Relationships between neuropsychological and antisaccade measures in multiple sclerosis patients

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Background The Stroop test is frequently used to assess deficits in inhibitory control in people with multiple sclerosis (MS). This test has limitations and antisaccade eye movements, that also measure inhibitory control, may be an alternative to Stroop. **Objectives** The aim of this study was twofold: i) to investigate if the performance in and antisaccade task is altered in patients with multiple sclerosis and ii) to investigate the correlation between performances in neuropsychological tests the Stroop test and the antisaccade task. **Methods** We measured antisaccades parameters with an infrared eye tracker (SMIRED 250Hz) using a standard antisaccades paradigm. Thirty-eight subjects diagnosed with MS and 38 age and gender matched controls participated in this study. Neuropsychological measures were obtained from the MS group. **Results** Patients with MS have higher error rates and prolonged latency than controls in the antisaccade task. There was a consistent association between the Stroop performance and antisaccades latency. Stroop performance but not antisaccades latency was associated with other neuropsychological measures in which the MS group showed deficits. **Conclusions** Our findings suggest that antisaccades may be a selective and independent measure to investigate inhibitory control in patients with MS. More studies are necessary to confirm our results and to describe brain correlates associated with impaired performance in the antisaccade task in people diagnosed with MS.

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46   from the MS group.
47   **Results**
48   Patients with MS have higher error rates and prolonged latency than controls in the antisaccade
49   task. There was a consistent association between the Stroop performance and antisaccades
50   latency. Stroop performance but not antisaccades latency was associated with other
51   neuropsychological measures in which the MS group showed deficits.
52   **Conclusions**
53   Our findings suggest that antisaccades may be a selective and independent measure to
54   investigate inhibitory control in patients with MS. More studies are necessary to confirm our
55   results and to describe brain correlates associated with impaired performance in the
56   antisaccade task in people diagnosed with MS.

57

INTRODUCTION

Multiple Sclerosis (MS) is a neurodegenerative disease producing inflammation of the central nervous system characterized by loss of oligodendrocytes and axonal degeneration (Keegan & Noseworthy 2002). Cognitive deficit is often the first indicator of the disease progression and can involve a variety of functions such as processing speed, long-term memory, attention and executive functions (Chiaravalloti & DeLuca 2008; Patti et al. 2009; Ruggieri et al. 2003). Executive functions involve, amongst others cognitive processes, inhibitory control. Inhibitory control can be defined as the ability to suppress prepotent but incorrect responses and the ability to filter out irrelevant information within a stimulus set (Botvinick et al. 2001). Prepotent response inhibition summons processes that regulate the selection of a weaker, but task-relevant response, over a competing strong, but task-irrelevant, response (Miller & Cohen 2001). Antisaccades (AS) performance obtained with an AS task is considered a good indicator of inhibitory control in humans. For example, performance on this task is correlated with neuropsychological tests of executive functions in multiple neurodegenerative diseases (Heuer et al. 2013; Hutton 2008).

The AS task requires suppression of an automatic saccade towards a sudden-onset peripheral visual target (defined as prosaccade) and the generation of a volitional (endogenous) saccade towards the mirrored target location (antisaccade) (Hutton & Ettinger 2006; Munoz & Everling 2004; Olk & Kingstone 2003; Talanow et al. 2016). In humans the integrity of the dorsolateral prefrontal cortex (DLPFC) and anterior cingulate gyrus (ACC) seems to be critical for good AS performance (Pierrot-Deseilligny et al. 2003). Animal studies have shown that neural activity in correct AS (looking away from the target) in regions such as the prefrontal and anterior cingulate cortices is higher than when a wrong decision (prosaccade, or looking at the target) is made (Amador et al. 2004; Johnston & Everling 2011).

Programming antisaccades requires inhibitory and generation phases involving activation of the ventrolateral and dorsolateral cortices (Asaad et al. 2000; Miller & Cohen 2001).These areas are also involved in, for example, decision making (Ettinger et al. 2008). A detailed description of the process can be found in the referred literature. In brief, the structures involved are thought to be mediating processes of vector transformation - for AS the locations of the stimulus and the saccadic goal are not the same and, in the transition from stimulus encoding to the initiation of the response, the stimulus vector must be inverted 180° into the movement vector (Moon et al. 2007; Talanow et al. 2016).

There are different opinions concerning the cognitive mechanisms underlying AS performance, a good summary can be found in a publication by Talanow (Talanow et al. 2016).

92   In his publication Talanow describes a theoretical model that is currently accepted to explain AS

93   performance in terms of parallel programming, often linked to a competition model or the horse-

94   race metaphor (Hutton 2008; Hutton & Ettinger 2006; Kristjansson 2007; Munoz & Everling

95   2004; Talanow et al. 2016). According to this model prosaccade and AS signs are generated

96   simultaneously in response to a visual stimulus. Both signs compete until one of them wins the

97   competition. Good AS performance thus requires efficient cognitive processes that are sensitive

98   to changes in brain structure occurring in neurodegenerative diseases or with age (Heuer et al.

99   2013; Lemos et al. 2016; Pa et al. 2014).

