Supplementary information

Neural Mechanism Underlying Task-specific Enhancement of Motor Learning by Concurrent Transcranial Direct Current Stimulation

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This supplementary information file includes:

Figs. S1 to S13

Captions for movies S1 to S10

Two-Sample T-Tests Allowing Unequal Variance

Other Supplementary Materials for this manuscript include the following:

Movies S1 to S10

Supplementary Figures and Figure Legends

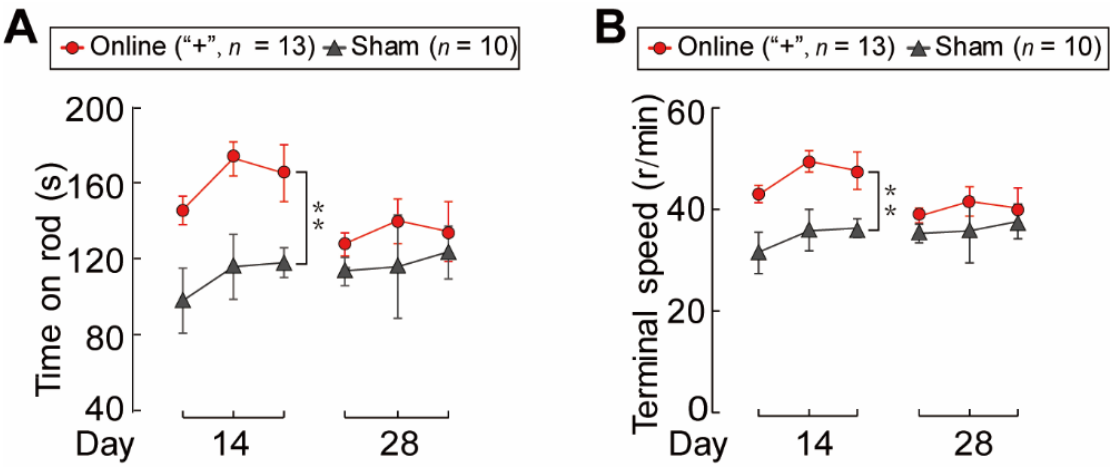


Fig. S1 Persistence of anodal tDCS-enhanced rotarod motor learning. **A** Average time spent on the rotarod during each of the three trials, tested at 14 and 28 days after 4 days of training with the online tDCS, for the same group of mice described in Fig. 1 C and D. Only the high rotation speed of 8–80 r/min was used in these re-tests. **B** Terminal rotation speed when mice fell off the rotarod during each trial. Error bars, SEM; ***P* <0.01, two-way ANOVA.

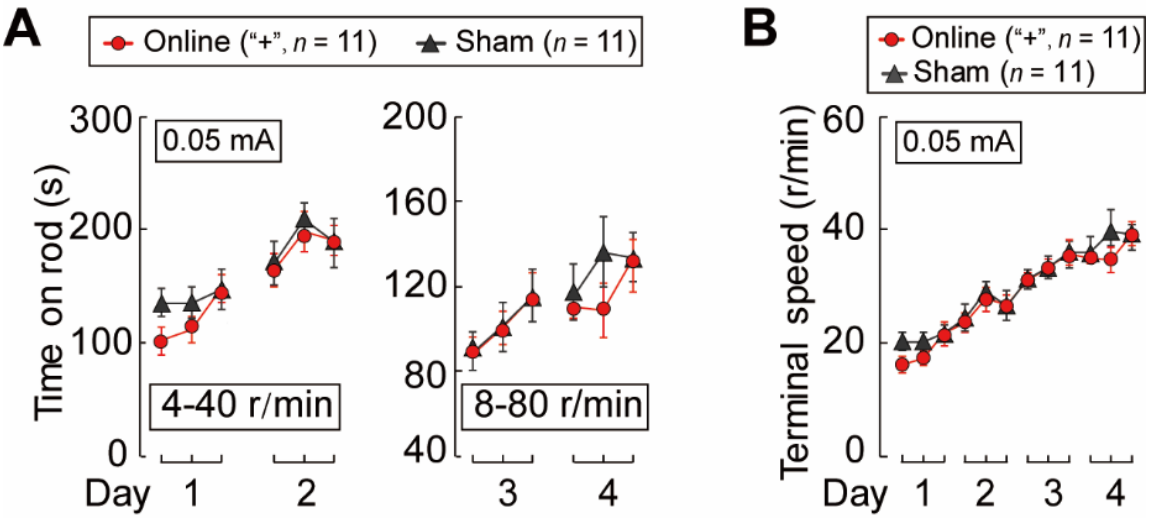


Fig. S2 No effect on motor learning of lower current density of anodal tDCS of primary motor cortex. **A** Average time spent on the rotarod during each trial with online anodal tDCS at 0.05 mA, using the same paradigm as that described in Fig. 1. **B** Terminal rotation speed when mice fell from the rotarod for the same group of mice as in **A**. Error bars, SEM; no significant difference, two-way ANOVA.

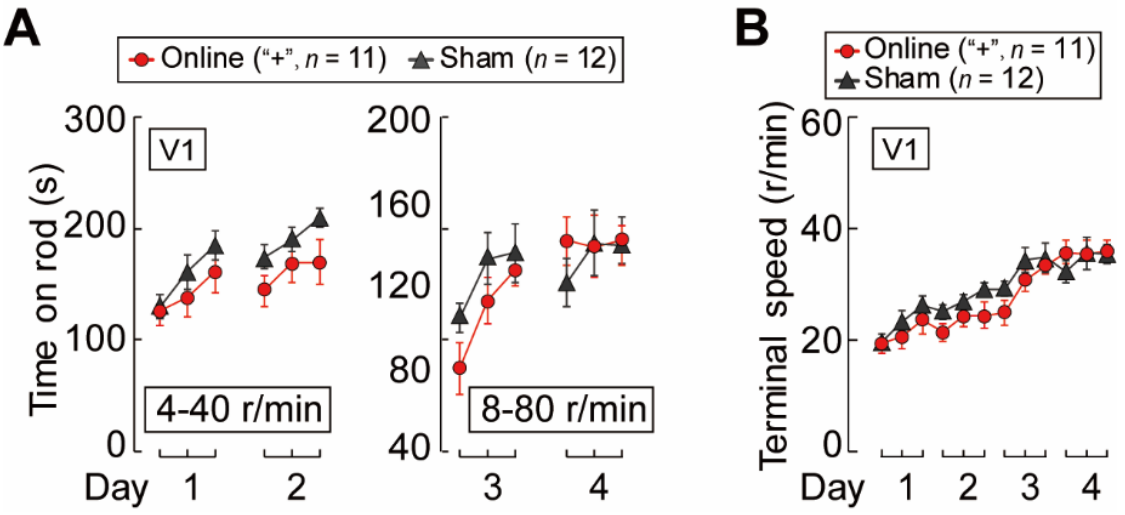


Fig. S3 Effects of online anodal tDCS on the primary visual cortex (V1) during rotarod training. **A** Average time spent on the rotarod during each trial with the online anodal tDCS applied to V1, using the same paradigm as that described in Fig. 1. **B** Terminal rotation speed when mice fell from the rotarod for the same group of mice as in **A**. Error bars, SEM; no significant difference, two-way ANOVA.

