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3 **BOLD response to multiple grip forces in MS: going beyond the main effect of**
4 **movement in BA 4a and BA 4p**
5

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28 **Short title:** Effects of forces on the sub-divisions of BA 4 in MS
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38 **Abstract:**

39 This study highlights the importance of looking beyond the main effect of movement to study
40 alterations in functional response in the presence of central nervous system pathologies such
41 as multiple sclerosis (MS). Data show that MS selectively affects regional BOLD (Blood
42 Oxygenation Level Dependent) responses to variable grip forces (GF). It is known that the
43 anterior and posterior BA 4 areas (BA 4a and BA 4p) are anatomically and functionally distinct.
44 It has also been shown in Healthy volunteers that there are linear (1st order, typical of BA 4a)
45 and non-linear (2nd-4th order, typical of BA 4p) BOLD responses to different levels of GF
46 applied during a dynamic motor paradigm. After modelling the BOLD response with a
47 polynomial expansion of the applied GFs, the particular case of BA 4a and BA 4p were
48 investigated in Healthy Volunteers (HV) and MS subjects. The main effect of movement (0th
49 order) analysis showed that the BOLD signal is greater in MS compared to healthy volunteers
50 within both BA 4 sub-regions. At higher order, BOLD-GF responses were similar in BA 4a but
51 showed a marked alteration in BA 4p of MS subjects, with those with greatest disability
52 showing the greatest deviations from the healthy response profile. Therefore, the different
53 behaviour in HV and MS could only be uncovered through a polynomial analysis looking
54 beyond the main effect of movement into the two BA 4 sub-regions. Future studies will
55 investigate the source of this pathophysiology, combining the present fMRI paradigm with
56 blood perfusion and non-linear neuronal response analysis.

57

58 **Key words:** BA 4p, BA 4a, fMRI, Force, Multiple Sclerosis

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62 **1. Introduction:**

63 The primary motor cortex, M1 or Brodmann area 4 (BA 4) is very important because of its
64 essential role in generating movement, a skill often affected by diseases such as Multiple
65 Sclerosis (MS). Interestingly, BA 4 has two sub-regions with distinct cytoarchitectonic
66 properties, anatomy, and neurochemistry both in humans and primates (Strick and Preston,
67 1982, Geyer et al., 1996, Kaas and Collins, 2002). Geyer et al showed that the two sub-divisions
68 have differences in transmitter binding sites and laminar density of neurons (Geyer et al. 1996).
69 In particular, they showed that BA 4a has more densely packed pyramidal cells. On the other
70 hand, BA 4p has higher laminar specific densities of different receptor and transmitter binding
71 sites.

72

73 Magnetic Resonance Imaging (MRI) functional studies have shown that in healthy volunteers
74 the blood oxygen level dependent (BOLD) signal response is modulated by attention
75 (Binkofski et al., 2002) and imagined forces (Sharma et al., 2008) in BA 4p, whilst BA 4a
76 responds to motor control (Alahmadi et al., 2016). In other words, BA 4a is predominantly
77 related to execution whereas BA 4p is predominantly related to higher-order cognitive tasks
78 (Sharma et al., 2008, Binkofski et al., 2002, Alahmadi, 2020, Alahmadi et al., 2015b, Alahmadi
79 et al., 2017, Alahmadi et al., 2016).

80

81 A recent study of healthy volunteers has revealed that the BOLD response to complex motor
82 tasks, involving different grip forces (GFs), is characterised by different MRI signal response
83 profiles, even during observation (Alahmadi et al., 2016, Casiraghi et al., 2019, Keisker et al.,
84 2009). Interestingly, the study reported a distinct behaviour of the BOLD-GF relationship
85 within the two sub-regions of BA 4 (Alahmadi et al., 2016). The BOLD-GF relationship

86 follows a distinct non-linear negative third order profile within BA 4p, while it is linear in BA
87 4a. Moreover, in healthy volunteers the BOLD signal within the two sub-regions has a distinct
88 response to motor complexity, when using the dominant or non-dominant hand while applying
89 different GFs (Alahmadi et al., 2015b). Therefore, the functional differences between BA 4a
90 and BA 4p provide an ideal case to compare complex BOLD responses going beyond the 0-
91 order (or main effect) of movement in a pathology like MS.

92

93 A question arises as to whether and if so how, these behaviours are affected by aging and by
94 diseases of the central nervous system, warranting a full characterization of the BOLD
95 behaviour in these two sub-regions, beyond a standard main effect of movement. Some
96 investigations have reported, indirectly, that there are distinct responses in BA 4p and BA 4a
97 in aging (Ward and Frackowiak, 2003) and in stroke patients (Ward et al., 2007). In particular,
98 they showed that BA 4p is affected by aging and that it is key to the functional integrity of the
99 cortical-spinal system and motor recovery in patients with stroke. Therefore, advanced analysis
100 of BA 4a and BA 4p could highlight mechanisms of functional alterations otherwise shadowed
101 in an undifferentiated analysis of BA 4.

102

103 On the bases of these considerations, the present study investigates the non-linear behaviour of
104 the BOLD response to different GFs within BA 4a and BA 4p, in healthy volunteers and in
105 people with MS, a neurological disease known to affect the motor system. MS has complex
106 disease mechanisms involving a number of pathophysiological components, including
107 demyelination, axonal loss and inflammation. Accumulation of sodium ions in tissue and a
108 redistribution of sodium channels along damaged axons alters conduction properties (Paling et
109 al., 2013, Cercignani et al., 2017), which could also be affected by alterations of tissue blood
110 perfusion (Bester et al., 2015, Rashid et al., 2004, Paling et al., 2014, Rovaris et al., 2002,

111 Lapointe et al., 2018, Rocca et al., 2007). fMRI studies in MS have shown altered patterns of
112 activations (Rocca et al., 2007, Filippi et al., 2013, White et al., 2009) and altered resting state
113 networks (Castellazzi et al., 2018), but were not designed to answer questions about complex
114 BOLD behaviour.