100   Some studies looked at cross-sectional (Fielding et al. 2009) and longitudinal alterations

101   (Fielding et al. 2012) in AS performance in people diagnosed with MS. These studies found that

102   in people with MS the number of errors and latency are associated with general cognitive

103   functioning. Others observed that AS performance was associated with structural and

104   microstructural changes in the cerebellum (Kolbe et al. 2014). However, given that performance

105   in AS tasks rely strongly in inhibitory control we hypothesise that performance in this task may

106   be associated with neuropsychological tests such as the Stroop test - that relies in a similar type

107   of cognitive control.

108   Like the AS task, the Stroop task requires overriding of the prepotent response or

109   prepotent response inhibition. In a Stroop colour-naming task participants have to name the ink

110   colour of a printed colour word. Greater conflict occurs for incongruent (e.g., the word **red** in

111   **green** ink) than for congruent (e.g., the word **red** in **red** ink) trials – this is the Stroop

112   interference effect (Vanderhasselt et al. 2009). Brain correlates indicate that the cognitive

113   control of this task involves cortico-subcortical circuit and that includes the ACC and the DLPFC

114   (Carter & van Veen 2007; MacDonald et al. 2000). A response conflict is generated when there

115   is a coactivation of incompatible stimulus-response processes, the conflicting signal is used to

116   recruit cognitive control in order to reduce the conflict and improve performance (Carter & van

117   Veen 2007; Johnston & Everling 2011). According to the prominent conflict-monitoring

118   hypothesis the occurrence of response conflict is signalled by the ACC leading to recruitment in

119   the DLPFC of more cognitive control for subsequent performance (Botvinick et al. 2001;

120   Vanderhasselt et al. 2009).

121   The current neurophysiological tests such as the Stroop test may have limitations to

122   assess inhibitory control. For example, Stroop interference performance can be related with

123   reading ability (Protopapas et al. 2007). Also, some studies reported effects of intelligence and

124   education in this test (Homack & Riccio 2004) and others failed to show differences in

125   performance between clinical groups and controls even if differences were supposed to occur

126   (Schwartz & Verhaeghen 2008). Another known factor that can interfere with the Stroop test

127   performance are colour vision defects that occur, for example, with normal aging or as a result

128   of diseases such as MS (Ben-David & Schneider 2010; Martinez-Lapiscina et al. 2014). These

129   facts inspired us to investigate if the AS task can be a reliable test that overcomes some of the

130   known limitations of the Stroop test but still provides a fine assessment of inhibitory control in

131   people with MS (Heuer et al. 2013; Sisco et al. 2016).

132   The aim of this study was to investigate antisaccades performance in patients with MS

133   and the association between AS and Stroop performances. We formulate two hypotheses: i) the

134   AS task is sensitive to capture subclinical functional impairment in inhibitory control in people

135   with MS and ii) AS performance is correlated with the performance in the Stroop task.

136   # METHODS

137   ## Participants

138   Thirty-eight patients diagnosed with MS were recruited by one of the authors (JJC) at Hospital

139   de Braga. An equal number of controls, age and gender matched, was recruited. The inclusion

140   criteria for the MS group were: age between 18 and 45 years old, relapsing remitting course,

141   early stages of disease severity as measured by the Expanded Disability Status Scale (EDSS)

142   ≤3 (Kurtzke 1983), and normal or corrected to normal visual acuity. Exclusion criteria were: on-

143   going relapse/relapse in the previous month, presence of clinically diagnosed cognitive

144   impairment, history of traumatic brain injury and/or stroke, depression (self-reported or detected

145   during the study using the Beck Depression Inventory (Beck et al. 1988)). Those with clinically

146   visible oculomotor abnormalities were not recruited (e.g., nystagmus or internuclear

147   ophthalmoplegia, see references (Frohman et al. 2005; Serra et al. 2018 (Table 1); Serra et al.

148   2003) for comprehensive lists. Controls were subject to equivalent exclusion criteria. The ethics

149   committee at University of Minho granted ethical approval to carry out the study, ref: SECVS

150   083-2013, and all participants were informed about the aim and procedures involved before

151   signing the informed consent. For a summary of demographic characteristics see Table 1 and

152   for neuropsychological results see Table 2.

153   ## Equipment and procedures

154   Eye movements were monitored using a binocular eyetracker running at 250Hz (RED250,

155   SMIGmb, Teltow, Germany). The eyetracker has a spatial resolution <0.4°, is controlled with

156   iView X software (v2.8, August 2011, SMIGmb, Teltow, Germany) and stimuli were presented

157   on a 22inch (1680x1050px) LCD monitor running at 60Hz (Dell P2210). The system used was

158   formed of two computers connected by a high-speed Ethernet. One computer controls the

159   eyetracker and other controls the stimulus presentation. A Matlab software development kit

160   provided by SMI and elements of the Psychophysics toolbox were used for running the

161   experiment (Pelli 1997). Code for data analysis was also written in Matlab v2010b (The

162   MathWorks, Inc., Natick, MA, USA).

163   Experiments were performed in a dimly lit room (~10 lux) and stimulus Michelson contrast

164   was 90%. One block of 8 practice trials were performed before data collection to make

165   participants familiar with calibration and with the task, a second block of practice was given

166   when participants did predominantly errors in the practice trials or expressed difficulties to

167   understand their task. A 5-point calibration procedure was applied, only participants with

168   calibration accuracy (mean deviation for the expected position) of 1degree or less in both x-axis

169   and y-axis were considered. Participants were seated 70cm from the monitor with their head

170   restrained with a headband attached to the seat.

