1   Title: The primary motor cortex is critical for the retention of implicit sensorimotor

2   adaptation

3   Li-Ann Leow[1*$], Welber Marinovic[1,2$], Timothy J Carroll[1], Stephan Riek[1]

4

5   [1] Centre for Sensorimotor Performance, School of Human Movement and Nutrition Sciences,
6   Building 26B, The University of Queensland, Brisbane QLD 4072, Australia.

7   [2] Centre of Clinical Research Excellent in Spinal Pain, Injury & Health, School of Health and
8   Rehabilitation Sciences, Therapies Building 84a, The University of Queensland
9   Brisbane QLD 4072, Australia.

10 [$] equal first authors.

11 Corresponding author: l.leow@uq.edu.au

12 Corresponding author address: Centre for Sensorimotor Performance, School of Human
13 Movement and Nutrition Sciences, Building 26B, The University of Queensland, Brisbane
14 QLD 4072, Australia.

15

16 **Highlights**

17 • Adaptation of movements to perturbations occurs through explicit and implicit processes.

18 • Here, explicit strategies were suppressed by shortening movement preparation time.

19 • Perturbing motor cortex (M1) with TMS selectively impaired retention but not acquisition
20   of sensorimotor adaptation.

21 • M1 plays a crucial role in retention of sensorimotor adaptation obtained via implicit
22   learning.

23 **Abstract**

24   Sensorimotor adaptation, or adaptation of movements to external perturbations, is

25 thought to involve the primary motor cortex (M1). In addition to implicit error-driven

26 remapping, explicit re-aiming strategies also contribute to sensorimotor adaptation. However,

27 no studies to date have examined the role of M1 in implicit learning in isolation from explicit

28 strategies. Because the application of explicit strategies requires time, it is possible to

29 emphasise implicit learning by controlling the time available to prepare movement. Here, we

30 examined M1's role in implicit adaptation to rotated visual feedback whilst suppressing the

31 use of explicit re-aiming strategies by limiting movement preparation times to less than

32 350ms. Perturbing M1 activity via single-pulse TMS during adaptation to 30° rotation of

33 visual feedback did not alter the rate or extent of error compensation, but elicited poorer

34 retention in post-adaptation trials with no perturbation. This work shows that M1 is critical in

35 the retention of new visuomotor maps as a result of implicit adaptation to a perturbation in

36 sensory feedback when strategic error correction processes are suppressed.

37 *Keywords*: motor cortex, sensorimotor adaptation, implicit learning

38   Humans display remarkable flexibility in their ability to adapt to external

39   perturbations, such as external force-fields (Shadmehr & Mussa-Ivaldi, 1994), or distortions

40   of visual feedback (Welch, 1978; Cunningham, 1989). This form of learning, termed

41   sensorimotor adaptation, is retained even up to a year (Shadmehr & Brashers-Krug, 1997;

42   Landi *et al.*, 2011).

43   Converging evidence implicates a role for the primary motor cortex (M1) in the

44   formation of motor memories during sensorimotor adaptation. For example, non-invasively

45   altering M1 excitability alters the retention of learning in adaptation to force-field (Cothros *et*

46   *al.*, 2006; Richardson *et al.*, 2006; Hunter *et al.*, 2009; Herzfeld *et al.*, 2014) and visuomotor

47   rotation (Galea *et al.*, 2011; Riek *et al.*, 2012; Leow *et al.*, 2014; Villalta *et al.*, 2015). One

48   recent study demonstrated that tagging M1 activity but not posterior parietal cortex activity

49   with tDCS allows the concurrent learning of two opposite force-fields, which suggests that

50   M1 is crucial to the formation of distinct motor memories. Similarly, neural recordings in M1

51   in non-human primates demonstrate that sensorimotor adaptation evokes a persistent change

52   in the preferred direction of motor cortex neurons (Gandolfo *et al.*, 2000; Li *et al.*, 2001).

53   This change in the preferred direction persists despite returning motor output to the

54   unadapted state with no-perturbation trials (Mandelblat-Cerf *et al.*, 2011) and subsequent

55   adaptation to the same perturbation (Paz *et al.*, 2003) or to an opposing perturbation (Zach *et*

56   *al.*, 2012). How M1 plays a role in the encoding and retrieval of motor memories is less well

57   understood, however, a recent study in non-human primates suggest that the motor cortices

58   provide error signals that drive sensorimotor adaptation(Inoue *et al.*, 2016). Two key findings

59   from this study support this proposal. First, neural recordings in the motor cortices showed

60   greater M1 firing at a critical time window of 100 ms post-movement during sensorimotor

61   adaptation, suggestive of a role of M1 in encoding errors post-movement. Second, when these

62   M1 neurons were stimulated immediately after movement execution but not after this 100ms

63   post-movement interval, errors during sensorimotor adaptation increased.

64   In humans, errors imposed by external perturbations can be reduced either through (1)

65   explicit mechanisms, for example by strategically re-aiming clockwise to a target to

66   compensate for a counterclockwise rotation in visual feedback (Uhlarik, 1973; Mazzoni &

67   Krakauer, 2006; Benson *et al.*, 2011)*, or through (2) implicit mechanisms, where errors are

68   incrementally reduced in an automatic fashion (Squire, 1992; Redding & Wallace, 1996).

