

17 **Abstract**

18 **Background:** Repeated sub-concussive head impacts are a growing brain health concern, but the
19 possible mechanisms of trauma and plausible biomarkers remain elusive. One impediment is the lack
20 of an experimental model to study the effects of sub-concussive head impacts on the brain.

21 **Objectives:** This work's objective was to provide an experimental model to study the acute effects of
22 sub-concussive head impacts on the brain. To do so, this study aimed to replicate previous work from
23 Di Virgilio et al. (2016) showing that head impacts from heading footballs acutely alter brain
24 excitability by increasing corticomotor inhibition.

25 **Methods:** Scores from the Rivermead Post-Concussion Questionnaire and measurements of cortical
26 silent period (CSP) duration – obtained using transcranial magnetic stimulation to assess corticomotor
27 inhibition in the central nervous system – were taken before and after participants performed 20 football
28 headings (Headings; n = 30) or control (Control; n = 30).

29 **Results:** The results revealed increased headaches and dizziness symptoms in the Headings as
30 compared to the Control group, revealing the qualitative experience of head impacts. The results then
31 revealed that CSP duration similarly lengthened in both the Headings and Control groups, suggesting
32 that head impacts did not cause the increased corticomotor inhibition.

33 **Conclusions:** The results show that head impacts from football headings did not acutely alter
34 corticomotor inhibition as compared to a control group that did not experience head impacts, suggesting
35 that excitability changes do not reflect acute sub-concussive brain injuries. Nonetheless, this work
36 suggests that football headings can be used as an experimental model to study the effects of sub-
37 concussive head impacts on brain health. Future work could use the present procedures to investigate
38 additional biomarkers of brain injury.

39

40 **Introduction**

41 Repeated sub-concussive head impacts are increasingly recognised to have detrimental effects on brain
42 health (Ntikas et al., 2022). For instance, neuroimaging studies have shown that repeated sub-
43 concussive head impacts negatively alter white matter tract integrity, reduce cortical thickness, and
44 impair cognitive functions (Koerte et al., 2015; Lipton et al., 2013). Moreover, a chronic history of sub-
45 concussive head impacts – such as those experienced by football players when heading footballs –
46 increases the risk of developing neurodegenerative diseases (Russell et al., 2021; Ueda et al., 2023).
47 Given that the socioeconomic burden of neurodegenerative diseases is increasing worldwide (Wong,
48 2020), and that head impacts are modifiable risk factors (Livingston et al., 2020), there is a pressing
49 need to characterize the full effects of sub-concussive head impacts on brain health using randomised
50 controlled experimental settings (Batty & Kaprio, 2022). The overarching objective of this work was to
51 provide an experimental model to study the acute effects of sub-concussive head impacts on the brain.
52 Similar to work on concussions (Feddermann-Demont et al., 2020), such systematic investigations
53 could ultimately lead to recommendations to attenuate (or manage) the detrimental effects of head
54 impacts on brain health.

55 One promising model is to use transcranial magnetic stimulation (TMS) to non-invasively
56 assess changes in brain excitability in response to performing football headings (Di Virgilio et al., 2016;
57 Ntikas et al., 2022). Specifically, Di Virgilio et al. (2016) used TMS to measure changes in the cortical
58 silent period (CSP) duration – defined as the suppression of voluntary electromyography activity, which
59 linearly reflects the extent of GABAergic corticomotor inhibition in the corticospinal tract (Ziemann et
60 al., 2015) – before and after participants performed 20 football headings over 10min. The authors found
61 that CSP duration lengthened immediately after performing the headings, suggesting that sub-
62 concussive head impacts acutely altered brain excitability by increasing corticomotor inhibition.
63 Although these results were crucially not compared to a control group that did not experience head
64 impacts, this approach remains a promising model because it allows to experimentally induce sub-
65 concussive head impacts in a controlled environment. Furthermore, changes in CSP duration constitute
66 a promising biomarker, as measuring CSP is non-invasive, can be easily and quickly measured, and its

67 utility to evaluate changes in brain excitability is supported by work on both concussive (Scott et al.,
68 2020) and sub-concussive head impacts (Ntikas et al., 2022). Overall, this evidence suggests that
69 measuring changes in CSP duration before and after performing football headings constitutes a
70 promising experimental model.

71 Therefore, the objective of this study was to replicate the results from Di Virgilio et al. (2016)
72 using a randomised controlled trial – by adding an appropriate control group – to ascertain that changes
73 in CSP duration reflect acute brain injuries. This work also sought to confirm that football headings can
74 be used as an experimental model to study the acute effects of sub-concussive head impacts on brain
75 functions. Here, scores from Rivermead Post-Concussion Questionnaire and changes in CSP duration
76 were assessed before and immediately after groups of participants performed (Headings; n = 30), or not
77 (Control; n = 30), 20 football headings. It was hypothesized that CSP would acutely lengthen in
78 response to the head impacts (as in Di Virgilio et al., 2016) *and* as compared to the control group.

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82 **METHODS**

83 **Participants**

84 Sixty physically and neurologically healthy young adults took part in this study (mean \pm 95%
85 confidence intervals (CIs) = 20.9 ± 0.7 years old; hereafter, all descriptive statistics represent the mean
86 \pm 95% CIs). All participants reported having no history of concussion in the previous 5 years, suggesting
87 preserved integrity of neurophysiological activity within the motor cortex (Tremblay et al., 2014). To
88 enhance the sample's ecological validity, participants with or without expertise playing in football were
89 recruited. It was reasoned that the effects of sub-concussive head impacts on the brain's excitability
90 should be apparent regardless of players' expertise (see Di Virgilio et al., 2022), so football headings
91 can be employed as a valid experimental model.

92 The participants were randomly allocated to a group that experienced sub-concussive head
93 impacts (Headings; $n = 30$; 5 females; 20.9 ± 1.1 years) and a group that did not (Control; $n = 30$; 5
94 females; 20.8 ± 0.9 years). Participants were screened for TMS contraindications (Rossi et al., 2009),
95 the major contraindications being the presence of metal particles/objects in the region of the head and
96 the presence of a personal history of epileptic seizures or convulsions. The study was approved by the
97 local research ethics committee of the University of Birmingham (project # ERN-182077AP10). All
98 participants provided written informed consent before their participation.