171   The paradigm shown in Figure 1 consisted of 2 steps. Step 1) subjects had to fixate a

172   centrally located target (cross) during a variable interval of 1250 or 1600 msec, intervals were

173   assigned in a random order. The fixation target for step 1 was only visible after fixating a gaze-

174   contingent box for 150 msec. Step 2) After the interval defined for step 1, a peripheral target

175   was presented at 5 or 10 degrees of visual angle at right or left side of the fixation target, all

176   locations were assigned in random order. Before data collection and during practice trials

177   subjects were clearly instructed to look to the empty side of the screen to a position equivalent

178   to where the target was visible (AS). They were also instructed to perform this movement as

179   quickly as possible. Each subject performed 40 trials and the duration of the task was

180   approximately 3 minutes.

181   =============== **Figure 1** ==============

182

183   **Neuropsychological tests**

184   A battery of tests was chosen to characterize the cognitive status of our participants with

185   MS. The neuropsychological tests used were: 1) Beck Depression Inventory (BDI)(Beck et al.

186   1988; Serra & Abreu 1973); 2) Montreal Cognitive Assessment (MOCA) (Freitas et al. 2010;

187   Nasreddine et al. 2005); 3) Digit Span (WAIS-III: Wechsler Adult Intelligence Scale - Version III)

188   and 4) Trail Making Test, Part A (Bucks 2013; Cavaco et al. 2013; Seabra-Santos et al. 2003;

189   Tombaugh 2004; Wechsler 1997); 5) Symbol Digit Modalities Test (SDMT) (da Costa Pinto

190 2004; Smith 1968); 6) Controlled Oral Word Association Test (COWAT) for phonetic fluency
191 (Cavaco et al. 2013; Heaton et al. 2004; Tombaugh et al. 1999); 7) Twenty Questions Test from
192 Delis-Kaplan Executive Function System (DKEFS 20Q)(Delis et al. 2001; Stephens 2014); 8)
193 Stroop Colour-Word test. All neuropsychological tests have normative data for the Portuguese
194 population (tests have been translated into the Portuguese language and administered to native
195 Portuguese speakers), results from the MS group are compared with the normative values in
196 Table 2. These tests were not performed by controls and were performed by MS participants
197 after the AS task.

198   The final score of the Stroop colour-word test corresponds to the number of correctly
199 reported colours in 45 seconds, the maximum possible score is 100 (Castro et al. 2003; Golden
200 1975). We scored the Stroop test using different methods that have been described by others
201 (Denney & Lynch 2009; Sisco et al. 2016) and formulas are specified in Table 3. We used
202 different formulas to cover different scenarios because there are reports in the literature
203 showing that two different formulas applied to the same data can lead to opposite conclusions
204 (Denney & Lynch 2009; Sisco et al. 2016).

Data analysis

206   Eye movements were collected binocularly with the point-of-regard (POR) information
207 being: $(POR_{right}+POR_{left})/2$, that is the mean POR of both eyes. AS latency is shown in Figure 1-
208 B and represents the period of time from target onset until the reaction of the eyes moving
209 towards the mirrored target location. For analysed trials, AS detection was performed after
210 smoothing xy positions with a 5-sample moving average. This method has been proposed and
211 used to reduce noise (Engbert & Kliegl 2003; Ferreira et al. 2017). After smoothing, eye velocity
212 and acceleration were computed, saccades were detected using the velocity threshold of
213 30deg/sec and/or acceleration threshold of $8500deg/sec^2$ with a minimum duration of 12msec
214 (Macedo et al. 2008; Macedo et al. 2011), microsaccades were ignored (Martinez-Conde et al.
215 2004).

216   In addition to the number of errors and peak-velocity, 3 latencies were computed: 1)
217 antisaccades, 2) prosaccades and 3) corrective saccades. When the first saccade is made
218 towards the target the movement is defined as a prosaccade and corresponds to an error.
219 When after a prosaccade participants made a saccade away from the target this is defined as
220 corrective saccade, the interval between the prosaccade and the corrective saccade is defined

221   as corrective saccade latency. Corrective saccades were considered when its amplitude was 2

222   degrees or more and performed away from the target.

223   **Statistical analysis**

224   The normality of the variables was tested using the Kolmogorov-Smirnov test. Differences

225   between neuropsychological results and normative values for the Portuguese population were

226   verified with t-test, the difference in number of errors between groups was evaluated with t-test

227   as well.

