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Age-related decline in cortical inhibitory tone strengthens motor memory

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Ageing causes a natural decline in cortical inhibitory tone and associated functional decrements. However, in young adults, experimentally lowering cortical inhibition during adaptation enhances retention. Here we tested the hypothesis that as sensorimotor cortex inhibitory tone decreases naturally with age, adaptation memory would increase. As predicted, older age was associated with lower γ-amino butyric acid (GABA), the inhibitory neurotransmitter, and stronger adaptation memory. Mediation analyses confirmed that the former explained the latter. To probe causality, brain stimulation was used to further lower sensorimotor cortical inhibitory tone during adaptation. Across individuals, stimulation enhanced or impaired memory, as a function of sensorimotor cortical excitation:inhibition ratio (E:I

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= Glutamate:GABA). Stimulation increased retention in individuals with low E:I, but disrupted it in those with high E:I. Thus, we identify a form of memory that improves naturally with age, depends causally on sensorimotor neurochemistry, and may be a potent target for neurorehabilitation.

Introduction

Motor capacities decline with age[1,2]. As the brain and body become older, movements lose speed[3,4], strength[5] and coordination[6]. This natural loss of function is exacerbated by motor disorders which rise sharply with age (e.g., stroke, sarcopenia, Parkinsonism). As the elderly population increases[7], there is a need for strategies to counteract and compensate for age-related motor decline.

During ageing, the motor system must adapt continuously to ongoing neuro-musculo-skeletal change. Brain plasticity enables this. Plasticity is essential to learn new motor skills, adapt and retain existing ones, and to rehabilitate functions impaired by disease[8,9]. Thus plasticity plays an important role in mitigating age-related motor decline[10,11].

Unfortunately, plasticity also declines with age[12], especially in the motor domain[13–15]. A major cause is the dysregulation of the finely tuned balance between cortical excitation and inhibition (E:I)[10]. Across cortex, E:I is disrupted because γ-aminobutyric acid (GABA), the major inhibitory neurotransmitter, declines with age, both in animals[16,17] and humans[15,18–26]. Regional decline of cortical GABA causes a loss of inhibitory tone, and this is associated with decrements in

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functions localized to the affected regions[27–29]. For example, in somatosensory cortex higher E:I is associated with poorer tactile discrimination, both in young and old adults [20,30]. In primary motor cortex (M1), age-related decline of inhibitory tone is associated with poorer upper-limb dexterity[23], postural imbalance[31,32], and impaired ability to suppress automatic responses[26].

By contrast, here we tested the hypothesis that, as M1 GABA declines with age, a specific form of upper limb functional plasticity would *increase*: adaptation memory. Across the lifespan, adaptation is that property of the motor system that enables individuals to compensate for perturbations by adjusting their movements to maintain motor success[33,34]. After a perturbation is removed, adaptation memory is expressed as an *after-effect* – a movement bias in the opposite direction. The strength of adaptation memory is indexed by the persistence of the after-effect. There is a wealth of evidence that adaptation is preserved (or somewhat impaired) in healthy ageing[35–45]. In our previous work, in young adults, we showed that experimentally lowering M1 inhibitory tone during adaptation increases persistence of the after-effect[46–48]. Here, we reasoned that if after-effect retention depends *causally* on M1 inhibitory tone, then owing to age-related M1 GABA decline, this form of memory would *increase naturally with age*.

This hypothesis was confirmed in a cross-sectional study of thirty-two healthy older adults (mean age: 67.46 years, *s.d.*: 8.07). Using magnetic resonance spectroscopy to quantify neurochemistry, we showed that M1 GABA declines with age. Using prism adaptation[49], we showed that retention increases with age. A mediation analysis subsequently confirmed that as GABA declines with age, memory increases, and the former explains the latter. To demonstrate causality,

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we intervened experimentally with excitatory anodal transcranial direct current stimulation - to try and further lower M1 GABA[50,51] and thus further increase memory. On average, stimulation did not increase memory. Rather, a moderation analysis showed that how stimulation changed memory depended on individuals' motor cortical E:I. In individuals with low E:I, stimulation increased retention; in individuals with high E:I, stimulation decreased retention.

Thus we identify a specific domain of motor functional plasticity that improves with age, as a natural consequence of motor cortical inhibitory decline. This memory function can be further enhanced by neurostimulation, but only in individuals least affected by age-related dysregulation of motor cortical E:I. These findings challenge the prevailing view of ageing as inevitable functional decline. Whereas learning of new motor skills may decline, the capacity to maintain adaptation of existing skills improves naturally with age. That adaptation memory is enhanced naturally with age indicates it may have untapped potential as a target for training strategies that aim to preserve, improve or restore motor function in healthy or pathological ageing[47].

Results

Retention increases with age. First we tested the prediction that adaptation memory increases with age. We used a cross-sectional correlational design to measure the continuous effect of ageing across a mid- to late- life sample. This avoids the confounds inherent in a between-groups "young vs. old" design caused by gross differences in body, brain and behaviour. In Experiment 1 thirty two healthy male volunteers aged between 49 and 81 (mean age: 67.46 years, *s.d.*: 8.07; Table

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S1) performed a session of prism adaptation (PA) with their dominant right hand. Only men were recruited to avoid potential confounds from cyclical variability in neurotransmitter concentration with the menstrual cycle in women[52,53] (see *Methods*).

The behavioural protocol was similar to previous work from our laboratory[47,54] (full details in *Methods*). All pointing error data were normalised by baseline (pre-adaptation) accuracy. Following adaptation, retention of the after-effect was assessed after a short (10 minutes) and long (24 hours) interval (Fig. S1). Effects were analysed statistically using linear mixed-effect models (LMMs) with maximal random structure. This allowed us to assess both the average lateral error across task blocks and the stability of the error within blocks, while controlling for random effects of inter-individual variation.

Fig.1a shows the pointing error data, plotted as changes from baseline accuracy. Throughout adaptation, participants made rapid pointing movements at a 10° left and right target, while wearing prism glasses that displaced their visual field 10° to the right. During prism exposure (Blocks E1-6) participants gradually corrected their errors. The learning and forgetting dynamics are visible within and across blocks. At prism onset participants exhibited a large rightward error (Fig.1a; Block E1, trial 1: mean 7.77°, s.e.m.: 1.05°, $t_{(31)} = 7.43,\ p < 0.001$) which was corrected gradually across trials and blocks (E1-6) until performance stabilized (E6) close to restored baseline accuracy (main effect of Trial within Block: $t_{(3185)} = -9.34, p < 0.001$; main effect of Block: $t_{(3185)} = -9.07, p < 0.001$; Table S2 - model 1).

As participants adapted gradually to the rightward visual shift, a consequent leftward after

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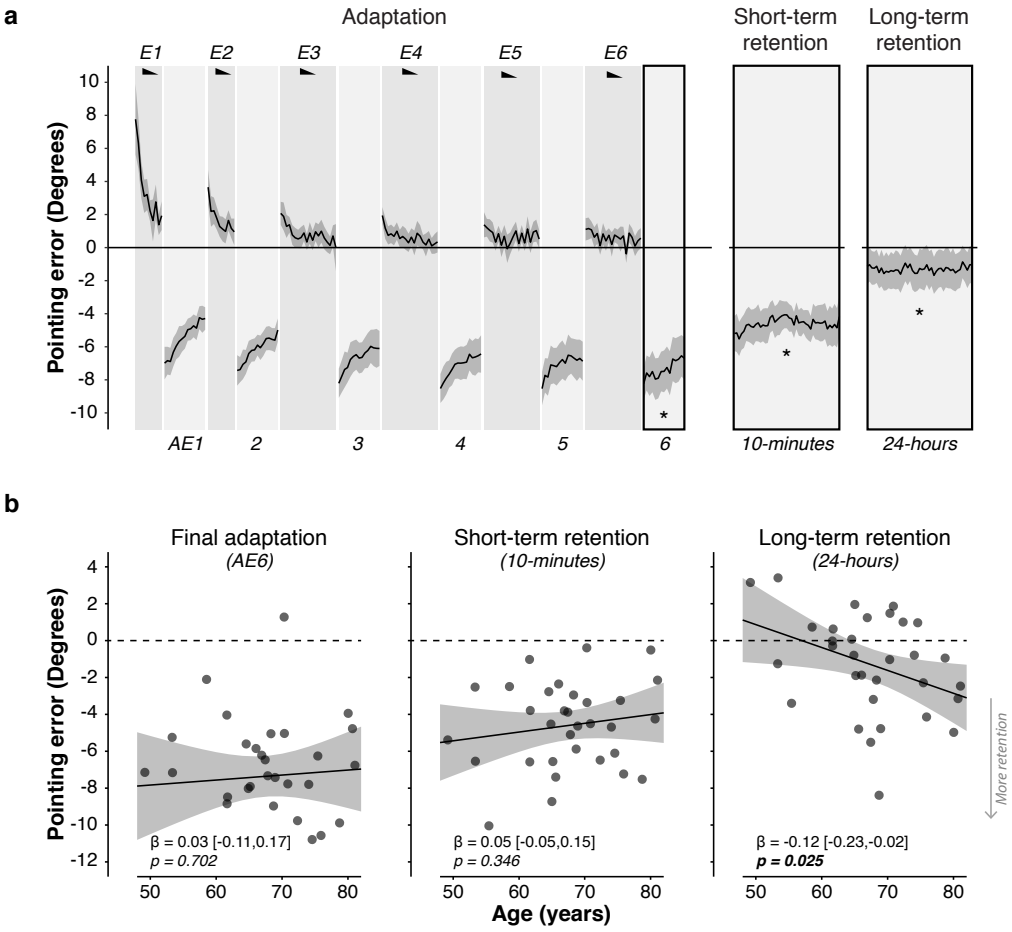

Figure 1: **Long-term retention of prism adaptation is higher in older adults. a.** Group mean pointing errors ($\pm$1 s.e.m.) expressed as change from baseline accuracy (y=0). Positive y-axis values are rightward errors, negative leftward. Black wedges indicate blocks in which prisms were worn. During right-shifting prism exposure (E1-6), visual feedback enabled participants to correct their rightward pointing errors across trials. Consequent leftward after-effects were measured in intervening blocks without visual feedback throughout adaptation (AE1-6). After-effect retention was measured post-adaptation after a short (10 minutes) and long (24 hours) interval. There was significant retention at both time points. **b.** Age had no effect on the after-effect magnitude acquired by the end of adaptation (block AE6), nor on short-term retention (10 minutes). The key finding was that older adults showed significantly greater long-term retention (24-hours). Full statistics are in Tables S2 & S3.

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effect developed, measured in interleaved blocks, critically without prisms and without visual feedback (Fig. 1a; Blocks AE1-6; mean normalised error: $-6.66°$, $t_{(2865)} = -16.94$, $p < 0.001$; Table S2 - model 2). This prism after-effect (AE) is the key experimental measure. On AE trials, the absence of visual feedback prevents error-based learning and requires participants to rely on internal representations of sensed limb position to guide their movements. Thus, the leftward AE expresses the visuomotor transformation acquired during prism exposure. Its persistence after prism removal is the measure of adaptation memory. The AE was measured after each block of prism exposure (AE1-6, Fig. S1). Initially memory was labile: on the first trial of the first block the AE was large ($-6.99°$), but across the 15 trials of the first block it decayed by $2.70°$ on average. Subsequent blocks of prism exposure led the AE to gradually stabilize, evidenced by the progressive flattening of slopes across blocks AE1-6 (interaction Trial $\times$ Block: $t_{(2865)} = -3.33$, $p = 0.001$; Fig. 1a; Table S2 - model 2). Thus, our protocol induced an adaptation memory trace that consolidated gradually across the Adaptation phase.

The critical measure of memory was AE retention post-adaptation (Fig. 1a-b). After 10 minutes of blindfolded rest there was significant short-term retention (mean error: $-4.61°$, s.e.m.: $0.41°$, $t_{(1434)} = -11.36$, $p < 0.001$; Table S2 - model 3). Long-term retention, measured 24 hours later, was also significant (mean error: $-1.30°$, s.e.m.: $0.48°$, $t_{(1434)} = -2.75$, $p = 0.006$; Table S2 - model 4). The AE was stable at both time points, indicated by no change in error across trials (main effect of Trial: both $p > 0.38$).

