MRI. Another highly popular alternative for rapid
128 T_1 relaxometry in the brain, although affected by imperfect RF spoiling and B_1^+ variations, is
129 the DESPOT1 method¹⁴, based on VFA and combined with 3D or 2D spoiled Gradient-
130 Recalled Echo (GRE) acquisitions.

131 More recently, there has been a growing interest towards the MP2RAGE (Magnetization
132 Prepared 2 Rapid Acquisition Gradient Echoes) sequence¹⁶, an extension of the
133 magnetization-prepared rapid gradient-echo (MPRAGE) sequence¹⁷. This new sequence
134 provides not only a 3D T_1 -weighted volume suitable for morphological analyses whose
135 contrast can be similar to the commonly-used MPRAGE, but also a so-called “uniform” T_1 -
136 weighted volume and a corresponding T_1 map. Although already largely used for brain
137 imaging^{16,18-22}, to the best of our knowledge, quantitative T_1 -mapping using MP2RAGE
138 acquisitions in the cervical spinal cord (SC) have only been reported at 7T²³ but never at
139 lower field (3T), where most clinical research studies are performed. As a matter of fact, T_1 -

140 mapping of the cervical SC at 3T reported so far relied either on VFA-based techniques^{24–26} or
141 on IR-based approach combined or not with echo-planar imaging (EPI) readout so as to
142 shorten the acquisition time^{26,27}.

143 In this study, our goal was to generate both 3D anatomical T_1 -weighted images and
144 reliable T_1 -mapping of the whole cervical cord by using the MP2RAGE sequence, while
145 ensuring clinically compatible scan time (T_{acq} less than 10 minutes) and sub-millimetric
146 resolution. To do so, MP2RAGE sequence parameters were optimized using Bloch equations
147 to render an optimal contrast between SC gray matter (GM) and white matter (WM) at 3T.
148 The accuracy of the proposed protocol was first assessed using a dedicated T_1 -phantom by
149 comparing the MP2RAGE-based T_1 values ($T_1^{MP2RAGE}$) to gold standard IR-SE-based T_1
150 values (T_1^{IRSE}). The resulting MP2RAGE protocol was then applied *in vivo* for cervical SC
151 explorations. Measurements reproducibility was evaluated through scan-rescan experiments.
152 The obtained T_1 values were also compared to SC T_1 values previously reported in the
153 literature at 3T^{24–28}. Finally, given that the knowledge of T_1 values could also help
154 characterizing age- and tract-related structural tissue changes^{29,30}, a preliminary MP2RAGE-
155 based cervical SC investigation on two age-groups and different regions-of-interest (ROIs)
156 was carried out. In addition, morphological measurements were performed to evaluate
157 possible age-related changes on both the T_1 -weighted GRE_{T12} volume and the $T_1^{MP2RAGE}$ map.

158

159 **2. Methods**

160 Experiments were performed on a whole body 3T system (MAGNETOM Verio, Siemens
161 Healthcare, Erlangen, Germany), using the body coil for RF transmission and the standard 12-
162 channel head, 4-channel neck and 24-channel spine matrix array coils for signal reception.

163

164 **2.1. Background**

165 The MP2RAGE sequence¹⁶ can be briefly described as an inversion recovery-based
166 sequence in which two 3D GRE volumes are acquired during the recovery period at two
167 different inversion times (TI_1 and TI_2) with two different flip angles (α_1 and α_2). By
168 combining these two GRE volumes acquired with identical sequence parameters except for
169 the flip angles and inversion times, a composite volume with a so-called “uniform” T_1 -
170 weighted contrast (MP2RAGE_{UNI}) can be generated with no volume co-registration needed,
171 according to:

172
$$MP2RAGE_{UNI} = Real\left(\frac{GRE_{TI1}^* GRE_{TI2}}{|GRE_{TI1}|^2 + |GRE_{TI2}|^2}\right) \quad (Eq.1)$$

173 where GRE_{TI1} and GRE_{TI2} are the complex signal intensities of the volumes acquired at TI_1
 174 and TI_2 and $*$ the complex conjugate operator; for further details see Appendix 1 of Marques
 175 et al.¹⁶. According to Eq. (1), the $MP2RAGE_{UNI}$ volume signal is limited within the [-0.5;
 176 +0.5] range and is to a large extent purely T_1 -weighted, i.e. with almost no proton density, T_2^*
 177 and B_1^- dependences¹⁶. Consequently, this volume provides an excellent basis for T_1
 178 estimation using Bloch-based signal simulations. Indeed, a Look-Up-Table (LUT) can be
 179 constructed by using the analytical signal model reported in¹⁶ and used to estimate the T_1
 180 value of each pixel.

181 However, since homogeneous flip angle prescription through the imaged volume may be
 182 hard to achieve due to transmit field (B_1^+) inhomogeneities, especially above 1.5T or if large
 183 volumes are excited as it is the case in SC acquisitions at 3T, the $MP2RAGE_{UNI}$ signal may
 184 suffer from B_1^+ bias and thus lead to inaccurate T_1 estimation depending on the amplitude of
 185 the flip angle deviation. To alleviate this potential B_1^+ bias, the $MP2RAGE$ prescribed flip
 186 angle values could be decreased, but at the expense of a contrast-to-noise ratio reduction¹⁶.
 187 Alternatively, another approach relying on additional B_1^+ mapping acquisition combined with
 188 a set of LUT accounting for B_1^+ variations (i.e. a 3D LUT) could be used during post-
 189 processing^{18,23}. In this study, a careful attention was directed toward the optimization of the
 190 flip angles (α_1 and α_2) in order to limit B_1^+ variation effects on T_1 measurements.
 191 Additionally, B_1^+ maps were acquired to further correct T_1 values from potential residual B_1^+
 192 variation effects.

193

194 **2.2. Sequence parameters optimization**

195 The first requirement for this study was to find the $MP2RAGE$ sequence parameters that
 196 provide an optimal SC GM/WM T_1 contrast (defined here as $MP2RAGE_{UNI}$ signal intensity
 197 difference) at 3T. To do so, the T_1 values reported by²⁶ for SC GM and WM (973 ± 33 ms and
 198 876 ± 27 ms, respectively) were used as a starting point to perform iterative Bloch simulations.
 199 A subset of sequence parameters was additionally fixed to ensure a total scanning time under
 200 8 min, a large spatial coverage including the whole cervical cord and a sub-millimetric spatial
 201 resolution (<1 mm³). Hence:

- 202 • The repetition times of the sequence ($MP2RAGE_{TR}$) and the GRE modules (GRE_{TR})
 203 were set to 4 s and 6 ms, respectively.

204 • A sagittal field of view (FOV) covering both brain and whole cervical spine and
205 placed at the isocenter was used (FOV: 243x300 mm², number of excitations per GRE
206 module (GRE_n): 176, phase/slice partial Fourier (PF) factor: 6/8; slice thickness: 1 mm),
207 with an in-plane resolution of 0.9x0.9 mm².