228   The effect of group, direction and eccentricity on AS parameters was tested with linear

229   mixed models in SPSS (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM

230   Corp) and R (lme4 package) (Bates et al. 2015; Ferreira et al. 2017; Kliegl et al. 2010). For this

231   analysis eye movements parameters reported were defined as "dependent variable" or

232   "response variable of interest" (e.g. latency). Participants were defined as "random factors" or

233   "group specific effects". Explanatory factors or "fixed factors" were: "group" (MS and Control),

234   "direction" (right and left), "eccentricity" (5 and 10 degrees) and "saccade type" (antisaccade,

235   prosaccade and corrective). Bonferroni correction was applied for multiple comparisons. Means

236   described in the text and shown in graphs are the estimated marginal means (EMM = mean

237   response for each variable, adjusted for any other variables in the model) and their standard

238   errors (SE) for the specified factors. Correlations between neuropsychological tests and AS

239   parameters were also tested.

240   **RESULTS**

241   Demographic characteristics of the 38 participants for each group are summarized in

242   Table 1. Table 2 summarizes the comparisons between the normative values for the

243   neuropsychological testes and the results for our participants. Table 3 summarizes the Stroop

244   test results scored in accordance with different formulas.

245   **=============== Table 1 ==============**

246

247   **=============== Table 2 ==============**

248

249   **=============== Table 3 ==============**

250   **AS errors**

251   There was no effect of eccentricity in any of the AS results analysed and therefore results

252   for 5 and 10 degrees are reported together. The mean proportion of directional errors was 26%

253   (SD=18) for the MS group and 16% (SD=11) for the control group. The mean difference of 10%

254   in the number of errors between the MS group and the control group was statistically significant,

255   t(60)=2.8 (p=0.007, unequal variances considered).

256   **AS latency**

257   Latency results are shown in Figure 2. A model (LMM) with 4 fixed factors was run (group:

258   patients or controls; saccade type: antisaccades, prosaccades or corrective; eccentricity: five or

259   ten degrees; direction of the eye movement: left or right) to compare latencies. Only statistically

260   significant effects are reported. There was a main effect of group $F_{(1, 88)}$=6.4 (p=0.013) and an

261   interaction group x saccade type $F_{(2,2382)}$=24 (p<0.001). There was also a main effect of

262   saccade type $F_{(2,2382)}$ = 1298 (p<0.001) and trial direction F=(1, 2367)= 25 (p<0.001). The

263   directional effect has been reported before and is likely to be explained by the higher frequency

264   of right-eye dominance (Vergilino-Perez et al. 2012).

265   AS latency was higher in the MS group than in controls, the estimated marginal mean

266   (EMM) in the MS group was 325 msec (SE=6.0) and 280 msec (SE=6.0) in the control group.

267   The difference between means was 45 msec, t(2362)=5.1 (p<0.001). The latency of

268   prosaccades was different from the latency of corrective saccades and both were different from

269   the latency of antisaccades. The mean latency for prosaccades was 209 msec (SE=5.0), for

270   corrective saccades was 161 msec (SE=5.0), the mean difference between means was 47

271   msec, t(2362) = -8.4 (p<0.001). The mean difference between eye movements performed to the

272   right (target presented at left) and to the left (target presented at right) was 10 msec, t(2362) = -

273   5.3 (p<0.001). Differences in latency for prosaccades and corrective saccades between groups

274   were not statistically significant, more details are given in supplemental files.

275   =============== Figure 2 ==============

276

277   **AS peak-velocity**

278   A model similar to that reported for latency was used to determine differences in peak-

279   velocity. Here we found two main effects: saccade type $F_{(2,2377)}$=98 (p<0.001) and trial

280   direction $F_{(1,2366)}$ = 24 (p<0.001). The mean difference in peak-velocity between right (EMM =

281   288 deg/sec, SE=7.0) and left (EMM = 275 deg/sec, SE=7.0) AS was 13 deg/sec, t(2362)=-4.84

282   (p<0.001). Corrective saccades (EMM= 321 deg/sec, SE=7.8) showed higher peak-velocities

283   than prosaccades (EMM = 260 deg/sec, SE= 7.9) and antisaccades (EMM= 263 deg/sec,

284   SE=6.9). The mean difference between prosaccades and corrective saccades was 60 deg/sec,

285   t(2362)=-11.51 (p<0.001) and between corrective and AS was 58 deg/sec, t(2362)= 13.81

286 (p<0.001). Differences in peak-velocity, for all types of saccades, between groups were not

287 statistically significant. More details are given in supplemental files.

288 **Correlations between neuropsychological tests and antisaccades**

289 Correlations between Stroop test scores and neuropsychological tests scores are

290 summarized in Table 4. The neuropsychological tests are categorized for the main cognitive

291 function assessed.

292

293 ==================== Table 4 =====================
294

295 Correlations between Stroop test and latency of AS, prosaccades and corrective

296 saccades, are summarized in Table 5. The correlations between scores of all other

297 neuropsychological tests, AS errors and latency were not statistically significant.