Our hypothesis was that AE retention would increase with age. Fig. 1b plots the results.

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Age had no effect on the AE magnitude acquired by the end of prism exposure (Block AE6), nor on short-term retention (both $p > 0.35$; Fig. 1b; Table S3 - models 1 & 2). However, older age was associated with greater long-term retention (Age $\times$ AE_{24hrs}: $t_{(1432)} = -2.24$, $p = 0.025$, Fig. 1b, Table S3 - model 3). This is the key finding. This association remained significant when controlling for the AE at the two preceding time points (AE6 and 10-min retention), and when controlling for average reaching speed during prism exposure (slower movements, expected in ageing, could arguably favour retention; Table S3 - models 4-6).

Motor cortical inhibitory tone declines with age. Next we tested for an expected decrease in motor cortical inhibitory tone with age. Three Tesla magnetic resonance spectroscopy was used to quantify neurochemical concentration in left sensorimotor cortex (labelled "M1"), and in a control region of occipital cortex (labelled "V1"; see *Methods*; Fig. S2). The metabolites of interest were GABA and Glutamix ("Glx"= Glutamate + Glutamine, since these two metabolites cannot be reliably distinguished at 3 Tesla). As expected, in both regions, age was associated with significant grey matter atrophy (both $p < 0.002$), which could indirectly lower neurochemical concentration estimates. Hence, all analyses of neurochemistry ruled out this potential confound by controlling for grey and white matter fractions within each region (see *Methods*). To minimize multiple comparisons, analyses focused on the ratio of excitation:inhibition (E:I = Glx:GABA). If an effect was significant, follow-up analyses assessed the individual contributions of Glx and GABA.

Figure 2 shows the results. Multiple linear regressions showed that sensorimotor cortex E:I increased with age (standardised $\beta_{age} = 0.66$, $t_{(18)} = 2.09$, $p = 0.051$; Table S4 - model 1). As

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predicted, across individuals, as age increased, M1 GABA concentration decreased (standardised $\beta_{age} = -0.74$, $t_{(17)} = -2.48$, $p = 0.024$; Table S4 - model 2). There was no such relationship with Glx (standardised $\beta_{age} = -0.23$, $t_{(17)} = -0.68$, $p = 0.51$; Fig. 2a, Table S4 - model 3).

In the anatomical control region (occipital cortex), there was a qualitatively similar pattern of age-related inhibitory decline, consistent with previous reports [55,56]. However this was not statistically significant, likely reflecting the impact of quality controls that reduced the size of the occipital dataset and consequently reduced power (Table S1). There was no significant relationship between neurochemistry and age in V1, not for E:I (standardised $\beta_{age} = 0.39$, $t_{(12)} = 1.46$, $p = 0.171$), GABA (standardised $\beta_{age} = -0.40$, $t_{(11)} = -1.57$, $p = 0.145$) or Glx (standardised $\beta_{age} = 0.04$, $t_{(11)} = .22$, $p = 0.832$; ; Table S4 - models 4-6).

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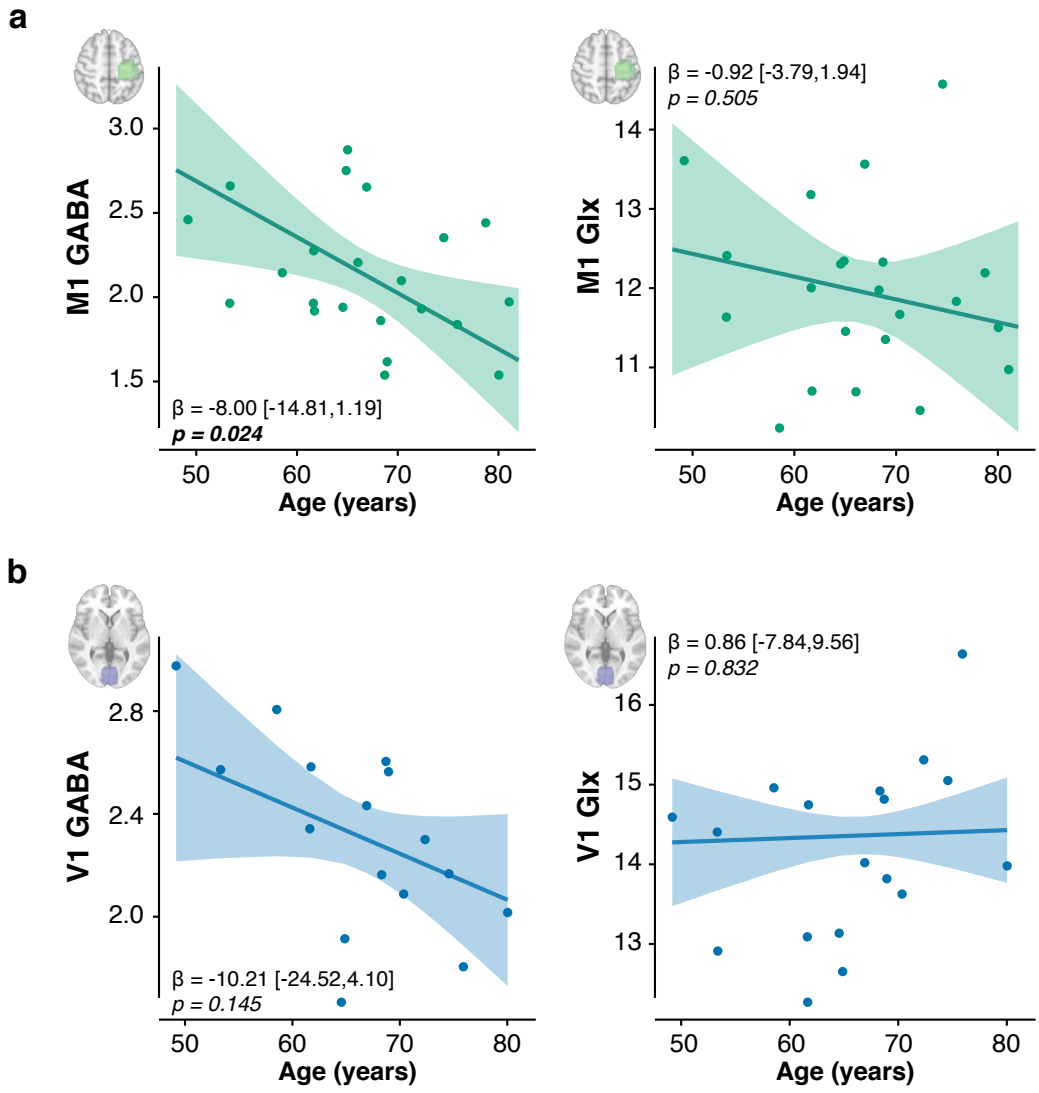

Figure 2: **Motor cortical inhibitory tone is lower in older adults. a.** The concentration of GABA but not Glutamix (Glutamate + Glutamine, Glx) was negatively associated with age in the left sensorimotor cortex (labelled "M1"). **b.** There was no significant association between age and neurochemical concentration in occipital cortex (labelled "V1"). For each voxel and neurotransmitter, relationships control for the fraction of grey matter and white matter, and the other neurotransmitter. Absolute concentrations are expressed in arbitrary units. Full statistical details are in Table S4.

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Lower motor cortical inhibitory tone is associated with greater long-term retention. Based on our previous work[47,48], we hypothesized that lower motor cortical inhibitory tone would be associated with greater retention. Results confirmed this prediction (Fig. 3). Across individuals, higher sensorimotor cortex E:I was associated with a larger prism AE at retention 24-hours after adaptation ($t_{(980)} = -5.40$, $p < 0.001$; Table S5 - model 1). This relationship was driven by GABA: individuals with lower M1 GABA concentration showed greater retention ($t_{(978)} = 5.04$, $p < 0.001$; Fig. 3a, Table S5 - model 2). There was no such relationship with M1 Glx ($t_{(978)} = 0.01, p = 0.99$; Fig. 3a, Table S5 - model 2). Thus, this memory effect was neurochemically specific (M1 GABA vs. M1 Glx: $z = 3.56$, $p < 0.001$). It was also anatomically specific (M1 GABA vs. V1 GABA: $z = 2.80$, $p = 0.005$): there was no relationship between retention and V1 metabolites - not for GABA, Glx or EI (all $p > 0.25$; Fig. 3b, Table S5 - models 5 & 6). As before, the results were unchanged when controlling for average movement time during prism exposure (S5 - models 3, 4, 7, 8).

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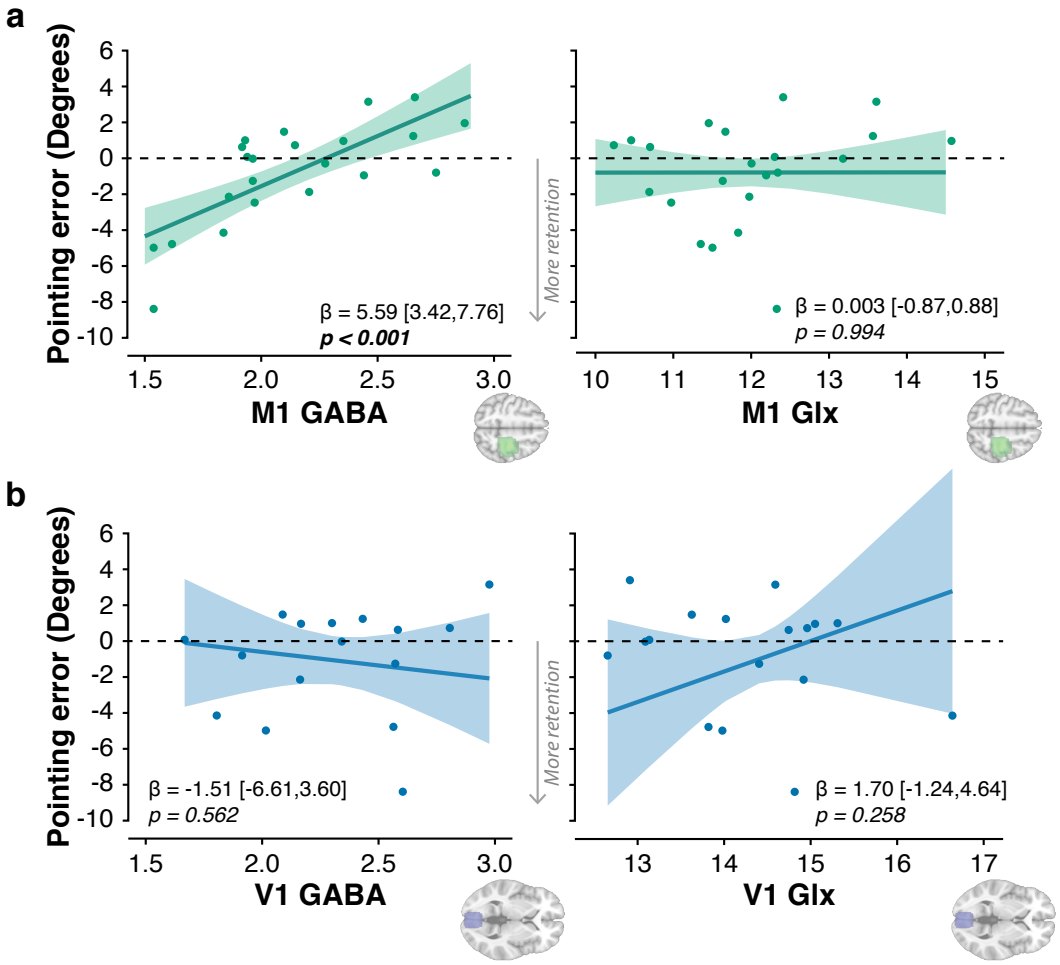

Figure 3: **Lower motor cortical inhibitory tone is associated with greater long-term retention.** Plot shows relationships between brain chemistry and the magnitude of prism after-effect retained 24 hours after adaptation. Negative values on the y-axis indicate retention. **a. Sensorimotor cortex ("M1")** Across individuals, lower GABA was associated with greater retention. There was no relationship with Glx (Glutamate + Glutamine). **b. Occipital cortex ("V1")** There was no relationship between GABA or Glx and 24-hour retention. For each voxel and neurotransmitter, relationships control for the fraction of grey matter and white matter, and the other neurotransmitter. Absolute concentrations are expressed in arbitrary units. Full statistics details are in Table S5.