69   Explicit adaptive processes are used to correct errors to ensure success in the task, and their

70   contribution to behavior is modulated to accommodate the current extent of implicit

71   adaptation (Taylor *et al.*, 2014; McDougle *et al.*, 2015). It is therefore difficult to assess how

72   a factor such as brain stimulation affects implicit adaptation from performance when explicit

73   contributions are allowed. Thus, despite growing evidence demonstrating the role of M1 in

74   the formation of motor memories during sensorimotor adaptation, it remains unclear whether

75   M1 plays a role in the implicit and/or explicit mechanisms known to be active during

76   sensorimotor adaptation (Taylor *et al.*, 2014). One possibility is that M1 is primarily involved

77   in implicit mechanisms during sensorimotor adaptation. For example, M1 anodal tDCS only

78   increased the persistence of adapted movements when there was no reason to recruit explicit

79   mechanisms for error reduction (i.e., in when all feedback of the movement trajectory was

80   hidden), but not when it is beneficial to use explicit mechanisms to reduce errors (i.e., when

81   errors were revealed via feedback of the movement trajectory) (Galea *et al.*, 2011). Similarly,

82   perturbing M1 activity reduced the persistence of adapted movements only when the

83   perturbation was gradually imposed but not when the perturbation was abruptly imposed

84   (Hadipour-Niktarash *et al.*, 2007). Gradual perturbations typically evoke small errors that

85   often escape conscious awareness (Kagerer *et al.*, 1997), and thus are thought less likely to

86   evoke explicit processes such as strategic re-aiming. The role of M1 in the rate or extent of

87   implicit learning during visuomotor adaptation is not fully understood, as the majority of

88   studies do not explicitly dissociate between implicit and explicit learning during sensorimotor

89   adaptation. One way to isolate the contribution of implicit mechanisms in sensorimotor

90   adaptation is to suppress the use of explicit mechanisms by reducing the amount of time

91   available to prepare the movement (Haith *et al.*, 2015; Leow *et al.*, 2016). Although this

92   procedure does not necessarily eliminate explicit processes when there is deliberate intention

93   to re-aim, it appears sufficient to suppress the inclination to re-aim when targets are presented

94   in a large angular range (Leow *et al.*, 2016), such that the rate of error compensation when

95   movement preparation time is reduced is comparable to that of implicit learning estimated by

96   subtracting self-reported aiming directions from actual movement directions (Taylor *et al.*,

97   2014). In this study, we examine the role of M1 in acquisition and retention of sensorimotor

98   adaptation whilst suppressing the recruitment of explicit processes by reducing the amount of

99   time available for movement preparation. We hypothesized that perturbing M1 excitability

100   would affect retention but not the acquisition of sensorimotor adaptation when we suppressed

101   the use of explicit strategies.

102   **Methods**

103   Thirty-two right-handed participants (22 female, aged between 18-25 years old) with

104   no recent wrist, elbow or shoulder injuries volunteered for the study. Right-handedness was

105   confirmed with Edinburgh Handedness Inventory (Oldfield 1971). A medical questionnaire

106   was used to screen the participants for neurological disorders and contraindications in relation

107   to the application of TMS. The study was approved by the Medical Research Ethics

108   Committee of The University of Queensland. All participants were briefed on the

109   experimental procedures and gave written informed consent prior to the experiment, which

110   conformed to the Declaration of Helsinki.

111   **Experimental design**

112   Participants were randomly assigned into a 'STIM' or 'SHAM' groups in a counter-

113   balanced manner. In each group, the participants performed at total of 520 trials of isometric

114   force aiming tasks toward 8 radial targets on a computer screen located about 1 m away with

115   their right hand. Their right forearms were secured into a custom-made manipulandum,

116   described previously (de Rugy *et al.*, 2012), in the neutral wrist position (midway between

117   pronation and supination). Both elbows were kept at 110° with the forearm parallel to the

118   table and supported by the manipulandum. The wrists were fixed by a series of twelve

119   adjustable metal clamps contoured around the metacarpal-phalangeal joints and around the

120   wrist proximal to the radial head. Wrist forces in abduction-adduction and flexion-extension

121   directions were recorded via a six degree-of-freedom force transducer (JR3 45E15A-163-

122   A400N60S, Woodland, CA) attached to each manipulandum. Force data were sampled at a

123   rate of 2 kHz via two 16-bit National Instruments A/D boards (NI BNC2090A, NI USB6221,

124   National Instruments Corporation, USA). The forces exerted in flexion-extension and

125   abduction-adduction directions were displayed as a cursor that moved in two dimensions (x =

126   flexion-extension, y = abduction-adduction) on the computer screen via a custom written

127   Matlab program. During the experiment, participants received 240 stimuli at the left motor

128   cortex for the 'STIM' group or at the vertex for the 'SHAM' group out of the 520 trials of

129   isometric force aiming tasks. Stimulation onset was timed to when the movement extent

130   reached the distance between the start and the target, similar to previous work where TMS

131   onset was timed to the trial end (Hadipour-Niktarash *et al.*, 2007)

132   **Isometric force aiming tasks**

133   Participants were required to move a cursor that represented the resultant force

134   exerted at the wrist joint in 2 degrees of freedom (ab-adduction versus flexion-extension) to 8

135   radial targets which were 45° apart for 520 trials. Target location was randomized. Before

136   starting the experiment, participants completed at least 16 trials (2 trials per target) to

137   familiarize themselves with the target-aiming task. The experiment consisted of 4 blocks,

138   which proceeded as follows. (1) **Pre**-adaptation: 160 trials with no rotation of visual feedback.

139   (2) **Adaptation**: 240 trials with a 30° of rotation of visual feedback of the cursor position,

140   either clockwise or counter-clockwise relative to the center of the start circle. For example,

141   when an upward movement was made toward a target with radial deviation in the wrist,

142   participants in the clockwise rotation group experienced a rightward deviation of the visual

143   feedback of the cursor for a clockwise 30° visual rotation. Likewise, participants in the

144   counterclockwise rotation group experienced a leftward deviation of the cursor for a counter-

145   clockwise 30° visual rotation for the same upward target. (3) **No-feedback**: 40 trials with no

146   visual feedback of cursor position, and no rotation of visual feedback—unlike in our recent

147   work (Leow *et al.*, 2016), participants were not informed that the rotation was removed, in

148   order to ensure close adherence to the previous M1 TMS study (Hadipour-Niktarash *et al.*,

149   2007), and (4) **Post-**adaptation: visual feedback of cursor position with no rotation.