298 ==================== Table 5 =====================

299 # DISCUSSION

300 Deficits in inhibitory control, that is the ability to suppress prepotent but incorrect

301 responses, are expected in people diagnosed with MS. The Stroop interference task and the

302 antisaccade task both require good inhibitory control and; therefore, the performance of patients

303 with MS in these tasks is likely to be impaired (Carter & van Veen 2007; Johnston & Everling

304 2011). In this study we hypothesize that the antisaccade task can be a quantitative surrogate

305 marker of neural damage leading to impairment in inhibitory control in people with MS. In the

306 present study we investigated whether Stroop and AS performances are altered in people with

307 MS and if both performances are correlated. In line with our hypotheses AS and Stroop

308 performances were impaired and correlated. These findings are in agreement with other studies

309 and are particularly relevant because the disease in our group of patients was at early stages –

310 median EDSS 1.5 in a 0-10 scale, see Table 1 (Kolbe et al. 2014; Ting et al. 2015). The AS task

311 remains a research tool because measuring and analysing eye movements requires specialized

312 equipment and training that still note available to use in clinics. Also, the extraction of results is

313 time-consuming and data recording protocols need standardization (Antoniades et al. 2013).

314 Although, we must acknowledge that we found the results reported in the literature, from

315 different laboratories and acquired with different equipment, remarkably consistent. For a good

316 review of the clinical utility of AS task and other eye movements metrics in MS we recommend a

317 recent review by Serra (Serra et al. 2018).

318 Our results show a consistent association between the interference Stroop test

319 performance and AS latency. Of note, Stroop performance - but not AS latency - was

320   associated with other cognitive measures such as processing speed (measured with the SDMT)

321   and verbal fluency/executive function (measure with COWAT) (Whiteside et al. 2016). These

322   findings suggest that the Stroop performance can be influenced by unspecific cognitive deficits

323   (Denney & Lynch 2009). In contrast, AS performance in our study was not correlated with

324   cognitive, visual and socio-demographic aspects. Nevertheless, the AS task is likely to be

325   affected by reduced visual acuity, poor comprehension of the instructions or slow cognitive

326   response. To our knowledge, our study is the first to show the association between Stroop and

327   AS tasks in patients with MS. Although, there is a recent study by Ting showing a equivalent

328   association in patients with mild traumatic brain injury and concussion (Ting et al. 2015).

329   Previous studies also pointed that different methods to compute Stroop scores would lead to

330   different conclusions about the cognitive status of patients with neurological diseases (Sisco et

331   al. 2016). To cover this aspect, our comparison included Stroop interference scores computed

332   using different formulas. Notwithstanding the approach tested, the association between Stroop

333   interference scores and AS latency was consistent, further suggesting its biological significance.

334   Below we discuss in detail the consistency and the implications of our AS findings.

335   AS performance was worse in patients with MS than in controls. These results are in line

336   with other studies investigating AS in MS (Fielding et al. 2012; Fielding et al. 2009; Kolbe et al.

337   2014) and other neurological diseases (Crawford et al. 2005; Peltsch et al. 2008; Ting et al.

338   2015). We noticed that the number of directional errors in our control group was higher than the

339   values reported by Fielding that reported only about 10% (Fielding et al. 2009). However,

340   according to Hutton, healthy individuals can have a proportion of directional errors up to 20% in

341   AS tasks (Hutton 2008). In our study, the latency values in both groups are in good agreement

342   with previous studies (Aichert et al. 2012; Fielding et al. 2009). Increased latency in the MS

343   group can be explained considering the competition model described before. Poor performance

344   in the AS task in MS is likely to occur because of a slow cognitive response that is due to

345   increased neural recruitment that is necessary to compensate for reduced neural transmission

346   (Amador et al. 2004; Chiaravalloti & DeLuca 2008; Crawford et al. 2002). We speculate that MS

347   is reducing the cognitive ability to process the vector inversion (suppression a prosaccadic

348   reflex (Edelman et al. 2006)) to program a volitional saccade. The possible consequences of a

349   reduced cognitive ability to process the vector inversion are: 1) an increased time to perform the

350   AS or 2) a prosaccade wins the competition (error). Impaired neural transmission that increases

351   latency and probably also error rates is likely to occur in areas of the frontal cortex such as

