**Figure S1.** The full experimental protocol consisted of initial training and 6hr retest sessions on the same day. The main text presents data from the initial training session only. RS fMRI = resting state functional magnetic resonance imaging. MRS = magnetic resonance spectroscopy of right sensorimotor cortex (contralateral to hand used to perform motor task). MSL = motor sequence learning. tDCS = transcranial direct current stimulation. DWI = diffusion weighted imaging.
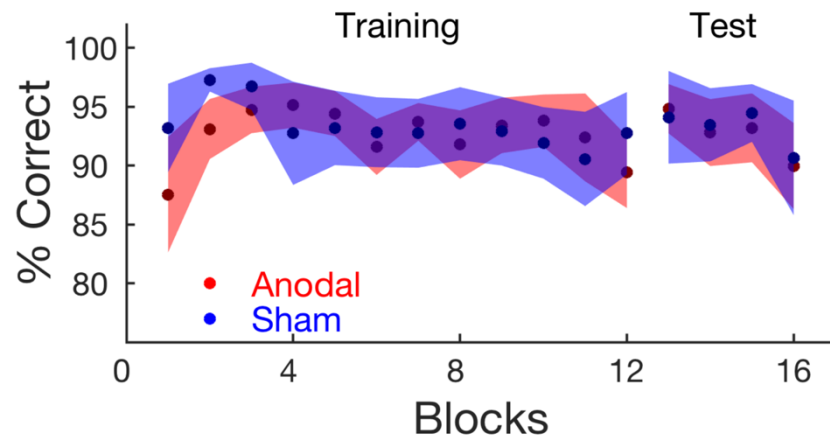
Figure S2. Percentage of correct transitions, reflecting sequence accuracy, plotted for the two experimental groups across the 12 blocks of training and the 4 blocks of the immediate post-test. Red and blue depict anodal and sham tDCS groups, respectively. Shaded regions represent SEM. A 12 (Block) x 2 (Group) ANOVA on the training run revealed no significant Group main effect ($F_{1,34}=0.046$, $p=0.83$), Block main effect ($F_{4.61,156.81}=1.86$, $p=0.11$) or Group by Block interaction ($F_{4.61,156.81}=1.32$, $p=0.26$). Similarly, there were no significant Group ($F_{1,34}=0.015$, $p=0.90$), Block ($F_{1.37,46.57}=1.22$, $p=0.29$), or Block by Group effects during the immediate post-training test ($F_{1.37,46.57}=0.065$, $p=0.87$).
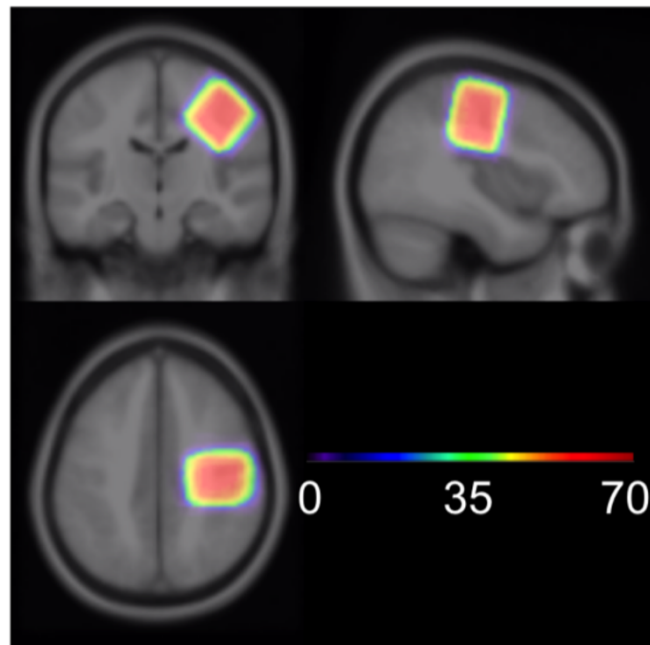
Figure S3. The T1-weighted structural images and MRS voxel masks were normalized to MNI space in SPM12. The normalized and binarized voxel masks (n=72; 36 participants x 2 sessions) were then summed and overlaid on an MNI template to depict the spatial overlap of the MRS voxels. The high spatial overlap indicates high consistency in voxel placement across participants and timepoints.