115

116 The hypothesis of this study is therefore that, in MS, there is (1) an altered functional response
117 in BA 4 compared to healthy volunteers during a motor fMRI task and that (2) this alteration
118 is region-specific. Given the involvement of BA 4p in higher-order motor control and its
119 modulation by attention and task complexity we also hypothesised that (3) area BA 4p may
120 show more severe abnormalities than BA 4a in the presence of MS pathology when compared
121 to healthy volunteers. If this is true, then the BOLD-GF relationship in BA 4a and BA 4p may
122 show different regional patterns of alteration compared to Healthy volunteers, offering new
123 insights in the pathology of MS.

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127 2. **Methods:**

128 2.1. Subjects:

129 14 right-handed healthy volunteers (9 female, 5 male; mean age 31 (\pm 4.64) years) and 14 right-
130 handed relapsing remitting MS (RRMS) patients (10 female, 4 male; mean age 35 (\pm 5.36)
131 years; median (range) expanded disability status score (EDSS) 3.5 (1.5-6.5)); median (range)
132 9-Hole Peg Test (9-HPT) = 20.05 (14.7-33.1) were recruited. The handedness of subjects were
133 assessed according to the Edinburgh handedness scaling questionnaire (Oldfield, 1971). All
134 subjects gave informed consent and the study was approved by the local research and ethics
135 committee.

136

137 2.2. MRI acquisition:

138 A 3.0 T MRI scanner Philips Achieva system (Philips Healthcare, Best, The Netherlands) and
139 a 32-channel head coil were used. The imaging acquisition protocol included the following:
140 T1-weighted volume (3DT1): 3D inversion-recovery prepared gradient-echo (fast field echo)
141 sequence with inversion time (TI) = 824ms, echo time (TE)/repetition time (TR) = 3.1/6.9ms,
142 flip angle = 8° and voxel size = 1mm isotropic; BOLD sensitive T2*-weighted echo planar
143 imaging (EPI): TE/TR = 35/2500ms, voxel size = 3×3×2.7 mm³, inter-slice gap of 0.3 mm,
144 SENSE factor = 2, number of slices = 46, acquired with descending order, field of view =
145 192×192mm², number of volumes = 200, number of dummy scans = 5, flip angle = 90°.

146

147 2.3. fMRI paradigm:

148 The experimental design was a *visually* guided event-related fMRI paradigm, where subjects
149 used their right (dominant) hand to squeeze a rubber ball with varying GF levels. The design
150 comprised 5 GF targets (20, 30, 40, 50 and 60% of subjects' maximum voluntary contraction
151 (MVC)) interleaved with rest intervals, each repeated randomly 15 times. This paradigm has
152 been validated previously in studies of Healthy volunteers (Alahmadi et al., 2015b, Casiraghi
153 et al., 2019, Alahmadi et al., 2017, Alahmadi et al., 2016).

154

155 Before the fMRI session, subjects were given a 6 minute training session including learning,
156 watching and performing a similar but not identical paradigm. During the fMRI session,
157 participants lay supine on the scanner bed throughout the experiment and were instructed to
158 extend both of their arms in a relaxed comfortable position. A support hand pad was provided
159 for each subject to ensure comfort and compliance.

160

161 2.4. Image pre-processing and analyses:

162 Data processing was performed using statistical parametric mapping (SPM12
163 (www.fil.ion.ucl.ac.uk/spm) implemented in Matlab14b (Mathworks, Sheborn, MA). The pre-
164 processing steps for each subject adopted the following fMRI pipeline: Slice time corrections;
165 Spatial volume realignments; (iii) Co-registration with the 3DT1 volume; (iv) Normalization
166 with the tissue probability maps of SPM12; (v) Smoothing of the functional volumes with an
167 8mm isotropic full-width half maximum (FWHM) Gaussian kernel.

168 2.5. Statistical analysis:

169 *Within-subjects (first level analysis):* Signal changes were modelled using a polynomial
170 expansion as described in (Alahmadi et al., 2016) and according to (Buchel et al., 1998, Buchel
171 et al., 1996). Briefly, for each subject, a fixed effects analysis was performed. To test efficiently
172 for linear and nonlinear effects, a parametric design was chosen. The parametric design
173 included a set of orthogonalised polynomial orders (up to the fourth order) and was specified
174 by the integral of the forces for each subject. This step creates five regressors of interest: 0th
175 order represents the main effect of gripping regardless of the applied force, 1st order represents
176 any linear changes between BOLD signals and the applied forces, and 2nd – 4th orders represent
177 nonlinear effects modelling the relationships between BOLD signals and the applied forces.
178 These covariates were then convolved with a canonical hemodynamic response function (HRF)
179 for standard SPM and general linear model (GLM) analysis (Friston et al., 1995). The
180 movement related realignment parameters were included as regressors of no interest in each
181 GLM (Friston et al., 1996). At this within-subject level, *t*-statistics were used to test for the
182 effects of the polynomial coefficients.

183 *Between-subjects (second level analysis):* Contrast images from the within-subject analysis for
184 the five polynomial orders were entered into random effects analyses, testing for non-linear

185 effects within and between groups, with the appropriate *t*-tests (i.e. one sample *t*-test for the
186 within group tests and two sample *t*-test for between group comparisons). Significant voxels
187 were defined using $P < 0.05$, corrected for multiple comparisons (FWE). Importantly, the
188 number of comparisons (voxels) performed in this study was within the sub-regions of BA 4,
189 thus the numbers of comparisons were low as compared to the whole brain.