99 The number of participants was based on a sample size analysis using G*Power (v.3.1.9.4).
100 Namely, the smallest effect size of interest for the context of this study was a Cohen's d of 0.8 (large
101 effect size). Assuming the use of an independent t-test (to evaluate differences between Headings and
102 Control), 80% statistical power, and a significance threshold of 0.05, the results of the analysis revealed
103 that two groups of 26 participants are required. To ensure a statistical power greater than 80%, two
104 groups of 30 participants were thus recruited. Furthermore, a post-hoc achieved power analysis using
105 G*Power was conducted to determine if the statistical power of this study would be enough to replicate
106 the acute lengthening in CSP duration from Di Virgilio et al. (2016), which reported a Cohen's d_z of
107 1.125 (above-large effect size). Assuming this effect size, a significance threshold of 0.05, and a sample

108 size of 30 participants, the achieved power in the present study was 100%. Overall, these results suggest
109 that two groups of 30 participants are sufficient to detect meaningful differences between the Headings
110 and Control groups and replicate the results from Di Virgilio et al. (2016).

111 **Study protocol**

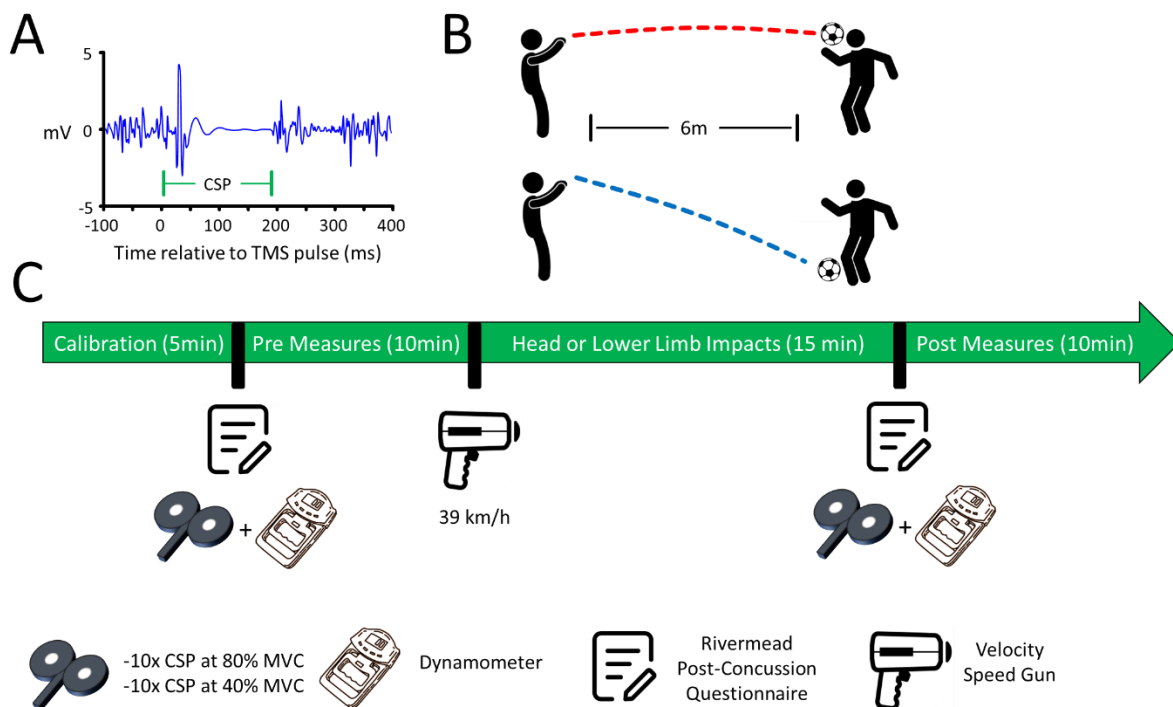
112 An overview of the study protocol is provided in Figure 1. The Rivermead Post-Concussion
113 Questionnaire, the CSP (Figure 1A) and the exerted force during a voluntary contraction were measured
114 before (Pre Measures) and after (Post Measures; Figure 1C) participants either headed the ball or used
115 their foot to control the ball (Figure 1B). In both groups, participants experienced 20 throws, at a rate
116 of 1 throw every 30 sec (as in Di Virgilio et al., 2016). Trials where participants missed the ball were
117 redone to ensure that a total of 20 contacts were experienced with the ball. Participants of the Headings
118 group were instructed to head the football to redirect it perpendicularly in the direction of their right-
119 hand side (rotational headers; as in Di Virgilio et al., 2016). Participants of the Control group were
120 instructed to use their dominant lower limb (preferably the foot) to control the ball. This method of
121 control was used to prevent head impacts and match the level of exercise performed between the two
122 groups. All parts of the procedures involving footballs were performed outdoors.

123 The ball consisted of a standard football (400g, 70 cm circumference; 8 psi) and was manually
124 thrown by an experienced (> 10 years) football player from a 6m distance (Di Virgilio et al., 2016)
125 towards the head (Headings) or dominant lower limbs (aiming at the foot; Control). The rationale for
126 manually throwing the ball was to best approximate practice and game conditions, as an effort to
127 enhance ecological validity. The ball was thrown at an average speed of 39km/h (Di Virgilio et al.,
128 2016), which was assessed upon each throw with a hand-held Velocity Speed Gun (Bushnell, model
129 101911). Ball speed for the Headings and Control groups was 39.15 ± 0.16 km/h and 39.09 ± 0.14 km/h,
130 respectively, confirming that the 39km/h target was successfully achieved. Globally, 40min were
131 required to complete the protocol (including the questionnaires and TMS procedures; Figure 1C).

132

133 Questionnaire

134 To assess sub-concussive head impact symptoms, the Rivermead Post-Concussion Questionnaire was
135 used (Potter et al., 2006). The Rivermead Questionnaire consists of 16 items (see Table 1 for a list),
136 each evaluated using the following scores: 0 (not experienced at all), 1 (no more of a problem), 2 (a
137 mild problem), 3 (a moderate problem), and 4 (a severe problem). Conceivably, some items are
138 unspecific in evaluating symptoms of acute sub-concussive head impacts (e.g., feeling frustrated, sleep
139 disturbance), but other items arguably appear well-suited to do so (e.g., headaches, feeling of dizziness).
140 In the absence of a validated questionnaire specifically developed for that purpose, it was reasoned that
141 the Rivermead Questionnaire would be sensitive enough to evaluate some of the symptoms of sub-
142 concussive head impacts. The results provide post-hoc support for this contention.