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Retention increases with age because motor cortical GABA concentration declines. Our key prediction was that as M1 GABA concentration declines with age, adaptation memory would increase, and the former would explain the latter. We used mediation analysis to formally test this hypothesis. Mediation analysis is well suited to a situation in which the independent variable (Age) may not directly influence the dependent variable (Long-term retention), but is instead hypothesized to do so indirectly via its influence on candidate mediators (M1 E:I, GABA, Glx). The extent to which the relationship between the independent and dependent variable is influenced by a mediator is termed the indirect effect. We tested the significance of indirect effects using a bootstrap estimation approach with 10,000 samples (see *Methods*).

Figure 4 shows that, as hypothesized, the effect of age on long-term retention was mediated by motor cortical E:I ($ab_1 = -0.41$, 95%CI: $[-1.45, -0.08]$, $p = 0.017$). More specifically, the indirect effect was driven by M1 GABA and not Glx. M1 GABA was a significant mediator ($ab_1 = -0.50$, 95%CI: $[-1.46, -0.16]$, $p = 0.0086$), accounting for 64% of the variance between age and long-term retention (Fig. 4, Table S6), while M1 Glx showed no such effect ($ab_2 = 0.018$, 95%CI: $[-0.095, 0.31]$, $p = 0.74$). When M1 neurochemistry was controlled for, age was no longer a significant predictor of 24-hour retention ($c' = -0.28,\ p = 0.38$), consistent with full mediation. Thus, age-related decline in sensorimotor GABA explains why adaptation memory increases with age. Once again, results were unchanged when controlling for average movement time during prism exposure (Table S7).

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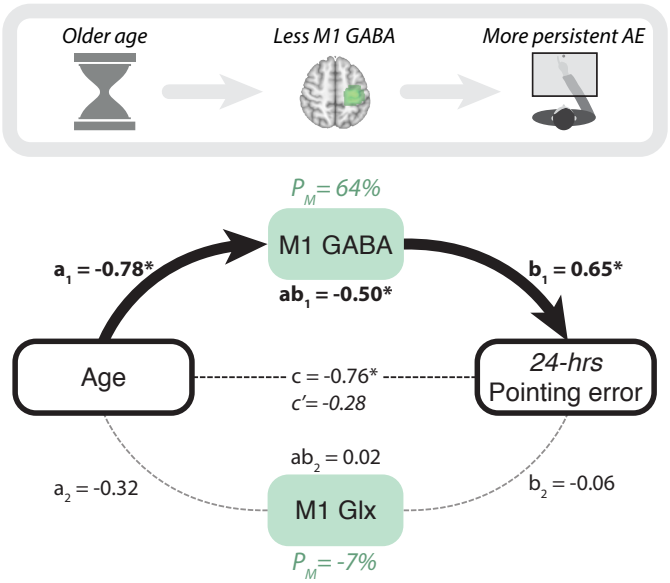

Figure 4: **Adaptation memory increases with age because motor cortical inhibitory tone declines.** A mediation model tested whether M1 neurochemistry explained the relationship between age and retention. Consistent with our mechanistic hypothesis, GABA, but not Glx, mediated the positive relationship between age and 24-hour retention, explaining 64% of the variance. Standardised regression coefficients are reported next to the corresponding paths. Asterisks indicate significance ($p < 0.05$). Full statistics: Table S6. Independent variable: Age. Dependent variable: AE 24-hours post-adaptation. Mediators: M1 GABA and Glx (controlling for grey and white matter tissue fractions).

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How stimulation changes memory depends on motor cortical E:I. The mediation model indicated that the M1 GABA decline caused the memory increase in older adults. However, the cross-sectional study design precludes direct causal inference[57]. Hence, to more directly test causation, we intervened experimentally with anodal transcranial direct current stimulation (a-tDCS). M1 a-tDCS has been shown to increase motor cortical E:I in young[58] and older[51] adults. In addition, we have previously shown in young adults that M1 a-tDCS during adaptation increases short- and long- term retention, in proportion to the stimulation-induced increase in E:I [47].

However, given our finding in Experiment 1 that M1 E:I is already naturally high with age (Fig. 2), we expected M1 a-tDCS (which increases E:I) to be consequently less effective overall in older adults. Homeostatic mechanisms constrain cortical excitability changes to within physiological range. Hence, if E:I is already near ceiling in older adults, this is likely to limit, or even reverse the direction of, the excitability increase that can be induced experimentally by a-tDCS [59–63]. For our hypothesis, that retention depends causally on M1 E:I, this predicts an inverted U-shape stimulation effect in older adults: improved memory in individuals with low E:I (who have capacity for an excitability increase), impaired memory in those with high E:I (who are near ceiling), and little or no change for those in between (Fig. 6a).

To test this hypothesis, a sub-set of twenty-five participants from Experiment 1 (mean age: 69.6 years, *s.d.*: 8.4; Table S1) consented to undergo a follow-up study (Experiment 2), in which tDCS (anodal/sham, counterbalanced) was applied in two weekly test sessions to left M1 during adaptation, and retention was assessed after 10 minutes and 24 hours (see *Methods*, Fig. S1).

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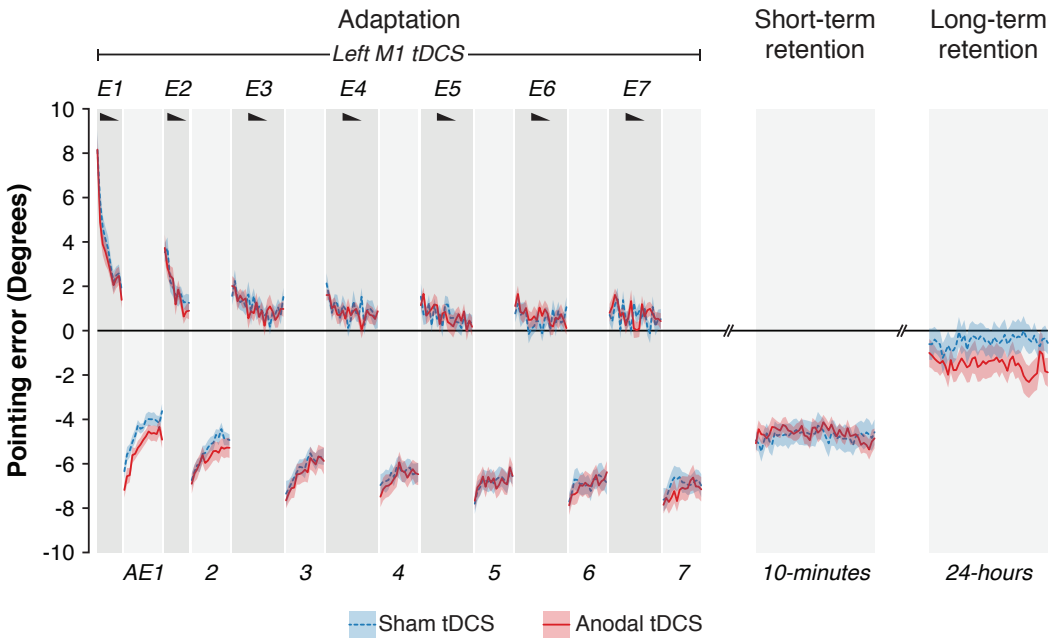

Figure 5: **No increase in retention across older adults with excitatory stimulation of M1 during adaptation.** Timecourse of pointing errors for the same behavioural paradigm and graph conventions as in Figure 1, except that stimulation (anodal or sham tDCS) was applied to left M1 throughout the adaptation phase. Errors are normalised to baseline (pre-adaptation) accuracy. Negative values on the y-axis indicate prism after-effects. Unlike our previous work in young adults, on average, there was no significant increase in retention after excitatory stimulation of M1 during adaptation.

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Figure 5 shows the results for the group average. Stimulation had no effect on short-term retention ($t_{(2235)} = 0.22$, $p = 0.83$; Table S8 - model 1). Although long-term retention increased numerically, this was not significant ($t_{(2235)} = -1.35$, $p = 0.18$; Table S8 - model 4). The lack of a significant memory gain from stimulation across the group contrasts with our previous findings in young adults[47,48].

To test our key hypothesis, that motor cortical E:I would causally influence the direction of stimulation-induced memory change, we conducted a moderation analysis. For all participants who had undergone Experiment 1 ($n = 17$, data shown in Fig. 2) we added their M1 Glx:GABA levels to the linear mixed model analyses of the effect of stimulation on retention. As predicted, for long-term retention stimulation interacted significantly with motor cortical E:I (E:I $\times$ a-tDCS: $t_{(1419)} = 2.40$, $p = 0.009$, one-tail; Fig. 6; Table S8 - model 5). Fig. 6 shows how the induced memory change varied as a function of M1 E:I. In those individuals with low E:I, stimulation enhanced memory; in individuals with high E:I, stimulation impaired memory. A similar trend was observed for short-term retention ($t_{1419} = 1.86$, $p = 0.064$, one-tail; Table S8 - model 2).

A follow-up LMM assessed the moderating roles of M1 GABA and Glx separately. Both Glx (Glx $\times$ a-tDCS: $t_{(1415)} = 2.57$, $p = 0.005$, one-tail) and GABA (GABA $\times$ a-tDCS: $t_{(1415)} = -1.73$, $p = 0.042$, one-tail) moderated the stimulation effect, each in opposite directions (Table S8 - model 6). Across individuals, stimulation increased retention in those with higher GABA and/or lower Glx, and impaired retention in those with lower GABA and/or higher Glx. This result was unchanged when controlling for average movement speed during prism exposure (Table S9), and

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was not observed within the anatomical control voxel placed over V1 (Table S10).

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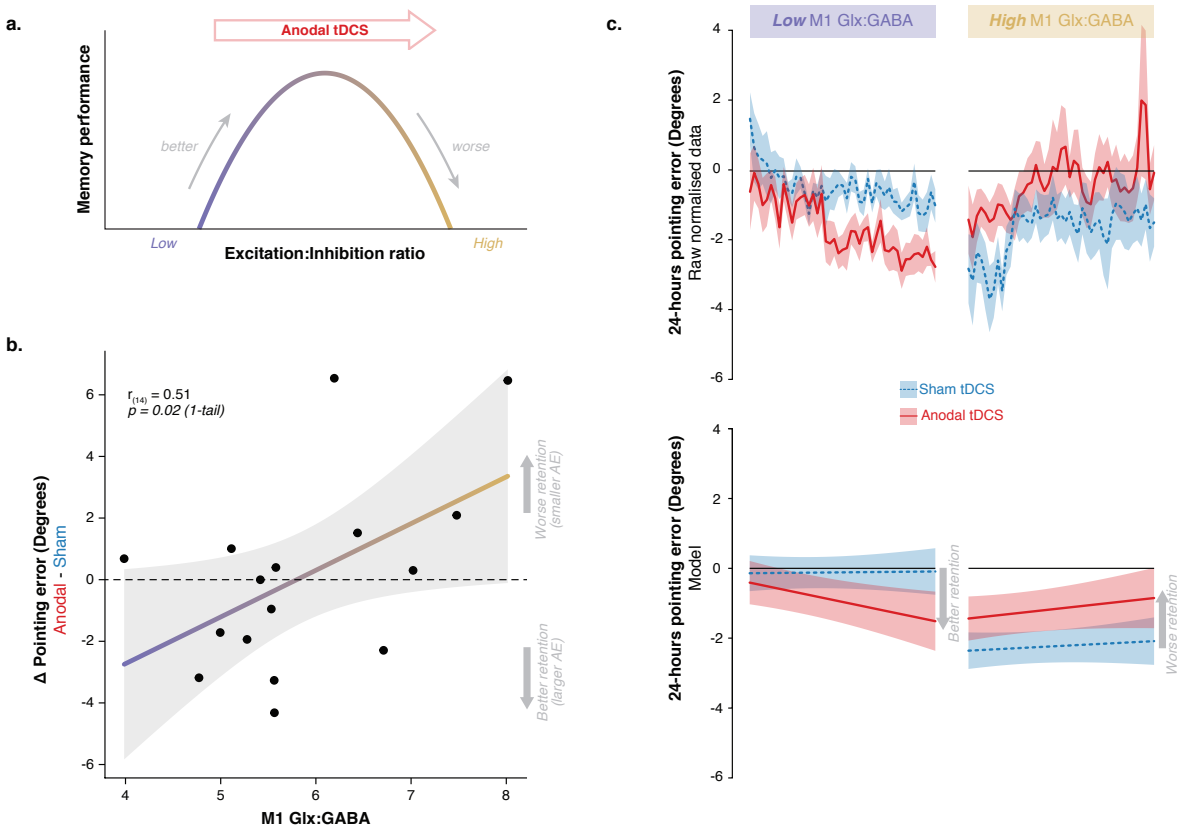