190

191 In addition to the above whole brain SPM analysis, BA 4 was defined and subdivided according
192 to (Eickhoff et al., 2005) as guided by (Geyer et al., 1996) (figure 1). The study by Eickhoff et
193 al. 2005 introduced a new probabilistic cytoarchitectonic map of different brain regions
194 (including BA 4), which significantly improved the accuracy of anatomical labelling. The new
195 cytoarchitectonic map measured the probability of a single voxel falling within an area based
196 on 10 post-mortem brains normalized to the MNI single subject template. Only voxels (or
197 clusters) having the highest probability of falling within the MNI sub-division of BA 4 were
198 considered for each *post mortem* subject and were used to define a map of BA 4 in MNI space.
199 Since this is an *a priori* predefined region of interest (ROI), we used this anatomical ROI for
200 each subject and performed small volume correction (SVC) to increase our sensitivity when
201 looking at individual subject behaviours and parametric effects. To assess the relationship
202 between BOLD signal and GF, we plotted the average group signal estimate over voxels within
203 these predefined ROIs for either BA 4a or BA 4p. Each plot therefore reports the maximum
204 likelihood estimates of the mapping between the different applied GF and BOLD signals based
205 on the polynomial expansion (Alahmadi et al., 2016).

206

207 To better understand the effect of disability on BOLD responses to GF, we divided the MS
208 group based on their EDSS score into two sub-groups of low ($EDSS \leq 3$) and high disability
209 ($EDSS > 3$). The 9-HPT was highly correlated with the EDSS scores and thus the two groups

210 could be sub-divided similarly to low (9 HPT \leq 21.7) and high disability (9-HPT $>$ 21.7).
211 Following the sub-division of the MS group, the low EDSS group comprised 8 subjects (4
212 female, 4 male; mean age 33.8 (\pm 4.5) years; median (range) EDSS 1.5 (1.5 – 2.5); median
213 (range) 9-Hole Peg Test (9-HPT) = 19.1 (14.7-22.1)), while the high EDSS group comprised
214 the remaining 6 subjects (5 female, 1 male; mean age 36.1 (\pm 7.8) years; median (range) EDSS
215 6 (3.5 – 6.5); median (range) 9-Hole Peg Test (9-HPT) = 25 (22.3-33.1)).

216

217 In order to characterise the response profile of each sub-division in each group, we used the
218 relative contribution of each polynomial term as described in Alahmadi et al. 2015b. We
219 therefore classified the effect of GF at each voxel within BA 4a and BA 4p based on the
220 polynomial order that showed the highest standardised group effect size (i.e. the most
221 significant group difference).

222

223 3. **Results:**

224 All subjects were able to perform the task correctly (table.1).

225 In this study, we report five major findings:

226 3.1. Main effect of movement:

227 Both groups activated BA 4a and BA 4p (figure 2 and figure 3). RRMS patients showed
228 increased and greater activation extent compared to Healthy volunteers in both BA 4a and BA
229 4p sub-regions (figure 2 & figure 3) (p-value=0.001). RRMS patients also showed increased
230 activations as their EDSS increased within BA 4p only (p-value=0.001) (figure 4).

231 3.2. Average BOLD relationship with GF in BA 4a:

232 In both groups, significant relationships were detected between BOLD signal and GF. There
233 were no differences detected between MS subjects and Healthy volunteers in terms of the
234 relationship between BOLD signal and GF within BA 4a (figure-5).

235 3.3. Average BOLD relationship with GF in BA 4p:

236 In patients with low EDSS, the BOLD-GF relationship was very similar to Healthy volunteers
237 (mainly negative 3rd order), whereas at higher EDSS, the predicted BOLD versus GF deviated
238 from the Healthy volunteers pattern and the BA 4a pattern (figure 6, 3rd column).

239 3.4. GF Response profile in single subjects:

240 Interestingly, the profile of the mean BOLD signal versus GF was very similar across
241 individual subjects, when grouping subjects by disease stage. Figure 5-6 show the maximum
242 likelihood estimates of the mapping between the applied GF and BOLD signals based on the
243 polynomial expansion at the group and subject levels.

244

245 3.5. Categorizing effect sizes:

246 Performing a post-hoc analysis to identify the polynomial order showing the highest effect size,
247 we showed that in both groups (healthy and MS) the 1st order effect was predominant within
248 BA 4a. On the other hand, the predominant effect within BA 4p was different in the two groups.
249 In Healthy volunteers, a negative 3rd order effect was predominant while in MS a positive (U-
250 shaped) 2nd order effect was predominant, with BOLD signal increasing with the highest force
251 in MS, which elicited the lowest BOLD response in Healthy volunteers. See supplementary
252 material.

253

254 **4. Discussion:**

255 In this study, we demonstrate the importance of characterising high-order BOLD responses to
256 GF as opposed to simply assessing group differences in the main effect of movement (0-order)
257 between patients and healthy controls in pathologies such as MS. This characterisation was
258 performed within the sub-divisions of the primary motor region (BA 4a and BA 4p), which
259 present different order response to the intensity of movement (Alahmadi et al., 2015a,

260 Alahmadi, 2020, Alahmadi et al., 2016). The main findings are that there are distinct grip (i.e.
261 main effect) and force level effects within these two sub-regions in healthy and MS subjects.
262 We further demonstrated that the main effect of gripping and the non-linear BOLD-GF
263 relationship within BA 4p changes with disease progression.