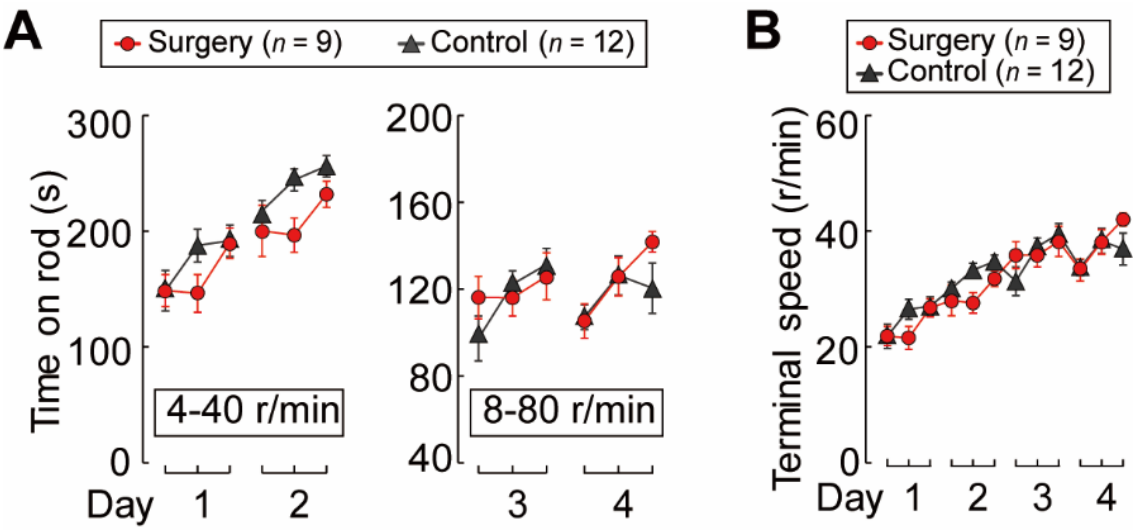


Fig. S4 tDCS electrode implantation surgery has no effect on mice learning the rotarod task. **A** Average time spent on the rotarod during each trial (Surgery, mice with tDCS electrode implantation; Control, mice without tDCS electrode implantation). **B** Terminal rotation speed when mice fell from the rotarod for the same group of mice as in **A**. Error bars, SEM; no significant difference, two-way ANOVA.

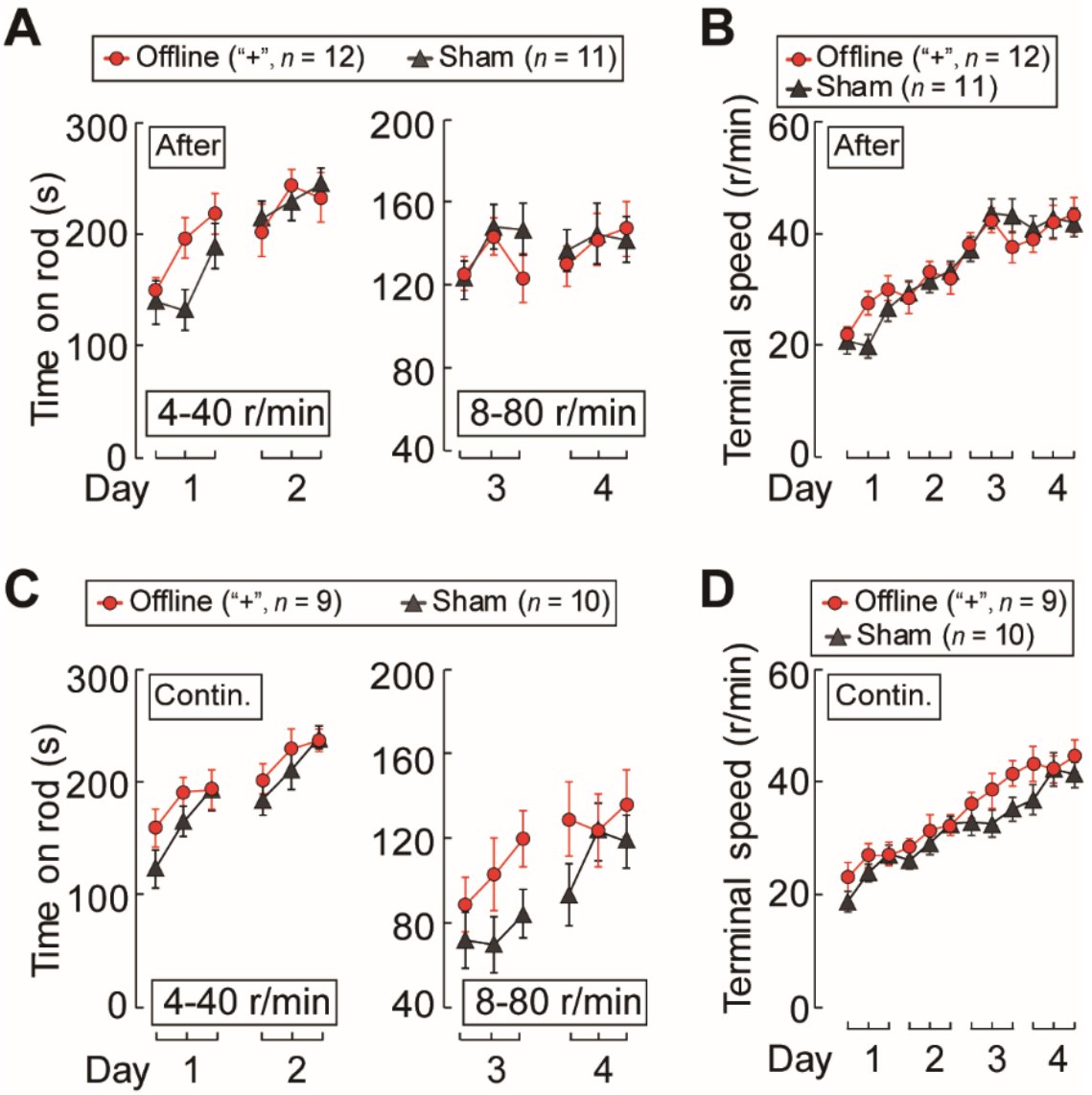


Fig. S5 Effects of offline anodal tDCS on rotarod learning. **A** Average time spent on the rotarod during each trial with offline anodal tDCS (0.1 mA) (After, average values when tDCS is applied during the ITI after each trial). **B** Terminal rotation speed when the mice fell from the rotarod for the same group as in **A**. **C** Average time spent on the rotarod during each trial with offline anodal tDCS (Contin, 20-min tDCS continuously applied before training). **D** Terminal rotation speed when the mice fell from the rotarod for the same group as in **A**. Error bars, SEM; no significant difference, two-way ANOVA.