208 The other sequence parameters (flip angles and inversion times) were investigated as follows:

209 • TI₁ and TI₂ were varied from (GRE_n*PF*GRE_{TR}/2) to MP2RAGE_{TR}-
210 (GRE_n*PF*GRE_{TR}/2) in steps of 50 ms while keeping the condition TI₂-
211 TI₁>(GRE_n*PF*GRE_{TR}) true.

212 • The flip angles of the two GRE modules (α_1 and α_2) were independently varied from 1
213 to 15 degrees, by 1-degree steps.

214 Bloch simulations were performed using Matlab (MATLAB R2014a, MathWorks,
215 Natick, MA, USA) for all possible combinations of TI₁, TI₂, α_1 and α_2 . Optimal sequence
216 parameters were chosen so that:

217 • the difference between MP2RAGE_{UNI} signal values in SC GM and WM was
218 maximum, and

219 • the relationship between the MP2RAGE_{UNI} signal and T₁ values was bijective for the
220 [500 ms – 3000 ms] T₁ value range, which was motivated by pathological T₁ values
221 found in the literature (ranging from 1200±400 ms to 1500±200 ms in multiple sclerosis
222 (MS) lesions ⁵) while also considering the surrounding cerebrospinal fluid and
223 intervertebral disk T₁ values at 3T (in the order of 2500ms¹⁶, 1140±76 ms (*nucleus*
224 *pulposus*) and 706±44 ms (*annulus fibrosus*)³¹, respectively).

225 Although the main objective of the study was not to provide a MP2RAGE sequence
226 immune from B₁⁺ inhomogeneities, the choice of the MR parameters was nonetheless aimed
227 to find a minimal influence on MP2RAGE_{UNI} signal and T₁^{MP2RAGE} values within the range of
228 T₁ of interest. This would allow for simpler and shorter post-processing if one does not need
229 to perform the B₁⁺ correction.

230

231 **2.3. T₁ measurements and B₁⁺ correction validation on phantom**

232 To validate the accuracy of T₁^{MP2RAGE} measurements with the chosen MR parameters, a
233 phantom (Eurospin II test objects, Diagnostic Sonar, Livingston, Scotland) containing 12
234 different tubes filled with gadolinium-doped agar gel with theoretical T₁ values mimicking

235 those expected in the healthy and pathological SC and ranging between 500 ms and 1700 ms
236 was used (see *Fig. 3b*).

237 The optimized MP2RAGE protocol was therefore run with the following parameters (TR
238 4 s; TE: 2.48 ms; GRAPPA: 2, α_1/α_2 : 4/5°, TI₁/TI₂: 650/2000 ms, TR-FOCI inversion
239 pulse³², FOV: 243x300 mm² placed at the isocenter, GRE_n: 176, phase/slice PF factor: 6/8;
240 voxel size of 0.9x0.9x1 mm³, standard B₀ shimming). To correct for potential residual B₁⁺
241 inhomogeneity effects on MP2RAGE_{UNI} image and T₁^{MP2RAGE} map (see paragraph 2.5 for
242 correction method details), a sagittal 2D magnetization-prepared turbo-FLASH B₁⁺-mapping³³
243 sequence was additionally acquired (TR: 5 s; TE: 3.42 ms; slice thickness: 5 mm; FOV:
244 250x250 mm; matrix size: 320x320; voxel size 1.5x1.5mm²; preparation flip angle: 45°, sinc
245 shape, T_{acq}: 1 min).

246 As a reference, the phantom was also scanned using a conventional single-slice inversion-
247 recovery spin-echo (IR-SE) sequence with nine different inversion times (TR: 9 s; TI:
248 [0.1/0.2/0.4/0.5/0.7/0.8/0.9/1.1/1.3 s]; slice thickness: 2 mm; FOV: 256 mm; matrix size: 128;
249 T_{acq} ~20 min per TI). Afterwards, T₁^{IRSE} values were calculated on a pixel-wise basis using a
250 mono-exponential fit of the signal recovery curve, computed on Matlab.

251 For quantitative analysis, twelve regions of interest were drawn on the T₁^{IRSE} map, then the
252 B₁⁺-corrected T₁^{MP2RAGE} and B₁⁺ maps were resliced to the T₁^{IRSE} map using c3D (linear
253 interpolation) (c3D, ITK-SNAP, University of Pennsylvania, PA, USA) so that the same
254 twelve ROIs could be used to extract their mean and standard deviation values per tube.
255 T₁^{MP2RAGE} and T₁^{IRSE} reproducibility was evaluated by repeating the whole experiment at
256 three different timepoints.

257 To evaluate the efficiency of the custom B₁⁺-correction method on T₁^{MP2RAGE} measurements,
258 6 MP2RAGE acquisitions were performed while varying the reference voltage from 70% to
259 120% of its nominal value, in steps of 10%. These values were chosen so as to cover the
260 minimal and maximal variations (69% and 119%, respectively) observed *in vivo* (see *Fig. 6*)
261 in the cervical spinal cord using our system. For each tube, mean and standard deviation
262 T₁^{MP2RAGE} values from all 6 MP2RAGE acquisitions were computed before and after B₁⁺
263 correction. To evaluate the efficacy of the B₁⁺ correction, the Root Mean Squared Error
264 (RMSE) was evaluated for each tube and each acquisition and an averaged RMSE value
265 across all tubes from all 6 acquisitions was subsequently computed.

266

267 **2.4. In vivo T₁ relaxometry**

268 As a preliminary application of the SC-dedicated MP2RAGE sequence, data from ten
269 healthy volunteers (5 young subjects, 28.8±4.3 years old; 5 elderly subjects, 60.2±2.9 years
270 old) were acquired to assess age- and tract-related structural changes in cervical SC tissues.
271 The protocol was approved by the local institutional ethics review board and written informed
272 consent from all subjects was obtained before participation. The 3D MP2RAGE sequence
273 covering the whole brain and the SC from C₁ to C₇ was acquired using the previously chosen
274 parameters and the same main parameters than used for the phantom experiment described
275 above. Acquisition time was 7:18 min.

276 Similarly, the 2D sagittal magnetization-prepared turbo-FLASH B₁⁺ mapping sequence³³
277 covering the cervical SC (TR: 5 s; TE: 3.42 ms; slice thickness: 5 mm; FOV: 250x250 mm;
278 matrix size: 320x320; preparation flip angle: 45°, sinc shape, 3 slices, voxel size 1.5x1.5mm²,
279 T_{acq}: 1 min) was acquired for B₁⁺-correction of T₁^{MP2RAGE} maps.