150   Each trial began with a visual word cue "Relax" to prompt participants to relax their

151   right forearm. A cursor corresponding to their wrist forces was displayed within a red circle

152   indicating the origin of the computer screen. The participants were provided with 4 beats of

153   audio cue and required to initiate cursor movement toward the target as quickly as possible

154   on the 4th beat. The targets appeared as green circles at 75 % of the distance from the origin

155   to the edge of the computer screen (10 cm) at 300 ms prior to the 4th audio beat. Participants

156   were given post-trial feedback on whether the movements were too early (<150ms) or too late

157   (>350ms) to encourage consistent movement onsets and, as a result, consistent preparation

158   time. We selected 350ms as a cutoff because piloting showed that it was very difficult to

159   move accurately to the target using the wrist manipulandum at briefer cutoff times. The

160   cursor gain was set such that 20 N was required to reach the edge of the screen. The 8 targets

161   appeared in a random order within every cycle of 8 trials to prevent participants from

162   anticipating the location of upcoming target.

163   **Surface electromyography recordings**

164 Electromyography (EMG) signals were recorded from the flexor carpi radialis (FCR)

165 and extensor carpi radialis brevis (ECRb) of the right forearm. Standard skin preparation was

166 performed after the muscles were located and marked. Bipolar Ag/AgCl surface electrodes

167 placed on the belly of the forearm muscles with an inter-electrode distance of 2 cm (centre to

168 centre). The EMG signals were amplified with a gain of 500 ~ 1000 with Grass P511

169 amplifiers (Grass Instruments, AstroMed, West Warwick, RI) and band-pass filtered (10 Hz -

170 1 kHz).

171

Transcranial magnetic stimulation

173 Single-pulse TMS was used to elicit motor evoked potential (MEP) responses from

174 the FCR and ECR muscles via a 70 mm outer diameter figure-of-eight magnetic coil

175 (Magstim 200, Magstim, UK) over the forearm area of the left motor cortex. The magnetic

176 coil was held tangentially on the scalp with the handle pointing backwards and 45° away

177 from mid-sagittal axis inducing a current in the posterior-anterior direction. An optimal

178 location that elicited the largest and most consistent MEP response was determined and

179 marked with felt-tip pen to ensure consistent stimulation throughout the experiment. The

180 resting motor threshold (RMT) for each participant was determined as the minimum stimulus

181 intensity that produced a MEP response of approximately 50 µV in 5 out of 10 consecutive

182 trials while the right forearm was at rest in the manipulandum. The testing intensity was set to

183 120% of each participant's RMT.

Data analysis

185 Data reduction was performed using custom Matlab software (Mathworks). Forces in

186 *x* and *y* axes were transformed to screen coordinates (e.g. cursor position) and filtered using a

187 low-pass 2nd order Butterworth filter with a cut-off frequency of 10Hz. Movement onsets

188 were estimated from the tangential speed time series (Teasdale *et al.*, 1993). Movement

189 direction was computed as the angle between the initial position of the cursor at movement

190 onset and its position 100 ms later, which is sufficient to prevent the use of online feedback

191 mechanisms to correct cursor trajectory (Desmurget & Grafton, 2000).

192 Statistical analyses were run on cycle-averaged data, with 1 cycle defined as 8 trials.

193 The dependent variable of interest was directional error, which was defined as the difference

194 between movement direction and target direction. Intersubject differences in baseline

195 directional biases (Ghilardi *et al.*, 1995), are known to affect adaptation behaviour [36-38].

196 Directional errors were bias-corrected by subtracting directional errors from the mean of the

197 first 6 cycles (we did not bias correct using the last baseline cycle (Cycle 20) here, because

198 previous research has shown that directional biases reduce with training. Unlike previous

199 studies which had fewer baseline cycles than in this study(Taylor *et al.*, 2014). Qualitatively

200 similar results were obtained when we estimated baseline biases from baseline cycle 6, which

201 is what we did in our previous work (Leow *et al.*, 2016). In the adaptation, no-feedback, and

202 washout blocks, only movements that were within 120° of the target (i.e., 60° clockwise or

203 counterclockwise of the target) were included for statistical analyses. This outlier removal

204 procedure excluded less than 2.5% of the data for each phase. Stimulation (sham, stimulation)

205 x Rotation Direction (CCW, CW) x Cycle ANOVAs were run on each block. For all

206 ANOVAs, we used generalized eta-squares (rather than partial eta-squares) to estimate effect

207 sizes. Eta-squares between 0.01 to 0.06 were considered small, eta-squares greater than 0.06

208 but smaller than 0.14 were considered medium, and eta-squares in excess of 0.14 were

209 considered large (Cohen, 1988).

210   To examine the rate of adaptation without the possible confound of intrinsic bias in

211 movement direction (Hardwick & Celnik, 2014), we also fit cycle-averaged movement

212 directions for each dataset to a single-rate exponential function (Zarahn *et al.*, 2008), as

213 follows:

$$y = y_0 \, e^{-kx} + a$$

214   where y is the movement direction, x is the trial number, k is the rate constant that

215 indicates the rate with which movement direction changes, a is the movement direction at

216 which performance reaches asymptote, and y0 + a is the hypothetical y value when x is zero.

217 One dataset failed to fit to the curve. Independent samples t-tests were used to compare rate

218 constants from the sham and the stimulation group.

219   We also examined the rate of decay in the no-feedback block by fitting trial-by-trial

220 movement directions for the no-feedback block to a straight line, as follows:

221   y= YIntercept + slope*x

222   where y is the movement direction, x is the trial number, slope is the rate constant that

223   indicates the rate with which movement direction changes, and YIntercept is the hypothetical

224   y value when x is zero. We did not use cycle-averaged movement directions for the no-

225   feedback block as there were only 5 no-feedback cycles, and this was insufficient data to fit

226   to the straight line. Independent samples t-tests were used to compare slopes from the sham

227   and the stimulation group.