**Table S1.** MRS data quality and voxel composition metrics for the 2 experimental groups and 2 timepoints.

	Anodal		Sham		Statistical Contrasts		
	Pre	Post	Pre	Post	Session	Group	S x G
GABA+ FE (%)	4.3 ± 1.2	4.0 ± 1.3	4.0 ± 1.1	4.5 ± 1.7	$F_{(1,34)}=0.22$	$F_{(1,34)}=0.04$	$F_{(1,34)}=2.59$
GABA+ SNR	24.9 ± 4.6	23.8 ± 4.1	25.2 ± 4.0	23.6 ± 4.0	$F_{(1,34)}=3.72$	$F_{(1,34)}=0.01$	$F_{(1,34)}=0.12$
NAA LW (Hz)	9.7 ± 0.9	10.4 ± 1.6	10.1 ± 1.1	11.8 ± 2.6	$\mathbf{F_{(1,34)}=14.69\#}$	$F_{(1,34)}=3.23$	$F_{(1,34)}=3.15$
SD Freq Off	0.76 ± 0.23	0.87 ± 0.36	0.70 ± 0.23	0.80 ± 0.35	$\mathbf{F_{(1,34)}=4.12*}$	$F_{(1,34)}=0.54$	$F_{(1,34)}=0.02$
GM (%)	29.5 ± 5.1	29.1 ± 5.3	27.3 ± 4.7	27.9 ± 4.8	$F_{(1,34)}=0.06$	$F_{(1,34)}=1.14$	$F_{(1,34)}=1.18$
WM (%)	54.7 ± 7.8	55.8 ± 6.9	58.9 ± 6.0	57.5 ± 7.1	$F_{(1,34)}=0.03$	$F_{(1,34)}=1.89$	$F_{(1,34)}=1.57$
CSF (%)	15.8 ± 5.9	15.1 ± 5.2	13.8 ± 2.9	14.6 ± 3.9	$F_{(1,34)}=0.01$	$F_{(1,34)}=0.70$	$F_{(1,34)}=1.57$

FE = Fit error; SNR = signal-to-noise ratio; NAA LW = linewidth (LW), quantified as the full-width half-maximum

(FWHM) of the modelled N-acetylaspartate (NAA) signal. SD Freq Off = standard deviation of the frequency offset;

GM=gray matter; WM = white matter; CSF = cerebrospinal fluid. S x G = session x group interaction. n = 17 and 19

