Supplementary Information

Supplementary Introduction (osf.io/y4xar)

In addition to the behavioral aspects, overlearning recently has been suggested to be associated with a change in the ratio of excitation and inhibition (E/I ratio) (Shibata et al., 2017). The E/I ratio shifted towards a more inhibitory dominant state after overlearning, in this case in a simple visual task and superseded learning. The balance between excitation and inhibition controls the temporal organization of neuronal avalanches which can be considered as a robust feature of spontaneous neuronal activity and are approximated by a power law (Shriki et al., 2013). Human resting-state magnetoencephalography (MEG) (Shriki et al., 2013) and electroencephalography (EEG) (Palva et al., 2013) consist of neuronal avalanches, suggesting that it is a critical state, which is typically measured via a branching parameter. These branching parameters indicate the degree to which a signal propagates between clusters of neurons, with a branching parameter of 1 indicating criticality. Therefore, the present study also investigated if neuronal avalanches could serve as an electrophysiological marker for arithmetic learning and overlearning, and if the effect of tRNS is predicted by the individuals' neuronal avalanches. We reasoned that a branching parameter of neuronal avalanches (in this case, kappa, κ) different from 1 may be seen amongst overlearners, due to increased inhibition as was shown elsewhere using magnetic resonance spectroscopy (Shibata et al., 2017). Considering that tRNS increases excitation, it is expected that overlearning in combination with tRNS leads to a scaling component closer to a critical system, that is, less branching. Learning and tRNS may also impact the branching parameter due to compounded excitation following learning and active tRNS. It was also expected that learning and sham tRNS would show less branching than learning and tRNS (κ is closer to 1).

Supplementary Results

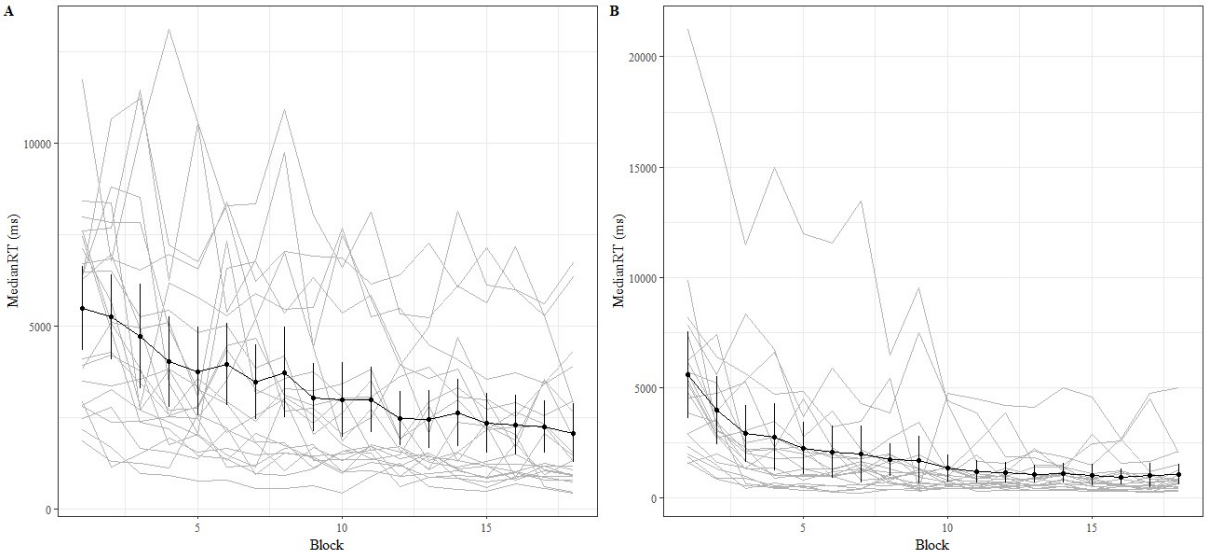

Figure S1| Individual learning curves of the RTs of the learning and overlearning task. **A)** The individual learning curves of the participants who received sham stimulation (n=22) during learning shows a linear gradient. **B)** The individual learning curve of the participants who received sham stimulation (n=21) during overlearning. Bars indicate 95% confidence intervals.