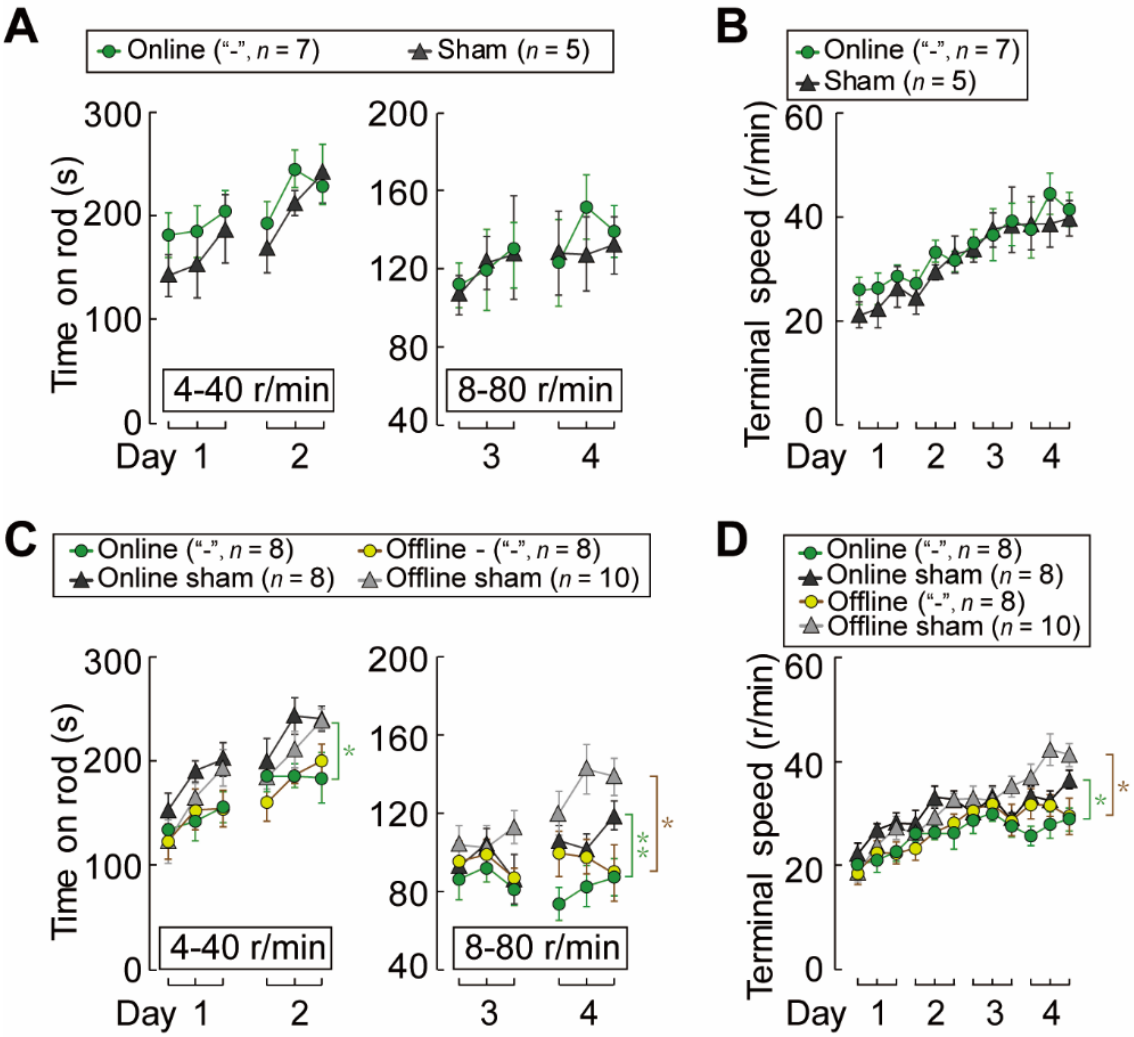


Fig. S6 Effects of online and offline cathodal tDCS on rotarod learning. **A** Average time spent on the rotarod during each trial with online anodal tDCS at 0.1 mA, using the same paradigm as that described in Fig. 1. **B** Terminal rotation speed when the mice fell from the rotarod for the same group of mice as in **A**. **C** Average time spent on the rotarod during each trial with online or pre-offline cathodal tDCS at 0.2 mA. **D** Terminal rotation speed when the mice fell from the rotarod for the same group as in **C**. Error bars, SEM; **P* <0.05, ***P* <0.01, two-way ANOVA.