264 A key finding is that the non-linear relationship between BOLD response and GF was
265 confirmed in both Healthy volunteers and MS groups and that in people with MS it was
266 distinctly altered only in BA 4p (figure 6). This result can be supported by the fact that the
267 neuronal and cellular structures as well as chemistry of these two sub-regions are different
268 (Strick and Preston, 1982, Geyer et al., 1996, Kaas and Collins, 2002). Thus, pathologies such
269 as MS could additionally affect these two sub-regions differentially. Focusing on BA 4a and
270 4p enabled us to characterise the BOLD signal response based on its relationship to increasing
271 GF, assessing its sensitivity to pathological changes at a sub-regional level.

272 BA 4 as a whole responded to the main effect of grip in both MS and healthy control groups.
273 This is in line with previous motor gripping studies that showed the role of BA 4 in motor
274 generation and function (Rocca et al., 2007, White et al., 2009, Kutzt-Buschbeck et al., 2008,
275 Kutzt-Buschbeck et al., 2001, Keisker et al., 2009).

276 This distinct functional segregation and parametric responses was effectively captured by this
277 study. Indeed, our findings regarding the main effect of movement show that both sub-regions
278 are activated within both groups. The responses though are different within the two sub-regions
279 as the BOLD signal was significantly increased in MS compared to the healthy group,
280 especially in BA 4p (figure 2 & 3). Previous reports of motor functional studies illustrate
281 different outcomes in the main effect of movement (within BA 4 as a whole) in MS, with the
282 BOLD signal response shown to either increase (Lee et al., 2000, White et al., 2009, Reddy et
283 al., 2000) or to have no difference compared to Healthy volunteers (White et al., 2009, Mancini

284 et al., 2009). Unfortunately, most of these studies used automated anatomical labelling that
285 relies on a template (e.g. the Talairach) or manual eye assessment labelling, both of which are
286 highly prone to inaccuracy and difficult to generalize. The other limiting factor is that most of
287 these studies were not anatomically specific for BA 4a or BA 4p (e.g. both M1 and S1 (primary
288 sensory area) were labelled as “sensorimotor cortex” or SMC). This makes comparisons
289 between our results and earlier studies difficult. The inconsistency among earlier studies can
290 be attributed to factors such as differences in paradigms, number of subjects, threshold values,
291 and MS subtypes. The last factor, i.e. MS subtype, may be very significant in affecting outcome
292 as functional activations have been shown to be altered during disease progression (Rocca et
293 al., 2005) (Rocca et al., 2005). More importantly, it has been suggested that MS patients during
294 the early stage of the disease tend to have normal patterns of activation (Pantano et al., 2015).

295 This study goes beyond the main effect of movement and shows that the non-linear relationship
296 BOLD-GFs within BA 4 is complex and region-specific. In previous studies of Healthy
297 volunteers we have demonstrated the complexity of BOLD signals as a function of GF in
298 different motor, sub-motor, associative and cerebellar areas (Alahmadi et al., 2015b, Alahmadi
299 et al., 2017, Alahmadi et al., 2016, Casiraghi et al., 2019). These were seen as well in action
300 observation and execution networks (Casiraghi et al., 2019). In the present study, we show that
301 pathology affects the BOLD-GF relationship differently in BA 4a and BA 4p and that the
302 altered behaviour was indicative of high EDSS. What was particularly striking was the
303 consistency of the profile between individual subjects, reflecting the group level findings
304 (figures 5 & 6).

305

306 The observation that the BOLD response to different GFs within BA 4p was similar to that of
307 Healthy volunteers in patients with low EDSS, while it was consistently altered at higher EDSS

308 poses interesting mechanistic questions, suggesting that differences not only in
309 cytoarchitecture but also in chemoarchitecture and myeloarchitecture of these two sub-regions
310 may translate into differences in their susceptibility to MS pathology. For example, these
311 architecture properties as well as differences in the distribution of neural cells densities within
312 the two sub-regions (Geyer et al., 1996) could be altered or be the cause of alterations in
313 myelination, axonal loss, vascular or neuronal activity in MS. One could speculate that given
314 the rich density of neurotransmitters in BA 4p compared to BA 4a (Geyer et al., 1996), our
315 observations could reflect an impaired neuronal response. With the present data, though, it is
316 not possible to link the present functional findings to tissue microstructure alterations, nor to
317 infer a causal relationship between an impaired functional response to a complex task and blood
318 perfusion, microvascular response, or even sodium channels malfunction, all of which are
319 known to be regionally affected and potentially responsible for our observations. Furthermore,
320 these differences could be due to differences in their structural connectivity to other brain
321 regions that could potentially drive this different behaviour. Thus, our findings underline the
322 need for multi-modal and longitudinal studies that could include other quantitative techniques
323 such as diffusion weighted imaging (DWI) (to assess tissue microstructure and connectivity),
324 MR spectroscopy (to assess metabolic changes), sodium imaging (to assess effects of sodium
325 ions tissue distribution essential for neurotransmission), grey matter sensitive sequences (to
326 assess grey matter atrophy and lesions) and perfusion imaging to pin down mechanistic
327 hypothesis and deliver sensitive and specific *in vivo* imaging biomarkers of the functional
328 substrate of MS alterations; we believe that it is really important to go beyond reporting the
329 main effect of movement, i.e a change in BOLD signal amplitude.