280 Three subjects were explored at three different time points for reproducibility assessment.

281

282 **2.5. Image processing**

283 The first step of the data post-processing was to build the LUT linking the MP2RAGE_{UNI}
284 signal intensities to T₁ values¹⁶. The LUT was built under Matlab for different values of T₁
285 ranging from 0.1 to 3.5 s with steps of 10 ms and for relative B₁⁺ variation (flip angle
286 variation) ranging from 50% to 150% of the nominal reference value (100%/45°). The 3D
287 LUT was then used to generate the corrected T₁^{MP2RAGE} map based on the B₁⁺-map and the
288 MP2RAGE_{UNI} reconstructed by the scanner based on the acquired GRE_{T11} and GRE_{T12}
289 volumes. Finally, a denoising step was performed on the reconstructed T₁^{MP2RAGE} map using
290 BM4D³⁴ for better visualisation.

291 The subsequent *in vivo* ROI-based T₁ quantification largely relied on the Spinal Cord
292 Toolbox³⁵ and dedicated SC templates (MNI-Poly-AMU³⁶, AMU₄₀³⁷, WM pathways³⁸),
293 allowing for automatic regional T₁^{MP2RAGE} quantification at all cervical levels from C₁ to C₇,
294 as described in Massire et al.²³. Automated GM/WM segmentation and WM tracts delineation
295 in the common reference template space were obtained after the binarization (threshold: 0.5)
296 of the probabilistic GM/WM AMU₄₀ and WM pathways atlases, respectively. T₁^{MP2RAGE}
297 values were then extracted from anterior GM horns (aGM), lateral motor (corticospinal and
298 rubrospinal) tracts (LMT), posterior sensory (gracile and cuneate) tracts (PST) and whole
299 WM for all slices. Average values per cervical level from C₁ to C₇ were computed. Total GM

300 ROI was not considered for T_1 evaluation due to potential partial volume effects (PVE) in the
301 dorsal horns.

302 Cord cross-sectional areas (CSA) were extracted using automatic cord segmentation
303 (PropSeg³⁵) on both anatomical T_1 -weighted GRE_{T12} (with MPRAGE-like contrast) and
304 T_1^{MP2RAGE} map in the subject space to demonstrate the CSA measurement feasibility on the
305 T_1^{MP2RAGE} map. Slice-by-slice and average values per cervical level from C₁ to C₇ were
306 computed. Compliance between CSA values from GRE_{T12} and T_1^{MP2RAGE} map was evaluated
307 and preliminary evaluation of age-related morphological changes was conducted.

308

309 **2.6. Statistical analyses**

310 All statistical analyses were performed using JMP9 (SAS institute, Cary, NC, USA),
311 considering p -values less than 0.05 for statistical significance.

312 Correlation between B_1^+ -corrected T_1^{MP2RAGE} and T_1^{IRSE} values was assessed using linear
313 regression and Bland-Altman plot was performed for further evaluation of measurement bias.
314 To assess the *in vitro* reproducibility of the T_1 measurement methods, T_1^{IRSE} and B_1^+ -
315 corrected T_1^{MP2RAGE} coefficient of variations (COV, in %) were computed as $\text{COV}=100.\rho/\mu$,
316 where μ is the inter-scan mean and ρ the standard deviation for the T_1 measurements in each
317 tube of the phantom over three time points. Then, global COV for each technique was
318 computed by averaging all COV. *In vivo* reproducibility of the B_1^+ -corrected T_1^{MP2RAGE}
319 measurements was also evaluated by quantifying the COV per ROI (aGM, LMT, PST and
320 whole WM) at each cervical level of the 3 participants over three time points. Then, global
321 COV per ROI was computed by averaging values from all three subjects at all levels.

322 To assess T_1^{MP2RAGE} sensitivity to age- and tract-related structural changes, T_1^{MP2RAGE}
323 values were analyzed using a multi-factorial analysis of variance (ANOVA) testing the effect
324 of age group, ROIs and cervical levels followed by Steel-Dwass all pairs tests when the
325 effects were significant. Bonferroni correction for multiple comparisons were performed
326 when needed.

327 Correlation between GRE_{T12} and T_1^{MP2RAGE} -based slice-by-slice CSA measurements was
328 evaluated using linear regression and Bland-Altman plot. Preliminary evaluation of age-
329 related morphological changes was performed using Wilcoxon rank tests on CSA values from
330 the two age-groups at each cervical level, corrected for multiple comparisons.

331

332 3. Results

333 3.1. Sequence parameters optimization and phantom validation

334 *Fig. 1* presents the results of the MP2RAGE_{UNI} GM/WM contrast optimization as a
335 function of α_1 , α_2 , TI_1 and TI_2 . *Fig. 2* shows the simulated MP2RAGE_{UNI}- T_1 relationship for
336 the protocol with optimal parameters (protocol #1) resulting from parameter optimization and
337 the protocol with the chosen parameters (protocol #2), along with the B_1^+ -variation effect on
338 each protocol.

339 As seen on *Fig. 1a*, the maximal GM/WM contrast achievable while ensuring the
340 constraints mentioned in paragraph 2.2 was 0.08, obtained with $\alpha_1/\alpha_2=8^\circ/5^\circ$ and $TI_1/TI_2=650$
341 ms/2000 ms (Protocol #1), which led to a flatter MP2RAGE_{UNI} signal curve than with
342 Protocol#2 (*Fig. 2a*). However, when introducing B_1^+ variation into the Bloch simulations
343 (see *Fig. 2b*), this parameter set led to a high dependence on B_1^+ inhomogeneities ($\pm 6\%$ of T_1
344 values for B_1^+ variations within [70%-120%] range) and a non-bijective MP2RAGE_{UNI}- T_1
345 relationship for a relative B_1^+ value of 70% (typically observed at lower cervical levels) that
346 would affect T_1^{MP2RAGE} measurements in CSF ($T_{1\text{CSF}} \sim 2500\text{ms}$) or in pathological tissue
347 ($T_{1\text{MS}_{\text{lesion}}} \sim 1200$ to 1700ms^5).

348 The optimum protocol limiting this B_1^+ variation effect while providing reasonable
349 WM/GM signal difference (0.06) was obtained with $\alpha_1/\alpha_2=4^\circ/5^\circ$ and $TI_1/TI_2=650$ ms/2000
350 ms (Protocol #2, *Fig. 2.c* and *2.d*). The consecutive error on the T_1 estimation due to B_1^+
351 variations within [70% to 120%] range corresponds to $\pm 3\%$ of the T_1 values (± 30 ms and ± 20
352 ms in the GM and WM, respectively) for this protocol. For example, a SC GM voxel ($T_{1\text{GM}}$
353 $\approx 973 \text{ms}^{26}$) associated with a relative B_1^+ variation of +20% (i.e. 120%), has a signal intensity
354 of 0.052; which without B_1^+ correction would result in an underestimated $T_{1\text{GM}}$ of about 948
355 ms ($\sim -3\%$). Same illustration goes for a voxel of SC WM ($T_{1\text{WM}} \approx 876 \text{ms}^{26}$) associated with a
356 B_1^+ variation of -30% for instance (i.e. 70%), leading to an overestimated $T_{1\text{WM}}$ of about 910
357 ms ($\sim +3\%$). Given these relatively small errors on T_1 estimation for the range of B_1^+ variation
358 observed *in vivo*, one could consequently choose to apply the B_1^+ correction for potential
359 residual effect or not.