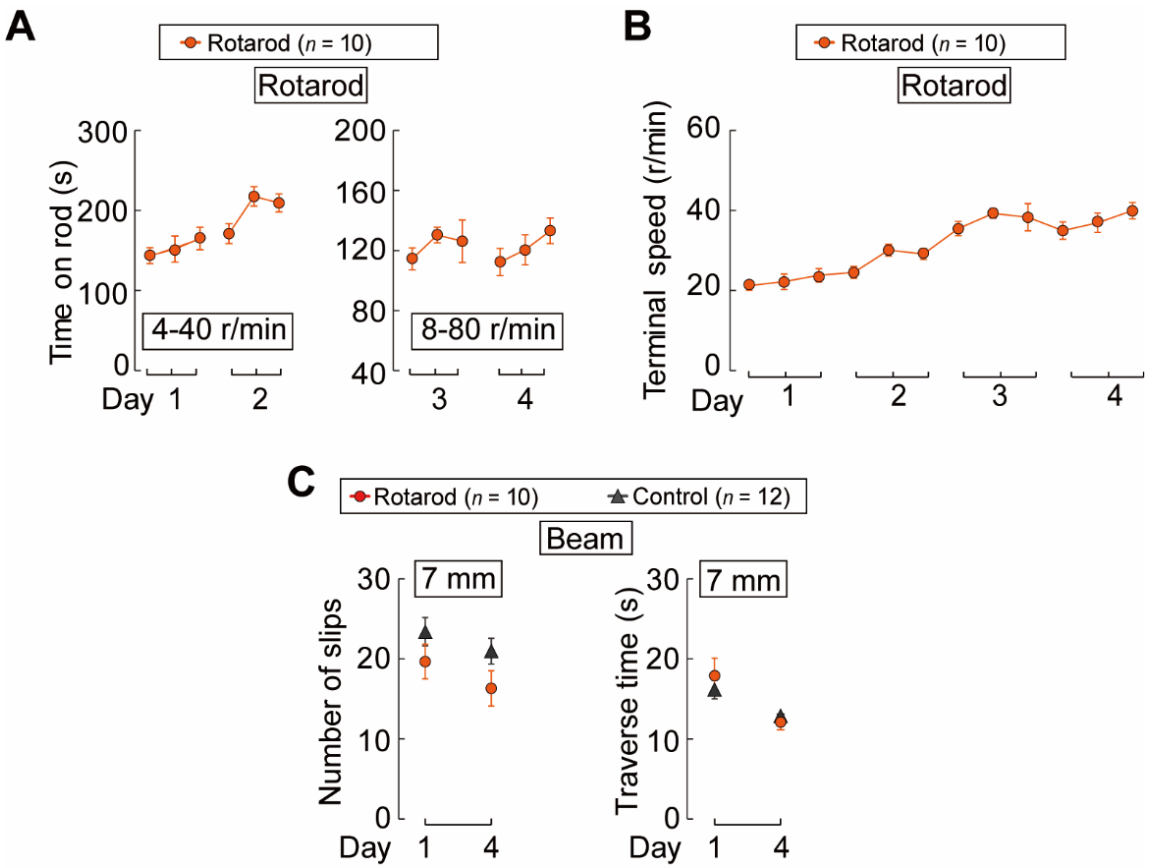


Fig. S7 No transfer of motor learning from rotarod running to beam walking in the absence of tDCS. **A** Average time spent on the rotarod during each trial. Mice performed one trial of walking on a 7-mm beam before and after 4 days of rotarod training. **B** Terminal rotation speed when the mice fell from the rotarod in each trial, for the same group of mice as in **A**. **C** Number of slips and traverse time when mice walked on the beam at days 1 and 4. Data for the Rotarod group that underwent rotarod training as described in **A** and **B**, and the Control group that were not trained for rotarod running. Error bars, SEM; no significant difference, unpaired *t* test.

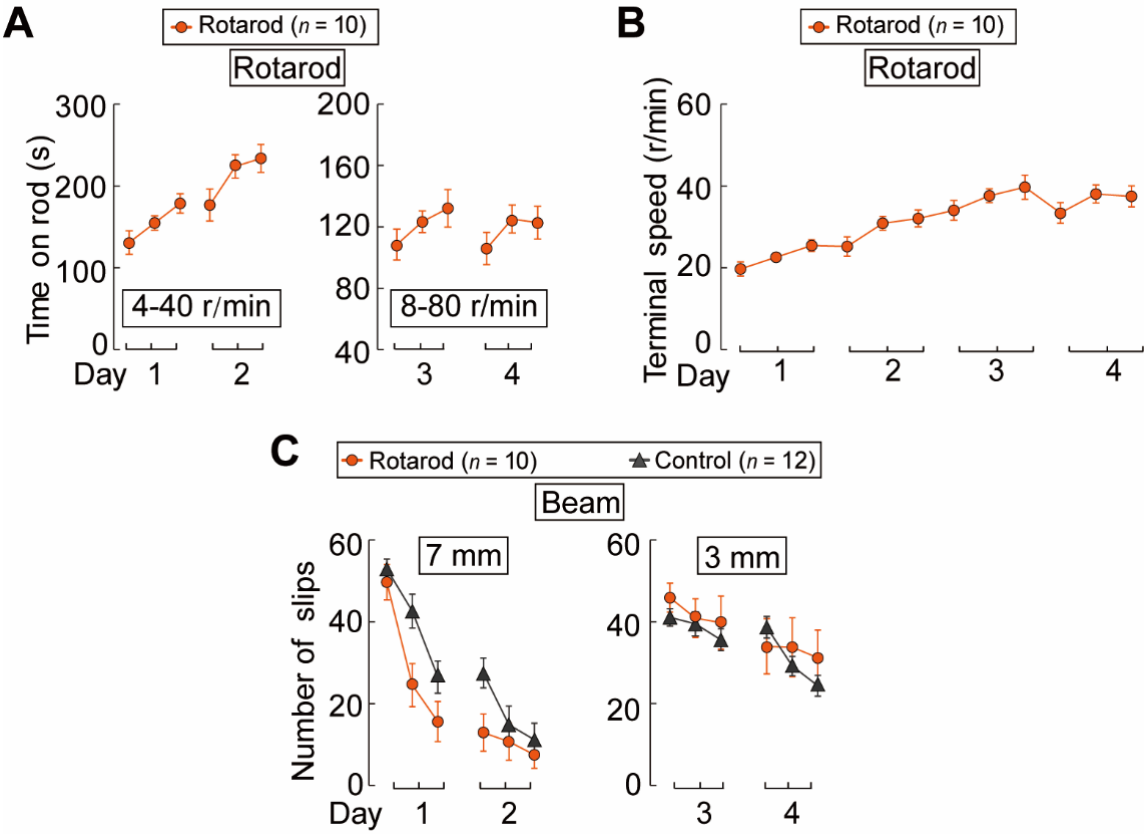


Fig. S8 No transfer from rotarod learning to beam walking learning in the absence of tDCS. **A** Average time spent on the rotarod during each trial. Mice performed beam walking learning each day after 4 days of rotarod learning. **B** Terminal rotation speed when mice fell from the rotarod in each trial for the same group of mice as in **A**. **C** Travers time and average number of hindlimb slips during beam walking learning for the Rotarod group that underwent rotarod training as described in **A** and **B**, and the Control group that were not trained for rotarod running. Error bars, SEM; no significant difference between mice with and without rotarod training, two-way ANOVA.

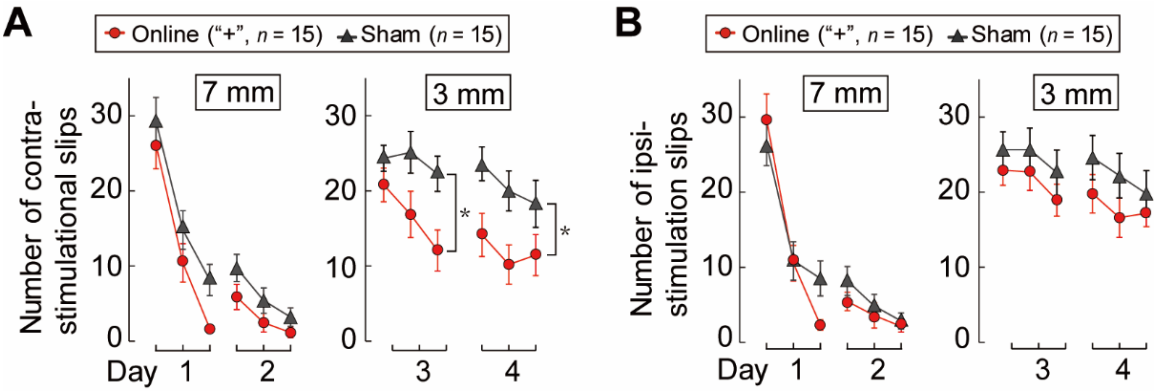


Fig. S9 Effects of contralateral and ipsilateral tDCS on learning the beam-walking task. **A** Average number of contralateral hindlimb slips with online anodal tDCS at 0.1 mA of M1. **B** Average number of ipsilateral hindlimb slips with the same online anodal tDCS as in **A** for the same group of mice.

Comparing **A** and **B**, note that online M1 stimulation reduces the number of slips only for the contralateral hindlimb. Error bars, SEM; *P <0.05, two-way ANOVA.