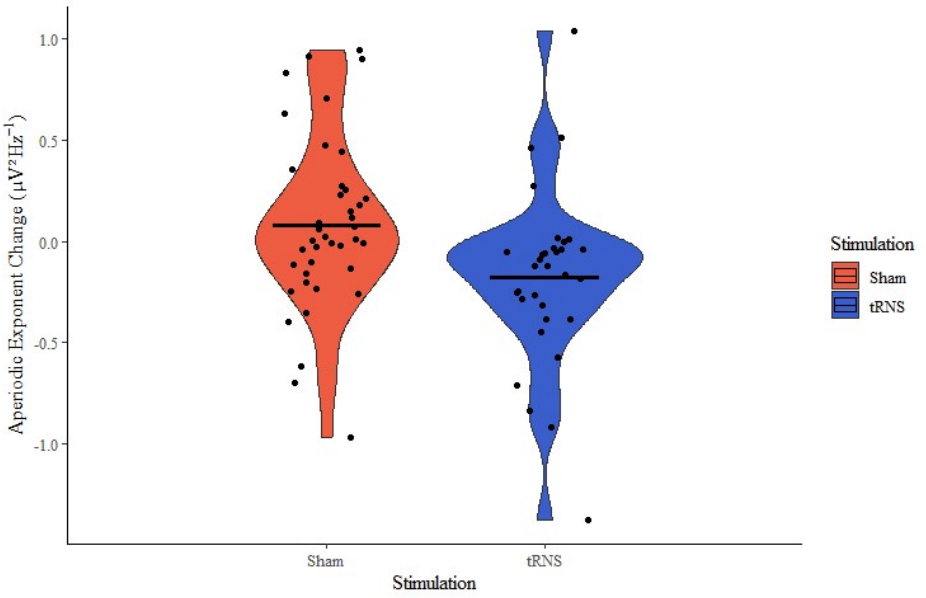

Figure S2| Individual change in aperiodic exponent for both stimulation groups. Individual data points indicate the aperiodic exponent change (post-pre) for the sham stimulation group (in red) and the tRNS group (in blue). Means are indicated with a solid black line. Note that this figure is based after the exclusion of outliers.

Bayesian ANCOVA

Task (learning/overlearning) and stimulation (tRNS/sham) were included as a fixed factor and the individual plateau as a covariate. The model with only stimulation included was the most probable model ($P(M|data)=0.41$, $BF_{M=}6.15$, $BF_{10}=3.75$) compared to the null model after observering the data. To account for model uncertainty, we looked at the Baysian model averaging, which tested the effects of both predictors (i.e., stimulation and task) and showed that the data were 3.20 more likely under models containing stimulation as a predictor compared to all models ($BF_{incl=}3.20$). The data was only 0.35 times as likely for task as a predictor and similarly for the interaction between stimulation and task compared to all models ($BF_{incl}=0.85$). This complementary analysis strengthens the conclusions that tRNS impacts the aperiodic exponent (mean change effect= -0.11, 95% credible interval (CrI; posterior distribution that contains 95% of the data)) tRNS [-0.21, -0.01], 95% CrI Sham [0.01, 0.21]), while task has no effect (mean change effect=0.02, 95% CrI learning [-0.12, 0.06], 95% CrI overlearning [-0.07, 0.12]).

Model Comparisons for Learning and Overlearning (RTs)

We implemented several model comparisons with the leave-one-out cross-validation (LOO) based on our brms model to determine the best fit for the learning and overlearning task data. First, we determined our starting model with the thought that the RTs should decrease over blocks and be dependent on the task paradigm.

Syntax:

Mod1 <- brm(medianRT ~ Block * Task + (1 + Block|Participant), family=lognormal, iter=5000, data=df_learning, save_pars=save_pars(all=TRUE)))

Next, we added levels of complexity by adding our predictors stimulation and baseline aperiodic exponent to see if the fit with our data increases.