228   To additionally guard against the possibility that individual differences in intrinsic

229   movement biases influenced statistical comparisons between the stim and the sham TMS

230   groups, we also quantified adaptation behaviour as a change in performance within each

231   block. For directional error data, this was quantified as the difference in directional errors

232   from the first cycle of each block to the last cycle of each block.

233   To test whether participants complied to the preparation time constraints throughout

234   learning, we evaluated temporal error for the preparation time, defined as the error in time

235   between target appearance and the time of movement onset. To guard against the possibility

236   that any differences between the stim and the sham TMS groups resulted from individual

237   differences in baseline ability to adhere to the preparation time constraints, we also quantified

238   the change in preparation time within each block as the difference in preparation time from

239   the first cycle to the last cycle of each block. Group differences on these change scores were

240   tested using unequal variance t-tests (Welch's t-test) to compare changes in directional error

241   and preparation time across all phases of the experiment. The 95% confidence intervals (CI)

242   were used for pairwise differences.

243   **Results**

244   We first used t-tests to verify whether initial performance between groups differed in

245   terms of directional error or preparation time at the beginning of each phase of the

246   experiment. We found no differences in terms of mean directional error (all $Ps > 0.38$) or

247   preparation time (all $Ps > 0.13$) across all experimental phases. These results indicate the

248   sham and the stim groups did not differ reliably in initial performance at the beginning of the

249   experimental phases.

250   Figure 1 shows cycle-averaged directional errors for each phase (1 cycle=8 trials).

251   Each block was split into 2 sub-blocks for analysis (early (cycles 1-15) and late (cycles 15-

252   30). Stimulation (sham, stimulation) x Rotation Direction (CCW, CW) x Cycle x Phase (early,

253   late) ANOVAs were run. There was no significant main effect of stimulation, and stimulation

254   did not interact significantly with any other factor (all p>0.3). Analyses of rate constants

255   obtained from fitting the single-exponential function to individual datasets yielded similar

256   results (see Figure 1B)—sham and stim M1 TMS groups did not differ reliably in rate of

257   error reduction in the adaptation phase t(29) =0.17, p=.86, cohen's d=.06, small effect size).

258   In the first cycle of the no-feedback phase, movements remained in the adapted state,

259   resulting in large errors in the opposite direction to the rotation indicating retention of the

260   learning. Consistent with previous findings(Kitago *et al.*, 2013), despite the absence of visual

261   feedback of the movement, and despite the experimenter not providing explicit knowledge

262   that the rotation had been removed, movements decayed with increasing cycles, as there was

263   a significant main effect of cycle F(4,112)=6.60, p <0.001, eta-squared = .17, as movements

264   decayed across cycles. M1 stimulation resulted in poorer retention in this no-feedback block,

265   as shown in faster decay of movements to the unadapted state in the retention block (see

266   Figure 1C), as a Stimulation (Sham, Stim) by Cycle (1-5) by Rotation Direction (CW, CCW)

267   ANOVA revealed a significant Stimulation x Cycle interaction. F(4, 112)=2.79, p = .03, eta-

268   square=.09 (moderate effect size). The rate of decay in the no-feedback phase (as shown by

269   slopes fit to trial-by-trial data) was faster for the stimulation group than the sham stimulation

270   group, as slopes were significantly more negative in the stimulation group than the sham

271   stimulation group, t(30) = 2.83, p =.005, cohen's d= 0.96 (large effect size). After the no-

272   feedback phase, visual feedback was returned in the post-adaptation phase.

273   Close inspection of Figure 1A suggests that unlike participants who received sham

274   TMS, participants who received M1 TMS showed an apparent rebound in errors upon return

275   of visual feedback in the washout phase (i.e., despite the greater decay of adaptation in the

276   no-feedback block, they showed a return to movements that were more adapted to the

277   rotation). One possibility is that the return of visual feedback acted as a contextual cue for

278   retrieval of the motor memory that transiently decayed as a result of disrupting M1 activity.

279   To test this, we ran a Stimulation (Sham, Stim) by Cycle (Last No Feedback cycle, First

280   Washout Cycle) by Rotation Direction (CW, CCW) ANOVA. However, the Cycle x

281   Stimulation interaction was not reliable, F(1, 28)=2.67, p =.11, although this was a moderate

282   effect size (eta-squared =.07), and the main effect of stimulation was also not reliable (p=.38),

283   To evaluate the effect of stimulation for the washout block, we ran a Stimulation (Sham, Stim)

284 by Cycle (1-10) by Rotation Direction (CW, CCW) ANOVA. The main effect of stimulation