143 **Figure 1. Overview of the protocol.** (A) Visual depiction of a cortical silent period (CSP). A silent
144 period in the surface electromyography (EMG) follows the delivery of a TMS pulse when participants
145 are voluntarily contracting hand muscles. The CSP duration is thought to positively covary with the
146 extent of GABAergic inhibition in the corticospinal tract (Ziemann et al., 2015). (B) Visual depiction
147 of the Headings and Control groups. An experienced experimenter manually threw the ball from a 6m
148 distance at a set speed of 39 km/h (measured using a velocity speed gun on every throw). The ball was
149 thrown a total of 20 times (once every 30 sec). Participants either headed the ball (Headings; upper
150 panel) or controlled the ball using their dominant lower limb (Control; lower panel), therefore
151 preventing any head impacts. (C) Timeline of the protocol. The experiment began with the calibration
152 of the surface EMG, TMS and dynamometer. Then, participants filled out the Rivermead Questionnaire
153 and a total of 20 valid CSP trials were collected (10 trials at 80% of the maximum voluntary contraction
154 (MVC) and 10 trials at 40% MVC). Subsequently, participants performed the headings or lower limb
155 ball contacts. Finally, the Rivermead Questionnaire was filled and a total of 20 valid CSP trials were
156

157 collected a second time (10 trials at 80% MVC and 10 trials at 40% MVC). The measures were collected
158 in this specific order for every participant.

159 **TMS, EMG, and Dynamometer**

160 The following procedures were performed indoors, in a temperature-controlled environment. TMS was
161 delivered using a 70mm figure-of-eight D-Alpha flat coil connected to a Magstim 200² stimulator
162 (Magstim, Whitland, UK). TMS pulses were delivered to the cortical representation of the first dorsal
163 interosseus (FDI) muscle of the left M1 (McNabb et al., 2020). The surface electromyography (EMG)
164 data of the FDI muscle of the right hand were recorded using bipolar electrodes connected to a Delsys
165 ® Bagnoli system, itself connected to a Power 1402 data acquisition interface (Cambridge Electronic
166 Design ®). The data were digitised at 10,000 Hz for 500ms epochs (100ms pre-trigger time; bandpass
167 filtered between 20 and 450 Hz) and recorded using Signal (v6.05; Cambridge Electronic Design ®).
168 The reference electrode was located on the proximal portion of the right ulnar bone.

169 Participants wore a rubber swimming cap, which was used to mark the location of the hotspot
170 (Hamel et al., 2020). Vertical lines were additionally drawn at the junction of participants' skin and
171 swimming cap, to ensure that the swimming cap did not move when performing the headings. The
172 hotspot location, defined as the cortical location where motor-evoked potentials (MEPs) in the FDI were
173 reliably induced, was found by delivering suprathreshold pulses. Then, the stimulator intensity was
174 adjusted to obtain MEPs of $\pm 1\text{mV}$ at rest. For all participants ($n = 60$), the stimulator intensity was set
175 at $55 \pm 2\%$. This was defined as the test stimulus intensity and was kept constant for the experiment's
176 duration for a given participant.

177 A total of 10 valid CSP trials per level of maximum voluntary contraction (MVC; see below)
178 for both Pre and Post Measures were recorded. This number of trials was chosen because measuring
179 more than 10 CSP trials does not enhance the reliability of CSP duration estimation (Garvey et al.,
180 2001). To induce CSP, participants squeezed a hand dynamometer (Kuptone ®) at 80% and 40% of
181 their MVC (defined as the greatest force generated out of three trials). Although arguably non-specific
182 to the FDI muscle, the use of a dynamometer was to provide an effective, inexpensive, and accessible
183 means to induce voluntary contractions and measure MVC of the hand muscles for on-the-field and

184 experimental settings. Participants were instructed to reach and maintain a contraction at their
185 individualised 80% and 40% MVC targets for ~3sec, allowing sufficient time for the experimenter to
186 deliver a single TMS pulse upon reaching those targets. The peak generated force (in kg) was assessed
187 for each CSP trial and kept for analysis. The results revealed that all participants ($n = 60$) exerted peak
188 forces that represented $80 \pm 0.1\%$ and $43 \pm 0.1\%$ of their MVC. A target of 100% MVC was not used
189 (unlike Di Virgilio et al., 2016), as it generated important fatigue and limited the number of CSP trials
190 that could be recorded. CSP trials were collected at 80% and 40% MVC to determine if similar CSP
191 lengthening could be observed at different MVC (as supported by Kojima et al., 2013). The case being,
192 it would suggest that CSP duration lengthening in response to head or lower limb impacts (Figure 1)
193 could be observed using low MVC, making it convenient to collect multiple trials to reliably evaluate
194 the effects of sub-concussive head impacts on CSP duration.

195 **Dependent variables**

196 The main dependent variables were CSP duration and the Rivermead Questionnaire scores. Using a
197 custom-designed algorithm in MatLab, CSP duration was measured as the time difference (in ms)
198 between the delivery of the TMS pulse and the return of the EMG of voluntary muscle activity (CSP
199 offset). Specifically, the CSP offset was determined as the moment when the SD of a 2.5ms sliding
200 window exceeded 50% of the SD of the EMG background activity, calculated over the 100ms that
201 preceded TMS pulse delivery, for at least 5ms (similar to Goodall et al., 2010; Hamel et al., 2022). The
202 EMG data were not rectified. To normalise the CSP duration, the CSP data (in ms) from the Post
203 Measures were divided by the CSP data (in ms) from the Pre Measures. This was done separately for
204 each level of Contraction Levels (80%, 40% MVC). The Rivermead Questionnaire scores (0 to 4) were
205 averaged across groups for each item (see Table 1).

206 **Statistical analysis**

207 To analyse the results, mixed ANOVAs were used. The within-subject factors were Measures (Pre, Post)
208 and Contraction Levels (80% MVC, 40% MVC). The between-subject factor was Groups (Headings,
209 Control). If the data violated the assumptions of sphericity ($p < 0.05$, Mauchly test), the Greenhouse-

210 Geiser correction was applied. If data deviated from normality ($p < 0.05$; Shapiro-Wilk test) upon
211 pairwise comparisons, non-parametric pairwise comparisons were conducted (Wilcoxon rank test rather
212 than dependent t-test for within-subject comparisons; U Mann-Whitney test rather than independent t-
213 test for between-subject comparisons). The Benjamini-Hochberg (1995) correction was used to control
214 for inflated type 1 error upon multiple comparisons. The statistical significance threshold was set at
215 0.05. All descriptive statistics reported in this work represent the mean \pm 95% CIs. The open-access
216 software JAMOVI was used to conduct the statistical analyses.