Mod2 <- brm(medianRT ~ Stimulation + Block + Aperiodic_Baseline + Task + (1 + Block|Participant, family=lognormal, iter=5000, data=df_learning, save_pars=save_pars(all=TRUE))

Mod3 <- brm(medianRT ~ Stimulation * Block + Aperiodic_Baseline + Task + (1 + Block|Participant, family=lognormal, iter=5000, data=df_learning, save_pars=save_pars(all=TRUE))

Mod4 <- brm(medianRT ~ Block + Stimulation * Aperiodic_Baseline + Task + (1 + Block|Participant), family=lognormal, iter=5000, data=df_learning, save_pars=save_pars(all=TRUE))

Mod5 <- brm(medianRT ~ Block * Aperiodic_Baseline + Task + Stimulation + (1 + Block|Participant), family=lognormal, iter=5000, data=df_learning, save_pars=save_pars(all=TRUE))

Mod6 <- brm(medianRT ~ Stimulation * Block * Aperiodic_Baseline * Task + (1 + Block|Participant), family=lognormal, iter=5000, data=df_learning, save_pars=save_pars(all=TRUE))

Mod7 <- brm(medianRT ~ Stimulation * Block * Aperiodic_Baseline + Task + (1 + Block|Participant), family=lognormal, iter=5000, data=df_learning, save_pars=save_pars(all=TRUE))

Mod8 <- brm(medianRT ~ Block + Aperiodic_Baseline * Task * Stimulation + (1 + Block|Participant), family=lognormal, iter=5000, data=df_learning, save_pars=save_pars(all=TRUE))

Mod9 <- brm(medianRT ~ Block + Aperiodic_Baseline + Task * Stimulation + (1 + Block|Participant), family=lognormal, iter=5000, data=df_learning, save_pars=save_pars(all=TRUE))

Mod10 <- brm(medianRT ~ Block * Task * Stimulation + Aperiodic_Baseline + (1 + Block|Participant), family=lognormal, iter=5000, data=df_learning, save_pars=save_pars(all=TRUE))

Loo(Mod1, Mod2, Mod3, Mod4, Mod5, Mod6, Mod7, Mod8, Mod9, Mod10)

Model	elpd_diff	ee_diff
Mod8	0.0	0.0
Mod10	-0.1	1.2
Mod6	-0.2	1.3
Mod9	-0.4	0.3
Mod7	-0.4	0.5
Mod3	-0.5	0.3
Mod1	-0.9	0.6
Mod2	-1.0	0.3
Mod5	-1.3	0.6
Mod4	-1.6	0.3