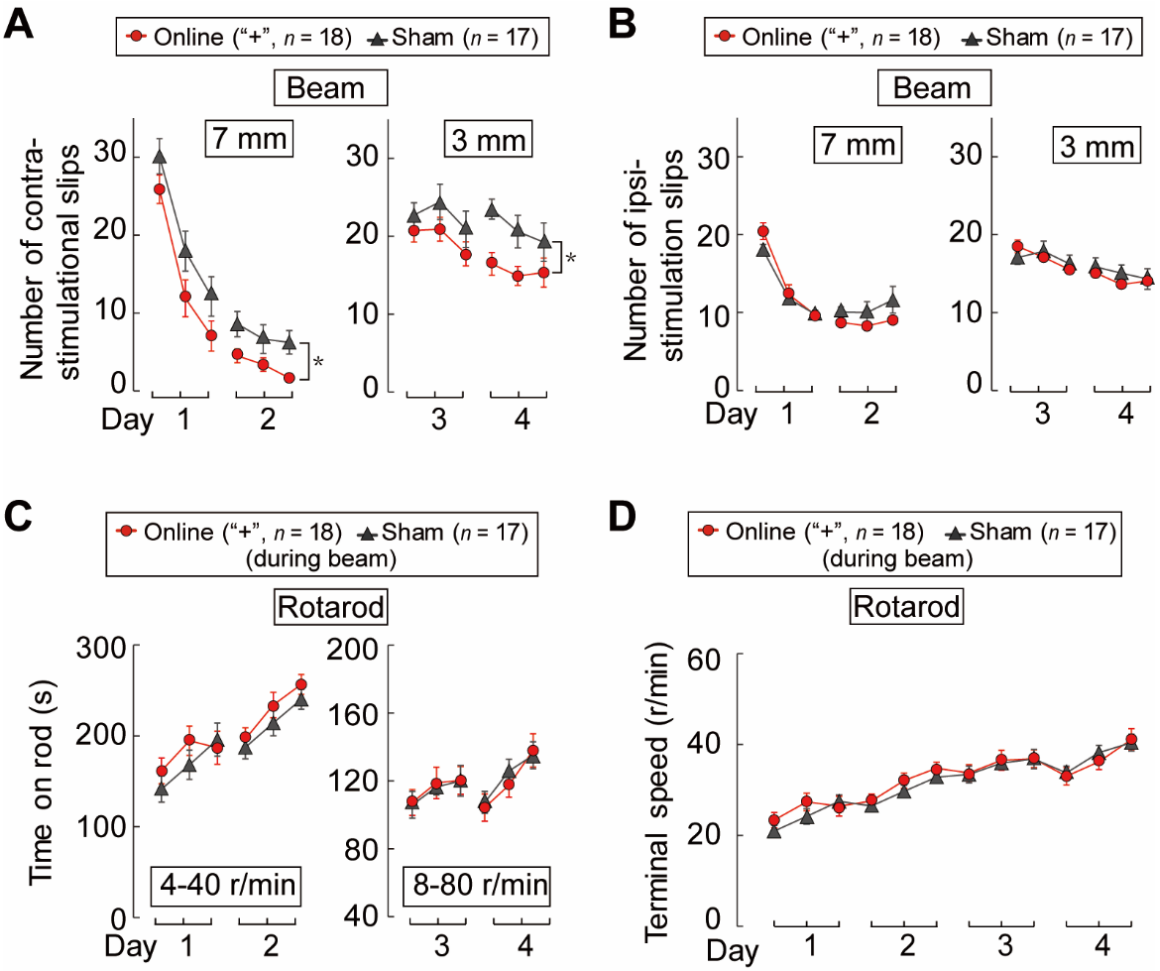


Fig. S10 Enhancement of learning beam walking by online anodal tDCS has no effect on rotarod learning. **A** Average number of contralateral hindlimb slips with online anodal tDCS at 0.1 mA at M1. **B** Average number of ipsilateral hindlimb slips with online anodal tDCS for the same group as in **A**. **C** Beam-walking learning is followed by a rotarod training task each day in the absence of tDCS. The average time spent on the rotarod during each trial followed by beam-walking learning for the same group as in **A**. **D** Terminal speed when mice fall from the rotarod in the same group as in **A**. Error bars, SEM; *P <0.05, two-way ANOVA.

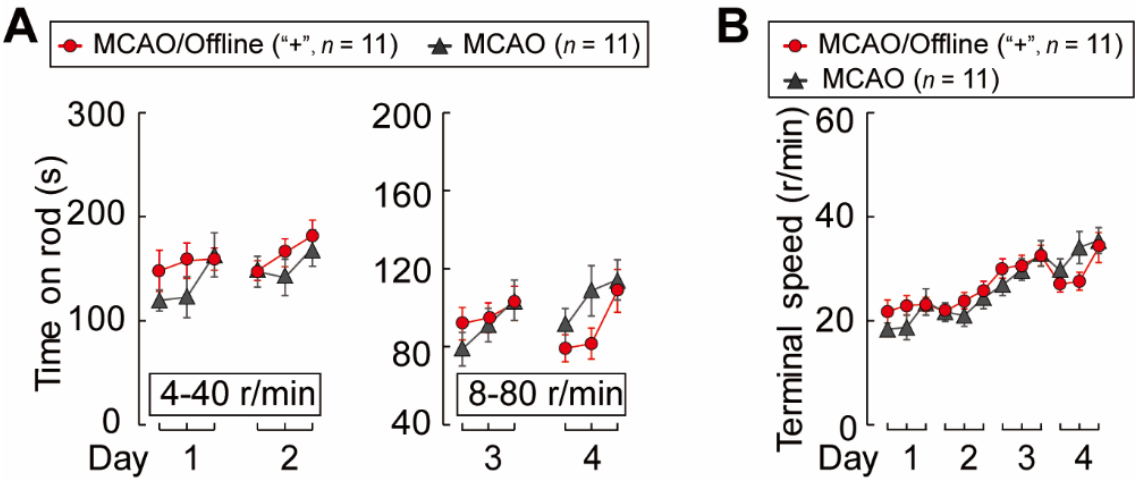


Fig. S11 No effects of offline anodal tDCS on rotarod learning of MCAO mice. **A** Average time spent on the rotarod during each trial by MCAO mice with offline anodal tDCS at M1 ipsilateral to the infarct site. **B** Terminal rotation speed when the mice fell from the rotarod for the same group of mice as in **A**. Error bars, SEM; no significant difference, two-way ANOVA.