217

218 **Results**

219 **Increased headaches and dizziness symptoms after football heading**

220 The data from the 16-item Rivermead Questionnaire were analysed using 2 Measures (Pre, Post) * 2
221 Groups (Headings, Control) mixed ANOVAs. See Table 1 for a complete report of the group
222 questionnaire data. Overall, the results below show that heading footballs significantly increased
223 headaches and dizziness symptoms as compared to the control group, providing post-hoc support to
224 using the Rivermead Questionnaire to assess some of the symptoms of sub-concussive head impacts.

225 For headaches symptoms (Figure 2A), the results revealed a Measures * Groups interaction
226 ($F_{(1,58)} = 23.460, p < 0.0001, \eta_p^2 = 0.288$). Breakdown of the Measures * Groups interaction revealed
227 that headaches symptoms increased from Pre to Post for the Headings group ($p = 0.0008$, Cohen's $d_z =$
228 0.845), but not for the Control group ($p = 0.3458$, Cohen's $d_z = 0.263$). The Headings and Control
229 groups did not differ at Pre ($p = 0.3128$, Cohen's $d_z = 0.265$), but the Headings showed greater
230 headaches symptoms than the Control group at Post ($p < 0.0001$, Cohen's $d = 1.239$). This analysis
231 indicates that participants experienced headaches symptoms when heading footballs but not in the
232 control group.

233 For dizziness symptoms (Figure 2B), the results also revealed a Measures * Groups interaction
234 ($F_{(1,58)} = 6.676, p = 0.0123, \eta_p^2 = 0.103$). A breakdown of the interaction revealed that dizziness
235 symptoms increased from Pre to Post for the Headings ($p = 0.0166$, Cohen's $d_z = 0.561$), but not for
236 the Control group ($p = 1.000$, Cohen's $d = 0.183$). The Headings and Control groups did not differ at
237 Pre ($p = 1.000$, Cohen's $d = 0.000$), but Headings showed greater dizziness symptoms than the Control
238 group at Post ($p = 0.0122$; Cohen's $d = 0.667$). This result confirms that participants experienced
239 dizziness symptoms when heading footballs but not in the control group.

240 Concerning the remaining symptoms (Table 1), the results selectively revealed an effect of
241 Groups ($F_{(1,58)} = 4.372, p = 0.0409, \eta_p^2 = 0.070$) and a marginal Measures * Groups interaction ($F_{(1,58)} =$
242 $2.866, p = 0.0959, \eta_p^2 = 0.047$) for "Taking longer to think" symptoms. Whilst the effect of Groups
243 revealed that participants in the Headings group "took longer to think" than in the Control group ($p =$

244 0.0409, Cohen’s $d = 0.540$), the marginal Measures * Groups suggests that this difference was apparent
 245 at Post ($p = 0.0430$, Cohen’s $d = 0.584$) but not at Pre ($p = 0.5702$, Cohen’s $d = 0.151$). Analyses of all
 246 the other symptoms revealed no effect of Measures (all $F_{(1,58)} < 2.000$, all $p > 0.1626$, all $\eta_p^2 < 0.033$),
 247 no effect of Groups (all $F_{(1,58)} < 1.851$, all $p > 0.1789$, all $\eta_p^2 < 0.031$), and no Measures * Groups
 248 interaction (all $F_{(1,58)} < 2.610$, all $p > 0.1116$, all $\eta_p^2 < 0.043$). Overall, these results suggest that heading
 249 footballs made participants “take longer to think” as compared to the control group. Furthermore, they
 250 confirm the absence of meaningful differences within and between groups in the remaining items of the
 251 Rivermead Questionnaire.

	Headings		Control	
	Pre	Post	Pre	Post
Headaches	0.033 (0.065)	0.767 (0.292)	0.100 (0.109)	0.033 (0.065)
Feeling of dizziness	0.000 (0.000)	0.300 (0.191)	0.000 (0.000)	0.033 (0.065)
Nausea/vomiting	0.000 (0.000)	0.100 (0.144)	0.000 (0.000)	0.000 (0.000)
Noise sensitivity	0.000 (0.000)	0.033 (0.065)	0.033 (0.065)	0.000 (0.000)
Sleep disturbance	0.167 (0.165)	0.133 (0.155)	0.133 (0.155)	0.100 (0.144)
Fatigue, tiring more easily	0.167 (0.165)	0.200 (0.146)	0.133 (0.155)	0.100 (0.144)
Irritable, easily angered	0.033 (0.065)	0.033 (0.065)	0.000 (0.000)	0.000 (0.000)
Depressed or tearful	0.067 (0.091)	0.033 (0.065)	0.100 (0.196)	0.067 (0.131)
Frustrated or impatient	0.033 (0.065)	0.033 (0.065)	0.100 (0.109)	0.033 (0.065)
Forgetfulness, poor memory	0.067 (0.091)	0.067 (0.091)	0.033 (0.065)	0.033 (0.065)
Poor concentration	0.100 (0.109)	0.233 (0.180)	0.200 (0.173)	0.133 (0.155)
Taking longer to think	0.067 (0.091)	0.200 (0.173)	0.033 (0.065)	0.000 (0.000)
Blurred vision	0.000 (0.000)	0.033 (0.065)	0.000 (0.000)	0.000 (0.000)
Light sensitivity	0.033 (0.065)	0.033 (0.065)	0.000 (0.000)	0.000 (0.000)
Double vision	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
Restlessness	0.000 (0.000)	0.067 (0.131)	0.100 (0.109)	0.100 (0.109)