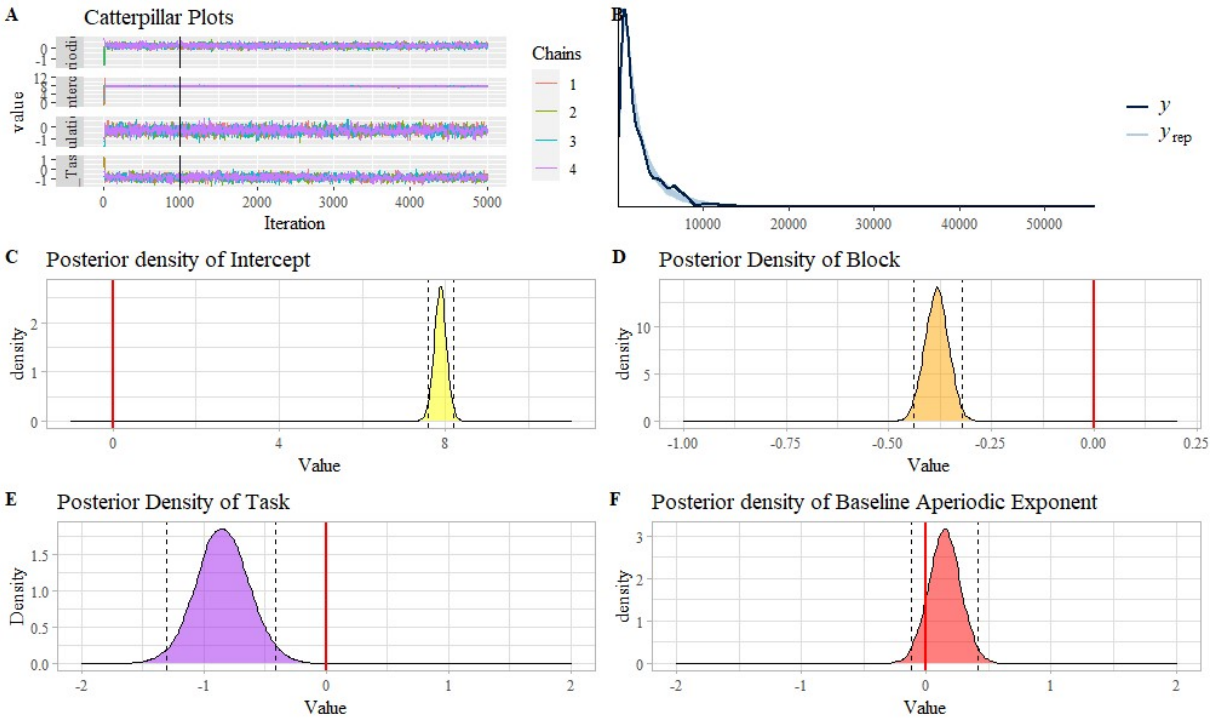

Figure S3| Output of the Bayesian mixed effects model with the three-way interaction. **A)** The hairy caterpillar plots showing that convergence was reached in all four chains. **B)** Comparison of the observed outcomes (y) and the kernel density estimate of the replications of y from the posterior predictive distribution (y_{rep}). This posterior predictive check shows a good fit. **C)** The posterior density of the intercept. **D)** The posterior density of block. **E)** The posterior density of task. **F)** The posterior density of the baseline aperiodic exponent (87% of the distribution is above zero).

Model Comparison for Learning and Overlearning (Accuracy)

Similarly to our brms models with RTs, we implemented several model comparisons with the leave-one-out cross-validation (LOO) to determine the best fit for the learning and overlearning task data. Note that this additional analysis served as a check since we do not expect any reliable results due to the instructions that were given to the participants. Namely, the participants were urged to avoid errors and there was no time limit present. This means that the accuracy should be consistent during the task. Note that the mean accuracy was calculated per block.

Syntax:

Mod1.A <- brm(meanRTACC ~ Block * Task + (1 + Block|Participant), family=lognormal, iter=5000, data=df_ACC, save_pars=save_pars(all=TRUE)))

Next, we added levels of complexity by adding our predictors stimulation and baseline aperiodic exponent to see if the fit with our data increases.

Mod2.A <- brm(meanRTACC ~ Stimulation + Block + Aperiodic_Baseline + Task + (1 + Block|Participant, family=lognormal, iter=5000, data=df_ACC, save_pars=save_pars(all=TRUE))

Mod3.A <- brm(meanRTACC ~ Stimulation * Block + Aperiodic_Baseline + Task + (1 + Block|Participant, family=lognormal, iter=5000, data=df_ACC, save_pars=save_pars(all=TRUE))

Mod4.A <- brm(meanRTACC ~ Block + Stimulation * Aperiodic_Baseline + Task + (1 + Block|Participant), family=lognormal, iter=5000, data=df_ACC, save_pars=save_pars(all=TRUE))

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Mod5.A <- brm(meanRTACC ~ Block * Aperiodic_Baseline + Task + Stimulation + (1 +
Block|Participant), family=lognormal, iter=5000, data=df_ACC,
save_pars=save_pars(all=TRUE))

Mod6.A <- brm(meanRTACC ~ Stimulation * Block * Aperiodic_Baseline * Task + (1 +
Block|Participant), family=lognormal, iter=5000, data=df_ACC,
save_pars=save_pars(all=TRUE))

Mod7.A <- brm(meanRTACC ~ Stimulation * Block * Aperiodic_Baseline + Task + (1 +
Block|Participant), family=lognormal, iter=5000, data=df_ACC,
save_pars=save_pars(all=TRUE))

Mod8.A <- brm(meanRTACC ~ Block + Aperiodic_Baseline * Task * Stimulation + (1 +
Block|Participant), family=lognormal, iter=5000, data=df_ACC,
save_pars=save_pars(all=TRUE))

Mod9.A <- brm(meanRTACC ~ Block + Aperiodic_Baseline + Task * Stimulation + (1 +
Block|Participant), family=lognormal, iter=5000, data=df_ACC,
save_pars=save_pars(all=TRUE))