in the anodal and sham groups, respectively. #p<0.01; *p<0.05.
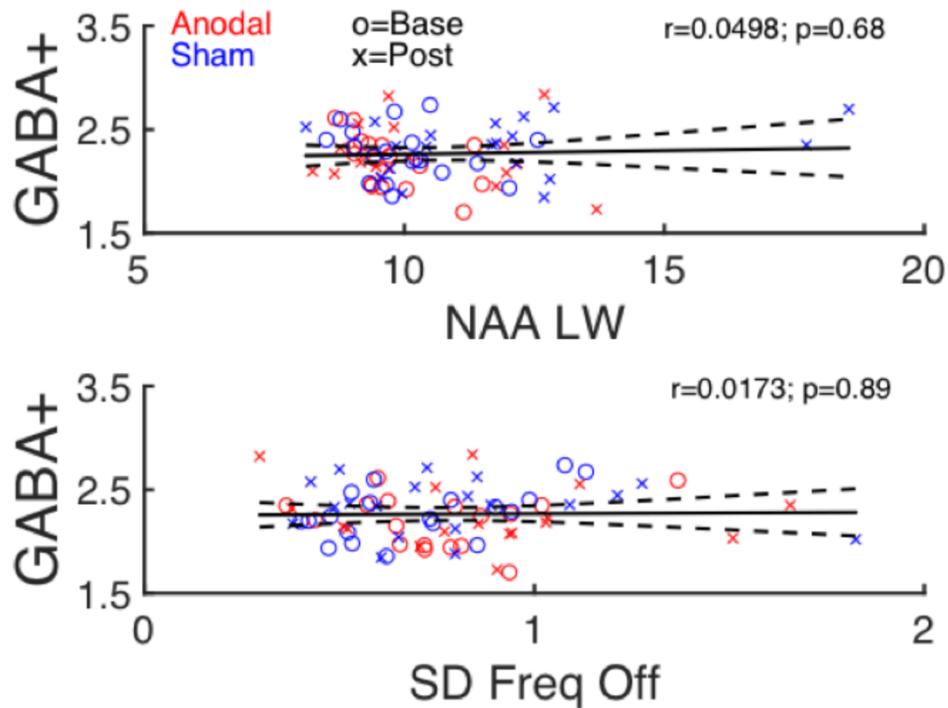
Figure S4. GABA+ levels (institutional units; i.u.) plotted as a function of NAA Line Width (Hz; top) and the SD of the Water Frequency Offset (bottom). Correlations were conducted across the two experimental groups and MRS timepoints (n=72), but the groups and timepoints are depicted in the scatterplot (red=Anodal; blue=Sham; circles = baseline session; crosses = post learning/stimulation session). Solid dark lines represent linear regression fits; dashed lines depict 95% prediction intervals of the linear function. Results indicate that there were no significant relationships between data quality metrics and GABA+ levels.

Table S2. Head motion during the resting state acquisition sequences for the 2 experimental groups and 2 timepoints.

	Anodal		Sham		Statistical Contrasts		
	Pre	Post	Pre	Post	Timepoint	Group	T x G
Translation	0.52 ± 0.40	0.69 ± 0.42	0.43 ± 0.21	0.52 ± 0.40	$F_{(1,31)}=2.30$	$F_{(1,31)}=1.02$	$F_{(1,31)}=0.03$
Rotation	0.57 ± 0.46	0.57 ± 0.29	0.55 ± 0.37	0.67 ± 0.37	$F_{(1,31)}=0.68$	$F_{(1,31)}<0.01$	$F_{(1,31)}=1.11$

Means and SD of the absolute value of the individuals' **maximum** translation and rotation (units of mm and degrees,

respectively) during the resting state scans are provided on the left. Values based on n = 16 and n = 17 in the anodal

and sham groups, respectively. Results from corresponding statistical analyses are shown on the right. There were no