Figure 6: **How stimulation changes memory depends on motor cortical E:I.** Predicted effect of stimulation on memory as a function of motor cortical E:I - enhanced retention in individuals with low E:I and impaired retention in individuals with high E:I. **b.** Results confirmed this hypothesis. Individual M1 E:I (M1 Glx:GABA) is plotted against the stimulation effect (anodal - sham difference in normalised pointing error at 24-hour retention). On the y-axis, negative values indicate greater retention with anodal tDCS compared to sham. Positive values indicate the opposite. Across individuals, stimulation enhanced retention in those with low E:I and impaired it in those with high E:I. **c.** A linear mixed-effect model moderation effect further confirmed that stimulation changed memory as a function of sensorimotor E:I (M1 Glx:GABA $\times$ tDCS : $t_{1419} = 2.40$, $p = 0.017$). For visualisation purposes, this interaction is illustrated using a median split on the M1 E:I data. The data (top) and model fit (bottom) are plotted separately for individuals with low/high M1 E:I, and show opposing effects of excitatory stimulation on adaptation memory depending on E:I.

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Discussion

This study investigated the relationship between sensorimotor cortical GABAergic inhibition and retention of the prism adaptation after-effect in the ageing brain. In line with our predictions, older age was associated with reduced tonic GABAergic inhibition within sensorimotor cortex (Fig. 2), and larger long-term (24-hours) retention of the AE following prism adaptation (Fig. 1). Crucially, a mediation analysis revealed that the former explained the latter (Figs. 3 & 4). The causal nature of this link was investigated further by manipulating the EIB within sensorimotor cortex[51,58] using a-tDCS in Experiment 2. At the group level, neurostimulation had no significant influence on long-term retention (Fig. 5). However, when investigating the determinants of individual responses, participants with lowest excitation:inhibition ratio were found to benefit the most from a-tDCS, while those with higher excitation:inhibition showed the opposite. Taken together, our data provide converging evidence for a role of motor cortical inhibition in the persistence of sensorimotor adaptation in the ageing brain.

Previous studies investigating the effect of ageing on sensorimotor adaptation have predominantly reported an age-related decline in the rate of adaptation[35,37,38,40,41,43,44]. Findings are mixed, however, with regards to the influence of ageing on the subsequent retention of the acquired visuomotor adaptation. Some studies have reported no change[36–39,43,44], while others have observed larger after-effects[35,42,45]. In part, these discrepant results can be accounted for by differences in the adaptation paradigm used (e.g. walking adaptation vs. reaching adaptation) and timescales considered (within-session vs. between-sessions retention). For example, in the present study,

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only long-term (24-hours) persistence was enhanced in older participants.

Changes in tonic GABAergic signalling in ageing is a well documented phenomenon[15,18–26]. Typically, the down-regulation of inhibition within sensorimotor regions has been reported to be detrimental for sensorimotor performance[10,20,23,24,26]. To our knowledge, however, the consequences of reduced GABAergic inhibition on the persistence of sensorimotor adaptation had never been elucidated. In this study, we provided evidence that, consistent with its role in younger adults, lower motor cortical GABA is actually beneficial for the maintenance of the newly acquired visuomotor map, presumably because it promotes local plasticity[47]. This mechanistic link explained why older adults showed enhanced long-term adaptation memory. In other words, normal ageing could be seen as a process similar to M1 anodal transcranial stimulation. That is, ageing is a natural process that releases inhibition, thus promoting sensorimotor plasticity. This idea of a more plastic ageing brain might appear at odd with existing theories[12]. However, based on our data alone, it is difficult to conclude whether this phenomenon is good or bad for real-world function. For example, it may prove to be maladaptive by inducing a certain rigidity in situations in which perturbations are volatile and require the agent to quickly learn and forget visuo-motor transformations[64]. A higher GABAergic tone (in younger adults) might allow for a more selective release of the inhibition blanket[30] and therefore promote retention of motor memories that are the most likely to be beneficial in the future. Therefore, the degree to which reduced GABAergic tone can be deemed to be adaptive depends on the specific context and task.

The current study relied on magnetic resonance spectroscopy to measure metabolite concen-

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trations in the living brain. This technique typically suffers from relatively low signal-to-noise (SNR) ratio, forcing us and others to collect data from a large ($2 \times 2 \times 2$ cm^3) region of interest. By increasing the size of the region-of-interest, however, adjacent regions of somatosensory cortex were also included in the measure of motor cortical metabolites, therefore reducing regional specificity. Spatial resolution is a common methodological limitation of MRS studies[30,47,50,51,65,66]. Similarly, although the transcranial stimulation used in experiment 2 was centered on the motor cortex this neurostimulation technique is known to operate in a diffuse manner. Moreover, the spatial distribution of the intracranial electric field is known to be shaped by the underlying gyro-sulcal architecture[67–69]. Although we cannot rule out the contribution of other parts of the sensorimotor network to our results, the convergence of many studies pointing towards a key role of the motor cortex in the consolidation of adaptation memory[47,70–77], suggesting that this region is likely to play a predominant role.

The past two decades have witnessed a growing interest for the use of non-invasive brain stimulation as an adjuvent to conventional post-stroke neuro-rehabilitation techniques0[47,78–81]. This body of work has highlighted a large inter-individual variability in the response to stimulation[82], which is likely to be responsible, at least in part, for the limited translation to a clinical setting. Better understanding the factors driving this inter-individual variability has therefore become a priority for the field. Here, we investigated the role of basal GABAergic inhibition in an age group that more closely match the clinical population likely to benefit from the protocol used in this study–post-stroke neglect patients[47]. We demonstrated that the basal level of GABAergic inhibition in the primary motor cortex was a significant moderator of inter-individual behavioural

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response to motor-cortical anodal transcranial stimulation. That is older adults with lower basal inhibition were less likely to show the expected stimulation-induced enhancement of adaptation memory. This finding has important translational value because it implies that the therapeutic potential of our intervention is constrained by patients' neurochemical profile.

The moderating influence of basal inhibitory tone raises the idea that the influence of neurostimulation on behaviour may, in part, be dependent on *metaplasticity* – a set of mechanisms engaged to maintain neural activity within a normal range[60]. Individuals with a higher basal excitation:inhibition ratio (lower basal GABA) are likely to be in a state that is closer to the threshold at which negative metaplastic feedback mechanisms are engaged. In this particular scenario the use of an excitatory intervention such as anodal transcranial stimulation could have the paradoxical effect of initiating metaplastic processes, thus reducing the excitation:inhibition ratio (e.g., by increasing upregulating GABAergic inhibition). Consistent with this idea, a recent study reported that the behavioural effect of motor cortical anodal transcranial stimulation could be enhanced in older adults by pre-conditioning the stimulated cortex with cathodal stimulation, which is hypothesised to increase inhibitory tone[83]. That is, reducing the excitation:inhibition ratio prior to applying an excitatory stimulation in order to limit the engagement of negative metaplasticity. This provides a potential solution to the neurochemical constraint identified in by our results. This further supports a person-centred approach to neurorehabilitation, suggesting that inter-individual differences in basal neurochemistry may drive response to therapy.

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Conclusion. In the present study, we provided evidence that older age promotes long-term persistence of adaptation after-effects by lowering GABAergic inhibition within the primary motor cortex. In this population, further lowering motor cortical inhibition by means of anodal transcranial direct stimulation enhanced the memory trace of the adaptation. However, this effect was restricted in individuals with lower basal GABAergic inhibition, indicating that a person-centred approach to neurostimulation is required. Taken together, our results are consistent with a maintained involvement of primary motor cortex neurochemistry in the consolidation of adaptation memory that is responsible for age-related behavioural changes.

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Materials and Methods

Participants. This study was approved by the local ethics committee (Oxford A Research Ethics Committee; REC reference number: 13/SC/0163), and written informed consent was provided by all participants. Thirty two right handed men aged between 49 and 81 (mean age: 67.5 years, *s.d.*: 8.1) without any personal or family history of neurological or psychiatric disorder participated in this study. This study comprised two experiments. In the first experiment ($n = 32$), participants completed a PA session to measure short (10-minutes) and long-term (24-hours) retention of the prism adaptation after-effect. A sub-sample of these participants underwent a magnetic resonance spectroscopy scan to measure neurochemical concentrations in a volume of interest centered on the left sensorimotor cortex ($n = 22$) and in an anatomical control volume centred bilaterally on mid-line occipital cortex ($n = 20$; Fig. S2). Exp. 1 was designed to investigate the cross-sectional relationships between age, neurochemistry, and adaptation memory. In Exp. 2, participants ($n = 25$) completed four behavioural sessions to characterise the effect of left M1 a-tDCS on the persistence of the prism AE. Details of which measurements were obtained for each individual are in Table S1.

Prism adaptation protocol. In both experiments, PA was performed on a purpose-built automated apparatus (Fig. S1a). The task was programmed in MATLAB version 2014b (MathWorks; `https://uk.mathworks.com`) using Psychtoolbox[84] version 3, run on a MacBook Pro laptop. Participants sat with their head fixed in a chin-rest. They were instructed to perform reaching movements with their right hand to one of three targets presented on a 32-inch horizontal LCD

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screen embedded in a table in front of them. There were two lateral targets situated either 10 cm to the left or right of a central target. The distance between participants' eyes and the central target was approximately 57 cm. In both experiments, retention of the prism AE was measure after 10 minutes (day 1) and 24 hours (day 2; Fig. S1).

The PA procedure comprised two trial types: closed-loop pointing (CLP) and open-loop pointing (OLP). On closed-loop trials, participants made rapid reaching movements (mean duration: 452 ms, *s.d.*: 119 ms) with their right index finger to either the left or right target in a pseudo-randomised order. Participants were instructed to be as accurate as possible whilst maintaining a "ballistic" hand movement throughout the entire trial. Similar to previous experiments[47,54,85], visual feedback was limited to the last two-thirds of the reaching movement in order to limit strategic adjustments and "in-flight" error correction[86,87]. Because movement speed during prism exposure is known to influence adaptation[88], all analyses of inter-individual differences in PA performance were also run while controlling for CLP duration (averaged across all trials for a participant). On open-loop trials, participants pointed at a comfortable speed (mean duration: 799 ms, s.d.: 135 ms) to the central target. Open-loop instructions emphasised pointing accuracy rather than speed. The target location was occluded by an opaque shutter screen upon initiation of the reaching movement, thereby requiring participants to rely on proprioception alone to guide their movement. Thus participants received no visual feedback of the reaching movement, terminal error, or return movement on this type of trial. This procedure enabled measurement of the AE due to lack of visual feedback, which ensured participants would not de-adapt.

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During PA sessions, participants initially performed closed-loop and open-loop pointing to measure their baseline accuracy on these two trial types. The adaptation phase consisted of six (Experiment 1) or seven (Experiment 2) blocks of prism exposure, alternating closed- and open-loop pointing trials (Fig. S1). Real or sham neurostimulation was applied throughout this phase in experiment 2. Persistence of the AE was then probed 10-minutes and 24-hours after completion of the PA protocol. All participants underwent Exp.1 1 first, which served as a "familiarisation" session. In Exp. 2, the order of the two sessions (anodal/sham stimulation) was counter-balanced across participants.