Mod10.A <- brm(meanRTACC ~ Block * Task * Stimulation + Aperiodic_Baseline + (1 +
Block|Participant), family=lognormal, iter=5000, data=df_ACC,
save_pars=save_pars(all=TRUE))
```

Loo(Mod1.A, Mod2.A, Mod3.A, Mod4.A, Mod5.A, Mod6.A, Mod7.A, Mod8.A, Mod9.A, Mod10.A)

Model	elpd_diff	se_diff
Mod3.A	0.0	0.0
Mod5.A	-2.1	2.8
Mod10.A	-2.8	2.6
Mod1.A	-3.1	3.5
Mod2.A	-3.4	3.4
Mod7.A	-3.6	2.6
Mod6.A	-3.9	2.9
Mod4.A	-4.2	4.4
Mod8.A	-4.5	4.3
Mod9.A	-5.2	4.4

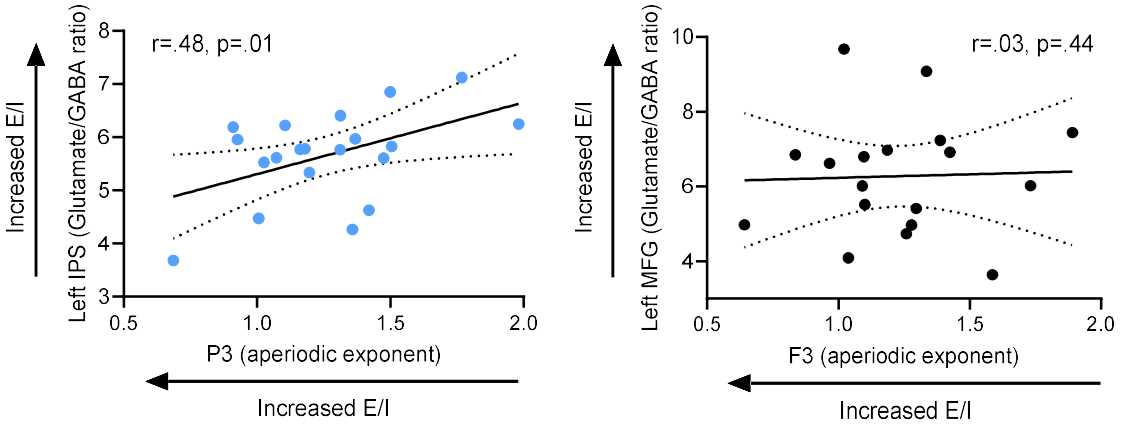

Figure S4| Correlations between MRS-based E/I and EEG-based E/I. On the left a positive correlation between the left IPS and electrode P3, which is placed approximately above this region, showing that an increase in E/I in the IPS as it is based on glutamate/GABA is associated with a decreased E/I as indicated by aperiodic exponent (r=0.48, 95% CI [0.05, 0.76], p=.01 (one-tailed)). On the right, a non-significant correlation between the left MFG E/I and the electrode nearly this region (r=0.03, 95% CI [-0.44, 0.49], p=.44 (one-tailed)). These results are in line with our prediction that both measures characterise different aspects of E/I, and in contrast to the view that both measures reflect a similar quantification of E/I, which should have been characterised by a negative correlation.

Neuronal Avalanches (osf.io/y4xar)

When comparing the presence of neuronal avalanches for the different groups for the pre and the post resting-state (rs) EEG, the neuronal avalanches were plotted against a standard power law (see **Figure S5**). There is no difference between the four groups regarding the pre rs-EEG. However, for the post rs-EEG there is a small diversion in the power law for the overlearning X tRNS condition. This diversion was explored further in the statistical regression analysis using κ for post rs-EEG values during overlearning. Note that we removed an additional 4 outliers in the overlearning group compared to the sample in the main manuscript. Predictors included median RT baseline, stimulation (tRNS x sham), plateau (i.e. amount of overlearning), and branching (κ) values in the pre rs-EEG and learning rate. We found that κ values during pre rs-EEG significantly predicted the κ values during post rs-EEG (β=.05^{-1}, SE=0.02^{-1}, t(27)=2.17, p=0.03). The predictor model was able to account for 10% of the variance of κ values in the post rs-EEG (F(5,27)=1.72, p=.16, R^2= .10). However, no interaction with stimulation and individual plateau was found (β=.05^{-1}, SE=0.05^{-1}, t(27)=.98 , p=.33).

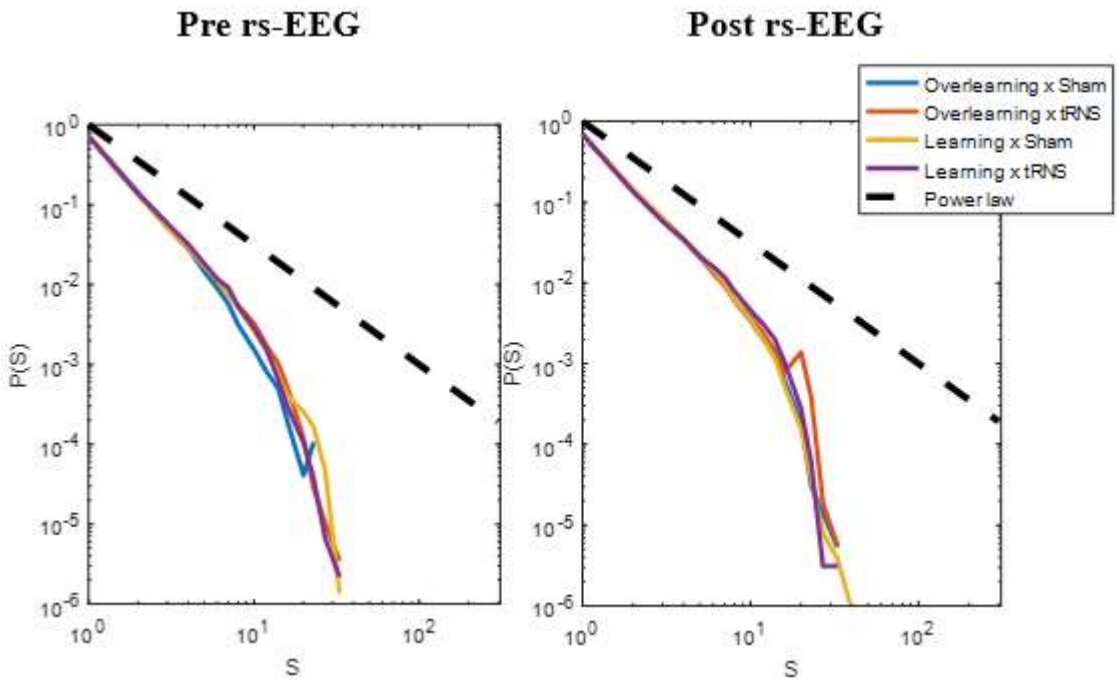

Figure S5| Neuronal avalanches for the pre and post rs-EEG for the four conditions following power laws. Cascade size distributions are shown on the x-axis plotted against the probability on the y-axis for the pre rs-EEG (left) and the post rs-EEG (right) using Δt=6 ms. The dashed black line represents a perfect power law with an exponent of -3/2. The different line colors in both plots indicate the four condition (task: learning x overlearning; stimulation: tRNS x sham).

Table S1| Sensations between the tRNS and the sham stimulation group as tested with the Mann-Whitney's U test (n=102)