significant Group, Timepoint or Timepoint by Group (T x G) effects on either movement parameter (all p>0.10).
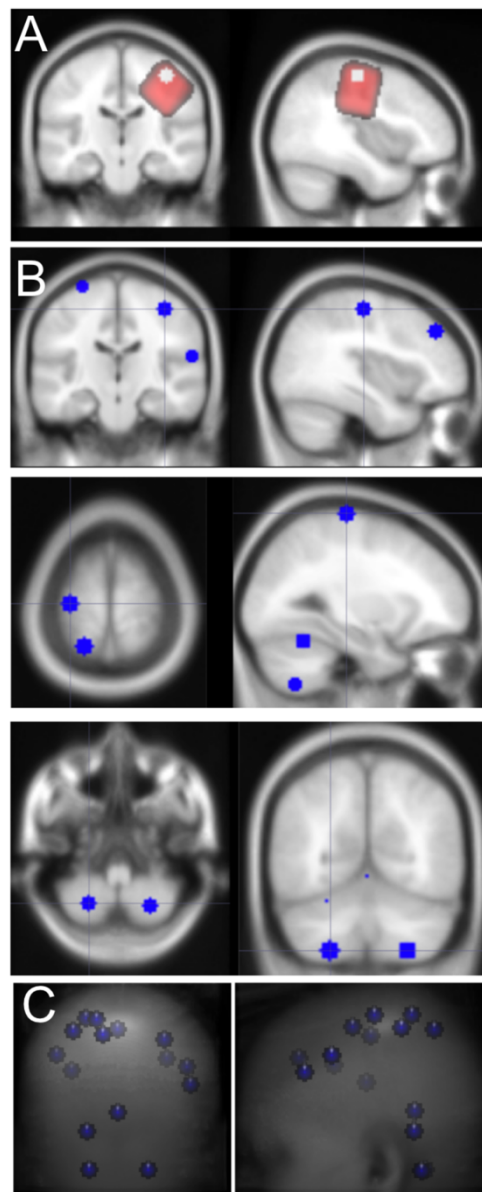
Figure S5. **A**. Right M1 seed (white sphere) identified based on task-related brain activity and used in subsequent functional connectivity analyses is located within the MRS voxel (red). **B.** Seed regions that demonstrated a significant increase in connectivity with the right M1 seed as a function of performance improvements are overlaid on an MNI template. See Table 5-2 for MNI coordinates of the 15 seeds identified and used in subsequent resting state functional connectivity analyses. Note that slices were selected in order to offer a representative depiction and thus not every single seed is visible in the images shown. **C.** All seeds are depicted in a glass brain from posterior (left) and sagittal views (right). Images were created using the software MRIcro and MRIcroGL (https://www.mccauslandcenter.sc.edu/crnl/tools).

Table S3. Regions of interest used in the resting state functional connectivity analyses.

Label	x	y	z	t-score
R motor cortex	38	-20	54	5.96
L motor cortex	-28	-24	72	3.50
SMA	-2	-6	62	4.34
L Sup Parietal (1)	-18	-54	72	3.95
L Sup Parietal (2)	-38	-46	62	4.55
L Precuneus	-12	-72	60	4.34
R Rol Op	60	-16	16	4.02
R Inf Frontal (Rol Op)	56	10	30	4.55
L Inf Frontal (Rol Op)	-54	10	40	4.29
R Mid Frontal	38	38	36	3.86
L Inf Frontal	-42	32	26	3.69
R CB Lobule VIII	26	-64	-54	3.27
L CB Lobule VIII	-24	-62	-54	3.70
L CB Lobule VI	-26	-56	-22	4.01
CB Vermis	0	-56	-6	3.41

x, y and z are the MNI coordinates of the identified seed regions. t-scores extracted from the PPI connectivity analyses assessing regions that exhibited significant increase in connectivity with right M1 as a function of performance improvements. R = right; L = left; SMA = supplementary motor area; Sup = superior; Inf = inferior; Rol Op = Rolandic Operculum, CB = cerebellum. See main text for details on the identification of these seeds.
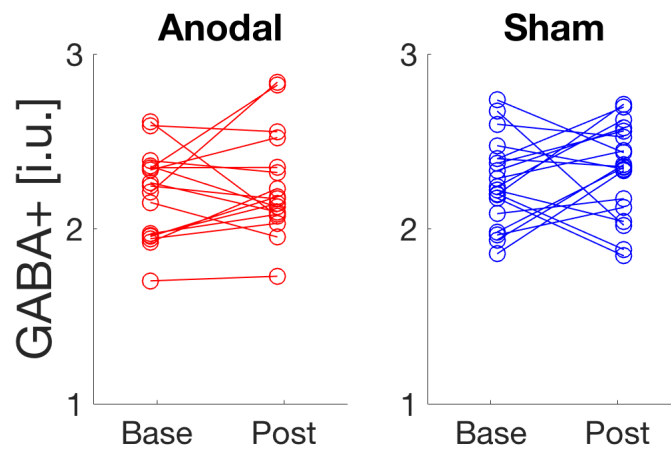
Figure S6. Individual GABA+ levels (institutional units; i.u.) for the baseline and post-learning/stimulation sessions are plotted pairwise for the anodal (red) and sham (blue) stimulation groups (small open circles).