MRI acquisition protocol. MR data were acquired on a 3T Siemens Trio. High resolution T1-weighted structural MR images (224 $\times$ 1 mm axial slices; TR/TE = 3000/4.71 ms; flip angle = 8 deg; FOV = 256; voxel size = 1 mm isotropic; scan time = 8 minutes 48 seconds) were acquired for magnetic resonance spectroscopy (MRS) voxel placement and registration purposes. MRS data were acquired from two volumes of interest in two consecutive acquisitions. The first volume-of-interest was centred on the left motor knob[89] and included parts of the pre- and post- central gyrus (Fig. S2a). The second (anatomical control) volume-of-interest was centred bilaterally on the calcarine sulcus in the occipital lobe (visual cortex)[65,66,90] (Fig. S2c). B0 shimming was performed using a GRE-SHIM (64 $\times$ 4.2 mm axial slices, TR = 862.56 ms, TE1/2 = 4.80/9.60 ms, flip angle = 12 deg, FOV = 400, scan duration = 63 secs). MR spectroscopy data were acquired using the semi-LASER sequence (TR/TE = 4000/50 ms, 64 scan averages, scan time = 264 secs) [91,92].

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Transcranial direct current stimulation. In Exp. 2, stimulation was delivered by a battery driven DC stimulator (Neuroconn GmbH, Ilmenau, Germany) connected to two 7 $\times$ 5 cm sponge electrodes soaked in a 0.9% saline solution. The protocol was identical to our previous work[47]. Electrodes were positioned immediately before stimulation onset and removed as soon at the stimulation ended. The anode electrode was centred over C3 (5 cm lateral to Cz) corresponding to the left primary motor cortex according to the international 10-20 System[93]. The reference electrode (cathode) was placed over the right supraorbital ridge. During anodal stimulation, the current intensity was set to 1 mA for 20 minutes with a ramp-up and ramp-down period of 10 seconds. During sham stimulation, the current also ramped up and down for 10 sec but no stimulation was delivered during the 20 minutes. Instead, small current pulses (110 μA over 15 ms) occurred every 550 ms to simulate the tingling sensation associated with real anodal stimulation. Both experimenters and participants were blinded to the stimulation condition.

Behavioural data analysis. Statistical analyses of behaviour were performed in R[94]. Unless specified otherwise, all statistical tests were two-tailed. Analyses were performed using linear regression models. Linear mixed-effects models (LMMs) were used for analyses comprising a longitudinal/repeated-measures component by including intercepts and slopes as participant random effects. This approach had two advantages compared to traditional analyses of variance (ANOVAs): it allowed us to consider the within-block rate of change in addition to the mean error, and to dissociate between random sources of inter-individual variability from meaningful ones.

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Because GABA is synthesised from glutamate, the concentrations of the two neurotransmitters are typically correlated in the brain[95,96]. Therefore, when analysing the relationship between the absolute concentration in GABA or Glx within a voxel and outcome, the concentration of the other neurotransmitter (GABA or Glx) was also included in the model. In addition, grey and white matter concentrations were also included as covariates of no interest in all models including neurochemical data.

A mediation analysis was used to characterise the "mechanistic" links underlying the observed correlations between age, neurochemistry, and retention. This was performed using the R package *mediation* for causal mediation analysis[97]. Mediation was conducted using regression with nonparametric bootstrapping (10,000 resamples) to ascertain whether the level of M1 tonic inhibition accounts for the link between age and long-term retention of the prism adaptation aftereffect. It included age as the independent variable (X), M1 GABA and Glx absolute concentrations as mediators (M_1, M_2), and the block mean error on OLP 24 hours after PA normalised by the baseline (pre-PA) deviation as the dependent variable (Y), and controlled for the fraction of GM and WM in the M1 voxel (C_1, C_2). The percentage mediation (P_M) was calculated as the fraction of total effect (c) accounted by indirect effects (ab_1 or ab_2).

MRS data analysis. Metabolites were quantified using LCModel[98–100] performed on all spectra within the chemical shift range 0.5 to 4.2 ppm. The model spectra were generated based on previously reported chemical shifts and coupling constants by VeSPA Project (Versatile Stimulation, Pulses and Analysis). The unsuppressed water signal acquired from the volume of interest was

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used to remove eddy current effects and to reconstruct the phased array spectra[101]. Single scan spectra were corrected for frequency and phase variations induced by subject motion before summation. Glutamix (Glx) was used in the current study due to the inability to distinguish between glutamate and glutamine using a 3T MRI scanner. To avoid biasing the sample towards high concentration estimates, an expected relative Cramér-Rao Lower Bound (CRLB) was computed for each individual dataset given the concentration estimate and assuming a constant level of noise across all measurements (see *SI* for detailed methods). Datasets for which the Pearson residual between the expected and observed relative CRLB exceeded 2 were excluded from subsequent analysis. Using this quality filtering criterion for γ-Aminobutyric acid (labelled GABA), Glutamix (Glutamine+Gutamate, labelled Glx) and total Creatine (Creatine + Phosphocreatine, labelled tCr), four V1 MRS datasets were discarded and no M1 MRS dataset was discarded.

Tissue correction is an important step in the analysis of MRS data, especially in older adults due to atrophy[102]. The output of LCmodel represents the metabolite concentration in the entire volume of interest. Therefore, if the fraction of neural tissue containing the metabolite of interest decreases, due to atrophy[103], the concentration of this metabolite in the MRS voxel will necessarily be lower. However, this depletion does not reflect a reduction in the metabolite concentration *per se*, but rather is a by-product of atrophy. Several tissue correction techniques have been proposed to account for this possible confound, with currently no consensus in the literature[104,105]. Most of these techniques make assumptions about the distribution of the metabolite of interest within the different tissue compartments. Such assumptions may not hold across the lifespan, as the normal ageing process may affect some compartments more so than others. To avoid this potential caveat,

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all analyses reported in this paper used non-tissue corrected concentration estimates and included the percentage of grey matter (GM) and white matter (WM) present in the voxel as confounding variables of no interest (see, for example, method used in [106]). This partial volume correction approach makes no assumption regarding the distribution of GABA and Glx within the different tissue types, which makes it more valid in the context of ageing. The percentage grey matter, white matter, and cerebrospinal fluid present in the volume of interest were calculated using FMRIBs automated segmentation tool[107].

Across individuals, the total creatine concentration estimate was negatively correlated with age in the M1 voxel ($r_{(22)} = -0.46, p = 0.04$) but not in the V1 voxel ($r_{(18)} = -0.06, p = 0.81$; Fig. S2c). The relationship with age in the M1 voxel is, therefore, problematic when using tCr for internal referencing. Throughout the study, we therefore report absolute concentration estimates, rather than ratios to tCr, for GABA and Glx.

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Supplementary Information

Supplementary methods

MRS data filtering procedure. Some authors have warned against the usage of *%CRLB* for MRS quality filtering because it could lead to wrong or missed statistical findings[108,109]. For equivalent metabolite concentrations, large levels of noise caused by bad quality acquisition (e.g. too small voxel size, not enough averages, bad shimming) or bad quality spectrum fitting (e.g. inappropriate basis files) would result in an increase in *%CRLB*, in a way that truly reflects estimation uncertainty. Thus, in this scenario, it would be valid to mistrust the data based on a high *%CRLB*. However, because of the *relative* nature of the *%CRLB*, this metric also strongly depends on its denominator, i.e. the estimated metabolite concentration. Thus, for equivalent levels of noise, a true decrease in the metabolite concentration would also be associated with an increase in *%CRLB*. In this scenario, however, it is no longer valid to reject such datasets. When measuring a change in GABA concentration following an intervention (e.g. anodal transcranial direct current stimulation, a-tDCS), this selection bias may artificially inflate the chances of detecting a reduction post-intervention, simply by virtue of regression towards the mean[110,111]. Additionally, it might be the case that individuals with higher baseline GABA levels are more likely to respond to a-tDCS than those with low basal GABA levels.

To avoid this methodological caveat we took into consideration the concentration estimate when rejecting datasets with high *%CRLB*. Datasets might have a high *%CRLB* because of a low

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concentration estimate rather than an excessive level of noise. We suggest that such datasets should not be excluded. Alternatively, datasets might have low *%CRLB* merely because the concentration estimate is high. However, it might be the case the the level of *%CRLB* is excessively high, given the metabolite concentration. Such datasets should be excluded.

We propose the following method as an alternative to standard *%CRLB*-based quality filtering. First, the following model is fitted to the "concentration estimate $\times$ *%CRLB*" relationship:

$$Expected\ \%CRLB_i = \frac{N_i}{C_i} \tag{1}$$

where N_i represents a group noise constant and C_i the concentration estimates for a metabolite i. If this simple model can explain most of the variance in the observed relationship between concentration estimates and *%CRLB*, it means that the level of noise is relatively constant across all measurements. However, any deviation from this model reflects an *unusual* level of noise compared to the other measurements. For each measurement, deviation from the model can be expressed as the Pearson residual as follows:

$$e_i = \frac{r}{\sqrt{MSE}} \tag{2}$$

where r_i is the raw residual (i.e. difference between the *%CRLB* and *expected %CRLB* for a certain measurement) and MSE is the mean squared error (i.e. mean deviation of all measurements from

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the model). The greater the Pearson residual for a given measurement, the noisier it is in regard to the rest of the data, irrespective of the concentration estimate. Note that this method did not reject the lower tail of the distribution entirely and therefore does not induce a selection bias towards high concentration estimates.

Supplementary Results

Effect of BDNF polymorphism on stimulation effect. Brain-derived Neurotrophic Factor (BDNF) is important for synaptic plasticity induction and is known to mediate the effect of direct current stimulation[112]. Individuals with the BDNF val66met polymorphism exhibit reduced behavioural and neural markers of motor cortical plasticity [113–116]. The val66met polymorphism causes a partial reduction in activity-dependent BDNF secretion, a factor involved in long-term potentiation[112,117,118]. Augmentation of BDNF-dependent synaptic plasticity is a candidate mechanism of action of sensorimotor cortex anodal-tDCS in mice and humans[114]. Plastic enhancement of motor skill learning via anodal-tDCS, however, is reduced in Met allele carriers [114]. In this supplementary analysis, we tested whether BDNF polymorphism type moderates the state-dependent effect of anodal-tDCS on after-effect retention. Identification of individual predictors of responsiveness to stimulation is crucial for both the mechanistic understanding of the effect of tDCS and the tailoring of interventions on an individual basis. Genotyping was acquired for 24/25 participants in Exp. 2. Genomic DNA was extracted from buccal cells using the ChargeSwitch® gDNA Buccal Cell Kit (ThermoFisher Scientific, UK) and samples were genotyped in duplicate by LGC Genomics (LGC Group, UK). Rs6265 was the only polymorphism examined.

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In agreement with the known allele distribution in the Caucasian population[119], 5/24 participants (21% of our sample) carried the Met allele. BDNF polymorphism had no significant influence on the effect of a-tDCS on either short-term (BDNF $\times$ stim $\times$ trial $= -0.001$, 95% CI $[-0.014,\ 0.012]$, $t_{(2141)} = -0.206$, $p = 0.84$), Fig. S3) or long-term retention (BDNF $\times$ stim $\times$ trial $= -0.01$, 95% CI $[-0.02,\ 0.002]$, $t_{(2141)} = -1.61$, $p = 0.11$), Fig. S3). This does not support the hypothesis that augmentation of BDNF-dependent synaptic plasticity is a contributory mechanism mediating behavioural plasticity induction via sensorimotor cortex anodal tDCS.