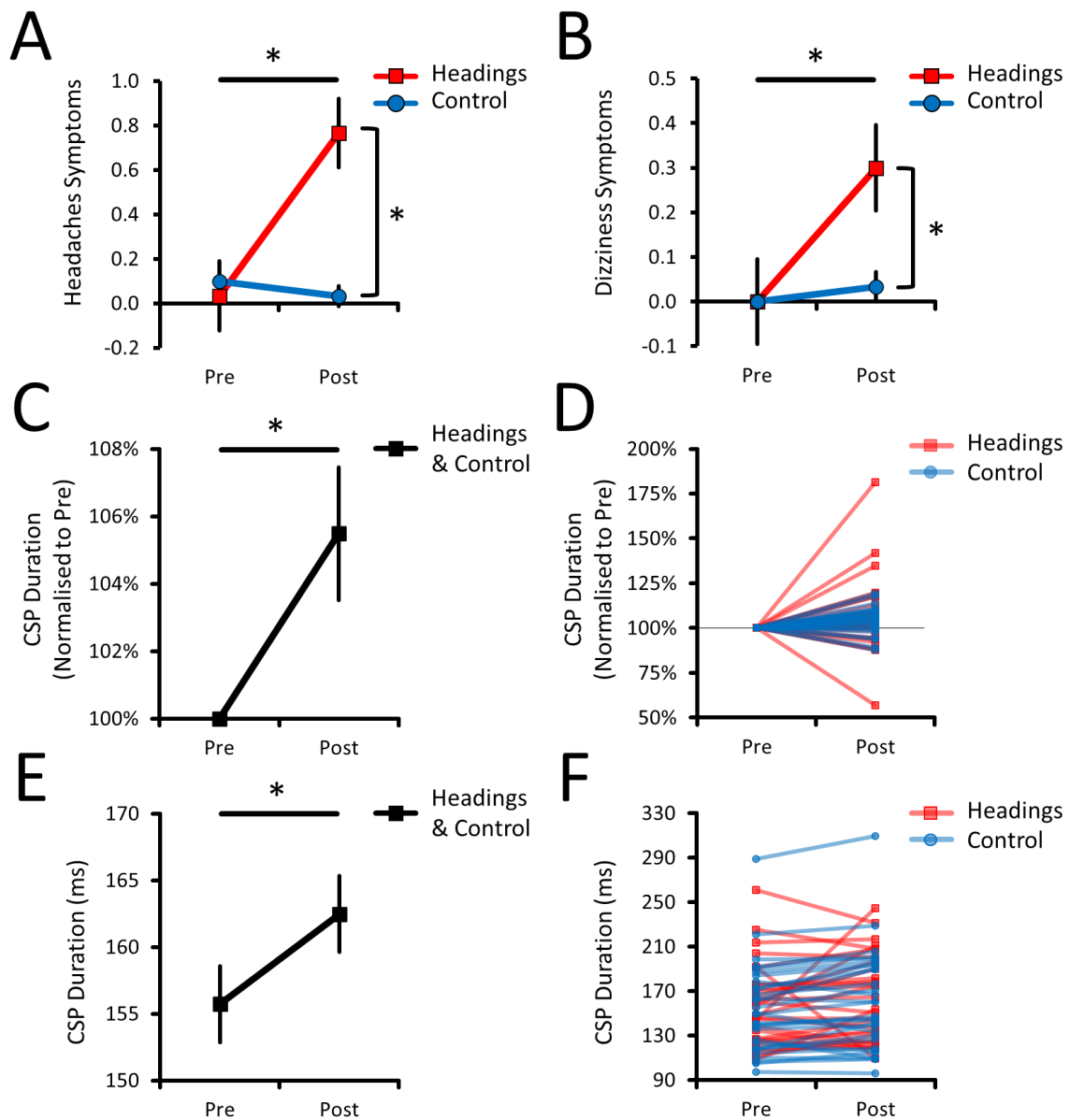
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253 **CSP duration lengthened in both the Headings and Control groups**

254 A 2 Measures (Pre, Post) * 2 Contraction Levels (80% MVC, 40% MVC) * 2 Groups (Headings,
255 Control) mixed ANOVA was conducted to analyse normalised CSP duration (Figure 2C and D). The
256 results revealed a main effect of Measures ($F_{(1,58)} = 7.363, p = 0.0087, \eta_p^2 = 0.113$), but no Measures *
257 Groups interaction ($F_{(1,58)} = 0.212, p = 0.6472, \eta_p^2 = 0.004$). This shows that CSP duration similarly
258 lengthened from Pre to Post for both the Headings ($6.4 \pm 3.7\%$) and Control groups ($4.6 \pm 1.3\%$). Also,
259 there was no effect of Contraction Levels ($F_{(1,58)} = 2.527, p = 0.1173, \eta_p^2 = 0.042$), no effect of Groups
260 ($F_{(1,58)} = 0.212, p = 0.6472, \eta_p^2 = 0.004$), no Measures * Contraction Levels interaction ($F_{(1,58)} = 2.527,$
261 $p = 0.1173, \eta_p^2 = 0.042$), no Contraction Levels * Groups interaction ($F_{(1,58)} = 0.231, p = 0.6325, \eta_p^2 =$
262 0.004), and no three-way interaction ($F_{(1,58)} = 0.231, p = 0.6325, \eta_p^2 = 0.004$). Overall, this analysis
263 shows that heading footballs increased CSP duration, but not more so than in a control group that did
264 not head the ball. It also shows that CSP duration did not differ between the different Contraction Levels.

265 To confirm that these results were not a by-product of normalising the CSP duration data, the
266 same mixed ANOVA was conducted on the non-normalised CSP duration data (in ms; Figure 2E and
267 F). The results also revealed a main effect of Measures ($F_{(1,58)} = 5.308, p = 0.0248, \eta_p^2 = 0.084$) and no
268 Measures * Groups interaction ($F_{(1,58)} < 0.001, p = 0.9850, \eta_p^2 < 0.001$). This analysis shows that CSP
269 duration similarly lengthened for both groups from Pre to Post (Headings: $6.8 \pm 10.8\text{ms}$; Control: $6.7 \pm$
270 3.9ms). Also, both groups did not differ at Pre (Headings: $157.3 \pm 13.1\text{ms}$; Control: $154.1 \pm 14.7\text{ms}$; p
271 $= 0.6865$, Cohen's $d = 0.082$) or Post (Headings: $164.1 \pm 13.7\text{ms}$; Control $160.8 \pm 16.2\text{ms}$; $p = 0.7576$,
272 Cohen's $d = 0.079$), suggesting that both groups had homogenous CSP duration. The results also
273 revealed no effect of Contraction Levels ($F_{(1,58)} = 0.009, p = 0.9245, \eta_p^2 < 0.001$), no effect of Groups
274 ($F_{(1,58)} = 0.104, p = 0.7477, \eta_p^2 = 0.002$), no Measures * Contraction Levels ($F_{(1,58)} = 2.671, p = 0.1076,$
275 $\eta_p^2 = 0.044$), no Contraction Levels * Groups interaction ($F_{(1,58)} = 1.626, p = 0.2073, \eta_p^2 = 0.027$), and
276 no three-way interaction ($F_{(1,58)} = 0.004, p = 0.9509, \eta_p^2 < 0.001$), confirming that the above results are
277 not a by-product of normalising the CSP duration data.