330

331 The fact that these behaviours reflected EDSS association is also of interest. Previous studies
332 have suggested that changes in functional activations in MS are possibly related to

333 compensatory mechanisms (Staffen et al., 2002, Audoin et al., 2003, Lenzi et al., 2007,
334 Mainero et al., 2004b), which could also be advocated to explain the higher activations
335 observed in the main effect of gripping in MS compared to healthy volunteers. It should be
336 noted, however, that previous studies showed that BA 4p is involved in executive motor
337 function tasks compared to BA 4a. The predominant reported factors for BA 4p involvement
338 were attention (Binkofski et al., 2002), complexity (Alahmadi et al., 2016) and imagination
339 (Sharma et al., 2008). In the current study, these factors, especially attention and complexity,
340 are all invoked by task execution. The use of an increased GF increases the complexity of
341 performance and the use of visual feedback to reach (as quickly as possible) and maintain (as
342 precisely as possible) forces at a specific level (especially low and high GFs) requires increased
343 attention. Previous reports showing that in MS there are functional alterations with task
344 complexity (Filippi et al., 2002) and attention (Mainero et al., 2004a), may support our finding
345 of increased functional changes in BA 4p. A positive correlation of the BOLD effect with
346 EDSS, though, indicates that this increased activity may either be a failed compensatory
347 mechanism or may not be compensatory after all, but rather maladaptive. Advanced network
348 modeling of the functional signal behavior may assist in understanding the source of these
349 alterations (Friston et al., 2003). Dynamic causal modeling (DCM) would be a possible analysis
350 to perform to help understand the precise nature of the non-linearity in the BOLD response at
351 the neuronal or hemodynamic level. Given that the most interesting hemodynamic
352 nonlinearities emerge over a timescale of seconds, due to hemodynamic saturation effects
353 (Friston et al., 2003, Friston et al., 1996, Friston et al., 1998, Friston et al., 2000), a revised
354 paradigm that includes variations in the GF duration as well as strength would be desirable for
355 future experiments.

356

357

358 *Limitations and methodological considerations*

359 In this study, there are some methodological considerations to disclose. The number of subjects
360 could be considered to be relatively low. This is especially relevant when sub-dividing the MS
361 subjects into two different groups. However, the subdivision of patients into two groups is
362 exploratory and the study was not powered for it. Given the striking results, though, it is
363 important to report these findings, which could drive future larger studies. Another limitation
364 of this study is the inability to investigate lesions within the targeted ROIs (i.e. BA 4). Grey
365 matter lesions in MS have been reported using MRI (Sethi et al., 2012, Geurts et al., 2011).
366 However, grey matter lesion sequences were not planned here, although the authors advocate
367 the need to include a grey matter lesion sequence in future functional MRI studies of MS.

368

369

370 **5. Conclusion:**

371 BA 4 has two sub-divisions (BA 4p and BA 4a) that are anatomically and functionally distinct;
372 therefore, BA 4 lends itself very well to the investigation of sub-regional differences in
373 functional response to complex motor tasks in healthy and MS subjects. Here, we demonstrated
374 region-specific alternations in the BOLD response to movement, showing that we should
375 investigate beyond the main effect to unveil altered non-linear coupling between the BOLD
376 signals and GFs, especially within BA 4p. The information obtained from studying the BOLD-
377 GF relationship could reflect not only disease activity, but also the degree of morbidity.
378 Furthermore, the alteration of the BOLD-GF profile in high EDSS patients compared to
379 Healthy volunteers is a very interesting finding that opens an entire new set of avenues to study
380 the mechanisms of neurological diseases *in vivo*. For example, the consistent alteration of the

381 BOLD-GF profile in BA 4p in the advanced stage of MS could be explained by many factors:
382 perhaps demyelination of BA 4p itself, which could make it unable to support an efficient
383 functional response? Or perhaps white matter fibers subtending BA 4p have redistributed
384 sodium channels that – instead of supporting functionality – impair efficient
385 neurotransmission? Or perhaps in MS the microvascular response is impaired with a regional
386 specificity that makes BA 4p responding differently than BA 4a? These questions highlight the
387 need for multi-modal cross-sectional and longitudinal studies that aim at disentangling the
388 contribution to functional alterations of many factors, in order to pin down sensitive and
389 specific *in vivo* imaging biomarkers.

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407 **7. References:**

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Table 1 Grip force task performance, showing the average (\pm SD) MVC (%) and duration (s) of squeeze for healthy and MS subjects.

	20%	30%	40%	50%	60%
A. Healthy volunteers					
MVC %	21.23 \pm 2.28	30.23 \pm 1.68	39.26 \pm 2.29	51.23 \pm 1.28	59.11 \pm 1.32
Duration (s)	2.89 \pm 0.34	3.19 \pm 0.33	3.11 \pm 0.14	2.99 \pm 0.51	3.13 \pm 0.22
B. MS					
MVC %	22.32 \pm 1.89	31.32 \pm 1.34	40.17 \pm 2.15	51.32 \pm 1.49	60.39 \pm 1.29
Duration (s)	3.04 \pm 0.75	2.97 \pm 0.95	3.25 \pm 0.093	3.08 \pm 0.693	3.32 \pm 1.12

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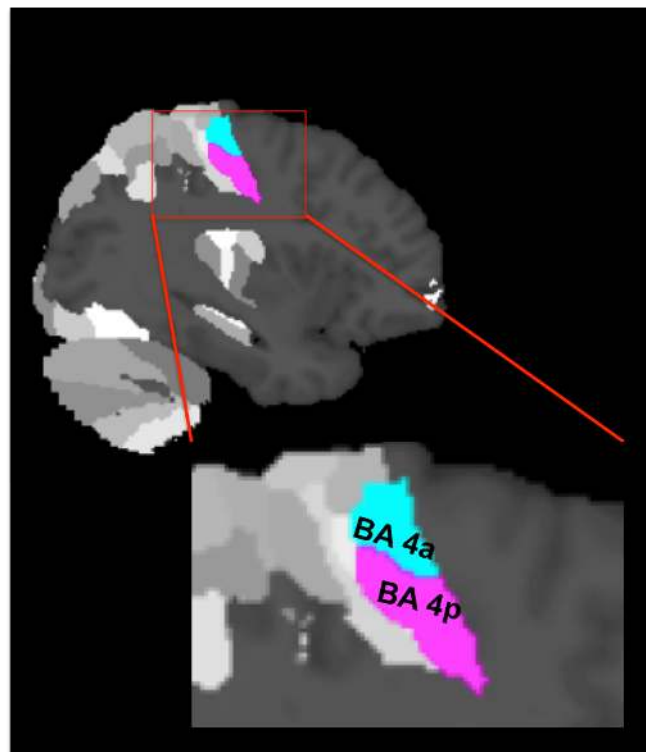


Figure 1: The cytoarchitectonic assignments of BA 4a and BA 4p projected onto the maximum probability map of the brain as provided by the SPM anatomy toolbox.