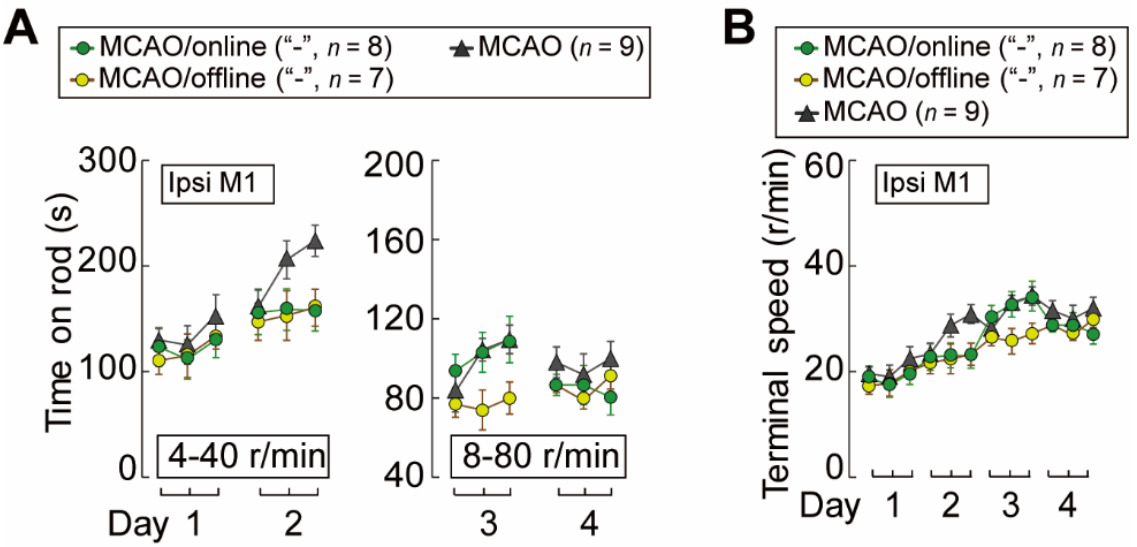


Fig. S12 Effects of online or offline cathodal tDCS on rotarod learning by MCAO mice. **A**, **B** Average time spent on the rotarod (**A**) and the terminal speed when the mice fell from the rotarod (**B**) during each trial when MCAO mice received online or offline cathodal tDCS at 0.2 mA of M1 ipsilateral to the infarct site (Ipsi M1). Error bars, SEM; no significant difference; two-way ANOVA.

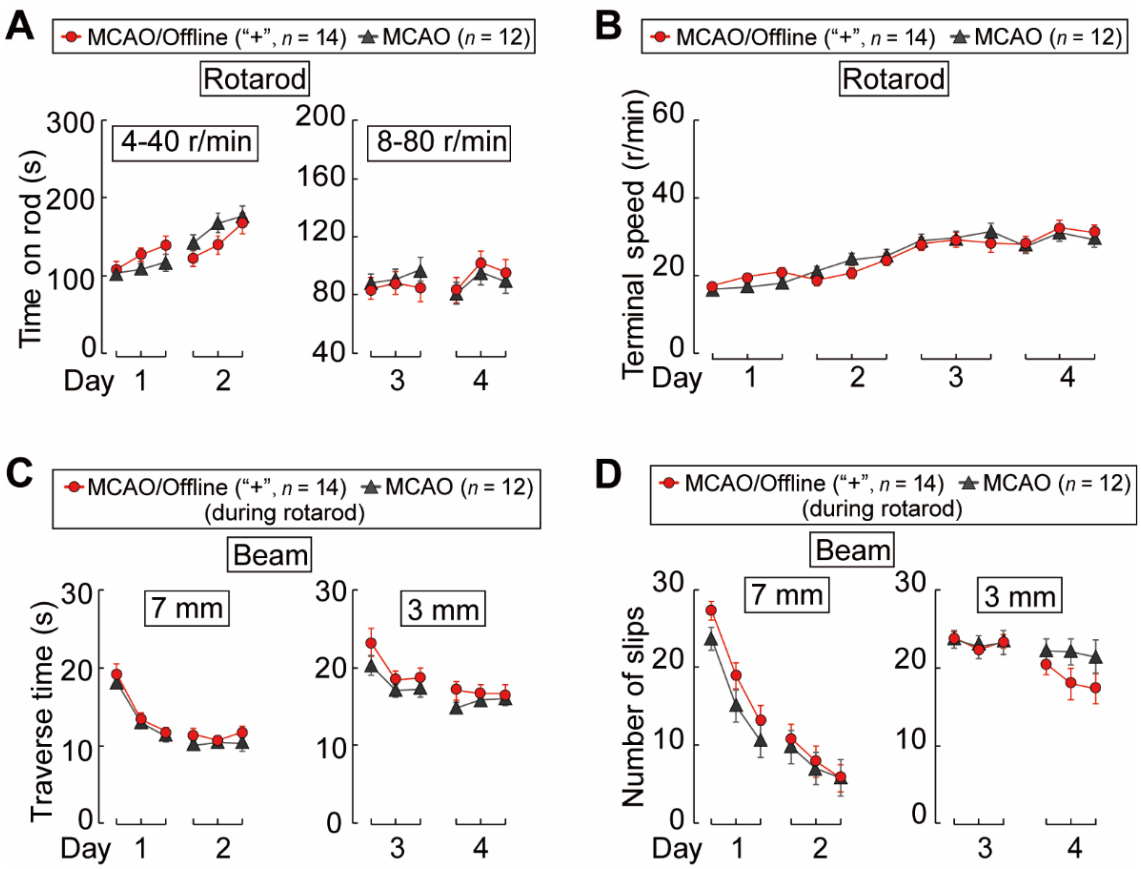


Fig. S13 No effect of offline anodal tDCS on learning either rotarod or beam walking in MCAO stroke mice performing dual-task training. **A** and **B** Rotarod learning with offline anodal tDCS at M1 ipsilateral to the infarct site, using the same dual-task paradigm as described in Fig. 2. **C** and **D** Beam-walking learning as measured by the traverse time and contralateral hindlimb slips, for the same group of mice as in **A**. Error bars, SEM; no significant difference; two-way ANOVA.

Movies Attached

Movies S1 and S2. Video images of mice performing rotarod running at day 1 (S1) and day 4 (S2) of training. Each movie shows one mouse subjected to online anodal tDCS (0.1 mA), and the other subjected to sham treatment (0 mA).

Movies S3 to S6. Video images of mice performing beam walking at day 1 (S3, S5) and day 4 (S4, S6) of training. The movies show the performance of the same mouse that received online anodal tDCS (0.1 mA, S3 and S4) and another mouse that received sham treatment (0 mA, S5 and S6).

Movies S7 and S8. Video images of fluorescence changes in a population of M1 neurons in a Thy-1 transgenic mouse expressing GCaMP6s in response to two episodes of anodal (S7) and cathodal (S8) tDCS, monitored by *in vivo* two-photon imaging of a head-fixed mouse running on a rotating treadmill.

Movies S9 and S10. Video images of MCAO stroke mice performing rotarod running at day 1 (S1) and day 4 (S2) of training. Each movie shows one mouse subjected to online anodal tDCS (0.1 mA), and the other subjected to sham treatment (0 mA).