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Supplementary Figures

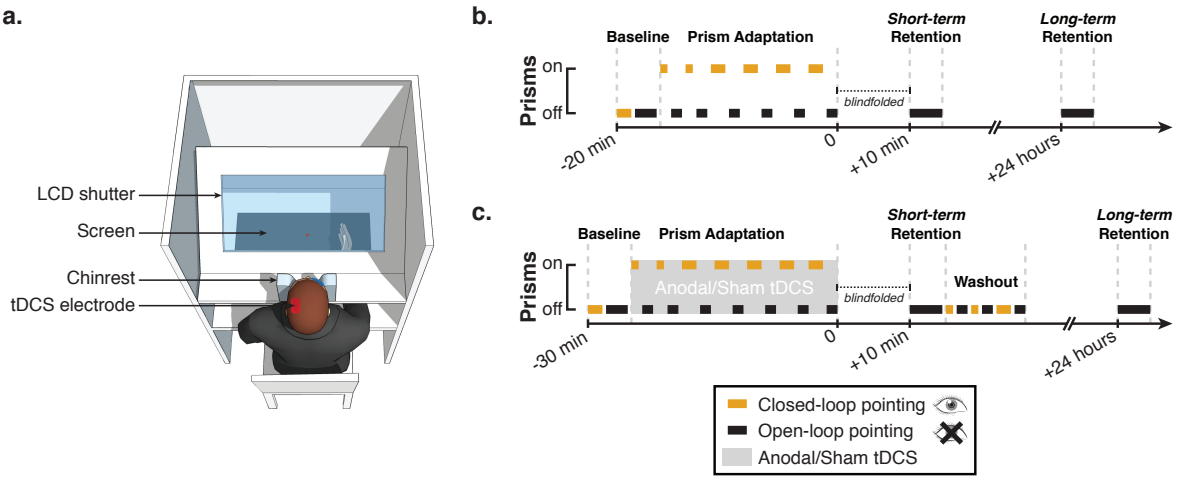

Figure S1: **Prism adaptation protocol. a. Experimental setup.** For both Experiment 1 and 2, participants sat in a chinrest viewing a horizontal 32-inch touchscreen through a liquid crystal shutter. The touchscreen was used to present visual targets and record reach endpoints. The liquid crystal display shutter was used to control visual feedback by turning opaque during reaching movements to conceal endpoint performance. **b. Procedure for Experiment 1.** Baseline accuracy was measured without prisms during blocks of closed-loop (continuous visual feedback) and open-loop (no visual feedback) pointing. During adaptation, participants alternated between blocks of prism exposure (closed-loop, glasses on) and after-effect measurement (open-loop, prisms off). Retention of the after-effect was measured 10 minutes and 24 hours post-adaptation. **c. Procedure for Experiment 2.** The procedure for Experiment 2 was the same as Experiment 1, except that left M1 anodal tDCS (real/sham) was applied throughout adaptation (grey shading). Short-term retention was followed by washout, during which participants observed and corrected their leftward errors (closed-loop pointing blocks, no prisms), interleaved with open-loop measures to confirm after-effect decay back to baseline.

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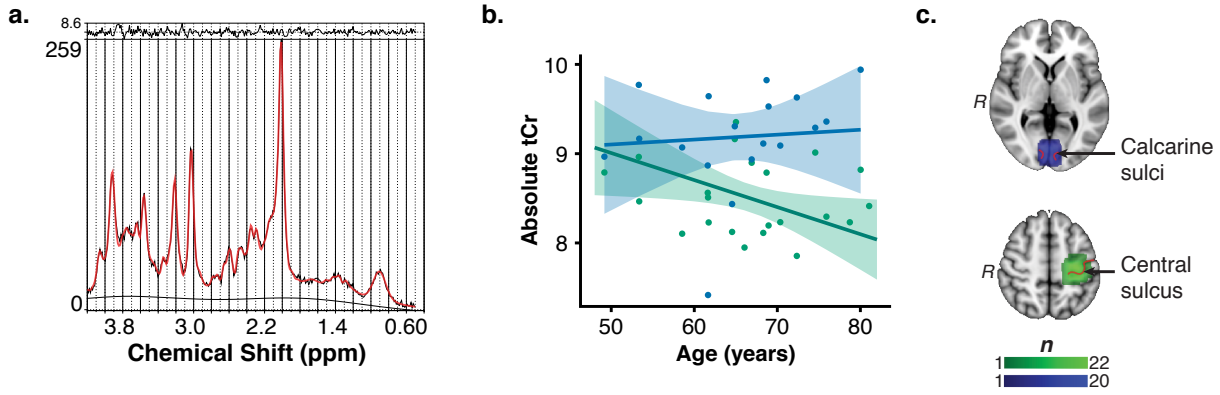

Figure S2: **Magnetic resonance spectroscopy data quality.** This figure shows the quality of MRS data collected for experiment 1. **a.** Example raw MRS spectrum and LCModel fit from one participant. The fitted LCModel (in red) is plotted overlaid on the raw data (in black). The difference between the data and model (residuals) is shown at the top and the baseline is shown at the bottom. **b.** This panel presents the association between age and total Creatine (tCr), controlling for the fraction of WM and GM, in the M1 voxel (in green) and the V1 voxel (in blue). Shading indicate 95% confidence intervals. This panel shows that tCr estimate was negatively correlated with age in M1, but not in V1. Because of this relationship, we use absolute conentrations of GABA and Glx throught the paper, rather than using tCr for internal referencing. **c.** Magnetic resonance spectroscopy voxels group overlap map. The M1 voxel was centred on the left central sulcus in 22 participants (in green, MNI coordinate z = 52). The control V1 voxel was centred on the bilateral calcarine sulcus in 20 participants (in blue, MNI coordinate z = 2). Colour bar represents the degree of overlap. All images are displayed in radiological convention (i.e. left side of the image corresponds to the right side of the brain).

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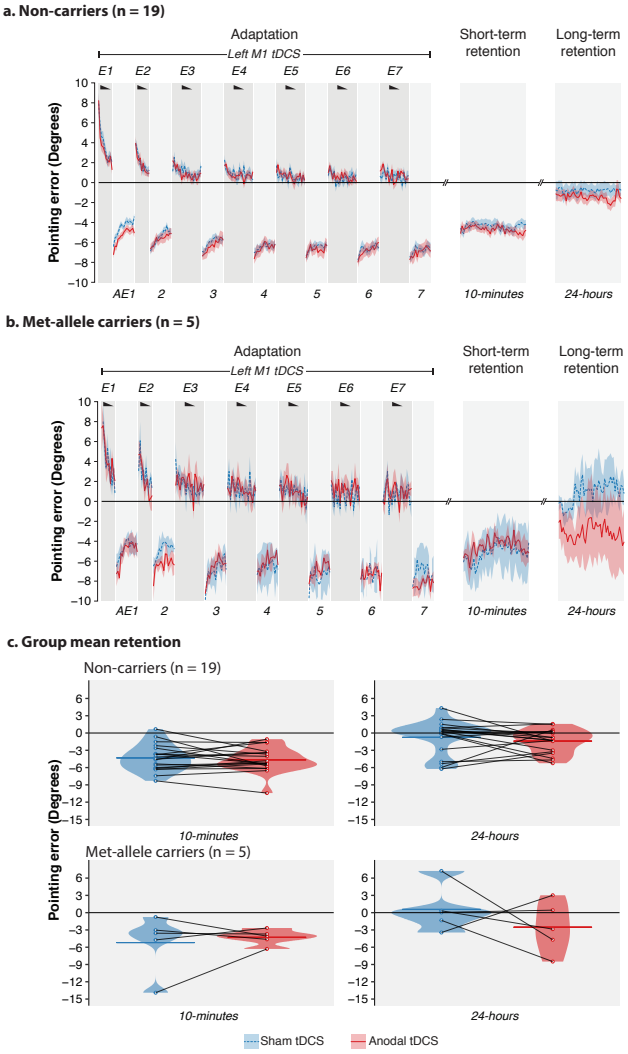

Figure S3: **The BDNF Val66Met polymorphism does not influence the effect of anodal tDCS on retention.** Here, we investigated the relationship between BDNF genotype and retention as a function of a-tDCS condition. **a.** Prism adaptation pointing behaviour is plotted for non-carriers. **b** Prism adaptation pointing behaviour is plotted for Met-allele carriers. **c.** Mean short-term (10-min) and long-term (24-hour) retention is plotted for non-carrier and Met-allele carriers. Pointing performance are normalised by the baseline (pre-exposure) accuracy. We used linear mixed models to examine whether BDNF polymorphism influenced the effect of a-tDCS on short-term (10min) and long-term (24hr) retention. BDNF polymorphism did not influence short (BDNF $\times$ a-tDCS: $t_{(2141)} = 1.00$, $p = 0.320$) or long-term retention (BDNF $\times$ a-tDCS: $t_{(2141)} = -1.61$, $p = 0.107$).

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Supplementary Tables

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		Experiment 1		Experiment 2
Participant	**Age**	*PA session*	*MRS*	*PA+tDCS sessions*
1	81.1	✓	(✓)	✓
2	78.7	✓	(✓)	✓
3	80.0	✓	✓	✓
4	75.9	✓	✓	✓
5	74.6	✓	✓	✓
6	72.3	✓	✓	✓
7	68.7	✓	✓	✓
8	66.9	✓	✓	✓
9	64.6	✓	✓	
10	53.3	✓	✓	
11	68.9	✓	✓	✓
12	80.7	✓		✓
13	70.4	✓	✓	✓
14	66.0	✓	✓	
15	61.7	✓	✓	✓
16	68.3	✓	✓	
17	58.5	✓	✓	✓
18	53.4	✓		
19	64.9	✓	✓	
20	61.7	✓	✓	✓
21	49.2	✓	✓	
22	61.6	✓	✓	✓
23	65.0	✓	✓	✓
24	65.6	✓	✓	✓
25	75.4	✓		✓
26	74.1	✓		✓
27	67.8	✓		✓
28	65.2	✓		✓
29	55.4	✓		✓
30	67.4	✓		✓
31	70.3	✓		✓
32	70.9	✓		✓

Table S1: **Participant demographics and inclusion details.** Experiment 1 included a behavioural experiment (prism adaptation, PA; with retention probe the next day) and a MR Spectroscopy scan. Experiment 2 included two PA sessions with retention probe 24 hours later, separated by a week. Inclusion of participants in every part of the experiments is indicated by a tick. In the MRS column, parentheses indicate incomplete data due to technical difficulties (missing control voxel).

	Dependent variable:			
	Normalised angular error			
	Closed-loop pointing	Open-loop pointing	10-min retention	24-hour retention
	(1)	(2)	(3)	(4)
Intercept	1.06^{***} (0.84, 1.27)	-6.66^{***} (-7.43, -5.89)	-4.61^{***} (-5.40, -3.81)	-1.30^{***} (-2.23, -0.37)
Trial	-0.08^{***} (-0.10, -0.07)	0.14^{***} (0.11, 0.17)	0.01 (-0.01, 0.03)	0.002 (-0.02, 0.02)
Block	-0.42^{***} (-0.51, -0.33)	-0.36^{***} (-0.55, -0.18)		
Trial:Block	0.05^{***} (0.04, 0.06)	-0.02^{***} (-0.03, -0.01)		
Observations	3,200	2,880	1,440	1,440
Bayesian Inf. Crit.	13,771.00	10,153.74	5,183.54	4,949.39

Table S2: **Experiment 1: Prism adaptation behaviour.** All LMMs use the normalised pointing error as the dependent variable (i.e. error *minus* baseline error). Model (1) assesses the reduction of CLP errors throughout prism exposure (blocks E1-6), while model (2) captures the development of an after-effect on OLP trials (blocks AE1-6). Models (3) and (4) assess the persistence (intercept) and stability (main effect of Trial) of the after-effect (OLP) 10-minutes and 24-hours after PA. $^{*}p<0.1$; $^{**}p<0.05$; $^{***}p<0.01$ (all two-tailed).