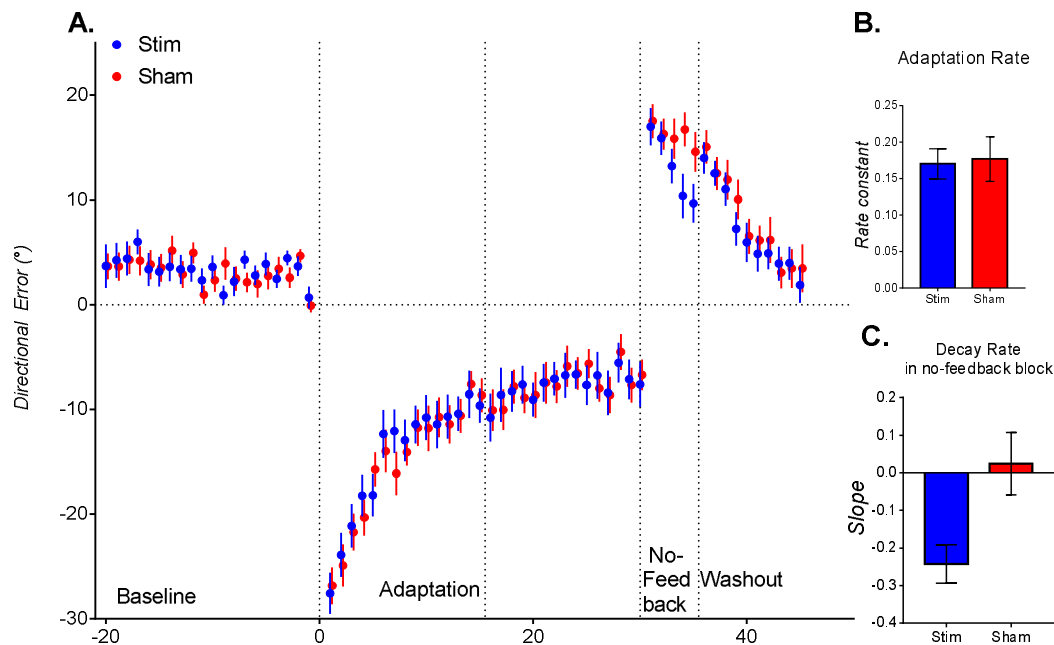285 was not reliable, and did not interact reliably with any factor (all p>0.3s).

286

287 **Figure 1. A:** Group mean cycle-averaged directional errors for the group who encountered
288 sham TMS pulses (1 cycle= 8 trials). Error bars indicate standard errors of the mean. Data
289 from the clockwise rotation group were sign-transformed to allow statistical comparisons
290 between clockwise and counterclockwise groups. Data from the adaptation phase, no-
291 feedback phase, and the washout phase were bias corrected by subtracting estimated intrinsic
292 bias (i.e., mean movement direction from the first 6 baseline cycles). Note that for the no-
293 feedback block, greater decay was evident in the STIM group who received single-pulse
294 TMS on the estimated location of M1 during adaptation than the SHAM group who received
295 TMS on the vertex. **B:** Adaptation rate, as estimated by rate constants fit to cycle-averaged
296 individual data from the adaptation block. **C.** Decay rate of trial-by-trial errors from the no
297 feedback block for the sham group (blue bar) and the stim group (red bar). Error bars indicate
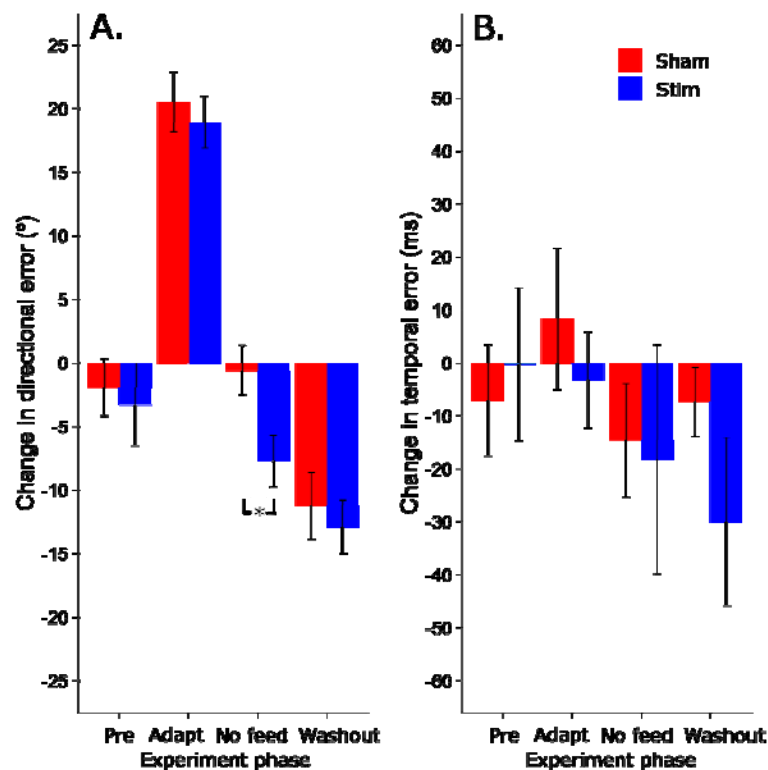298 standard errors of the mean.

299

300

Figure 2A - Mean changes in directional error (mean of first 4 trials – mean of last 4 trials) across all phases of the experiment. **2B** - Mean changes in temporal errors in preparation time (First 4 trials - Last 4 trials) across all phases of the experiment. *Mark statistically significant differences between groups. Error bars are 95% CI.

As shown in Figure 2A, the analysis of change in directional error detected a statistically reliable difference between groups in the Retention block ($t_{29.96} = 2.57$, $p = 0.015$, 95% CI [-1.49, 12.87]), indicating that the group receiving TMS stimulation retained less of the original adaptation without visual feedback of the cursor position. T-tests failed to detect statistically reliable differences between groups in all other phases of the experiment (Pre: $t_{26.74} = 0.34$, $p = 0.73$, 95% CI [-6.75, 9.43]; Adaptation: $t_{29.41} = 0.51$, $p = 0.61$, 95% CI [-4.77, 7.93]; Post: $t_{28.66} = 0.49$, $p = 0.62$, 95% CI [-5.17, 8.51]). These results suggest M1 disruption sped up the decay of adapted movements, suggesting that M1 impaired retention of sensorimotor adaptation.

Figure 2B shows the changes in temporal errors for preparation time across all phases of the experiment. T-tests found no reliable differences between groups for any pairwise comparisons on preparation time (Pre: $t_{27.28} = 0.37$, $p = 0.71$, 95% CI [-43.38, 29.88]; Adaptation: $t_{26.39} = 0.71$, $p = 0.48$, 95% CI [-21.60, 44.69]; Retention: $t_{21.88} = 0.14$, $p = 0.88$,

319   95% CI [-46.46, 53.53]; Post: $t_{19.85} = 1.32$, $p = 0.20$, 95% CI [-13.04, 58.23]). These results

320   indicate that any differences - or the lack thereof - in terms of directional error are unlikely to

321   be due to differences in ability to adhere to the preparation time restraints.