352   DLPFC and ACC (Amador et al. 2004; Johnston & Everling 2011; Miller & Cohen 2001).

353 However, some studies mention lesions in other areas of the brain associated with reduced AS
354 performance (Kolbe et al. 2014; Ting et al. 2015)
355   Differences between groups for prosaccades and corrective saccades latency and peak-
356 velocities (for all types of saccades) were not statistically significant. Our interpretation of a lack
357 of statistically significant differences for the latency is that - the generation of reflexive
358 prosaccades and corrective saccades was not compromised in our MS participants. Our
359 findings for controls, are consistent with the results from Evdokimidis in which the mean value of
360 prosaccades latency from a sample of 2006 young men was 208 ms (Evdokimidis et al. 2002)
361 and corrective saccades is typically 130 ms (Tatler & Hutton 2007). These results are in line
362 with results from other studies in MS (Fielding et al. 2009) and in other neurological diseases
363 (Crawford et al. 2005). The lack of statistically significant differences in peak-velocities between
364 groups for all types of saccades (AS, pro and corrective saccades) is reassuring because this
365 indicates that internuclear ophthalmoplegia was unlikely in our participants with MS (Bird &
366 Leech 1976). Conversely, the difference in peak-velocities between AS and, for example,
367 corrective saccades was obtained as expected. Corrective saccades are associated with larger
368 amplitudes and it is known that peak-velocities increase with amplitude according with a
369 relationship defined by the main sequence (Leigh & Kennard 2004). In summary, latencies for
370 prosaccades and corrective saccades and peak-velocities indicate that the integrity of the
371 oculomotor plant was well preserved in our participants with MS.
372   One limitation of the current study is the lack of experimental data measuring
373 neurocognitive performance in controls and that reduces the strength of the neurocognitive
374 comparisons between groups. It has also been pointed during revision that the validity of our
375 findings may be limited because: a) differences between groups in the AS task may be driven
376 by other aspects of MS, rather than deficits in inhibitory control per se; and b) is unclear that AS
377 measures are selective for MS per se, or if the same differences would be present in other
378 comorbid conditions (excluded from the tested population). Concerning a) - we performed a
379 comprehensive characterization of the neurocognitive performance in the MS group and
380 measured its association with the results of Stroop and the AS task. These experimental
381 decisions were made to minimize this possible limitation imposed by multiples deficits caused
382 by MS. Concerning b) - comorbid conditions that can interfere with AS performance were
383 excluded in the sample tested.

CONCLUSIONS

385   Our findings suggest that antisaccades may be a selective and independent measure to
386 investigate inhibitory control in patients with MS. More studies are necessary to confirm our

387 results and to describe brain correlates associated with impaired performance in the AS task in

388 people diagnosed with MS.

389

390

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398 Denver, Colorado. In ARVO 2015 the number of participants was different because the
399 neuropsychological tests were only performed in a sub-group of participants with MS.

400

401

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579

Figure 1

The antisaccade paradigm and position traces of the eyes

A - The antisaccade paradigm. Step 1) subjects had to fixate a centrally located target (cross, 30mmx30mm, ref 23) during an interval of 1250 or 1600 msec which assigned in a random order. The fixation target for step 1 was only visible after fixating a gaze-contingent box (10mmx10mm) for 150 msec. Step 2) After the interval defined for step 1, a peripheral target was presented at 5 or 10 degrees of visual angle at right or left side of the fixation target, all locations were assigned in random order (the arrow points the direction of the expected antisaccade). B - Representation of changes in eye movement amplitude with time during 3 trials. Antisaccades where performed to the right when the target was presented at 10 deg from the central fixation target on left side of the screen. The thick-solid line shows a trial in which a corrective saccade was necessary after an initial error - amplitude has been computed using xy coordinates and that is why it never reaches 0 deg during the corrective saccade. The dotted line shows a correct AS with a good spatial precision when compared with the mirrored position of the target. The thin-solid line shows a correct AS with a large latency and poor spatial precision. In the thin-solid line the triangle shows the instant at which the AS was detected and the dot shows the instant when the AS ended.