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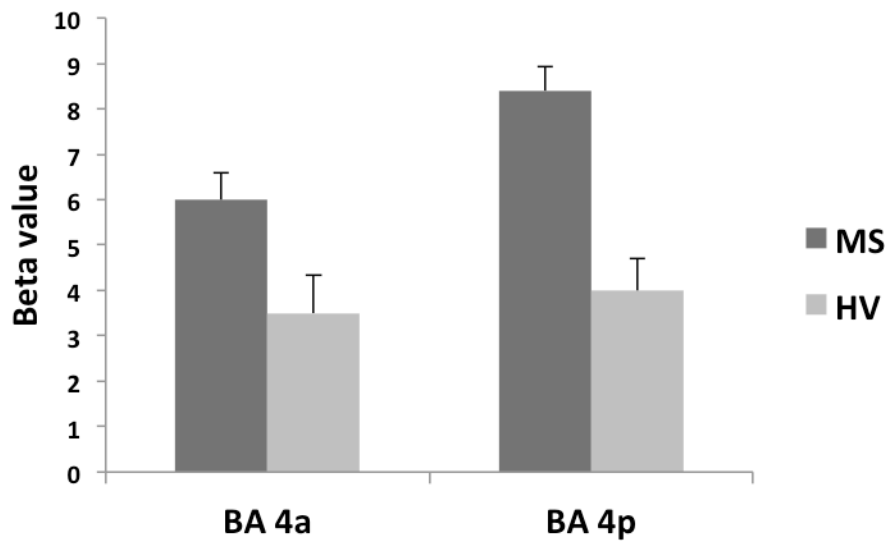


Figure 2: Mean of the beta values and their standard errors calculated at group level for the main effect of gripping for both groups and sub-regions. There are significantly higher betas in the MS compared to the Healthy volunteers within the sub-regions.

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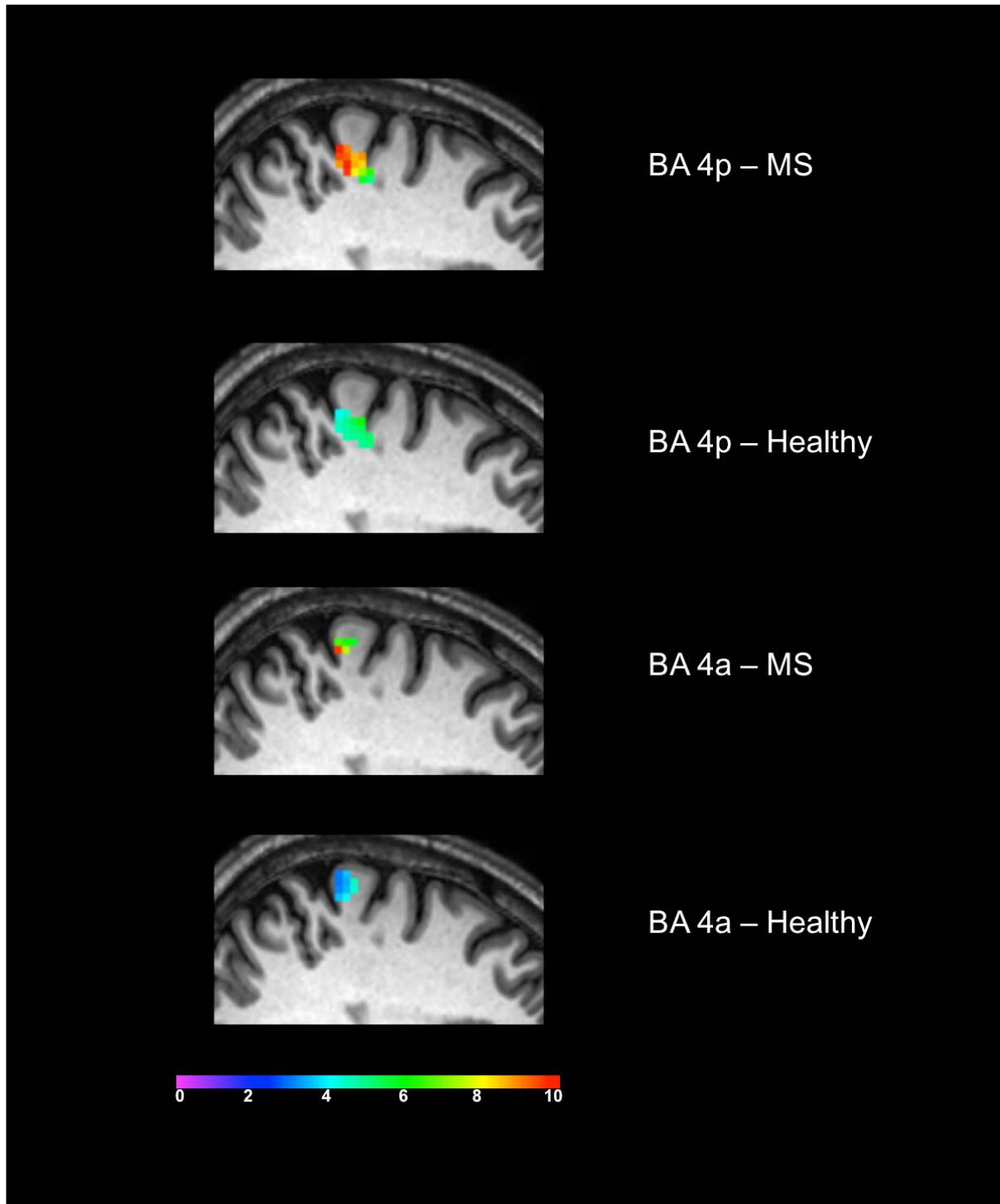