Discussion

323   Here, we show that when explicit re-aiming is likely suppressed by shortening

324   movement preparation time, perturbing M1 during sensorimotor adaptation does not affect

325   the rate and extent of error compensation, but elicits greater decay of adapted movements.

326   This shows that M1 plays a key role in *retention* of sensorimotor adaptation acquired via

327   implicit mechanisms. Although it is possible that constraining movement preparation time

328   alone might not be sufficient to completely eliminate the employment of explicit strategies,

329   our preparation time constraints appeared sufficient to reduce explicit strategy use to reveal

330   implicit sensorimotor adaptation, in line with previous work showing that constraining

331   preparation time results in slower error compensation typically indicative of reduced explicit

332   strategy use, revealing implicit mechanisms in sensorimotor adaptation (Fernandez-Ruiz *et*

333   *al.*, 2011; Haith *et al.*, 2015). This work adds to existing evidence (Hadipour-Niktarash *et al.*,

334   2007) supporting the hypothesis that M1 plays a role in implicit mechanisms of sensorimotor

335   adaptation. In that study, M1 TMS impaired the retention of adapted movements with a

336   gradually imposed perturbation, but not an abruptly imposed perturbation (note however that

337   the stimulation protocol differed for the gradual and the abrupt perturbation—stimulation was

338   applied in the pre-rotation phase for the abrupt condition but was applied in the rotation phase

339   for the gradual perturbation) (Hadipour-Niktarash *et al.*, 2007). Such gradual perturbations

340   evoke small errors which typically escape conscious awareness and thus are less likely to

341   evoke strategic re-aiming. The abrupt perturbation was likely to have evoked explicit re-

342   aiming, which should have sped up error compensation, and could have masked effects of M1

343   TMS on underlying implicit processes. However, the current data indicate that TMS to M1

344   does not impair acquisition of visuomotor adaptation even when explicit re-aiming is likely

345   suppressed by constraining preparation time conditions.

346   It is worth noting that gradual and abrupt perturbations not only evoke differences in

347   conscious awareness of the perturbation, but also differences in the amount of movement

348   repetition of the adapted movement, as gradually imposed perturbations necessarily result in

349   less repetition of the fully adapted movement than abrupt perturbations. One might argue that

350   our findings of faster decay of adaptation learning when M1 activity was disrupted by TMS

351   are driven by its effects on repetition-dependent effects (i.e., on use-dependent plasticity,

352   where repetition of a movement induces a bias in subsequent movement toward that repeated

353   movement) (Classen *et al.*, 1998). Previous studies have demonstrated that modulating M1

354   excitability selectively alters sensorimotor adaptation when repetition of the adapted

355   movements are allowed. For example, one previous study (Orban de Xivry *et al.*, 2011)

356   showed that the extent of error compensation was reduced by perturbing M1 excitability

357   when the perturbation schedule afforded extended repetition of the adapted movement, but

358   not when repetition was constrained. Another study showed that M1 excitability increased

359   when an abrupt perturbation schedule encouraged extended repetition of the adapted

360   movement, but not when a gradual perturbation schedule reduced repetition of the adapted

361   movement [39]. Similarly, altering M1 excitability with tDCS only changed the persistence

362   of adapted movements with extended repetition of the adapted movement, and not with

363   limited repetition of the adapted movement (Leow *et al.*, 2014)—this effect was

364   demonstrated when target manipulation was used to ensure extended repetition of the adapted

365   movement in a gradual perturbation schedule, suggesting that the effect of M1 stimulation in

366   that study was not due to the difference in the size of the error from abrupt and gradual

367   perturbation schedules, but rather due to the amount of repetition of the adapted movement.

368   However, although we cannot preclude the possibility that use-dependent plasticity effects

369   contributed to our current results, we suggest that our results are not solely driven by use-

370   dependent effects, as movements were made towards eight randomly presented targets,

371   limiting continuous repetition of the adapted movement towards any one target.

372   We note that M1 TMS may also affect processing in remote brain regions (Bestmann *et*

373   *al.*, 2004) known to be involved in sensorimotor adaptation such as the cerebellum, either

374   directly via functional interconnections (e.g.,(Ugawa *et al.*, 1991)), or indirectly due to

375   compensatory mechanisms (Lomber, 1999). However, given the weight of previous findings

376   from multiple paradigms (brain stimulation, neural recordings, neuroimaging) (Gandolfo *et*

377   *al.*, 2000; Li *et al.*, 2001; Paz *et al.*, 2003; Cothros *et al.*, 2006; Richardson *et al.*, 2006;

378   Hunter *et al.*, 2009; Galea *et al.*, 2011; Mandelblat-Cerf *et al.*, 2011; Riek *et al.*, 2012; Zach

379   *et al.*, 2012; Herzfeld *et al.*, 2014; Leow *et al.*, 2014; Panouilleres *et al.*, 2015; Villalta *et al.*,

380   2015) suggesting involvement of M1 in formation of motor memories in sensorimotor

381   adaptation, we think that the most parsimonious account of our findings is that M1 is

382   involved in retention of sensorimotor adaptation, even when explicit processes were

383   suppressed with short preparation times.

384   Finally, it is important to note that our findings that M1 contributes to implicit learning

385   do not preclude the possibility that M1 also contributes to explicit learning processes. For

386   example, in humans, increasing M1 excitability improves error compensation when using

387   large perturbations (60°) which engage explicit processes (Panouilleres *et al.*, 2015).