A

B

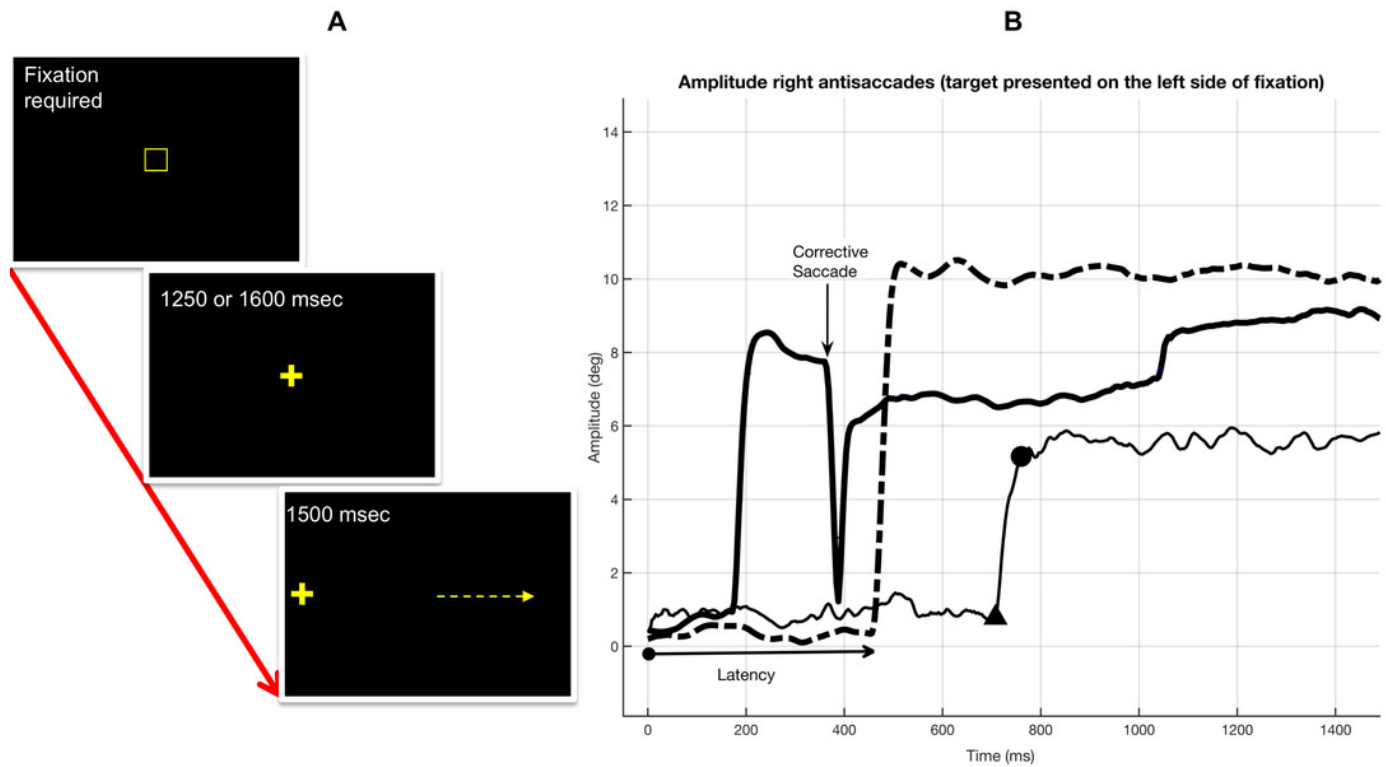
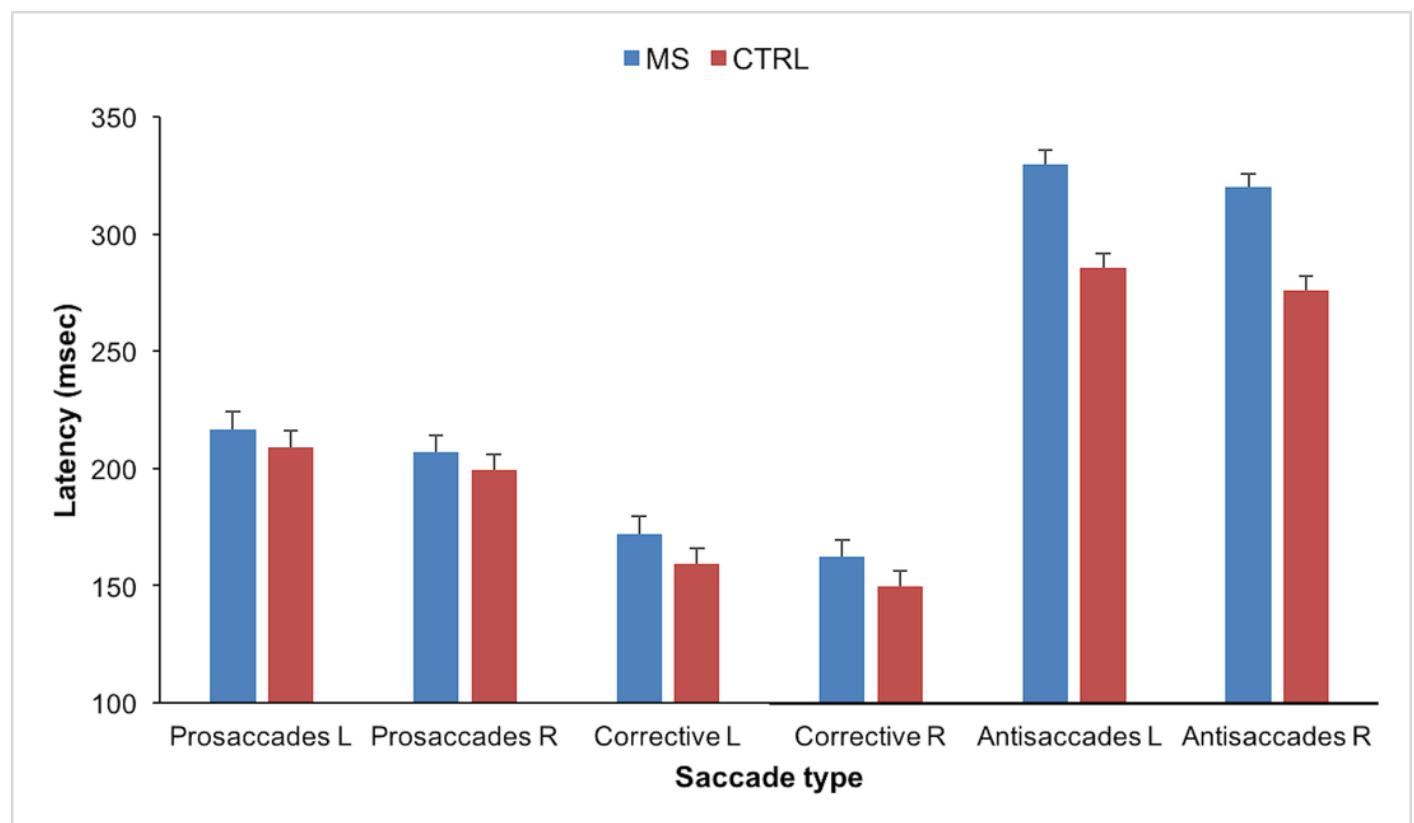



Figure 2

Bar graph showing the latency values per saccade type for both groups and for both directions of the expected antisaccades.