360 Curves illustrating the brain protocol proposed by Marques et al.¹⁶ at 3T together with the
361 same brain protocol but modified to cope with our SC needs are also shown to illustrate the
362 need for optimization when dealing with different anatomical regions (*Fig. 2a*). While such a
363 protocol led to a flatter signal curve and therefore would allow improved WM/GM signal

364 difference (maximal contrast of 0.09), it would have led to larger uncertainties for T_1 value
365 measurements above 1200 ms (due to non-bijective behavior), which potentially correspond
366 to pathological T_1 values or pixels contaminated by PVE with CSF.

367 *Fig. 3* illustrates the efficacy of the custom B_1^+ correction on the 6 MP2RAGE datasets
368 acquired with various voltages from 70% to 120% of the nominal reference value (100% \Leftrightarrow
369 45° , nominal flip angle value, targeted at C_3 level). The curves from the graph to the left
370 represent the uncorrected T_1^{MP2RAGE} values whilst those from the right represent the B_1^+ -
371 corrected values. For the sake of clarity, data from tubes #7, #9, #12, #13 and #17 are not
372 shown here. Within the 70%-120% range of B_1^+ variation investigated, T_1^{MP2RAGE} values from
373 all twelve tubes presented an average variation of +0.57% ([-4.5% to 5.6%], RMSE=32.84
374 ms) and -0.01% ([-1.3% to 1.0%], RMSE=5.30 ms) as compared to the nominal T_1^{MP2RAGE}
375 (100% of reference voltage) before and after B_1^+ correction respectively. In addition, the
376 [min-max] range of mean T_1^{MP2RAGE} value per tube across all 6 acquisitions (horizontal
377 shaded bars on *Fig. 3c* and *d*) were found greatly reduced after the B_1^+ correction. All these
378 results support the efficacy of the B_1^+ correction method.

379 To demonstrate the conformity of MP2RAGE-based T_1 values to gold standard T_1^{IRSE} , *Fig.*
380 *4a* shows a pixel-by-pixel plot of T_1^{MP2RAGE} and T_1^{IRSE} . The $T_1^{\text{MP2RAGE}}=T_1^{\text{IRSE}}$ line is shown in
381 dashed black. Linear regression (red line) showed the high degree of agreement between the
382 two techniques (slope: 1.0014, R^2 :0.997) confirmed by the Bland-Altman representation in
383 *Fig. 4b* showing a negligible bias (mean bias: 0.55) between the two measurements.

384 Finally, both *in vitro* T_1^{IRSE} and T_1^{MP2RAGE} measurements were found highly reproducible.
385 COV and in-ROI standard deviations were estimated to 2.94% (range: [2.21% - 3.75%]) and
386 0.66% (range [0.42% - 1.35%]), respectively for the T_1^{MP2RAGE} and 2.15% (range: [1.66% -
387 3.07%]) and 0.96% (range: [0.49% - 1.46%]), respectively for T_1^{IRSE} .

388

389 **3.2. In vivo T_1 measurements**

390 *Fig. 5* shows a representative dataset of the T_1 -weighted $\text{MP2RAGE}_{\text{UNI}}$ volume
391 reconstructed online and the corresponding T_1^{MP2RAGE} map (without B_1^+ correction) in all
392 three orientations (sagittal, coronal and axial) allowing clear visualization of vertebral bodies,
393 intervertebral disks and spinal cord. The butterfly-shaped GM, whose mask (derived from the
394 probabilistic atlas) is highlighted with the dotted line boundary in blue, can be distinguished
395 in both axial images as compared to the surrounding WM. The brain was also covered in the

396 acquisition but not exploited in the present study. *Fig. 6* illustrates a typical *in vivo* B_1^+ map
397 showing the variation pattern in the cervical SC when targeting flip angle (100% B_1^+) around
398 C_3 . Boxplots of the B_1^+ variation in all ten subjects show reduced B_1 efficiency at lower
399 cervical levels (69% at C_7) and slight increase at C_1 - C_2 level (119% at C_1). Without B_1 -
400 correction, $T_1^{MP2RAGE}$ values from the whole WM at C_7 across the subjects would vary from
401 (910ms \pm 51ms) and (1055ms \pm 139ms) with inter-individual mean $T_1^{MP2RAGE}$ values of
402 (955ms \pm 38ms) and mean in-ROI SD of 78ms (8.1% of the mean). After B_1 -correction, they
403 varied between (865ms \pm 40ms) and (1011ms \pm 134ms), with a mean of (915ms \pm 37ms) and
404 a mean in-ROI SD of 73ms (8.0% of the mean).

405

406 *In vivo* corrected $T_1^{MP2RAGE}$ measurements were found highly reproducible. All COV
407 values were found to be lower than 5% in all ROIs at all cervical levels (range [0.97-4.65]),
408 with highest COV values ($> 2.5\%$) mostly found at lower cervical levels (C_5 to C_7). *Table 1*
409 summarizes the global COV values, averaged across all cervical levels, for each ROI (aGM,
410 LMT, PST and whole WM), along with the mean in-ROI standard deviation values (expressed
411 as a ratio relative to mean $T_1^{MP2RAGE}$ value). In-ROI standard deviation values were found
412 higher than COV values.

413

414 **3.2.1. Level-, tract- and age-related variations of $T_1^{MP2RAGE}$ values**

415 Average $T_1^{MP2RAGE}$ group maps in the template space for both young and elderly subjects per
416 cervical level are presented in *Fig. 7*, along with examples of individual maps. The butterfly-
417 shaped GM can be visualized at each cervical level, particularly in the young group dataset.
418 The contrast between the LMT and PST tracts can also be clearly visualized. For detailed
419 $T_1^{MP2RAGE}$ data per ROI and cervical level from the two age groups, the reader is referred to
420 *Supplementary Table*.

421 Results of three-way ANOVA highlighted significant effects of age group, ROI and cervical
422 levels on $T_1^{MP2RAGE}$ values. No significant second or third effects were found. When
423 comparing the two age-groups, $T_1^{MP2RAGE}$ values decrease trends in elderly subjects as
424 compared to young ones were found in every ROI at every cervical level especially at upper
425 levels (C_1 - C_3) in the order of -3%. However, no statistical significance was found. As for the
426 cervical level influence, *Fig. 8* shows the pattern of $T_1^{MP2RAGE}$ variations along the cervical
427 cord for each age-group and ROI. Although no significant differences were found between
428 $T_1^{MP2RAGE}$ values from the different levels along the cord, a U-shaped trend can be observed

429 from upper to lower levels, particularly pronounced in the WM tracts. Finally, considering
430 ROI effect on T_1^{MP2RAGE} values, *Fig. 9* plots mean T_1^{MP2RAGE} values (n=10 subjects) per ROI
431 (aGM, LMT, PST) at each cervical level (C_1 to C_7). Results of Steel-Dwass all pairs tests
432 showed significant differences between aGM and LMT for all cervical levels
433 ($0.006 \leq p \leq 0.037$), but only differences at upper levels remained significant after Bonferroni
434 correction. T_1^{MP2RAGE} values from LMT were also found lower than that of PST at C_2
435 ($p=0.037$), C_3 ($p=0.013$) and C_4 ($p=0.019$) but the differences were not significant after
436 Bonferroni correction.