388   Similarly, reducing M1 excitability also slowed reaction times when participants were

389   reducing large errors and impaired error compensation when relearning the same perturbation

390   (i.e., impaired savings) (Riek *et al.*, 2012). Several studies have shown that altering M1

391   excitability alters savings (Richardson *et al.*, 2006; Panouilleres *et al.*, 2015), which has

392   recently been proposed to be at least partly driven by explicit mechanisms (Haith *et al.*, 2015;

393   Morehead *et al.*, 2015).

394

395 **References**

396 Benson, B.L., Anguera, J.A. & Seidler, R.D. (2011) A spatial explicit strategy reduces error
397   but interferes with sensorimotor adaptation. *J. Neurophysiol.*, **105**, 2843-2851.
398

399 Bestmann, S., Baudewig, J., Siebner, H.R., Rothwell, J.C. & Frahm, J. (2004) Functional
400   MRI of the immediate impact of transcranial magnetic stimulation on cortical and
401   subcortical motor circuits. *Eur. J. Neurosci.*, **19**, 1950-1962.
402

403 Classen, J., Liepert, J., Wise, S.P., Hallett, M. & Cohen, L.G. (1998) Rapid plasticity of
404   human cortical movement representation induced by practice. *Journal of*
405   *Neurophysiology*, **79**, 1117-1123.
406

407 Cohen, J. (1988) *Statistical power analysis for the behavioral sciences*. Lawrence Erlbaum
408   Associates, New Jersey.
409

410 Cothros, N., Ko, S., Dickie, E.W., Mirsattari, S.M. & Gribble, P.L. (2006) Proactive
411   Interference as a Result of Persisting Neural Representations of Previously Learned
412   Motor Skills in Primary Motor Cortex. *J. Cognit. Neurosci.*, 2167-2176.
413

414 Cunningham, H.A. (1989) Aiming error under transformed spatial mappings suggests a
415   structure for visual-motor maps. *J Exp Psychol Hum Percept Perform*, **15**, 493-506.
416

417 de Rugy, A., Loeb, G.E. & Carroll, T.J. (2012) Muscle Coordination Is Habitual Rather than
418   Optimal. *Journal of Neuroscience*, **32**, 7384-7391.
419

420 Desmurget, M. & Grafton, S. (2000) Forward modeling allows feedback control for fast
421   reaching movements. *Trends in Cognitive Sciences*, **4**, 423-431.
422

423 Fernandez-Ruiz, J., Wong, W., Armstrong, I.T. & Flanagan, J.R. (2011) Relation between
424   reaction time and reach errors during visuomotor adaptation. *Behav. Brain Res.*, **219**,
425   8-14.
426

427 Galea, J.M., Vazquez, A., Pasricha, N., de Xivry, J.J. & Celnik, P. (2011) Dissociating the
428   roles of the cerebellum and motor cortex during adaptive learning: the motor cortex
429   retains what the cerebellum learns. *Cereb. Cortex*, **21**, 1761-1770.
430

431 Gandolfo, F., Li, C.S.R., Benda, B.J., Padoa Schioppa, C. & Bizzi, E. (2000) Cortical
432   correlates of learning in monkeys adapting to a new dynamical environment.
433   *Proceedings of the National Academy of Sciences of the United States of America*, **97**,
434   2259-2263.
435

436 Ghilardi, M.F., Gordon, J. & Ghez, C. (1995) Learning a visuomotor transformation in a local
437   area of work space produces directional biases in other areas. *Journal of*
438   *Neurophysiology*, **73**, 2535-2539.
439

440 Hadipour-Niktarash, A., Lee, C.K., Desmond, J.E. & Shadmehr, R. (2007) Impairment of
441   Retention But Not Acquisition of a Visuomotor Skill Through Time-Dependent
442   Disruption of Primary Motor Cortex. *J. Neurosci.*, **27**, 13413-13419.
443

444 Haith, A.M., Huberdeau, D.M. & Krakauer, J.W. (2015) The influence of movement
445   preparation time on the expression of visuomotor learning and savings. *The Journal of*
446   *neuroscience : the official journal of the Society for Neuroscience*, **35**, 5109-5117.
447

448 Hardwick, R.M. & Celnik, P.A. (2014) Cerebellar direct current stimulation enhances motor
449   learning in older adults. *Neurobiol. Aging*, **35**, 2217-2221.
450

451 Herzfeld, D.J., Pastor, D., Haith, A.M., Rossetti, Y., Shadmehr, R. & O'Shea, J. (2014)
452   Contributions of the cerebellum and the motor cortex to acquisition and retention of
453   motor memories. *Neuroimage*, **98**, 147-158.
454

455 Hunter, T., Sacco, P., Nitsche, M.A. & Turner, D.L. (2009) Modulation of internal model
456   formation during force field-induced motor learning by anodal transcranial direct
457   current stimulation of primary motor cortex. *The Journal of physiology*, **587**, 2949-
458   2961.
459

460   Inoue, M., Uchimura, M. & Kitazawa, S. (2016) Error Signals in Motor Cortices Drive
461   Adaptation in Reaching. *Neuron*, **90**, 1114-1126.
462

463   Kagerer, F.A., ContrerasVidal, J.L. & Stelmach, G.E. (1997) Adaptation to gradual as
464   compared with sudden visuo-motor distortions. *Exp. Brain Res.*, **115**, 557-561.
465

466   Kitago, T., Ryan, S.L., Mazzoni, P., Krakauer, J.W. & Haith, A.M. (2013) Unlearning versus
467   savings in visuomotor adaptation: Comparing effects of washout, passage of time and
468   removal of errors on motor memory. *Frontiers in Human Neuroscience*.
469

470   Landi, S.M., Baguear, F. & Della-Maggiore, V. (2011) One Week of Motor Adaptation
471   Induces Structural Changes in Primary Motor Cortex That Predict Long-Term
472   Memory One Year Later. *The Journal of Neuroscience*, **31**, 11808-11813.
473