Table S4. Correlations among age, GABA+ measures and motor sequence learning magnitude within the 2 experimental groups.

	Age	Base GABA+	% GABA Δ	Learn Mag
Age	-	**-0.56 (0.013)**	**0.54 (0.017)**	-0.16 (0.52)
Base GABA+	-0.09 (0.72)	-	**-0.62 (0.004)**	0.32 (0.18)
% GABA Δ	0.32 (0.20)	-0.40 (0.11)	-	-0.48 (0.038)
Learn Mag	-0.44 (0.07)	0.23 (0.38)	-0.32 (0.21)	-

Below and above the diagonal contains Pearson's correlation coefficients and corresponding

uncorrected p-values (in parentheses) within the anodal (n=17) and sham (n=19) stimulation groups,

respectively. Those in bold remained significant after correction for multiple comparisons using the

false discovery rate (FDR) approach (adjusted critical p=0.0172 for sham group). Learn Mag =

magnitude of motor sequence learning in the initial training session. % GABA+ Δ = percent change

in GABA+ from the baseline to post-learning / stimulation MRS timepoints.

Table S5. Group (anodal / sham) differences in the correlations among age, GABA+ measures and motor sequence learning magnitude.

	Age	Base GABA+	% GABA Δ	Learn Mag
Age	-	-	-	-
Base GABA+	0.14	-	-	-
% GABA Δ	0.47	0.40	-	-
Learn Mag	0.38	0.79	0.61	-

Uncorrected p-values corresponding to group differences in the z-transformed within-group

Pearson's correlation coefficients. Note that the two groups did not statistically differ in the

relationship among any of these pairs of variables and thus the correlation coefficients presented in

the main text are collapsed across the two groups.

Table S6. Partial correlations among age, GABA+ measures and motor sequence learning magnitude controlling for the effects of NAA LW.

	Age	Base GABA+	% GABA Δ	Learn Mag
Age	-	-	-	-
Base GABA+	-0.32 (0.064)	-	-	-
% GABA Δ	**0.45 (0.006)**	**-0.49 (0.002)**	-	-
Learn Mag	-0.29 (0.087)	0.18 (0.293)	**-0.36 (0.035)**	-

Pearson's partial correlation coefficients and corresponding uncorrected p-values (parentheses) are provided for each pair of variables of interest with NAA Line Width from the post-learning MRS session entered as a covariate. Those in bold are significant at p=0.05. Learn Mag = magnitude of motor sequence learning in the initial training session. % GABA+ Δ = percent change in GABA+ from the baseline to post-learning / stimulation MRS timepoints. Correlations were conducted across the two experimental groups (n=36).

Table S7. Partial correlations among age, GABA+ measures and motor sequence learning magnitude controlling for the effects of the SD of the Frequency offset.

	Age	Base GABA+	% GABA Δ	Learn Mag
Age	-	-	-	-
Base GABA+	-0.32 (0.056)	-	-	-
% GABA Δ	**0.46 (0.006)**	**-0.50 (0.002)**	-	-
Learn Mag	-0.29 (0.090)	0.21 (0.226)	**-0.40 (0.017)**	-