437

438 **3.2.2. Morphological measurements**

439 CSA values extracted from automated SC segmentation on T_1^{MP2RAGE} map were found highly
440 correlated to those extracted from the anatomical T_1 -weighted $\text{GRE}_{\text{T}2}$ volume, confirmed by
441 linear fit results (*Fig. 10a*, slope: 0.83, $R^2=0.9$, $p<.0001$). However, the Bland-Altman plot
442 (*Fig. 10b*) shows evidence of a systematic bias (mean bias: -7.03, regression line $y=-0.14*x$
443 $+1.81$, $R^2=0.17$, $p=0.0005$) between the two measurements, i.e., CSA values from the
444 T_1^{MP2RAGE} map were systematically lower than that from $\text{GRE}_{\text{T}2}$ map by 7 mm^2 in average.
445 When used to preliminarily investigate age-related morphological changes at each cervical
446 level, $\text{GRE}_{\text{T}2}$ -based CSA values were found decreased in elderly subjects compared to young
447 ones (*Fig. 10c*). Nonetheless, no significant decrease was found from the Wilcoxon's rank
448 tests.

449

450 **4. Discussion**

451 The purpose of this study was to use the recently proposed MP2RAGE sequence to obtain
452 reliable volumetric T_1 -mapping of the whole cervical cord at 3T within a clinically acceptable
453 scan time at a sub-millimetric resolution. A validation experiment was performed on a
454 dedicated phantom by comparing T_1 -values obtained from the MP2RAGE sequence to the
455 gold-standard IR-SE protocol. The optimized MP2RAGE protocol was then applied *in vivo*
456 and combined with an automated processing pipeline based on dedicated SC MR templates
457 and atlases to probe regional microstructural organization of the healthy aging SC, while
458 avoiding bias associated to manual ROI delineation.

459

460 **4.1. MP2RAGE parameters in this study**

461 The range of T_1 values for which we wanted to ensure a bijective behavior and precise
462 T_1^{MP2RAGE} determination was motivated by the future use of the MP2RAGE sequence in a
463 clinical context. For instance, a previous study investigating multiple sclerosis reported values
464 of T_1 ranging from 500 to 1700 ms in the brain at $3T^5$. Our choice was also motivated by the
465 need for unambiguous T_1 determination within the CSF to avoid PVE contamination in pixels
466 at the interface of WM and CSF and in pathologies in which CSF leaks within the cord or
467 edema is present, such as in syringomyelia or *neuromyelitis optica* spectrum disorders. As an
468 example, the application of the brain protocol described in¹⁶ would have led to a non-bijective
469 behavior of the $\text{MP2RAGE}_{\text{UNI}}\text{-}T_1^{\text{MP2RAGE}}$ relationship, and therefore to a more ambiguous
470 estimation for T_1 values close to and above 1600 ms (see purple and magenta curves on *Fig.*
471 *2*). Another criteria that we chose to apply to our study was to limit the B_1^+ variation effect on
472 T_1^{MP2RAGE} measurements, motivated by the need for precise T_1 quantification along the
473 cervical cord allowing for characterization of microstructural changes encountered in diffuse
474 pathology. Overall, these choices and the resolution vs acquisition time compromise led to a
475 small possible range for the MP2RAGE parameters.

476 In the current study, under the maximum GM-WM signal difference criteria, the parameters
477 optimization led to protocol #1 ($\alpha_1/\alpha_2=8^\circ/5^\circ$ and $TI_1/TI_2= 650 \text{ ms}/2000 \text{ ms}$), but this protocol
478 potentially leads to subsequent ambiguous T_1 estimation in CSF and pathological tissues due
479 to non-bijective behavior with reduced B_1 efficiency up to 70%, a value that could be
480 observed *in vivo* at lower cervical levels ($C_6\text{-}C_7$). Furthermore, within the [70%-120%] range
481 of B_1^+ variation, this protocol led to $\pm 6\%$ of simulated T_1^{MP2RAGE} measurement errors in the
482 SC GM and WM. Therefore, when including the low sensitivity to B_1^+ variation criteria, the
483 optimum protocol was protocol #2, with a reduced α_1 of 4° as compared to protocol #1 (the
484 $\text{MP2RAGE}_{\text{UNI}}$ signal indeed shows a higher sensitivity to B_1^+ when the α_1 value is increased).
485 For protocol #2, the GM-WM signal difference was 0.06, and more importantly, the simulated
486 T_1^{MP2RAGE} measurement error was about $\pm 3\%$ for the range of B_1^+ variation observed *in vivo*
487 ([70%-120%]), making this protocol more suitable for *in vivo* application.

488 When applied for validation to a phantom, this protocol provided T_1^{MP2RAGE} values that were
489 found in a close to perfect pixel-wise agreement with the gold standard inversion-recovery
490 T_1^{IRSE} values (see *Fig. 4*, $R^2=0.997$) with a mean measurement bias of $0.55\pm 20.29\text{ms}$.
491 Furthermore, the phantom results were found highly reproducible, with an average COV of
492 2.94% and in-ROI standard deviations of 0.66% of mean values.

493

494 **4.2. B₁⁺ inhomogeneity and MP2RAGE imaging**

495 At high fields (≥ 3 T), the shorter radiofrequency wavelength leads to transmit field
496 inhomogeneities, i.e. flip angle/B₁⁺ variations across the imaged object, that could be
497 prejudicial to image signal homogeneity or quantitative parameters derived from biased
498 images³⁹. These inhomogeneities could be further accentuated in the SC by the proximity of
499 large bony structures and the flowing cerebrospinal fluid. In our *in vivo* 3T study, the
500 reference voltages were determined during automated scanner adjustments, and the range of
501 observed B₁⁺ relative variation throughout the cervical SC could reach up to [70% – 120%],
502 found at C₇ and C₁/C₂, respectively.

503 With protocol #2, the T₁^{MP2RAGE} measurement error within the observed range of B₁⁺ variation
504 was about $\pm 3\%$ for SC tissues. With this range of measurement error, this protocol could be
505 used without further B₁⁺ correction. However, we would still recommend correcting for B₁⁺
506 effects. Indeed, the custom LUT-based B₁⁺ correction method proposed here exhibited very
507 good efficacy when applied to *in vitro* data (B₁⁺ related T₁^{MP2RAGE} error measurements relative
508 to nominal T₁^{MP2RAGE} values before and after correction of +0.57% ([-5% to 6%],
509 RMSE=32.84 ms) and -0.01% ([-1% to 1%], RMSE=5.30 ms), respectively), motivating its
510 use for *in vivo* data.