PASS 15.0.5

Two-Sample T-Tests Allowing Unequal Variance

Numeric Results for Two-Sample T-Test Allowing Unequal Variance

Alternative Hypothesis: H1: $\delta = \mu1 - \mu2 \neq 0$

Target Power	**Actual Power**	**N1**	**N2**	**N**	**μ1**	**μ2**	**δ**	**σ1**	**σ2**	**Alpha**
0.9	1.00000			0	0.5	0.5	0.0	0.3	0.3	0.050
0.9	0.91255	9	9	18	0.5	1.0	-0.5	0.3	0.3	0.05
0.9	0.97266	4	4	8	0.5	1.5	-1.0	0.3	0.3	0.05
0.9	0.99278	3	3	6	0.5	2.0	-1.5	0.3	0.3	0.05
0.9	0.99992	3	3	6	0.5	2.5	-2.0	0.3	0.3	0.05
0.9	0.91255	9	9	18	1.0	0.5	0.5	0.3	0.3	0.05
0.9	0.91255			0	1.0	1.0	0.0	0.3	0.3	0.05
0.9	0.91255	9	9	18	1.0	1.5	-0.5	0.3	0.3	0.05
0.9	0.97266	4	4	8	1.0	2.0	-1.0	0.3	0.3	0.05
0.9	0.99278	3	3	6	1.0	2.5	-1.5	0.3	0.3	0.05
0.9	0.97266	4	4	8	1.5	0.5	1.0	0.3	0.3	0.05
0.9	0.91255	9	9	18	1.5	1.0	0.5	0.3	0.3	0.05

0.9	0.91255			0	1.5	1.5	0.0	0.3	0.3	0.05
0.9	0.91255	9	9	18	1.5	2.0	-0.5	0.3	0.3	0.05
0.9	0.97266	4	4	8	1.5	2.5	-1.0	0.3	0.3	0.05

References

Julious, S. A. 2010. Sample Sizes for Clinical Trials. Chapman & Hall/CRC. Boca Raton, FL.

Chow, S. C., Shao, J., and Wang, H. 2008. Sample Size Calculations in Clinical Research (Second Edition).

Chapman & Hall/CRC. Boca Raton, FL.

Machin, D., Campbell, M., Fayers, P., and Pinol, A. 1997. Sample Size Tables for Clinical Studies, 2nd Edition. Blackwell Science. Malden, MA.

Zar, Jerrold H. 1984. Biostatistical Analysis (Second Edition). Prentice-Hall. Englewood Cliffs, New Jersey.

Report Definitions

Target Power is the desired power value (or values) entered in the procedure. Power is the probability of rejecting a false null hypothesis.

Actual Power is the power obtained in this scenario. Because N1 and N2 are discrete, this value is often (slightly) larger than the target power.

N1 and N2 are the number of items sampled from each population.

N is the total sample size, N1 + N2.

$\mu 1$ and $\mu 2$ are the assumed population means.

$\delta = \mu 1 - \mu 2$ is the difference between population means at which power and sample size calculations are made.

$\sigma 1$ and $\sigma 2$ are the assumed population standard deviations for groups 1 and 2, respectively.

Alpha is the probability of rejecting a true null hypothesis.

Summary Statements

Group sample sizes of NA and NA achieve 100.000% power to reject the null hypothesis of equal means when the population mean difference is $\mu 1 - \mu 2 = 0.5 - 0.5 = 0.0$ with standard deviations of 0.3 for group 1 and 0.3 for group 2, and with a significance level (alpha) of 0.050 using a two-sided two-sample unequal-variance *t*-test.

Two-Sample T-Tests Allowing Unequal Variance

Dropout-Inflated Sample Size

				Dropout-Inflated Enrollment ------Sample Size------			Expected Number of ------Dropouts------		
Dropout Rate	N1	N2	N	N1’	N2’	N’	D1	D2	D
20%			0						
20%	9	9	18	12	12	24	3	3	6
20%	4	4	8	5	5	10	1	1	2
20%	3	3	6	4	4	8	1	1	2
20%	3	3	6	4	4	8	1	1	2
20%	9	9	18	12	12	24	3	3	6
20%			0						
20%	9	9	18	12	12	24	3	3	6
20%	4	4	8	5	5	10	1	1	2
20%	3	3	6	4	4	8	1	1	2
20%	4	4	8	5	5	10	1	1	2
20%	9	9	18	12	12	24	3	3	6
20%			0						
20%	9	9	18	12	12	24	3	3	6
20%	4	4	8	5	5	10	1	1	2

Definitions

Dropout Rate (DR) is the percentage of subjects (or items) that are expected to be lost at random during the course of the study and from whom no response data will be collected (i.e. will be treated as "missing").

N1, N2, and N are the evaluable sample sizes at which power is computed. If N1 and N2 subjects are evaluated out of the N1' and N2' subjects that are enrolled in the study, the design will achieve the stated power.

N1', N2', and N' are the numbers of subjects that should be enrolled in the study in order to end up with N1, N2, and N evaluable subjects, based on the assumed dropout rate. After solving for N1 and N2, N1' and N2' are calculated by inflating N1 and N2 using the formulas N1' = N1 / (1 – DR) and N2' = N2 / (1 – DR), with N1' and N2' always rounded up. (See Julious, S.A. (2010) pages 52-53, or Chow,

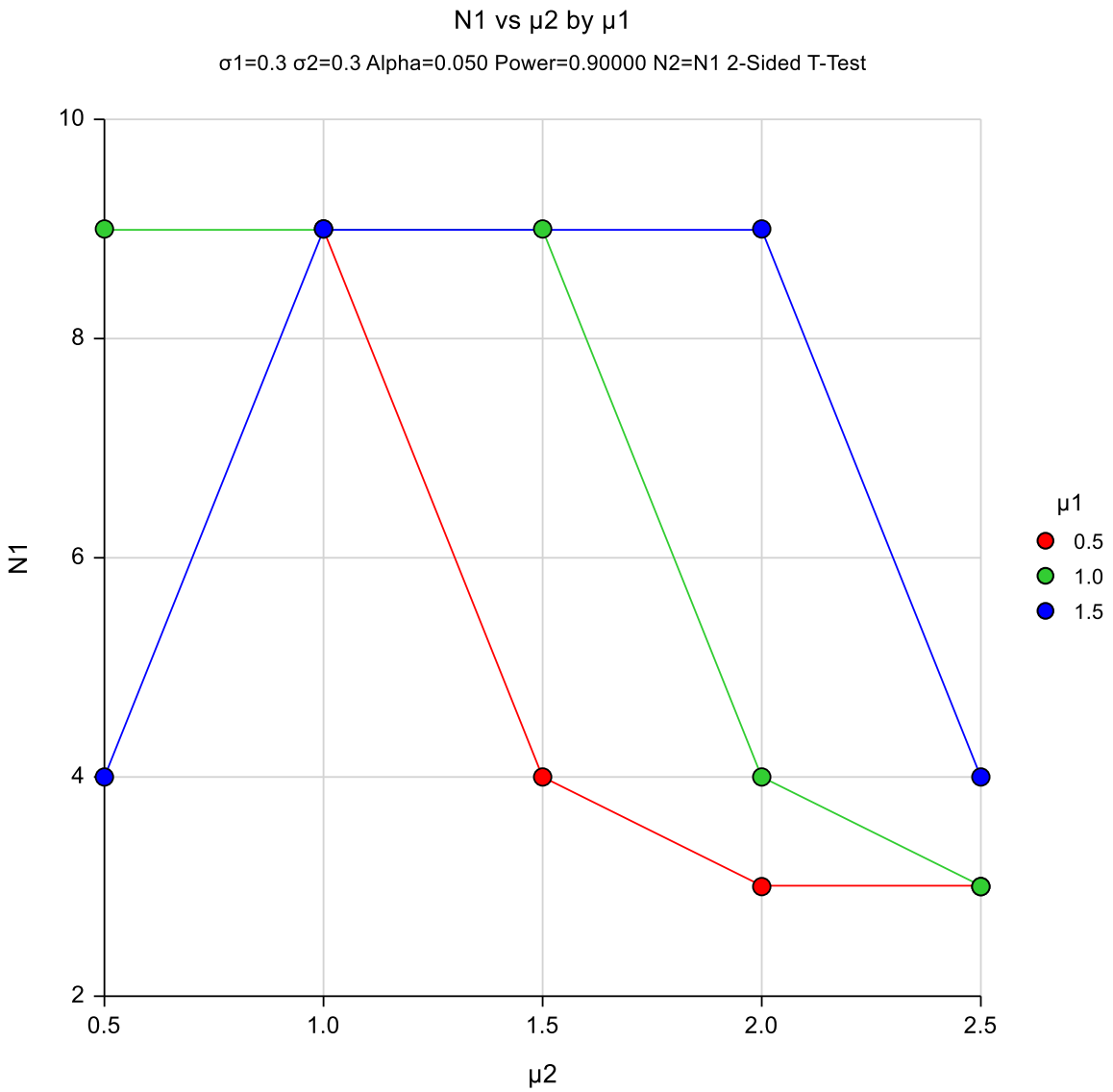
S.C., Shao, J., and Wang, H. (2008) pages 39-40.)