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	Dependent variable:					
	Normalised angular error					
	End of PA	10-min retention	24-hour retention	24-hour retention [c:AE6]	24-hour retention [controlling:10-min]	24-hour retention [c:mvtDuration]
	(1)	(2)	(3)	(4)	(5)	(6)
Intercept	−7.36*** (−8.46, −6.26)	−4.61*** (−5.39, −3.82)	−1.30*** (−2.16, −0.44)	−1.30*** (−2.16, −0.44)	−1.30*** (−2.16, −0.44)	−0.07 (−3.36, 3.21)
Trial	0.10*** (0.07, 0.14)	0.01 (−0.01, 0.03)	0.002 (−0.02, 0.02)	0.002 (−0.02, 0.02)	0.002 (−0.02, 0.02)	0.002 (−0.02, 0.02)
Age	0.03 (−0.11, 0.17)	0.05 (−0.05, 0.15)	−0.12** (−0.23, −0.02)	−0.13** (−0.24, −0.02)	−0.13** (−0.24, −0.02)	−0.12** (−0.23, −0.01)
Age:Trial	0.0005 (−0.004, 0.01)	0.002* (−0.0003, 0.004)	0.001 (−0.002, 0.004)	0.001 (−0.002, 0.004)	0.001 (−0.002, 0.004)	0.001 (−0.002, 0.004)
mean AE (end of PA)				0.11 (−0.14, 0.37)		
mean AE (10-min)					0.03 (−0.33, 0.40)	
Movement duration						−0.003 (−0.01, 0.004)
Observations	480	1,440	1,440	1,440	1,440	1,440
Bayesian Inf. Crit.	1,730.39	5,194.85	4,957.99	4,964.52	4,965.23	4,964.71

Table S3: **Experiment 1: Older participants show a more persistent prism after-effect.** The LMMs reported in this table examine the relationship between age and prism adaptation memory. They all use normalised OLP error as the dependent variable (i.e. error *minus* baseline error). Models (1), (2) and (3) examine the relationship between age and prism after-effect at the end of adaptation (block AE6), and at the 10-minutes and 24-hour retention time points respectively. Only 24-hours retention behaviour was related to age, such that older participants showed a larger (more negative) AE. The next three models assess the robustness of this result when controlling for the average AE at the end of the adaptation (model 4), the average AE at the 10-minutes retention block (model 5), and the average movement duration on CLP trials during prism exposure (model 6). The relationship between age and long-term adaptation memory survived controlling for all three factors, confirming that it was not an artefact of older participants adapting to a greater extent on the first day or pointing more slowly. *p<0.1; **p<0.05; ***p<0.01 (all two-tailed).

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	Dependent variable:					
	M1 E:I	M1 GABA	M1 Glx	V1 E:I	V1 GABA	V1 Glx
	(1)	(2)	(3)	(4)	(5)	(6)
Intercept	−0.00 (−0.41, 0.41)	0.00 (−0.14, 0.14)	0.00 (−0.39, 0.39)	−0.22 (−0.73, 0.30)	0.08 (−0.12, 0.27)	−0.34** (−0.63, −0.05)
Age	0.08* (0.005, 0.15)	−0.03** (−0.06, −0.01)	−0.03 (−0.11, 0.05)	0.05 (−0.02, 0.12)	−0.02 (−0.04, 0.004)	0.005 (−0.04, 0.05)
Glx		0.04 (−0.13, 0.21)			0.28* (0.003, 0.56)	
GABA			0.31 (−0.98, 1.61)			0.93* (0.01, 1.85)
GM	0.15 (−0.04, 0.34)	−0.07* (−0.14, −0.0003)	−0.07 (−0.27, 0.13)	−0.10 (−0.24, 0.05)	0.05* (−0.003, 0.10)	−0.12** (−0.20, −0.03)
WM	0.04 (−0.05, 0.13)	−0.03 (−0.07, 0.01)	−0.12** (−0.22, −0.03)	−0.11 (−0.24, 0.02)	0.06 (−0.01, 0.14)	−0.22*** (−0.29, −0.15)
Observations	22	22	22	16	16	16
Adjusted R^2	0.06	0.20	0.25	0.40	0.48	0.77

Table S4: **Experiment 1: Older participants have a higher excitation:inhibition ratio in sensorimotor cortex.** The linear regressions reported in this table examine the relationship between age and metabolite concentration within the motor (labelled "M1") and occipital (labelled "V1") cortex voxels. All models controlled for the fraction of grey and white matter within the MRS voxel, and included the MRS measure as the dependent variable. Model (1) shows the predicted significant positive relationship between age and E:I ratio (Glx:GABA). Models (2) and (3) decompose this relationship into its GABA and Glx constituents respectively. They highlight that the age-related increase in E:I was mainly due to a loss of inhibition. The final three models show a qualitatively similar, though not significant, pattern within the bilateral occipital cortex. *p<0.1; **p<0.05; ***p<0.01 (all two-tailed).

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	Dependent variable:							
	Normalised angular error							
	M1 E:I	M1 GABA and Glx	M1 E:I [c:mvtDuration]	M1 GABA and Glx [c:mvtDuration]	V1 E:I	V1 GABA and Glx	V1 E:I [c:mvtDuration]	V1 GABA and Glx [c:mvtDuration]
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Intercept	−0.79** (−1.53, −0.05)	−0.79** (−1.56, −0.03)	2.73 (−1.41, 6.87)	1.51 (−2.86, 5.87)	−0.44 (−2.07, 1.20)	0.08 (−1.75, 1.91)	10.95*** (4.23, 17.67)	11.12*** (4.14, 18.11)
Trial	0.01 (−0.03, 0.04)	0.01 (−0.03, 0.04)	0.01 (−0.03, 0.04)	0.01 (−0.03, 0.04)	−0.001 (−0.04, 0.04)	−0.003 (−0.04, 0.04)	−0.001 (−0.04, 0.04)	−0.003 (−0.04, 0.04)
EI	−2.07*** (−2.82, −1.32)		−2.07*** (−2.77, −1.37)		0.21 (−1.41, 1.82)		1.02 (−0.35, 2.39)	
GABA		5.59*** (3.42, 7.76)		5.58*** (3.49, 7.67)		−1.51 (−6.62, 3.60)		−3.32 (−7.63, 0.99)
Glx		0.003 (−0.87, 0.88)		−0.15 (−1.04, 0.74)		1.70 (−1.24, 4.63)		0.48 (−1.97, 2.94)
GM	0.24** (0.03, 0.45)	0.29*** (0.09, 0.50)	0.15 (−0.07, 0.37)	0.23** (0.01, 0.45)	0.28 (−0.11, 0.67)	0.44* (−0.05, 0.94)	0.20 (−0.10, 0.50)	0.23 (−0.17, 0.64)
WM	−0.01 (−0.15, 0.12)	0.11 (−0.05, 0.27)	−0.03 (−0.16, 0.10)	0.08 (−0.08, 0.24)	0.30 (−0.08, 0.69)	0.65* (−0.06, 1.36)	0.18 (−0.12, 0.48)	0.18 (−0.45, 0.80)
Trial:EI	−0.01 (−0.04, 0.02)		−0.01 (−0.04, 0.02)		0.01 (−0.02, 0.05)		0.01 (−0.02, 0.05)	
Trial:GABA		0.01 (−0.08, 0.10)		0.01 (−0.08, 0.10)		−0.03 (−0.13, 0.08)		−0.03 (−0.13, 0.08)
Trial:Glx		−0.004 (−0.03, 0.03)		−0.004 (−0.03, 0.03)		0.01 (−0.03, 0.05)		0.01 (−0.03, 0.05)
Movement duration			−0.01* (−0.02, 0.001)	−0.01 (−0.02, 0.01)			−0.03*** (−0.05, −0.01)	−0.03*** (−0.05, −0.01)
Observations	990	990	990	990	720	720	720	720
Bayesian Inf. Crit.	3,359.62	3,371.64	3,363.89	3,377.51	2,479.94	2,491.97	2,477.90	2,490.77

Table S5: **Experiment 1: Higher sensorimotor cortex excitation:inhibition ratio is associated with greater 24-hour retention**. The LMMs reported in this table examine the relationship between M1 and V1 neurochemistry and the magnitude of the AE at 24-hours. All models controlled for the fraction of grey and white matter within the MRS voxel. Model (1) shows that individuals with higher M1 E:I had a larger (more negative) AE at 24-hour. Model (2) decomposes this relationship into its GABA and Glx constituents respectively, highlighting that GABA but not Glx drives the previous relationship. Models (3) and (4) show that these findings were robust to controlling for the average movement duration on CLP trials during prism exposure. Finally, models (5) to (8) reproduce the same set of analyses using MRS data from the anatomical control voxel (V1). No relationship between neurochemistry and long-term adaptation memory was observed in the V1 voxel. *p<0.1; **p<0.05; ***p<0.01 (all two-tailed).

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	Dependent variable:					
	M_1 (M1 E:I)	M_2 (M1 GABA)	M_3 (M1 Glx)		Y (AE24hrs)	
	(1)	(2)	(3)	(4)	(5)	(6)
X (age)	0.66* (0.31)	−0.78** (0.28)	−0.31 (0.27)	−0.76** (0.29)	−0.35 (0.24)	−0.28 (0.28)
M_1 (M1 E:I)					−0.63*** (0.16)	
M_2 (M1 GABA)						0.65*** (0.20)
M_3 (M1 Glx)						−0.06 (0.20)
C_1 (GM)	0.54 (0.35)	−0.68** (0.31)	−0.31 (0.30)	−0.40 (0.32)	−0.07 (0.25)	0.02 (0.29)
C_2 (WM)	0.20 (0.26)	−0.53** (0.23)	−0.70*** (0.22)	−0.32 (0.23)	−0.19 (0.18)	−0.01 (0.25)
Observations	22	22	22	22	22	22
Adjusted R^2	0.06	0.24	0.28	0.22	0.56	0.48
F Statistic	1.46 (df = 3; 18)	3.16* (df = 3; 18)	3.72** (df = 3; 18)	2.99* (df = 3; 18)	7.81*** (df = 4; 17)	4.88*** (df = 5; 16)

Table S6: **Experiment 1: Motor cortical GABA mediates the relationship between age and long-term adaptation memory.** Model (1) shows a near-significant relationship between age and motor cortical E:I ($p = 0.051$, two-tailed; $p = 0.025$, one-tailed). Models (2) and (3) show that this relationship is driven by GABA ($p = 0.013$) and not Glx ($p = 0.27$). Model (4) shows that older age is associated with greater 24-hour retention ($p = 0.02$). Crucially, model (5) demonstrates that the association between age and 24-hour retention is no longer significant when accounting for M1 E:I. Further, model (6) shows that the mediation is specifically driven by GABA ($p = 0.004$) and not Glx ($p = 0.78$). Overall, these regression models provide support in favour of M1 GABA mediating the relationship between age and long-term retention, which was subsequently assessed formally. The results indicate a significant mediating effect of M1 GABA ($ab_1 = -0.50,\ 95\%CI : [-1.36, -0.14],\ p = 0.01$) but not M1 Glx ($ab_2 = 0.02,\ 95\%CI : [-0.09, 0.31],\ p = 0.73$; see Fig. 4).*p<0.1; **p<0.05; ***p<0.01 (all two-tailed).

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	Dependent variable:					
	M_1 (M1 E:I)	M_2 (M1 GABA)	M_3 (M1 Glx)		Y (AE24hrs)	
	(1)	(2)	(3)	(4)	(5)	(6)
X (age)	0.66* (0.31)	−0.78** (0.28)	−0.31 (0.27)	−0.68** (0.29)	−0.34 (0.24)	−0.28 (0.29)
M_1 (M1 E:I)					−0.60*** (0.17)	
M_2 (M1 GABA)						0.66** (0.23)
M_3 (M1 Glx)						−0.05 (0.21)
C_1 (GM)	0.54 (0.35)	−0.68** (0.31)	−0.31 (0.30)	−0.31 (0.32)	−0.04 (0.26)	0.02 (0.30)
C_2 (WM)	0.20 (0.26)	−0.53** (0.23)	−0.70*** (0.22)	−0.27 (0.23)	−0.18 (0.18)	−0.01 (0.27)
C_3 (Mvt duration)				−0.23 (0.20)	−0.09 (0.16)	0.02 (0.20)
Observations	22	22	22	22	22	22
Adjusted R^2	0.06	0.24	0.28	0.24	0.55	0.45
F Statistic	1.46 (df = 3; 18)	3.16* (df = 3; 18)	3.72** (df = 3; 18)	2.63* (df = 4; 17)	6.08*** (df = 5; 16)	3.81** (df = 6; 15)

Table S7: **Experiment 1: Mediation analysis controlling for average CLP duration.** This table presents the results of the mediation analysis, controlling for the average reaching movement duration on CLP trials during prism exposure. Overall, this table indicates that the results presented in Table S6 persist: M1 GABA, but not Glx, mediates the relationship between age and 24-hour retention.*p<0.1; **p<0.05; ***p<0.01 (all two-tailed).