Figure 3: Significant activations of the main effect of gripping within BA 4p and BA 4a in both MS and Healthy volunteers. Colours represents the T-value of the effects at a 0.05 FWE.

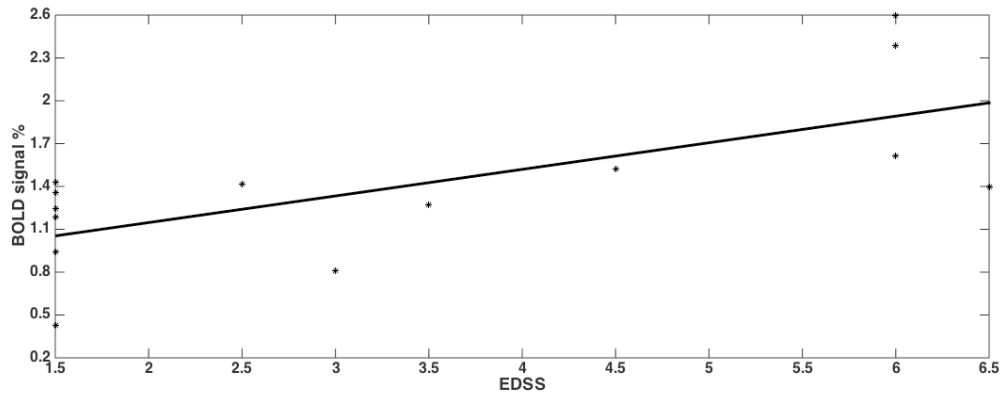
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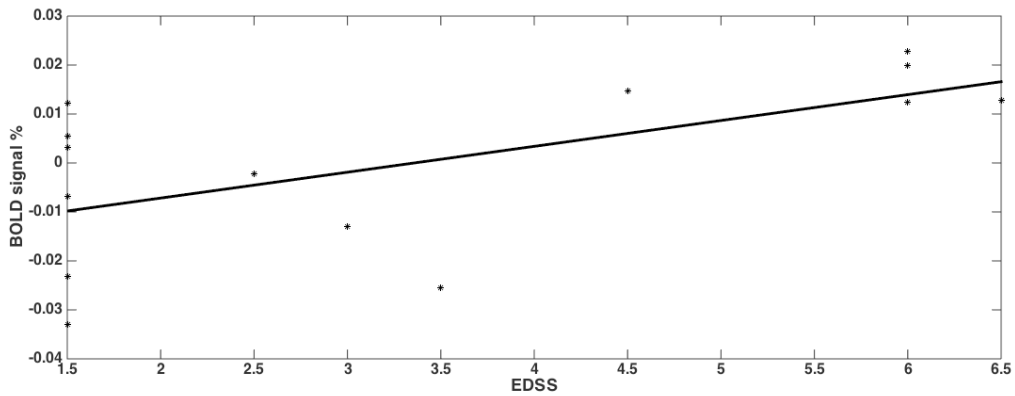
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Figure 4: RRMS patients showed increased activations as their EDSS increased within BA 4p (p-value=0.001;r=0.68) (top plot) and within BA 4a (p-value=0.001;r=0.61) (bottom plot) in the main effect of gripping (i.e. 0th order).

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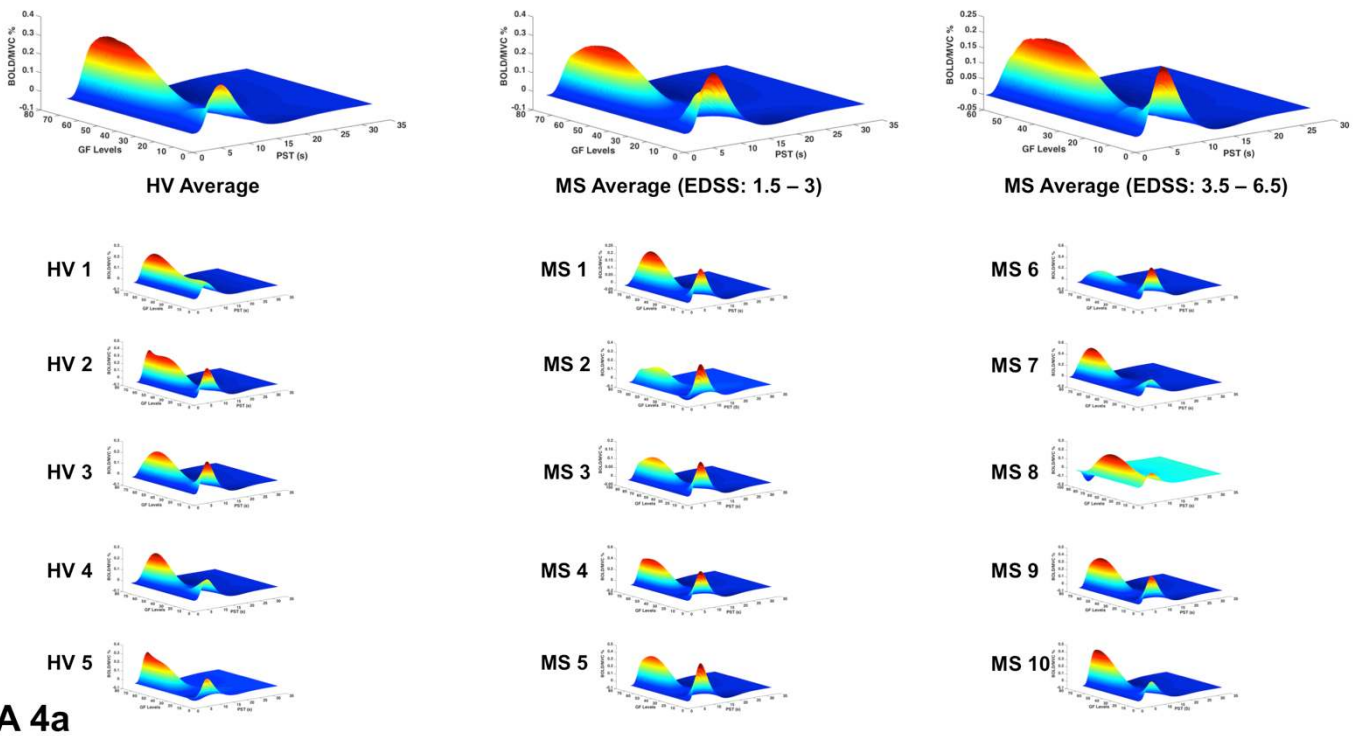
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BA 4a