D1, D2, and D are the expected number of dropouts. D1 = N1' – N1, D2 = N2' – N2, and D = D1 + D2.

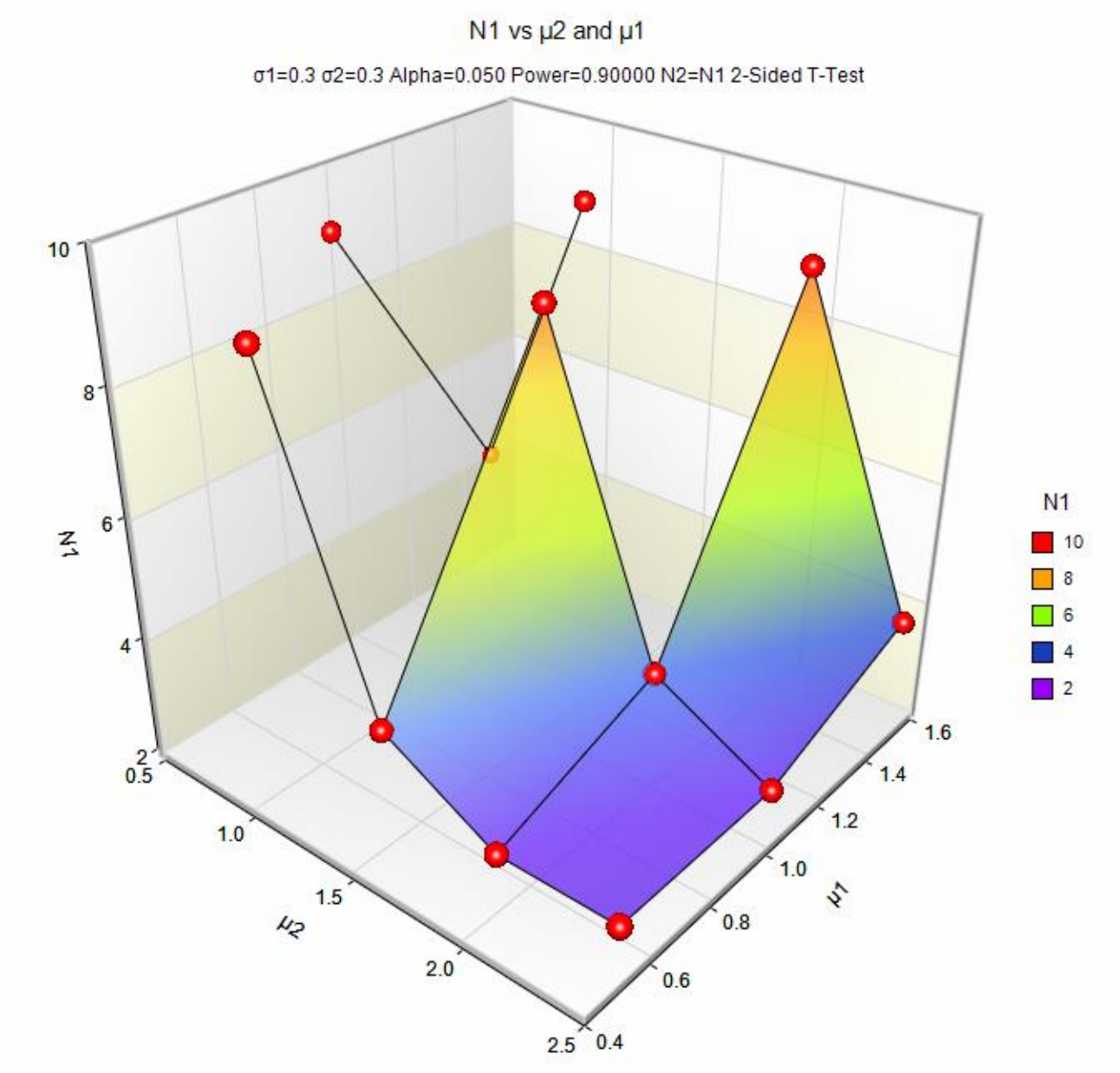
PASS 15.0.5

Two-Sample T-Tests Allowing Unequal Variance

Chart Section



Two-Sample T-Tests Allowing Unequal Variance



Two-Sample T-Tests Allowing Unequal Variance

Procedure Input Settings

Autosaved Template File

T-Tests Allowing Unequal Variance - Autosaved 2022_3_16-15_17_16.t389

Design Tab

Solve For:	Sample Size
Alternative Hypothesis:	Two-Sided
Power:	0.9
Alpha:	0.05
Group Allocation:	Equal (N1 = N2)
Input Type:	Means
μ1:	0.5 to 1.5 by 0.5
μ2:	0.5 to 2.5 by 0.5
σ1:	0.3
σ2:	0.3

The power for predicting the sample size of experimental results has been calculated for statistical analysis. According to previous relevant studies and our study of rotarod, the standard deviation (σ) is usually <0.3, the mean of the control group is usually between 0.5 and 1.5, and the mean of experimental group is usually between 0.5 and 2.5. The power is designed at 90%, which is considered to be largely valid. From the results of power calculation (by PASS, NCSS Inc.), our experiments satisfy the minimum sample size.