Pearson's partial correlation coefficients and corresponding uncorrected p-values (parentheses) are provided for each pair of variables of interest with SD of the Frequency Offset from the post-learning MRS session entered as a covariate. Those in bold are significant at p=0.05. Learn Mag = magnitude of motor sequence learning in the initial training session. % GABA+ Δ = percent change in GABA+ from the baseline to post-learning / stimulation MRS timepoints. Correlations were conducted across the two experimental groups (n=36).
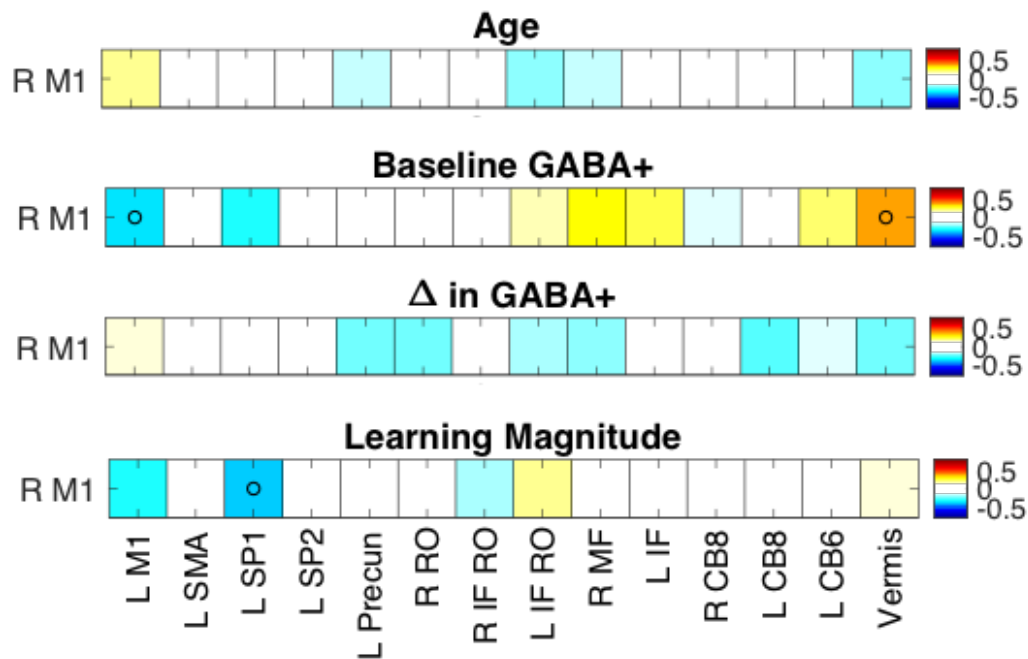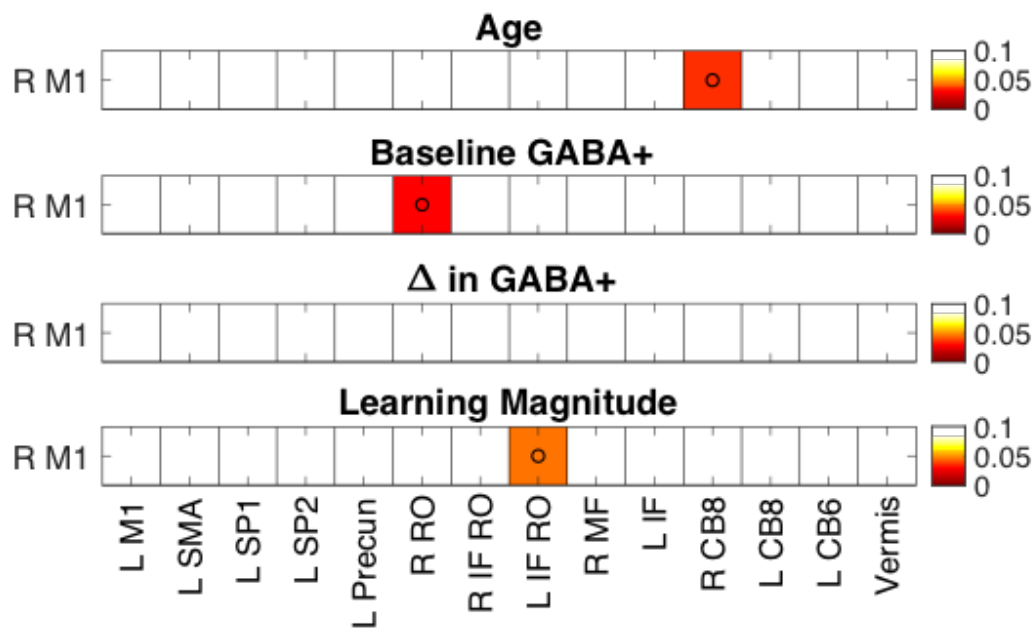
Figure S7. Relationships between baseline functional connectivity between the right M1 seed and the 14 motor task-relevant ROIs and age (top row), baseline GABA+ levels (2nd from top), change in GABA+ (2nd from bottom) and motor sequence learning magnitude (bottom row). Values represent Pearson's correlation coefficients conducted across the anodal and sham groups (n = 33). Tests of statistical significance were based on comparisons of the coefficients to a correlation of 0 and corrected for multiple comparisons with a False Discovery Rate (FDR) threshold set to 0.05. None of the seed/ROI pairs were significantly correlated with a variable of interest after correction for multiple comparisons. It is worth emphasizing that baseline connectivity between bilateral M1s did exhibit a negative relationship with baseline