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Figure 5: BOLD responses (Z-axis) of the fitted polynomial-orders of GF (Y-axis) at the defined post-stimulus time (PST) (X-axis) within BA 4a for Healthy volunteers (HV 1-5), MS patients with low (MS 1-5) and high EDSS (MS 6-10)—representing an estimate of the mapping between GF and BOLD based on all components of the polynomial expansion. The top row shows the average group effect while underneath examples of individual subjects are plotted.

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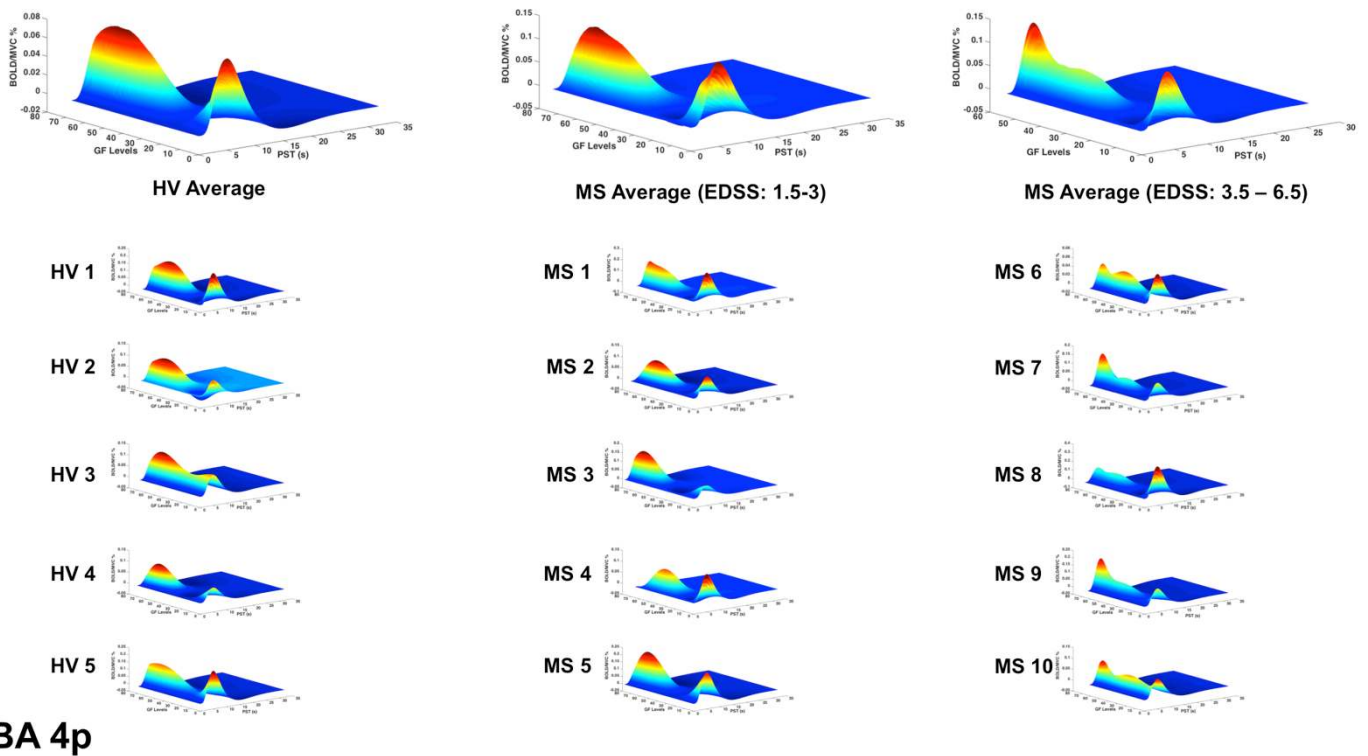
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Figure 6: BOLD responses (Z-axis) of the fitted polynomial-orders of GF (Y-axis) at the defined post-stimulus time (PST) (X-axis) within BA 4p for Healthy volunteers (HV 1-5), MS patients with low (MS 1-5) and high EDSS (MS 6-10)—representing an estimate of the mapping between GF and BOLD based on all components of the polynomial expansion. The top row shows the average group effect while underneath examples of individual subjects are plotted.

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