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	Dependent variable:					
	Normalised angular error					
	a-tDCS:10min	a-tDCS:EI (10min)	a-tDCS:GABA/Glx (10min)	a-tDCS:24-hour	a-tDCS:EI (24hrs)	a-tDCS:GABA/Glx (24hrs)
	(1)	(2)	(3)	(4)	(5)	(6)
Intercept	−4.7*** (−5.5, −3.8)	−5.0*** (−6.1, −4.0)	−5.0*** (−6.1, −4.0)	−1.0** (−1.8, −0.2)	−1.1*** (−1.7, −0.4)	−1.1*** (−1.7, −0.5)
Trial	−0.003 (−0.02, 0.01)	0.003 (−0.02, 0.02)	0.003 (−0.02, 0.02)	−0.001 (−0.02, 0.02)	−0.001 (−0.03, 0.03)	−0.001 (−0.03, 0.03)
GM		0.1 (−0.1, 0.3)	0.1 (−0.1, 0.3)		0.2*** (0.1, 0.4)	0.2*** (0.1, 0.4)
WM		−0.1 (−0.2, 0.04)	−0.04 (−0.2, 0.1)		0.1 (−0.05, 0.2)	0.1 (−0.1, 0.2)
a-tDCS	0.1 (−0.5, 0.6)	0.1 (−0.5, 0.7)	0.1 (−0.5, 0.7)	−0.5 (−1.3, 0.2)	0.04 (−0.6, 0.7)	0.04 (−0.6, 0.7)
E:I		−0.4 (−1.5, 0.6)			−0.9*** (−1.5, −0.3)	
GABA			0.6 (−2.4, 3.6)			2.8*** (1.0, 4.6)
Glx			−0.1 (−1.2, 1.0)			−0.3 (−1.0, 0.5)
Trial:a-tDCS	−0.01 (−0.02, 0.01)	0.000 (−0.01, 0.01)	0.000 (−0.01, 0.01)	−0.01 (−0.02, 0.005)	−0.01 (−0.02, 0.01)	−0.01 (−0.02, 0.01)
Trial:EI		0.01 (−0.01, 0.03)			0.02 (−0.01, 0.05)	
Trial:GABA			−0.03 (−0.1, 0.03)			−0.04 (−0.1, 0.04)
Trial:Glx			0.01 (−0.01, 0.03)			0.004 (−0.02, 0.03)
a-tDCS:EI		0.6* (−0.03, 1.2)			0.8** (0.1, 1.4)	
a-tDCS:GABA			−1.2 (−3.0, 0.6)			−1.5* (−3.3, 0.2)
a-tDCS:Glx			0.3 (−0.3, 0.8)			0.7** (0.2, 1.3)
Trial:a-tDCS:EI		0.01 (−0.01, 0.02)			0.01** (0.002, 0.02)	
Trial:a-tDCS:GABA			−0.01 (−0.1, 0.03)			−0.03 (−0.1, 0.01)
Trial:a-tDCS:Glx			0.01 (−0.003, 0.02)			0.004 (−0.01, 0.01)
Observations	2,250	1,440	1,440	2,250	1,440	1,440
Bayesian Inf. Crit.	7,470.8	4,830.3	4,858.5	8,378.3	5,400.6	5,425.6
Note:						*p<0.1; **p<0.05; ***p<0.01

Table S8: **Experiment 2: Association between a-tDCS, M1 neurochemistry, and adaptation memory.** All linear mixed-effect models use the normalised pointing error (at 10-min or 24-hours post-PA) as the dependent variable. Model (1) assesses the effect of left M1 anodal tDCS on the after-effect at 10-min. Model (2) assesses the interaction of left M1 E:I (Glx:GABA) with this effect. Model (3) decomposes the individual interaction of GABA and Glx with the behavioural effect of a-tDCS effect on short-term retention. Models (4), (5), (6) assess the same effects at the long-term retention time point (24-hours). The most important finding here is that M1 E:I significantly interacted with the effect of a-tDCS on long-term retention (a-tDCS:EI in Model 5), which could be decomposed into opposite interactions with GABA and Glx (a-tDCS:GABA and a-tDCS:Glx in Model 6). All models including MRS data also controlled for the fraction of grey and white matter within the voxel. *p<0.1; **p<0.05; ***p<0.01 (all two-tailed).

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	Dependent variable:			
	Normalised angular error			
	a-tDCS:EI (10min)	a-tDCS:GABA/Glx (10min)	a-tDCS:EI (24hrs)	a-tDCS:GABA/Glx (24hrs)
	(1)	(2)	(3)	(4)
Intercept	−1.9 (−7.6, 3.8)	−1.9 (−9.4, 5.6)	−4.3* (−9.0, 0.4)	−7.4** (−13.1, −1.7)
Trial	0.003 (−0.02, 0.02)	0.003 (−0.02, 0.02)	−0.001 (−0.03, 0.03)	−0.001 (−0.03, 0.03)
Mvt Duration	−0.01 (−0.02, 0.005)	−0.01 (−0.02, 0.01)	0.01 (−0.003, 0.01)	0.01** (0.001, 0.02)
GM	0.01 (−0.2, 0.3)	0.01 (−0.3, 0.3)	0.3*** (0.1, 0.5)	0.4*** (0.2, 0.6)
WM	−0.1* (−0.2, 0.02)	−0.1 (−0.3, 0.1)	0.1 (−0.03, 0.2)	0.1* (−0.01, 0.3)
a-tDCS	0.1 (−0.5, 0.7)	0.1 (−0.5, 0.7)	0.04 (−0.6, 0.7)	0.04 (−0.6, 0.7)
EI	−0.3 (−1.3, 0.7)		−1.0*** (−1.7, −0.4)	
GABA		0.2 (−2.9, 3.2)		3.6*** (1.7, 5.6)
Glx		−0.4 (−1.6, 0.8)		0.2 (−0.7, 1.0)
Trial:a-tDCS	0.000 (−0.01, 0.01)	0.000 (−0.01, 0.01)	−0.01 (−0.02, 0.01)	−0.01 (−0.02, 0.01)
Trial:EI	0.01 (−0.01, 0.03)		0.02 (−0.01, 0.05)	
Trial:GABA		−0.03 (−0.1, 0.03)		−0.04 (−0.1, 0.04)
Trial:Glx		0.01 (−0.01, 0.03)		0.004 (−0.02, 0.03)
a-tDCS:EI	0.6* (−0.03, 1.2)		0.8** (0.1, 1.4)	
a-tDCS:GABA		−1.2 (−3.0, 0.6)		−1.5* (−3.3, 0.2)
a-tDCS:Glx		0.3 (−0.3, 0.8)		0.7** (0.2, 1.3)
Trial:a-tDCS:EI	0.01 (−0.01, 0.02)		0.01** (0.002, 0.02)	
Trial:a-tDCS:GABA		−0.01 (−0.1, 0.03)		−0.03 (−0.1, 0.01)
Trial:a-tDCS:Glx		0.01 (−0.003, 0.02)		0.004 (−0.01, 0.01)
Observations	1,440	1,440	1,440	1,440
Bayesian Inf. Crit.	4,836.5	4,865.2	5,406.9	5,430.6
Note:				*p<0.1; **p<0.05; ***p<0.01

Table S9: **Experiment 2: Association between a-tDCS, M1 neurochemistry, and adaptation memory while controlling for average movement speed during prism exposure.** All linear mixed-effect models use the normalised pointing error (at 10-min or 24-hours post-PA) as the dependent variable, and control for the average movement duration of the CLP trials of the prism exposure. Model (1) assesses the modulation influence of left M1 E:I (Glx:GABA) on the behavioural effect of a-tDCS on the AE at 10-minutes. Model (2) decomposes the individual interaction of GABA and Glx with the behavioural effect of a-tDCS effect on short-term retention. Models (4) and (5) assess the same effects at the long-term retention time point (24-hours). There was no significant interaction between a-tDCS and E:I at any of the two retention time points. All models also controlled for the fraction of grey and white matter within the voxel. *p<0.1; **p<0.05; ***p<0.01 (all two-tailed).

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	Dependent variable:			
	Normalised angular error			
	a-tDCS:EI (10min)	a-tDCS:GABA/Glx (10min)	a-tDCS:EI (24hrs)	a-tDCS:GABA/Glx (24hrs)
	(1)	(2)	(3)	(4)
Intercept	−4.1*** (−5.4, −2.8)	−3.3*** (−4.7, −1.9)	−2.0*** (−3.2, −0.9)	−1.3** (−2.4, −0.2)
Trial	0.004 (−0.02, 0.03)	0.004 (−0.02, 0.03)	−0.01 (−0.04, 0.03)	−0.01 (−0.05, 0.02)
GM	0.2 (−0.1, 0.4)	0.3** (0.04, 0.5)	−0.2* (−0.5, 0.04)	−0.3*** (−0.6, −0.1)
WM	0.2 (−0.1, 0.4)	0.5*** (0.1, 0.8)	−0.3** (−0.5, −0.01)	−0.1 (−0.4, 0.2)
a-tDCS	0.3 (−0.5, 1.0)	0.2 (−0.5, 0.8)	0.4 (−0.5, 1.2)	0.3 (−0.6, 1.1)
EI	0.3 (−0.9, 1.5)		−1.3** (−2.4, −0.2)	
GABA		−2.6 (−6.5, 1.3)		4.9*** (1.9, 7.8)
Glx		0.8 (−1.0, 2.6)		0.4 (−1.2, 1.9)
Trial:a-tDCS	−0.001 (−0.02, 0.02)	−0.002 (−0.02, 0.02)	−0.002 (−0.02, 0.01)	−0.003 (−0.02, 0.01)
Trial:EI	−0.01 (−0.03, 0.02)		0.005 (−0.03, 0.04)	
Trial:GABA		0.01 (−0.1, 0.1)		−0.004 (−0.1, 0.1)
Trial:Glx		−0.000 (−0.03, 0.03)		0.01 (−0.03, 0.05)
a-tDCS:EI	−0.4 (−1.1, 0.3)		0.4 (−0.4, 1.1)	
a-tDCS:GABA		2.6** (0.5, 4.7)		−0.6 (−3.2, 1.9)
a-tDCS:Glx		0.5 (−0.2, 1.3)		0.5 (−0.4, 1.5)
Trial:a-tDCS:EI	−0.005 (−0.02, 0.01)		0.005 (−0.01, 0.02)	
Trial:a-tDCS:GABA		0.03 (−0.03, 0.1)		−0.000 (−0.05, 0.05)
Trial:a-tDCS:Glx		0.01 (−0.01, 0.03)		0.01 (−0.01, 0.03)
Observations	1,080	1,080	1,080	1,080
Bayesian Inf. Crit.	3,639.9	3,661.8	4,148.1	4,170.5
Note:				*p<0.1; **p<0.05; ***p<0.01

Table S10: **Experiment 2: Association between a-tDCS, V1 neurochemistry, and adaptation memory.** All linear mixed-effect models use the normalised pointing error (at 10-min or 24-hours post-PA) as the dependent variable. Model (1) assesses the modulation influence of left M1 E:I (Glx:GABA) on the behavioural effect of a-tDCS on the AE at 10-minutes. Model (2) decomposes the individual interaction of GABA and Glx with the behavioural effect of a-tDCS effect on short-term retention. Models (4) and (5) assess the same effects at the long-term retention time point (24-hours). There was no significant interaction between a-tDCS and E:I at any of the two retention time points. All models also controlled for the fraction of grey and white matter within the voxel. *p<0.1; **p<0.05; ***p<0.01 (all two-tailed).

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