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279

280 **Figure 2. Headaches, Dizziness, and CSP results.** (A) Headaches and (B) Dizziness symptoms, as
 281 evaluated with the Rivermead Questionnaire. The Headings group showed greater headaches and
 282 dizziness symptoms after headings were performed as compared to the Control group. This indicates
 283 that head impacts were experienced in the Headings, but not in the Control group. (C) Mean change
 284 and (D) individual data of CSP duration, depicted as a percentage change from the Pre Measures. The
 285 results revealed an effect of Measures ($p = 0.0087$, $\eta_p^2 = 0.113$), but no interaction between Measures
 286 and Groups ($p = 0.6472$, $\eta_p^2 = 0.004$), showing that CSP duration lengthened similarly in both groups.
 287 (E) Mean change and (D) individual data of CSP duration, depicted as values in milliseconds. The
 288 results also revealed an effect of Measures ($p = 0.0248$, $\eta_p^2 = 0.084$) and no interaction between
 289 Measures and Groups ($p = 0.9850$, $\eta_p^2 < 0.001$), confirming that the results in (C) and (D) are not a by-
 290 product of normalising the CSP duration. For panels (A), (B), (C), and (E), the mean \pm 95% CIs are
 291 shown. Asterisks (*) denote significant differences.

292

293 **Discussion**

294 This work's objective was to test the hypothesis that CSP would lengthen in response to football
295 headings (as in Di Virgilio et al., 2016) *and* as compared to a control group. However, the results do
296 not support this hypothesis. Namely, the results first revealed increased headaches and dizziness
297 symptoms in the Headings compared to the Control group, indicating that sub-concussive head impacts
298 were experienced in the Headings group only. The results then revealed that CSP duration similarly
299 lengthened in both the Headings and Control groups, suggesting that head impacts were not the cause
300 of the observed increase in CSP duration. One implication is that changes in brain excitability – as
301 assessed by CSP duration – are not a reliable indicator of acute sub-concussive brain injury.

302 **Increased headaches and dizziness symptoms after heading footballs**

303 One novel result of this work is that performing football headings (Headings group) increased subjective
304 headaches and dizziness symptoms as compared to hitting the ball with the dominant lower limb
305 (Control group), indicating that head impacts were experienced in the Headings group only.
306 Interestingly, the symptom “taking longer to think” was also marginally increased following the head
307 impacts as compared to control, aligning with previous work suggesting that football headings
308 deteriorate cognitive performance (Di Virgilio et al., 2016; Dioso et al., 2022). The present results
309 extend previous work (Di Virgilio et al., 2016, 2019; McNabb et al., 2020) by showing that sub-
310 concussive head impacts also induce subjective effects that can be monitored using the Rivermead
311 Questionnaire. Overall, these results indicate that head impacts were experienced in the Headings group
312 only and suggest that – in absence of a validated questionnaire developed for that purpose – the
313 Rivermead Questionnaire can be used to assess some of the subjective effects of sub-concussive head
314 impacts.

315

316 **CSP duration similarly lengthened for both groups**

317 The main result of this work is that CSP duration lengthened similarly for both the Headings and Control
318 groups, suggesting that acute head impacts are not the cause of the CSP lengthening. Moreover, CSP
319 duration was not affected by the strength of the muscle contraction performed by the participant (80%
320 MVC vs 40% MVC), aligning with previous work showing that different levels of voluntary muscle
321 contraction do not affect CSP durations (Kojima et al., 2013). Whilst the present results replicated the
322 CSP lengthening reported by Di Virgilio et al. (2016), the lack of difference between the Headings and
323 Control groups indicates that such CSP lengthening cannot be attributed to performing football headings
324 *per se*. Therefore, a factor unrelated to head impacts – but present in both the Headings and Control
325 groups – must account for the present CSP lengthening or obscure differences between the groups.

326 The Control group was designed to match the exercise levels – albeit low in absolute terms –
327 of the Headings group. Thus, one possibility is that the performing comparable exercise levels increased
328 CSP duration similarly for both groups. High and low-intensity aerobic exercise alone can reduce
329 intracortical inhibition in M1 (Stavrinos & Coxon, 2016; Yamazaki et al., 2019) but can also increase
330 GABA concentrations in cortical motor areas (Coxon et al., 2018). Although this evidence makes it
331 unclear if exercise should shorten or lengthen CSP duration, it nonetheless suggests that groups
332 performing similar levels of exercise will show similar changes in CSP duration. In opposition, Di
333 Virgilio et al. (2019) reported that three 3-min sparring bouts increased CSP duration as compared to a
334 mock-sparring control group, suggesting that head impacts should increase CSP duration even if
335 exercise levels are matched. However, Di Virgilio et al. (2016) did not control for exercise levels,
336 making it unclear if the increased CSP duration they reported was due to heading footballs or to the
337 exercise levels achieved during the intervention. Nonetheless, one obstacle for future studies will be to
338 isolate the effects of exercise from those of sub-concussive head impacts, as the two usually co-occur
339 and can therefore confound each other (Tremblay et al., 2018). Overall, one possibility is that raising
340 the exercise level above the resting (sedentary) state CSP are typically recorded in – alone – accounts
341 for the similar CSP lengthening in both the Headings and Control groups.

342 Another possibility is that changes in CSP duration between different groups can only be
343 observed – or enhanced – when groups have different history of concussions (for a review, see Scott et
344 al., 2020). For instance, De Beaumont et al. (2007) assessed CSP duration at rest in varsity athletes with
345 a concussion history (between 2 to 5 concussions, which occurred more than 9 months before testing)
346 and in control participants with no concussion history. Their results showed greater CSP duration in the
347 varsity athletes as compared to the control participants, suggesting that having a concussion history
348 could predispose to CSP duration lengthening in response to sub-concussive head impacts (see McNabb
349 et al., 2020). De Beaumont et al. (2007) also reported that concussion severity positively correlated with
350 CSP duration whereas the number of experienced concussions did not, suggesting that a history of
351 severe concussions is an important confounding factor. Here, the participants all reported having no
352 history of concussion in the 5 years before they participated in this study, which was shown to be
353 sufficient to restore levels of inhibition and excitation in M1 (Tremblay et al., 2014). As a result, one
354 possibility is that the present lack of difference in CSP duration stems from a similar lack of concussion
355 history between the Headings and Control groups.