Directions indicate the direction of the expected antisaccades (R directions the target was presented left and vice-versa for L directions). Bars represent the EMM (mean response for each factor, adjusted for any other variables in the model) and the error bars the SE of the EMM for the specified factors.

Summary of the demographic and clinical information of the final sample of participants for both groups (SD = standard deviation).

Differences in age and gender were not statistically significant.

1 **Table 1. Summary of the demographic and clinical information of the final sample of participants**
2 **for both groups (SD = standard deviation). Differences in age and gender were not statistically**
3 **significant.**

Variable	Group	
	MS (n=38)	**Control (n=38)**
Gender *[n (%)]*	24 females (63%)	21 females (55%)
Age *[mean (SD)]*	37 (6)	36 (6)
Time since diagnosis in months *[mean (SD)]*	96 (59)	-
EDSS *[median (IQR)]*	1.5 (2)	-

Summary of the psychological evaluation results of the final sample of MS patients and normalized data for the Portuguese population (SD = standard deviation)

1 **Table 2. Summary of the psychological evaluation results of the final sample of MS patients and**
2 **normalized data for the Portuguese population (SD = standard deviation)**

Variable	Group		
	MS (n=38) Mean (SD)	**Normative** Mean (SD)	*p-value*
MOCA Total	24.03 (2.7)	26.4 (2.2)	*p<0.001*
Digit Span (WAIS-III)	9.8 (2.2)	10 (3)	0.68
Trail making (Women)	111(64)	61 (37)	*p=0.001*
Trail making (Men)	108 (54)	52 (37)	*p=0.002*
SDMT (Women)	51.61 (8.4)	60 (10.1)	*p<0.001*
SDMT (Men)	51.07 (10.7)	65.9 (12.2)	*p<0.001*
COWAT	32.3 (10.25)	33.2 (9.6)	0.60
Twenty Questions (D-KEFS)	10.5 (3.3)	10 (3)	0.35

Summary of Stroop results for the multiple sclerosis group.

Formulas for each score are specified in the table (SD = standard deviation, IQR = interquartile range).

Table 3. Summary of Stroop results for the multiple sclerosis group. Formulas for each score are specified in the table (SD = standard deviation, IQR = interquartile range).

Stroop test score	Values	
	Mean (SD)	Median (IQR)
Word reading *(W)*	90.97(13.01)	95.00(5.00)
Colour naming *(C)*	69.50 (9.99)	69.00 (5.75)
Interference		
Raw (CW = correct colours in 45sec)	35.50 (9.04)	38.00 (5.75)
Golden = [(WxC)/(W+C)]-CW	-3.35 (7.50)	-0.55 (3.85)
Relative = [(C-CW/C]x100	48.08 (10.47)	43.28 (7.16)
Ratio = (C/CW) x 10	19.54 (3.74)	17.63 (2.56)

Correlations between the Stroop test and other neuropsychological measures ("Test" column).

1 **Table 4: Correlations between the Stroop test and other neuropsychological measures ("Test" column).**

Test	Measure	Stroop		Stroop Interference			
		Word reading	Colour naming	Raw	Golden	Relative	Ratio
MOCA (n=38)	General	r=0.32 p=0.05	ns	r=0.42 p=0.01	ns	ns	r=-0.32 p=0.050
WAISIII digit span (n=28♦)	Memory Attention	ns	ns	r=0.44 p=0.018	ns	ns	ns
Trail Making A (n=37)	Attention	r=-0.33 p=0.05	ns	ns	ns	ns	ns
SDMT (n=37♦)	Processing	ns	ns	r=0.48 p=0.003	r=0.37 p=0.024	r=0.36 p=0.028	ns
COWAT (n=38)	Executive	ns	ns	r=0.37 p=0.021	r=0.32 p=0.052	r=-0.47 p=0.003	r=-0.33 p=0.042
DKEFS 20Q (n=37♦)	Executive	ns	ns	ns	ns	ns	ns

2   ♦When n is less than 38 is because there was missing data from the neuropsychological test. Executive = Executive function; General = General
3   cognitive function; Processing = Processing speed measure. "ns" = the correlation is not statistically significant.

Correlation between antisaccades measures and Stroop interference scores.

Errors, prosaccades and corrective saccades are defined in the methods section.

1 **Table 5: Correlation between antisaccades measures and Stroop interference scores.**
2 **Errors, prosaccades and corrective saccades are defined in the methods section.**

Anti-saccades	Stroop interference			
	Raw	**Golden**	**Relative**	**Ratio**
Errors	ns	ns	ns	ns
AS latency	r=-0.35, p=0.034 (n=38)	r=-0.33, p=0.044 (n=38)	r=-0.41, p=0.01 (n=38)	r=-0.38, p=0.018 (n=38)
Prosaccade latency	ns	ns	r=-0.33, p=0.039 (n=38)	ns
Corrective latency	r=-0.43, p=0.011 (n=35)	ns	ns	ns