511 Another method to correct for B₁⁺ bias could be to introduce a third readout at a third
512 inversion time from which a B₁⁺ and a T₁ map would be calculated⁴⁰⁻⁴². This would require
513 additional and more complex sequence parameters optimization. Given the results obtained in
514 the present study, the LUT approach using an additional B₁⁺-map acquisition appears as a
515 good alternative to enable bias-free MP2RAGE acquisitions, adding only one minute of
516 acquisition time. Note that a 2D magnetization-prepared turbo-FLASH B₁⁺-mapping³³ method
517 was used in this study but other B₁⁺-mapping methods, such as the recently proposed
518 SA2RAGE (Saturation-prepared with 2 rapid Gradient Echoes)⁴³ or the AFI⁴⁴ (Actual Flip-
519 angle Imaging) could also be of great interest and should be considered in future studies. It is
520 also worth mentioning that, alternatively, an increased MP2RAGE_{TR} could also be used,
521 resulting in a reduction of the MP2RAGE_{UNI} signal sensitivity to B₁⁺ inhomogeneity (e.g. less
522 than $\pm 1.9\%$ T₁ measurements errors within the [70%-120%] range of B₁⁺ variation for
523 TR=5s), at the expense of a slight increase in the acquisition time (9:10 min for TR=5s vs.
524 8:18 min in our study with T₁ and B₁ measurements).

525

526 **4.3. Accuracy and reproducibility of *in vivo* T_1^{MP2RAGE} measurements**

527 Being able to measure T_1 values *in vivo* with the highest reproducibility and accuracy
528 available is very important since these values should closely reflect the underlying tissue
529 microstructure and their magnetic properties. IR-SE sequences are the gold standard methods
530 to perform tissue T_1 mapping but scan times for such acquisitions are prohibiting for potential
531 clinical applications (for example, in this study, acquisition time for the IR-SE on the
532 phantom was 20 min per TI for a single slice acquisition). Another emerging alternative
533 approach could be MR fingerprinting, but SC implementation has not yet been proposed
534 although applications to the brain are currently already available with in-plane spatial
535 resolution in the order of $1 \times 1 \text{ mm}^2$ ^{6,45}.

536 With a sub-millimetric spatial resolution suitable for SC investigation, a less than 10 min
537 acquisition, and a relatively smooth post-processing pipeline, the MP2RAGE sequence
538 allowed highly reproducible *in vivo* T_1 quantification in the cervical SC with mean and max
539 COV of 2% and 5% respectively, within the different ROIs (cf. *Table 1*). Moreover, the
540 MP2RAGE sequence parameters were optimized to allow unambiguous T_1 values
541 determination from 500 ms to 3000 ms (see *Fig. 2*), hence covering the full extent of T_1
542 values reported for human brain and SC at 3T, even in pathological contexts. The *in vivo* in-
543 ROI standard deviations were found in average in the order of 7% of mean T_1 values (see
544 *Table 1*), for all regions of interest.

545 *Table 2* summarizes T_1 values reported in this study and other studies of the literature
546 performed in the cervical SC at 3T using various techniques. The T_1 values obtained in this
547 study exhibited a similar pattern of T_1 values distribution across the different regions of the
548 cervical SC as the values reported by Smith et al.²⁶, i.e. $T_{1\text{ILMT}} < T_{1\text{WM}} < T_{1\text{PST}} < T_{1\text{GM}}$ using
549 both IR-EPI and GRE-based techniques. However, T_1 values measured in SC WM are longer
550 in the current study. This difference could arise from the fact that in²⁶, SC WM values were
551 obtained by averaging dorsal and lateral columns T_1 values, whereas our values were
552 extracted from the whole WM ROI, hence also including the anterior WM regions which were
553 reported by Lévy et al.²⁵ to have longer T_1 values of $1006.8 \pm 168 \text{ms}$ (vs. $971.6 \pm 64.5 \text{ms}$ in
554 lateral tracts). Furthermore, T_1 values in GM were found shorter in the present study. This
555 may arise from the difference between manual and template-based ROI delineation. This
556 more probably comes from the fact that in their study, whole GM was considered whereas in
557 the present study, only anterior GM was studied due to posterior GM potentially suffering
558 from GM/WM PVE. T_1 values reported in^{24,25,27} were found higher than²⁶ and our study in all

559 ROIs. However, in the study of Battiston et al., the similarity of GM/WM values as well as
560 larger standard deviations, which may come from the thicker slices, could induce some
561 CSF/WM and WM/GM partial volume effect in the rostro-caudal direction if not strictly
562 parallel to the cord axis, hence biasing values in the different compartments. For the studies
563 from Lévy et al. and Duval et al., the difference could likely be explained by the use of VFA-
564 based techniques, which usually overestimate T_1 values when not corrected for B_1^+
565 variations⁹. As for the study of Samson et al., their reported T_1 values using a multi-echo
566 FLASH are much higher than those reported in all other studies.

567

568 **4.4. MP2RAGE-based SC microstructural characterization**

569 Microstructural alterations of the aging brain have been previously largely investigated using
570 quantitative MRI^{6,29,30,46}. Characterization of the aging SC, on the other hand, generally lag
571 behind the brain and the few studies conducted so far have mostly focused on morphological
572 measurements reporting age-related atrophies of GM and whole SC^{37,47-49}. In the current
573 study, MP2RAGE acquisitions allowed to evaluate SC morphology using the automated SC
574 segmentation on T_1 -weighted GRE_{T12} image, whose contrast is similar to the MPRAGE,
575 commonly used in clinical studies and previously validated with regards to manual
576 delineation⁵⁰. The resulting CSA values exhibited decrease trends, without statistical
577 significance, in elderly subjects when compared to young ones, at all cervical levels from C₁
578 to C₇, suggesting SC atrophy, in line with previous reports. It is worth noting that CSA
579 extracted from SC segmentation based on quantitative T_1 ^{MP2RAGE} were highly correlated to
580 those from GRE_{T12}, but with a mean bias of -7 mm² relative to the CSA GRE_{T12}, suggesting
581 that conventional tool and default options for cord segmentation (PropSeg³⁵) have to be
582 slightly optimized for this new contrast. This optimization was beyond the scope of this study,
583 as CSA could be extracted from the GRE_{T12} volume.

584 Several studies have also used quantitative SC MRI, mostly diffusion tensor imaging^{48,51,52}
585 and magnetization transfer-based techniques^{25,48} to study microstructural changes occurring
586 with age, with evidences supporting hypotheses of demyelination and destructuration. To the
587 best of the authors knowledge, the study from²⁵ is the first study to evaluate SC T_1
588 relaxometry variation with age. This latter study did not find evidence of significant T_1
589 variations whereas brain studies reported age-related T_1 decrease in the GM⁵³ and T_1 increase
590 in the WM⁵⁴. In this preliminary work, slight trends of T_1 value decrease were found in both
591 anterior GM and WM tracts in the elderly group, however given the small number of

592 participants in our study, this should be further explored. Higher inter-subject variability of T_1
593 data were particularly noticed on both graphs and mean T_1 maps with less sharp GM/WM
594 boundaries than in younger subjects, which could express different degrees of ageing
595 impairments (linked to sport or professional activities for example).