Sensations	U-value	p
Itching	1058	.43
Pain	1142.5	.92
Burning	1090.5	.41
Warmth/Heat	1109.5	.70
Pinching	1089	.52
Iron Taste	1125	.30
Fatigue	1092.5	.74
Subjective performance	1080	.48

Supplementary Materials and Methods

Table S2| List of the 4 presented multiplication problems used as training and the 10 multiplication problems presented during the baseline task.

Operand 1	Operand 2
15	5
18	5
12	5
17	5
23	4
19	4
14	3
13	3
27	2
13	7
26	2
16	3
18	4
21	3

Table S3| List of one block of the presented multiplication problems used during the learning task and overlearning task.

Learning task		Overlearning task	
Operand 1	Operand 2	Operand 1	Operand 2
17	4	17	4
14	6	14	6
29	2	29	2
17	3	17	3
24	3	24	3
12	8		
29	3		
13	6		
16	6		
12	7		

Neuronal Avalanches Computation and Statistical Analysis (osf.io/y4xar)

Due to the comparison of neural activity between the pre and post rs-EEG, we decided to remove an additional three participants from the neuronal avalanches analysis with an excluded post rs-EEG recording according to our previous exclusion criteria. To identify neuronal avalanches, standardized z-scores were calculated for each channel. Hereafter, timepoints were identified in which each channel exceeded a z score of three standard deviations (our predefined threshold; Shriki et al., 2013). In other words, periods were identified in which each electrode contained elevated activity. Data was subsequently binned into time blocks of 6 ms. A neuronal avalanche is considered to be any length of time in which a superthreshold event occurs. In short, when 6 ms passes and no further events occur, the neuronal avalanche is over. The size of the neuronal avalanches is the number of superthreshold events (spikes) occurring before the 6 ms of time when no events is seen. Lastly, the size of the neuronal avalanches was computed, i.e., occurrence of the amount of spikes in the signal after a 6 ms time window (Shriki et al., 2013; Shew, Yang, Petermann, Roy, & Plenz, 2009). Regarding the statistical analysis of neural avalanches, the post rs-EEG was compared to the pre rs-EEG (baseline) and compared between the four conditions by means of a graphical illustration and a regression model regarding κ. Branching in neuronal avalanches was indexed using κ. The dependent variable included the branching (κ) for the post rs-EEG. Predictors included the baseline performance, κ for the pre rs-EEG, stimulation, and learning rate.

Material and Methods MRS

We recruited 22 healthy participants (16 males, mean age=26.05, standard deviation =6.5) who completed an MRI scan and EEG session for two different studies. All participants provided written, informed consent and the study was approved by the University of Oxford's Medical Sciences Interdivisional Research Ethics Committee (MS-IDREC-C2_2015_016).

298 ***MR data Acquisition and Pre-processing***

299 All MRI data were acquired at the Oxford Centre for Functional MRI of the Brain (FMRIB)
300 on a 3T Siemens MAGNETOM Prisma MRI System equipped with a 32 channel receive-only