GABA+ levels before correction for multiple comparisons (r = -0.35; p = 0.043). That is, higher baseline GABA+ levels were related to lower M1-M1 functional connectivity. Although this result is analogous to previous research in both young (Bachtiar, Near, Johansen-Berg, & Stagg, 2015; Stagg et al., 2014) and older adults (Antonenko et al., 2017), it should be interpreted with caution as it did not survive FDR correction. o = p(uncorrected)<0.05. L = left; R = right; M1 = primary motor cortex; SMA = supplementary motor area; SP = superior parietal; Precun = precuneus; RO = rolandic operculum; IF = inferior frontal; MF = medial frontal; CB = cerebellar; the numbers 6 and 8 represent the cerebellar lobules.
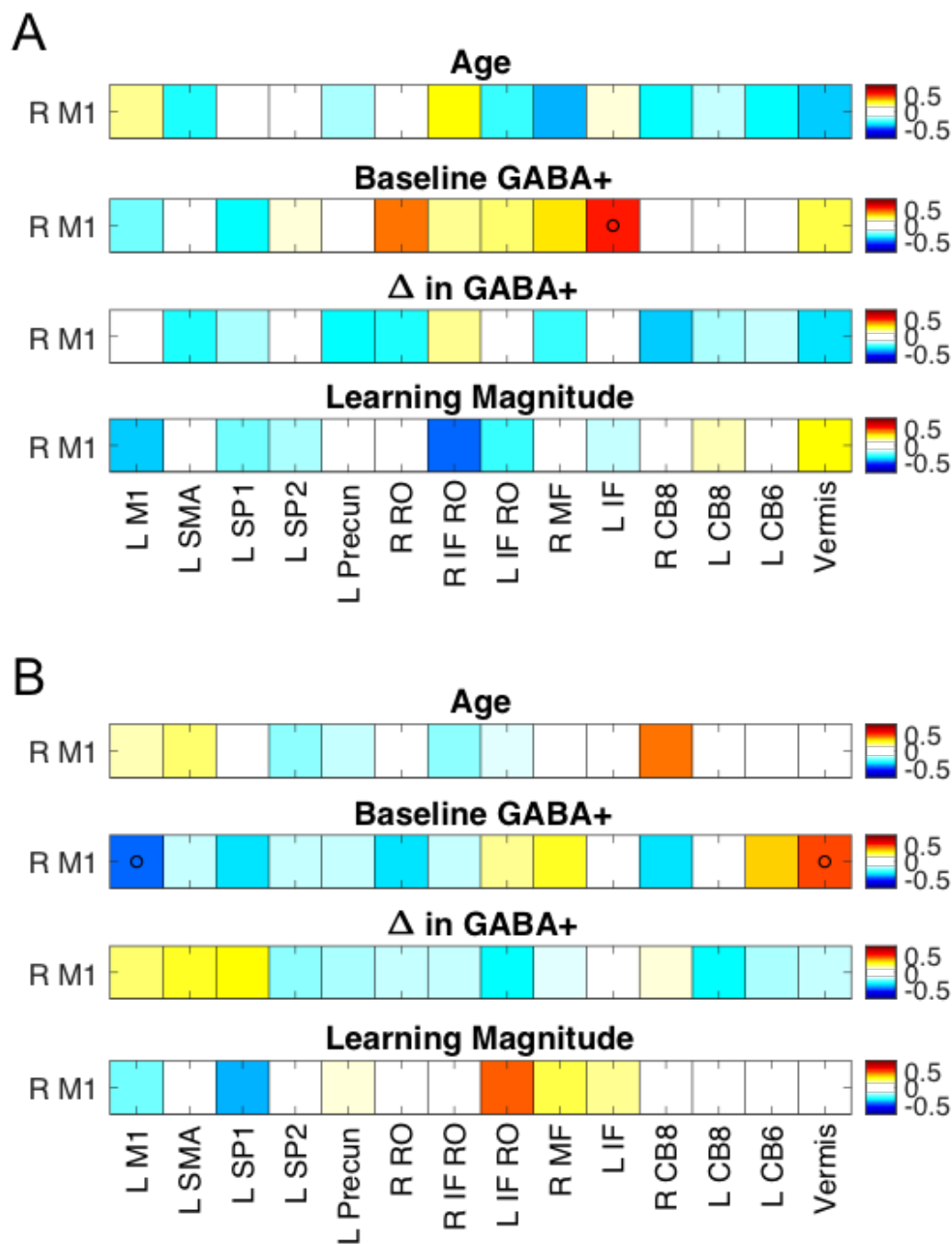
Figure S8. A. Relationships between the change in RS functional connectivity between the right M1 seed and the 14 motor task-relevant ROIs across the two RS timepoints and age, baseline GABA+ levels, change in GABA+ and motor sequence learning magnitude separately for the anodal (Panel A; n=16) and sham (B; n=17) stimulation groups. Values represent Pearson's correlation coefficients. Within-group tests of statistical significance were based on comparisons of the coefficients to a correlation of 0 and corrected for multiple comparisons with a False Discovery Rate (FDR) threshold set to 0.05. o = p(uncorrected)<0.05. No seed/ROI pair survived correction for multiple comparison.