596 Finally, our preliminary tract-based analysis also exhibited trends of lower T_1 values in LMT
597 (motor) tracts as compared to that in PST (sensory) tracts. This is in agreement with previous
598 studies reporting higher myelination in lateral motor tracts as compared to posterior sensory
599 tracts^{24,25,48}, which would lead to lower T_1 values, according to⁵⁵. As for the vertebral level
600 influence on T_1 values, a U-shaped pattern in the rostro-caudal direction could be observed,
601 although no statistical differences were found as it was the case in previous reports^{23,27}. As
602 mentioned above, these should also be reinvestigated while considering a larger cohort.

603

604 **5. Conclusion**

605 In this work, MP2RAGE-based T_1 -mapping was optimized for sub-millimetric SC
606 imaging for the first time at 3T. Using an additional B_1^+ correction method, 3D T_1 -weighted
607 anatomical imaging and unbiased T_1 -mapping of the entire cervical cord were obtained within
608 a scan time compatible with clinical research. Combined with an atlas-based approach, highly
609 reproducible and accurate T_1 measurements within specific regions of the cervical white
610 matter and anterior gray matter horns were obtained, highlighting preliminary regional and
611 age-related differences.

612 Benefiting from a very high reproducibility, the MP2RAGE protocol proposed here could be
613 of particular interest in the characterization of early and longitudinal SC microstructural
614 changes in different diseases involving the spinal cord such as Multiple Sclerosis and
615 Amyotrophic Lateral Sclerosis. Alone or associated with other quantitative and more specific
616 MR imaging such as diffusion tensor imaging, myelin imaging using either
617 conventional/inhomogeneous magnetization transfer or myelin water imaging in a multi-
618 parametric approach, this technique could help investigating the different underlying
619 pathophysiological mechanisms.

620

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628

629 **Conflict of interest:**

630 *Tobias Kober is an employee of Siemens Healthcare AG Switzerland, who provided sequence*
631 *support with the MR technique but was not involved in data acquisition and analysis. No*
632 *conflicts of interest are declared for the remaining authors.*

633

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825

ROI	aGM	LMT	PST	Whole WM
COV (%)	2.46 ± 0.67 [1.44 - 3.26]	3.08 ± 1.04 [0.97- 4.65]	2.34 ± 0.79 [1.21 - 3.98]	1.88 ± 0.30 [1.46 - 2.25]
In-ROI SD (%)	3.92 ± 1.21 [1.84 – 8.93]	9.74 ± 3.15 [4.65 – 18.44]	6.89 ± 3.88 [2.86 – 20.11]	7.92 ± 1.24 [4.76 – 10.91]

827 **Table 1:** In vivo $T_1^{MP2RAGE}$ reproducibility. Mean coefficient of variation (COV, in %) of $T_1^{MP2RAGE}$
828 values are presented together with mean in-ROI standard deviations (SD, computed as the ratio of
829 $T_1^{MP2RAGE}$ SD and mean values) from the different regions of interest (anterior gray matter (aGM),
830 lateral motor tracts (LMT), posterior sensory tracts (PST) and whole white matter (WM), all cervical
831 levels considered, from $n=3$ subjects examined at 3 different time-points.

<i>References</i>	Sequence type / Levels	Population age (years) (number)	Image resolution (mm³)	T₁ GM (ms)	T₁ WM (ms)	T₁ Dorsal column (ms)	T₁ Lateral column (ms)
<i>Smith et al. (2008)</i>	IR-EPI C ₃	31±6 (n=6)	1x1x5	972±36	876±27	900±17	863±23
<i>Smith et al. (2008)</i>	GRE VFA C ₃	31±6 (n=6)	1x1x4	994±54	838±54	853±68	830±47
<i>Samson et al. (2013)</i>	Multiecho-FLASH C ₁ -C ₅	36±12 (n=13)	1.28x1.28x3	1815±170	-	1735±205	1650±220
<i>Battiston et al. (2017)</i>	IR-ZOOM-EPI C ₁ -C ₇	[27-37] (n=5)	1x1x5	1136±91	-	1150±103	1109±80
<i>Duval et al. (2017)</i>	VFA-FLASH C ₂ -C ₅	29±14 (n=9)	0.8x0.8x5	-	1290±130	-	-
<i>Lévy et al. (2018)</i>	VFA-FLASH C ₂ -C ₄	57 [21-72] (n=16)	0.9x0.9x5	-	1011±61	1068±64	-
<i>This study</i>	MP2RAGE C ₃	29±4 (n=5)	0.9x0.9x1	927±23	911±29	914±26	874±36
<i>This study</i>	MP2RAGE C ₁ -C ₇	29±4 (n=5)	0.9x0.9x1	943±32	919±24	925±29	888±29

833 **Table 2:** Comparison of T₁ values measured in this study and those found in the literature for cervical
834 spinal cord at 3T. Values are reported in ms (mean ± standard deviation). Population age are
835 reported as mean± standard deviation or [min-max] range. **IR:** Inversion Recovery; **EPI:** Echo
836 Planar Imaging; **GRE:** Gradient Echo; **FLASH:** Fast low-angle shot; **ZOOM:** Zonally oblique-
837 magnified multi-slice; **VFA:** Variable Flip Angle; **MP2RAGE:** Magnetization Prepared 2 Rapid
838 Acquisition Gradient Echoes; **GM:** gray matter; **WM:** white matter.

840 **Figures**

841 **Figure 1: Contour plots showing the simulated SC GM-WM contrast** (signal intensity difference
842 between SC GM and WM) as a function of flip angles α_1 and α_2 (**left**) and inversion times TI_1 and TI_2
843 (**right**). Two protocols with different parameter subsets are identified in both graphs: Protocol #1
844 ($\alpha_1/\alpha_2=8^\circ/5^\circ$ and $TI_1/TI_2=650ms/2000ms$) rendering the maximal signal difference (0.08) but with
845 higher B_1^+ dependency (see Fig. 2b); and Protocol #2 ($\alpha_1/\alpha_2=4^\circ/5^\circ$ and $TI_1/TI_2=650ms/2000ms$)
846 rendering a signal difference of 0.06 but much less sensitive to B_1^+ variation (see Fig. 2c).