301 head coil. Anatomical high-resolution T1-weighted scans were first acquired (MPRAGE
302 sequence: TR=1900ms; TE=3.97ms; 192 slices; voxel size=1×1×1mm).

303 For MRS, spectra were measured with a semi-adiabatic localization by adiabatic selective
304 refocusing (semi-LASER) sequence (TE=32 ms; TR=3.5 s; 32 averages) (Deelchand et al.,
305 2015; Öz & Tkáč, 2011) with variable power RF pulses with optimized relaxation delays
306 (VAPOR), water suppression and outer volume saturation. Unsuppressed water spectra
307 acquired from the same volume of interest were used to remove residual eddy current effects
308 and to reconstruct the phased array spectra with MRspa
309 (https://www.cmrr.umn.edu/downloads/mrspa/). Two 20mm^3 voxels of interest were manually
310 placed centred on the left intraparietal sulcus (IPS) and centred on the left inferior/middle
311 frontal gyrus (FG) based on the individual's T1-weighted image while the participant lay down
312 in the MR scanner. Acquisition time per voxel of interest was 10-15 minutes including sequence
313 planning and shimming and B0 shimming.

314 Neurochemicals were quantified with an LCmodel (Provencher, 2001) using a basis set of
315 simulated spectra generated based on previously reported chemical shifts and coupling
316 constants based on a VeSPA (versatile simulation, pulses, and analysis) simulation library
317 (Soher et al., 2011). Simulations were performed using the same RF pulses and sequence
318 timings as in the 3T system described above. Absolute neurochemical concentrations were
319 extracted from the spectra using a water signal as an internal reference.

320 As in previous studies, the exclusion criteria for data was the Cramér-Rao bounds (Emir et
321 al., 2012). Neurotransmitters quantified with Cramér-Rao lower bounds (CRLB, the estimated

error of the neurotransmitter quantification) >50% were classified as not detected. Additionally, we excluded cases with an SNR beyond 3 standard deviations (per voxel of interest, per neurotransmitter), and neurotransmitter or WM capacity score that fallen beyond 3 standard deviations from the group mean. This led to the exclusion of 2 cases for the GABA measure of the frontal gyrus. For each participant, we calculated 4 (brain region (frontal, parietal) * neurochemical (GABA, glutamate)) neurotransmitter concentrations all of which were calculated as the ratios between the absolute neurotransmitter concentrations divided by the absolute concentration of total creatine (creatine+phosphocreatine). The neurotransmitter concentrations were referenced to total creatine for (i) creatine is a commonly used as a reference and it is widely accepted as an internal reference standard, (ii) its signal shares the same imperfections (e.g., frequency drift, phase drift, and subject motion) as the signal of the GABA and glutamate as all concentrations are acquired simultaneously (Cohen Kadosh et al., 2015) measure was similar to our description in the main text. For the correlation analysis one data was defined as an outlier (±3SD from the mean) and was removed from the analysis.