474   Leow, L.-A., Marinovic, W., Gunn, R. & Carroll, T.J. (2016) Estimating the implicit
475   component of visuomotor rotation learning by constraining movement preparation
476   time. *bioRxiv*, 082420.
477

478   Leow, L.A., Hammond, G. & de Rugy, A. (2014) Anodal motor cortex stimulation paired
479   with movement repetition increases anterograde interference but not savings. *The
480   European journal of neuroscience*, **40**, 3243-3252.
481

482   Li, C.S.R., Padoa-Schioppa, C. & Bizzi, E. (2001) Neuronal correlates of motor performance
483   and motor learning in the primary motor cortex of monkeys adapting to an external
484   force field. *Neuron*, **30**, 593-607.
485

486   Lomber, S.G. (1999) The advantages and limitations of permanent or reversible deactivation
487   techniques in the assessment of neural function. *Journal of Neuroscience Methods*, **86**,
488   109-117.
489

490   Mandelblat-Cerf, Y., Novick, I., Paz, R., Link, Y., Freeman, S. & Vaadia, E. (2011) The
491   neuronal basis of long-term sensorimotor learning. *Journal of Neuroscience*, **31**, 300-
492   313.
493

494   Mazzoni, P. & Krakauer, J.W. (2006) An implicit plan overrides an explicit strategy during
495   visuomotor adaptation. *Journal of Neuroscience*, **26**, 3642-3645.
496

497   McDougle, S.D., Bond, K.M. & Taylor, J.A. (2015) Explicit and implicit processes constitute
498   the fast and slow processes of sensorimotor learning. *The Journal of Neuroscience*, **35**,
499   9568-9579.
500

501   Morehead, J.R., Qasim, S.E., Crossley, M.J. & Ivry, R. (2015) Savings upon Re-Aiming in
502   Visuomotor Adaptation. *The Journal of neuroscience : the official journal of the*
503   *Society for Neuroscience*, **35**, 14386-14396.
504

505   Orban de Xivry, J.J., Criscimagna-Hemminger, S.E. & Shadmehr, R. (2011) Contributions of
506   the motor cortex to adaptive control of reaching depend on the perturbation schedule.
507   *Cereb. Cortex*, **21**, 1475-1484.
508

509   Panouilleres, M.T., Joundi, R.A., Brittain, J.S. & Jenkinson, N. (2015) Reversing motor
510   adaptation deficits in the ageing brain using non-invasive stimulation. *J Physiol*, **593**,
511   3645-3655.
512

513   Paz, R., Boraud, T., Natan, C., Bergman, H. & Vaadia, E. (2003) Preparatory activity in
514   motor cortex reflects learning of local visuomotor skills. *Nat. Neurosci.*, **6**, 882-890.
515

516   Redding, G.M. & Wallace, B. (1996) Adaptive Spatial Alignment and Strategic Perceptual-
517   Motor Control. **22**.
518

519   Richardson, A.G., Overduin, S.A., Valero-Cabré, A., Padoa-Schioppa, C., Pascual-Leone,
520   A., Bizzi, E. & Press, D.Z. (2006) Disruption of primary motor cortex before learning
521   impairs memory of movement dynamics. *Journal of Neuroscience*, **26**, 12466-12470.
522

523   Riek, S., Hinder, M.R. & Carson, R.G. (2012) Primary motor cortex involvement in initial
524   learning during visuomotor adaptation. *Neuropsychologia*, **50**, 2515-2523.
525

526   Shadmehr, R. & Brashers-Krug, T. (1997) Functional stages in the formation of human long-
527   term motor memory. *Journal of Neuroscience*, **17**, 409-419.

528

529 Shadmehr, R. & Mussa-Ivaldi, F.A. (1994) Adaptive representation of dynamics during
530 learning of a motor task. *Journal of Neuroscience*, **14**, 3208-3224.

531

532 Squire, L.R. (1992) Declarative and nondeclarative memory: Multiple brain systems
533 supporting learning and memory. *J. Cognit. Neurosci.*, **4**, 232-243.

534

535 Taylor, J.A., Krakauer, J.W. & Ivry, R.B. (2014) Explicit and implicit contributions to
536 learning in a sensorimotor adaptation task. *The Journal of neuroscience : the official*
537 *journal of the Society for Neuroscience*, **34**, 3023-3032.

538

539 Teasdale, N., Bard, C., Fleury, M., Young, D.E. & Proteau, L. (1993) Determining movement
540 onsets from temporal series. *Journal of motor behavior*, **25**, 97-106.

541

542 Ugawa, Y., Day, B.L., Rothwell, J.C., Thompson, P.D., Merton, P.A. & Marsden, C.D. (1991)
543 Modulation of motor cortical excitability by electrical stimulation over the cerebellum
544 in man. *Journal of Physiology*, **441**, 57-72.

545

546 Uhlarik, J.J. (1973) Role of cognitive factors on adaptation to prismatic displacement. *J Exp*
547 *Psychol*, **98**, 223-232.

548

549 Villalta, J.I., Landi, S.M., Fló, A. & Della-Maggiore, V. (2015) Extinction interferes with the
550 retrieval of visuomotor memories through a mechanism involving the sensorimotor
551 cortex. *Cerebral Cortex*, **25**, 1535-1543.

552

553 Welch, R.B. (1978) *Perceptual modification: Adapting to altered sensory environments*.
554 Elsevier.

555

556 Zach, N., Inbar, D., Grinvald, Y. & Vaadia, E. (2012) Single neurons in M1 and premotor
557 cortex directly reflect behavioral interference. *PLoS ONE*, **7**.

558

559 Zarahn, E., Weston, G.D., Liang, J., Mazzoni, P. & Krakauer, J.W. (2008) Explaining savings
560 for visuomotor adaptation: linear time-invariant state-space models are not sufficient.
561 *J. Neurophysiol.*, **100**, 2537-2548.

562

563