L = left; R = right; M1 = primary motor cortex; SMA = supplementary motor area; SP = superior

parietal; Precun = precuneus; RO = rolandic operculum; IF = inferior frontal; MF = medial frontal;

CB = cerebellar; the numbers 6 and 8 represent the cerebellar lobules.

Figure S9. Uncorrected p-values (thresholded at p = 0.1) corresponding to a test of group differences

in the relationships between the change in RS functional connectivity and age, baseline GABA+

levels, change in GABA+ and motor sequence learning magnitude (n=16 and 17 in the anodal and

sham groups, respectively). There were no significant differences between anodal and sham groups

after correction for multiple comparison (all p(FDR)>0.4) and thus the correlation coefficients

presented in the main text are collapsed across the two groups. o = p(uncorrected)<0.05. L = left; R

= right; M1 = primary motor cortex; SMA = supplementary motor area; SP = superior parietal; Precun

= precuneus; RO = rolandic operculum; IF = inferior frontal; MF = medial frontal; CB = cerebellar;

the numbers 6 and 8 represent the cerebellar lobules.

Supporting Information References

Antonenko, D., Schubert, F., Bohm, F., Ittermann, B., Aydin, S., Hayek, D., … Floel, A. (2017). tDCS-Induced modulation of GABA levels and resting-state functional connectivity in older adults. *The Journal of Neuroscience*, *37*(15), 4065–4073.

Bachtiar, V., Near, J., Johansen-Berg, H., & Stagg, C. J. (2015). Modulation of GABA and resting state functional connectivity by transcranial direct current stimulation. *ELife*, *4*.

Stagg, C. J., Bachtiar, V., Amadi, U., Gudberg, C. A., Ilie, A. S., Sampaio-Baptista, C., … Johansen-Berg, H. (2014). Local GABA concentration is related to network-level resting functional connectivity. *ELife*, *3*, e01465.