356 **Conclusion**

357 This work shows that heading footballs did increase CSP duration, but not more so than in a control
358 group that did not head footballs, suggesting that heading footballs did not account for the acute
359 increases in corticomotor inhibition. By the same token, this also suggests that changes in CSP duration
360 do not reliably reflect acute brain injuries, as confounding factors other than head impacts can cause
361 CSP to lengthen. Future studies should carefully control factors such as the levels of exercise performed
362 and the history (and severity) of concussion over (at least) the last 3 years (Tremblay et al., 2014).
363 Importantly, the present methods can be used as an experimental model to evaluate the effects of sub-
364 concussive head impacts on brain functions. Future studies could use the present design to collect data
365 in even larger cohorts ($n > 100$) and could include wet (inflammatory) biomarkers to assess additional
366 dimensions of brain injury in response to sub-concussive head impacts (see Sandmo et al., 2022).

367 **REFERENCES**

- 368 Batty, G. D., & Kaprio, J. (2022). Traumatic brain injury, collision sports participation, and
369 neurodegenerative disorders: Narrative power, scientific evidence, and litigation. *Journal of*
370 *Epidemiology and Community Health*, jech-2022-219061. [https://doi.org/10.1136/jech-2022-](https://doi.org/10.1136/jech-2022-219061)
371 219061
- 372 Benjamini, Y., & Hochberg, Y. (1995). Controlling the False Discovery Rate: A Practical and
373 Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society. Series B*
374 *(Methodological)*, 57(1), 289–300. JSTOR.
- 375 Coxon, J. P., Cash, R. F. H., Hendrikse, J. J., Rogasch, N. C., Stavrinou, E., Suo, C., & Yücel, M.
376 (2018). GABA concentration in sensorimotor cortex following high-intensity exercise and
377 relationship to lactate levels. *The Journal of Physiology*, 596(4), 691.
378 <https://doi.org/10.1113/JP274660>
- 379 De Beaumont, L., Lassonde, M., Leclerc, S., & Théoret, H. (2007). LONG-TERM AND
380 CUMULATIVE EFFECTS OF SPORTS CONCUSSION ON MOTOR CORTEX
381 INHIBITION. *Neurosurgery*, 61(2), 329.
382 <https://doi.org/10.1227/01.NEU.0000280000.03578.B6>
- 383 Di Virgilio, T. G., Hunter, A., Wilson, L., Stewart, W., Goodall, S., Howatson, G., Donaldson, D. I.,
384 & Ietswaart, M. (2016). Evidence for Acute Electrophysiological and Cognitive Changes
385 Following Routine Soccer Heading. *EBioMedicine*, 13, 66–71.
386 <https://doi.org/10.1016/j.ebiom.2016.10.029>
- 387 Di Virgilio, T. G., Ietswaart, M., Selvamorthy, R., & Hunter, A. M. (2022). The Reliability of
388 Transcranial Magnetic Stimulation-Derived Corticomotor Inhibition as a Brain Health
389 Evaluation Tool in Soccer Players. *Sports Medicine - Open*, 8(1), 7.
390 <https://doi.org/10.1186/s40798-021-00399-3>
- 391 Di Virgilio, T. G., Ietswaart, M., Wilson, L., Donaldson, D. I., & Hunter, A. M. (2019).
392 Understanding the Consequences of Repetitive Subconcussive Head Impacts in Sport: Brain

- 393 Changes and Dampened Motor Control Are Seen After Boxing Practice. *Frontiers in Human*
394 *Neuroscience*, 13. <https://www.frontiersin.org/articles/10.3389/fnhum.2019.00294>
- 395 Dioso, E., Cerillo, J., Azab, M., Foster, D., Smith, I., Leary, O., Goutnik, M., & Lucke-Wold, B.
396 (2022). Subconcussion, Concussion, and Cognitive Decline: The Impact of Sports Related
397 Collisions. *Journal of Medical Research and Surgery*, 3(4), 54–63.
398 <https://doi.org/10.52916/jmrs224081>
- 399 Feddermann-Demont, N., Chiampas, G., Cowie, C. M., Meyer, T., Nordström, A., Putukian, M.,
400 Straumann, D., & Kramer, E. (2020). Recommendations for initial examination, differential
401 diagnosis, and management of concussion and other head injuries in high-level football.
402 *Scandinavian Journal of Medicine & Science in Sports*, 30(10), 1846–1858.
403 <https://doi.org/10.1111/sms.13750>
- 404 Garvey, M. A., Ziemann, U., Becker, D. A., Barker, C. A., & Bartko, J. J. (2001). New graphical
405 method to measure silent periods evoked by transcranial magnetic stimulation. *Clinical*
406 *Neurophysiology*, 112(8), 1451–1460. [https://doi.org/10.1016/S1388-2457\(01\)00581-8](https://doi.org/10.1016/S1388-2457(01)00581-8)
- 407 Goodall, S., Ross, E. Z., & Romer, L. M. (2010). Effect of graded hypoxia on supraspinal
408 contributions to fatigue with unilateral knee-extensor contractions. *Journal of Applied*
409 *Physiology*, 109(6), 1842–1851. <https://doi.org/10.1152/jappphysiol.00458.2010>
- 410 Hamel, R., Demers, O., Boileau, C., Roy, M.-L., Théoret, H., Bernier, P.-M., & Lepage, J.-F. (2022).
411 The neurobiological markers of acute alcohol's subjective effects in humans.
412 *Neuropsychopharmacology: Official Publication of the American College of*
413 *Neuropsychopharmacology*. <https://doi.org/10.1038/s41386-022-01354-w>
- 414 Hamel, R., Fontaine, É. D. L., Bernier, P.-M., & Lepage, J.-F. (2020). Letter to the editor: No
415 influence of static magnetic stimulation applied for 30 minutes over the human M1 on
416 corticospinal excitability. *Brain Stimulation: Basic, Translational, and Clinical Research in*
417 *Neuromodulation*, 13(3), 594–596. <https://doi.org/10.1016/j.brs.2019.12.001>
- 418 Koerte, I. K., Lin, A. P., Willems, A., Muehlmann, M., Hufschmidt, J., Coleman, M. J., Green, I.,
419 Liao, H., Tate, D. F., Wilde, E. A., Pasternak, O., Bouix, S., Rathi, Y., Bigler, E. D., Stern, R.