847 **Figure 2: $T_1^{MP2RAGE}/MP2RAGE_{UNI}$ signal intensity relationship and effect of B_1^+ inhomogeneity on**
848 $T_1^{MP2RAGE}$ estimation. (a): Plots of $T_1^{MP2RAGE}$ values as function of $MP2RAGE_{UNI}$ image intensity estimated
849 using Bloch equations. The red dotted lines correspond to the expected SC GM and WM T_1 values
850 reported in ²⁶. **Green curve (Protocol #1):** Protocol with optimal sequence parameters providing the
851 maximum GM/WM contrast. **Blue curve (Protocol #2):** protocol used in this study; **the purple curve**
852 shows what would be obtained using optimal parameters proposed by Marques et al. for the brain in
853 ¹⁶($MP2RAGE_{TR}=5s$; $GRE_{TR}=7ms$; $GRE_n=160$ excitations per GRE module; $TI_1/TI_2=700ms/1500ms$;
854 $\alpha_1/\alpha_2=4^\circ/5^\circ$, spatial resolution = $1x1x1mm^3$) without further optimization. The **pink curve** indicates
855 what would be obtained with this latter protocol but modifying $MP2RAGE_{TR}$, GRE_{TR} and GRE_n to suit
856 our SC needs, i.e. sub-millimetric spatial resolution and T_{acq} less than 10 minutes. (b): Effect of B_1^+ -
857 variation (70% to 120%) on Protocol #1; (c): Effect of B_1^+ -variation (70% to 120%) on Protocol #2
858 (used in this study); (d): Zoom on the black box of Fig. (c) so as to better illustrate the B_1^+ effect on T_1
859 estimation in GM and WM using protocol #2.

860 **Figure 3: Phantom experiments to validate the custom B_1^+ correction method.** **Top:** from left to
861 right: Eurospin T_1 phantom containing twelve tubes filled with gadolinium-doped agar gel; theoretical
862 T_1 mean and standard deviation specifications (in ms) for each tube, provided by the manufacturer
863 (Temperature: $23^\circ C$, last phantom QA control in 2011); and the B_1^+ -corrected $T_1^{MP2RAGE}$ map. It is
864 worth noting that IR-SE and $MP2RAGE$ T_1 values were not compared to the values provided by the
865 constructor due to experimental temperature differences and potential time alteration of the phantom.
866 **Bottom left:** Curves corresponding to mean uncorrected $T_1^{MP2RAGE}$ values for 7 phantom tubes with the
867 prescribed B_1^+ variation (Tubes #7, #9, #12, #13 and #17 are not shown for easier visualization). The
868 range of B_1^+ variation considered here were chosen so as to cover the range of variation observed in
869 vivo ([69% - 119%], see Fig. 6). **Bottom Right:** Curves corresponding to B_1^+ -corrected $T_1^{MP2RAGE}$
870 values. Horizontal shaded bars correspond to [min-max] range of observed $T_1^{MP2RAGE}$ values for each
871 tube across the relative B_1^+ variation range. The dashed vertical line corresponds to the $T_1^{MP2RAGE}$
872 values obtained with 100% of nominal B_1^+ .

873 **Figure 4: $T_1^{MP2RAGE}$ measurement validation on phantom.** (a): Pixel-by-pixel plot of B_1^+ -corrected
874 $T_1^{MP2RAGE}$ and T_1^{IRSE} values from all tubes, over three different timepoints. The dashed black line
875 corresponds to $T_1^{MP2RAGE}=T_1^{IRSE}$. Linear regression fit between $T_1^{MP2RAGE}$ and T_1^{IRSE} is illustrated by the
876 red trend line and associated coefficient of correlation R^2 . (b): Bland-Altman plot of absolute
877 differences between $T_1^{MP2RAGE}$ and T_1^{IRSE} . Mean bias and 95% limits of agreement are shown with
878 horizontal green lines.

879 **Figure 5: Representative in vivo dataset including, from left to right, sagittal, coronal and zoomed**
880 axial view at C_2 of (from top to bottom) the acquired GRE_{TI1} and GRE_{TI2} volumes, the resulting T_1 -
881 weighted $MP2RAGE_{UNI}$ volume reconstructed online and the corresponding $T_1^{MP2RAGE}$ map without B_1^+
882 correction. A manually-drawn GM region boundary (in blue) has been added to the $MP2RAGE_{UNI}$
883 image for better visualization.

884

885 **Figure 6: In vivo B_1^+ variation in the cervical spinal cord.** *Left:* illustration of an acquired B_1 map
886 superimposed on an anatomical T_2 -weighted sagittal image for visualization, showing the B_1^+
887 variation pattern throughout the spinal cord and particularly the reduced B_1 efficiency observed at
888 lower cervical levels (C_6 - C_7). **Right:** Relative B_1^+ variation (expressed in % relative to the nominal B_1^+
889 flip angle (100%)) at each cervical level from all ten subjects. The box plots represent the minima,
890 first quartile (25%), the median, the third quartile (75%) and the maxima from bottom to top,
891 respectively. Average values per cervical level are also reported.

892 **Figure 7: Age-, tract- and cervical level-related T_1 variation in the cervical spinal cord.** *From left to*
893 **right:** Anatomical T_2^* -weighted AMU_{40} template image; average T_1 map for the young group; average
894 T_1 map for the elderly group; representative individual T_1 map of a young subject (Male; 22 years
895 old); representative individual T_1 map of an elderly subject (Female; 57 years old). Rows correspond
896 to the vertebral levels from C_1 to C_7 .

897 **Figure 8: Evolution of $T_1^{MP2RAGE}$ values (in ms) versus cervical level for every region of interest**
898 (anterior gray matter (aGM), lateral motor tracts (LMT) and posterior sensory tracts (PST)) for
899 young (left, $n=5$, <35 years old) and elderly (right, $n=5$, >50 years old). The box plots represent the
900 minima, first quartile (25%), the median, the third quartile (75%) and the maxima from bottom to top
901 respectively.

902

903 **Figure 9: Regional variation of $T_1^{MP2RAGE}$ values** (anterior gray matter (aGM), lateral motor tracts
904 (LMT) and posterior sensory tracts (PST)) at each cervical level, all subjects considered ($n=10$). The
905 boxplots represent the minima, first quartile (25%), the median, the third quartile (75%) and the
906 maxima from bottom to top respectively. P -values are reported when the results of Steel-Dwass all
907 pairs tests are statistically significant. P -values in light gray color indicate significant differences
908 between aGM and LMT, p -values in black, between LMT and PST. † indicates statistical differences
909 that survive Bonferroni correction for multiple comparisons (adjusted Bonferroni $p<0.0071$)

910 **Figure 10: MP2RAGE-based morphological measurements.** (a): Linear regression between
911 $T_1^{MP2RAGE}$ - and GRE_{T2} -based cross-sectional areas (CSA, in mm^2) showing the high degree of
912 correlation between the two measurements. (b): Bland-Altman plot showing absolute differences
913 between the two measurements. Mean bias and 95% limits of agreement are also reported with blue
914 lines. (c): GRE_{T2} -based SC CSA values in the two age-groups as a function of cervical level, from C_1
915 to C_7 . Boxplots represent the minima, first quartile (25%), the median, the third quartile (75%) and
916 the maxima from bottom to top respectively.