- 420 A., & Shenton, M. E. (2015). A Review of Neuroimaging Findings in Repetitive Brain
421 Trauma. *Brain Pathology*, 25(3), 318–349. <https://doi.org/10.1111/bpa.12249>
- 422 Kojima, S., Onishi, H., Sugawara, K., Kirimoto, H., Suzuki, M., & Tamaki, H. (2013). Modulation of
423 the cortical silent period elicited by single- and paired-pulse transcranial magnetic
424 stimulation. *BMC Neuroscience*, 14(1), 43. <https://doi.org/10.1186/1471-2202-14-43>
- 425 Lipton, M. L., Kim, N., Zimmerman, M. E., Kim, M., Stewart, W. F., Branch, C. A., & Lipton, R. B.
426 (2013). Soccer Heading Is Associated with White Matter Microstructural and Cognitive
427 Abnormalities. *Radiology*, 268(3), 850–857. <https://doi.org/10.1148/radiol.13130545>
- 428 Livingston, G., Huntley, J., Sommerlad, A., Ames, D., Ballard, C., Banerjee, S., Brayne, C., Burns,
429 A., Cohen-Mansfield, J., Cooper, C., Costafreda, S. G., Dias, A., Fox, N., Gitlin, L. N.,
430 Howard, R., Kales, H. C., Kivimäki, M., Larson, E. B., Ogunniyi, A., ... Mukadam, N.
431 (2020). Dementia prevention, intervention, and care: 2020 report of the Lancet Commission.
432 *The Lancet*, 396(10248), 413–446. [https://doi.org/10.1016/S0140-6736\(20\)30367-6](https://doi.org/10.1016/S0140-6736(20)30367-6)
- 433 McNabb, C., Reha, T., Georgieva, J., Jacques, A., Netto, K., & Lavender, A. P. (2020). The Effect of
434 Sub-Concussive Impacts during a Rugby Tackling Drill on Brain Function. *Brain Sciences*,
435 10(12), Article 12. <https://doi.org/10.3390/brainsci10120960>
- 436 Ntikas, M., Binkofski, F., Shah, N. J., & Ietswaart, M. (2022). Repeated Sub-Concussive Impacts and
437 the Negative Effects of Contact Sports on Cognition and Brain Integrity. *International
438 Journal of Environmental Research and Public Health*, 19(12), Article 12.
439 <https://doi.org/10.3390/ijerph19127098>
- 440 Potter, S., Leigh, E., Wade, D., & Fleminger, S. (2006). The Rivermead Post Concussion Symptoms
441 Questionnaire: A confirmatory factor analysis. *Journal of Neurology*, 253(12), 1603–1614.
442 <https://doi.org/10.1007/s00415-006-0275-z>
- 443 Russell, E. R., Mackay, D. F., Stewart, K., MacLean, J. A., Pell, J. P., & Stewart, W. (2021).
444 Association of Field Position and Career Length With Risk of Neurodegenerative Disease in
445 Male Former Professional Soccer Players. *JAMA Neurology*, 78(9), 1057–1063.
446 <https://doi.org/10.1001/jamaneurol.2021.2403>

- 447 Sandmo, S. B., Matyasova, K., Filipcik, P., Cente, M., Koerte, I. K., Pasternak, O., Andersen, T. E.,
448 Straume-Næsheim, T. M., Bahr, R., & Jurisica, I. (2022). Changes in circulating microRNAs
449 following head impacts in soccer. *Brain Injury*, *36*(4), 560–571.
450 <https://doi.org/10.1080/02699052.2022.2034042>
- 451 Scott, E., Kidgell, D. J., Frazer, A. K., & Pearce, A. J. (2020). The Neurophysiological Responses of
452 Concussive Impacts: A Systematic Review and Meta-Analysis of Transcranial Magnetic
453 Stimulation Studies. *Frontiers in Human Neuroscience*, *14*, 306.
454 <https://doi.org/10.3389/fnhum.2020.00306>
- 455 Stavrinou, E. L., & Coxon, J. P. (2016). High-intensity Interval Exercise Promotes Motor Cortex
456 Disinhibition and Early Motor Skill Consolidation. *Journal of Cognitive Neuroscience*, *29*(4),
457 593–604. https://doi.org/10.1162/jocn_a_01078
- 458 Tremblay, S., Beaulé, V., Proulx, S., Tremblay, S., Marjańska, M., Doyon, J., Lassonde, M., &
459 Théoret, H. (2014). Multimodal assessment of primary motor cortex integrity following sport
460 concussion in asymptomatic athletes. *Clinical Neurophysiology*, *125*(7), 1371–1379.
461 <https://doi.org/10.1016/j.clinph.2013.11.040>
- 462 Tremblay, S., Pascual-Leone, A., & Théoret, H. (2018). A review of the effects of physical activity
463 and sports concussion on brain function and anatomy. *International Journal of*
464 *Psychophysiology*, *132*, 167–175. <https://doi.org/10.1016/j.ijpsycho.2017.09.005>
- 465 Ueda, P., Pasternak, B., Lim, C.-E., Neovius, M., Kader, M., Forssblad, M., Ludvigsson, J. F., &
466 Svanström, H. (2023). Neurodegenerative disease among male elite football (soccer) players
467 in Sweden: A cohort study. *The Lancet. Public Health*, *8*(4), e256–e265.
468 [https://doi.org/10.1016/S2468-2667\(23\)00027-0](https://doi.org/10.1016/S2468-2667(23)00027-0)
- 469 Wong, W. (2020). Economic burden of Alzheimer disease and managed care considerations. *The*
470 *American Journal of Managed Care*, *26*(8 Suppl), S177–S183.
471 <https://doi.org/10.37765/ajmc.2020.88482>
- 472 Yamazaki, Y., Sato, D., Yamashiro, K., Nakano, S., Onishi, H., & Maruyama, A. (2019). Acute Low-
473 Intensity Aerobic Exercise Modulates Intracortical Inhibitory and Excitatory Circuits in an

474 Exercised and a Non-exercised Muscle in the Primary Motor Cortex. *Frontiers in Physiology*,
475 10. <https://www.frontiersin.org/articles/10.3389/fphys.2019.01361>
476 Ziemann, U., Reis, J., Schwenkreis, P., Rosanova, M., Strafella, A., Badawy, R., & Müller-Dahlhaus,
477 F. (2015). TMS and drugs revisited 2014. *Clinical Neurophysiology: Official Journal of the*
478 *International Federation of Clinical Neurophysiology*, 126(10), 1847–1868.
479 <https://doi.org/10.1016/j.clinph.2014.08.028>
480